Caloric Restriction and Intervention in Pancreatic Carcinogenesis in the Rat

B. D. Roebuck, Karen J. Baumgartner, and Denise L. MacMillan

ABSTRACT

In two experiments, the effects of caloric restriction during the postinitiation phase of pancreatic carcinogenesis were evaluated. Male Lewis rats were given injections of azaserine at 14 days of age and weaned to the postinitiation test protocols at 21 days of age. In the first experiment, the caloric content of the diets was restricted by 10, 15, 20, and 30% of the intakes of the ad libitum-fed rats. A sixth group was fed diet ad libitum for only 5-6 h/day; i.e., they were “meal-fed.” The development of putative preneoplastic lesions (henceforth termed foci) was evaluated by quantitative stereological (morphometric) analysis of the pancreas. Caloric restriction during the 4-month postinitiation phase resulted in a significant reduction in focal development beginning at 10% caloric restriction and increasing with more severe restriction. The caloric intake of the meal-fed group closely matched the caloric intake of the 10 or 15% caloric restriction groups and the focal response of the meal-fed rats was similar to the groups restricted in calories by 15 to 20%. In the second experiment, rats were initiated with azaserine and weaned to one of four groups: ad libitum; meal-fed; meal-fed for 2 months and ad libitum thereafter; or ad libitum for 2 months and meal-fed thereafter. Foci were evaluated at 2 and 4 months; neoplasm incidence and multiplicity were determined at 14 months postinitiation. Compared to the ad libitum group, the meal-fed group had significantly fewer foci at all times of evaluation and significantly fewer neoplasms. When rats were meal fed for 2 months and then switched to ad libitum feeding for the remainder of the experiment, the focal outcome at 4 months was similar to the group meal fed for all 4 months; and at 14 months the neoplastic outcome was intermediate between the ad libitum and the meal-fed group. Intervention in the ad libitum feeding regimen at 2 months by meal feeding for the remainder of the experiment resulted in a significant decrease in the focal and neoplastic development, as compared to the group fed ad libitum continuously. These two intervention groups were intermediate in response between the meal-fed and ad libitum-fed groups. These results indicate that the postinitiation phase of pancreatic carcinogenesis can be modulated by relatively simple dietary interventions such as moderate caloric restriction.

INTRODUCTION

In the United States, pancreatic cancer ranks fifth for cause of death due to cancer with approximately 24,000 deaths in 1988 (1). Pancreatic cancer usually is diagnosed late in the course of disease development thus precluding effective treatment. The only strong epidemiological evidence of causation is an association with the smoking of cigarettes (2, 3). Considering the paucity of information regarding the causation of pancreatic cancer and the advanced nature of this disease at diagnosis, the prevention of pancreatic cancer is of utmost importance.

A quantitative model of pancreatic cancer has been developed in the rat by treatment with the carcinogen, azaserine (4, 5). Following a single dose of azaserine, putative preneoplastic lesions (henceforth called “foci”) are reliably evaluated by 4 months (5-7) and cancers arise by approximately 1 year (5). Foci are uncommon (5, 6) and cancers are rare (8, 9) in non-carcinogen-treated rats. It is possible to separate the initiation phase when azaserine is given from the postinitiation (promotion) phase of carcinogenesis (9). In general agreement with models of mammary cancer, high levels of dietary polyunsaturated vegetable fat but not similar levels of saturated fat enhance pancreatic carcinogenesis as compared with low levels of total dietary fat (4, 10). As little as 10% food restriction is sufficient to inhibit pancreatic cancer (8, 9).

The present studies were undertaken to evaluate the dose-response relationship for caloric restriction and pancreatic carcinogenesis. Additionally, we evaluated the effectiveness of intervening during the postinitiation phase of carcinogenesis by withdrawing or providing calories.

MATERIALS AND METHODS

Two experiments were undertaken. Using our short-term model of pancreatic carcinogenesis (4, 11), the effects of various degrees of caloric restriction on carcinogen-induced focal development were determined. Second, a relatively mild caloric restriction was imposed by meal feeding early or late in the postinitiation phase; the effects of this dietary restriction on both focal and cancer development were evaluated.

Pancreatic Carcinogenesis Model. Suckling male Lewis rats (Charles River Breeding Laboratories, Wilmington, MA) were given injections i.p. at 14 days of age of a single dose of 30 mg azaserine/kg body weight or a similar volume of 0.9% NaCl. Azaserine (Calbiochem-Behring Corp., La Jolla, CA) was dissolved in 0.9% NaCl solution. The dams were fed ad libitum AIN-76A diet (12, 13), but without the antioxidant ethoxyquin. The pups were weaned at 21 days of age to the experimental groups and to their respective diets of 20% polyunsaturated fat (corn oil). From weaning all rats were housed individually in wire mesh-bottomed cages in an environmentally controlled facility with a 12-h light/12-h dark photoperiod. Except for the meal-fed groups indicated below, food and water were available ad libitum. All rats were weighed weekly during the first 4 months of the postinitiation phase and biweekly thereafter. At autopsy the entire pancreas was excised, fixed in Bouin’s fixative for 4 h, and transferred to a solution of 70% ethyl alcohol and 5% potassium acetate, completely embedded in paraffin by a standardized method for routine histology, sectioned, and stained with hematoxylin and eosin for light microscopy. From each pancreas, sections of approximately equal tissue area were cut such that all anatomical regions of the pancreas were represented. Quantitative stereological comparisons used splenic regions of the pancreases, representing approximately 150 mm² tissue area per rat. All other pancreatic slides were examined by light microscopy but were not analyzed by quantitative stereology. Additionally, all grossly abnormal tissues were excised for histopathological analysis.

At 2 and 4 months following azaserine initiation, pancreases were examined for putative preneoplastic, acinar cell lesions (i.e., foci). These acinar cell foci were identified and classified as acidophilic or acanthoplastic phenotype in accord with criteria of Rao et al. (14) and Roebuck et al. (15). Focal transactional areas were measured with a semiautomatic image analysis system (7). From the observed number and area of the focal transactions, the mean number and mean diameter of the foci were determined by the quantitative stereological methods of Pugh et al. (16) as applied to the pancreas (7). The focal volume % of pancreas occupied by foci is analogous to tumor burden and represents the most reliable data regarding the growth of foci (16).

At 14 months following azaserine, adenomas and adenocarcinomas of the pancreas were classified by the criteria of Longnecker (17), and their incidence and multiplicity were determined.

Calorie Restriction, Meal Feeding, and Focal Development. The effects of various degrees of calorie restriction on focal development following azaserine treatment were evaluated in 6 groups of 10 rats/group. Groups were restricted 10, 15, 20, and 30%, compared to the ad libitum-fed group. The mean food consumption of the ad libitum group was determined weekly by measuring food intakes for 2 successive 24-h periods. The diets of the 4 food restricted

Received 7/22/92; accepted 10/21/92.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by a grant from Best Foods/CPC International.

2 To whom requests for reprints should be addressed, at Department of Pharmacology and Toxicology, Dartmouth Medical School, 7650 Remsen, Room 522, Hanover, NH 03755-3835.
groups were formulated such that vitamins, minerals, protein, and fiber intake were approximately the same as the ad libitum group (Table 1). These adjustments were made at the expense of small changes in the carbohydrate levels (starch and sucrose). A meal-fed group (the sixth group) was allowed to eat the control diet ad libitum for between 5.5 and 6.0 h/day during the first 4 weeks of the postinitiation phase; thereafter, food was available for 5 h/day. Food was presented during the last segment of the dark cycle and removed when the light cycle began. Food consumption in the meal-fed group was monitored weekly as described for the ad libitum rats above. Meal feeding represented a convenient and practical method for instituting a 10 to 15% restriction in food intake and consequently calorie intake.

Intervention in Pancreatic Carcinogenesis. The effects of imposing food restriction early or later post-azaserine initiation were examined. This experimental protocol is schematically illustrated in Fig. 1. Based upon the results from experiment 1 above, meal feeding afforded a mild degree of caloric restriction. Therefore, groups were either fed ad libitum or a meal.

Analysis of Body Composition. Ten rats from each of the ad libitum group and the meal-fed group of experiment 1 were analyzed. At autopsy, carcasses were shipped in dry ice to Nutrition International, Inc., Dayton, NJ. The carcass was defined as the entire body minus the gastrointestinal tract, head, tail, and the entire pancreas. Proximate analysis was conducted by standard methods of the Association of Official Agricultural Chemists. Briefly, the water content was determined by lyophilization, followed by chemical assay of total fat and protein. Total ash was calculated gravimetrically following thermal decomposition at >500°C.

Statistical Analyses. The statistical tests used are identified in the text and footnotes to the figures and tables.

RESULTS

Calorie Restriction and Focal Development. The imposition of food restriction or “meal feeding” (ad libitum feeding limited to a specified number of hours per day) affected the growth rate of the rats (Fig. 2). The rate of growth was proportional to the degree of caloric restriction. As little as 10% restriction in calories led to suppression of growth of the rats which was observed as early as the third week of the postinitiation phase (P < 0.05, Student’s t test). Over the 4-month postinitiation period, the meal-fed rats averaged 16.3 ± 0.4 (SE) g food/day compared to 18.5 ± 0.3 g food/day for the ad libitum-fed rats. Over the entire experimental period, meal feeding represented a 12% restriction in food intake. Calorie-restricted rats regularly consumed all the food presented to them. The rats restricted to greater degrees than the 10% calorie restricted group ate all their food as a meal; i.e., they ate the food in the first few hours after its presentation.

As the degree of food restriction increased, body weights and organ weights became increasingly affected (Table 2). Restriction by as little as 10% significantly affected the final body weight [P < 0.05 (Table 2)]. For all calorie-restricted groups and the meal-fed rats, pancreases were significantly smaller than in the ad libitum group. Compared to the ad libitum-fed rats, perirenal fat depots were smaller at 15% calorie restriction, and epididymal fat depots showed significant decreases in weight at 10% restriction. Meal-fed rats compared most closely to the 15 or 20% calorie-restricted groups with respect to body weight and weights of these fat depots.

Few basophilic foci were observed. The mean number observed was 4.7/cm² pancreas and their mean size was 0.025 mm² in transsectional area. Because of the small numbers of basophilic foci, the quantitative stereological transformations to yield number of foci/cm³ and mean focal diameter cannot reliably be calculated (15); however, the volume % can still be calculated and evaluated (data not shown). This parameter was 5- to 30-fold smaller than for the acidophilic foci and was not consistently related to the dietary treatments. These observations on basophilic foci are similar to previous experiments (15, 18). Basophilic foci have a low growth potential (5, 14, 15, 18).

Table 1: Composition of diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Ad Libitum and meal-fed groups</th>
<th>Restricted groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td>Casein</td>
<td>23.47</td>
<td>26.08</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.35</td>
<td>0.39</td>
</tr>
<tr>
<td>Corn starch</td>
<td>10.34</td>
<td>9.44</td>
</tr>
<tr>
<td>Sucrose</td>
<td>34.47</td>
<td>31.46</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.86</td>
<td>6.51</td>
</tr>
<tr>
<td>Corn oil</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Vitamins/minerals</td>
<td>5.51</td>
<td>6.12</td>
</tr>
<tr>
<td>Gross energy (kcal/g)</td>
<td>4.55</td>
<td>4.49</td>
</tr>
</tbody>
</table>

* The amounts are expressed as percentage by weight, with the exception of gross energy which is expressed as kcal/g.
CALORIC RESTRICTION INHIBITS PANCREATIC CANCER

Table 2 Effects of caloric restriction and meal feeding on body weight of rats and on organ weights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Body wt (g)</th>
<th>Pancreas wt (g)</th>
<th>Perirenal fat pads (g)</th>
<th>Epididymal fat pads (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>8</td>
<td>498 ± 12b</td>
<td>1.11 ± 0.03b</td>
<td>11.3 ± 0.5b</td>
<td>6.7 ± 0.3b</td>
</tr>
<tr>
<td>10%</td>
<td>9</td>
<td>473 ± 4c</td>
<td>0.97 ± 0.02c</td>
<td>11.3 ± 0.4b</td>
<td>5.4 ± 0.2c</td>
</tr>
<tr>
<td>15%</td>
<td>10</td>
<td>441 ± 4d</td>
<td>0.97 ± 0.02c</td>
<td>8.4 ± 0.3c</td>
<td>4.1 ± 0.2c</td>
</tr>
<tr>
<td>20%</td>
<td>10</td>
<td>420 ± 4c</td>
<td>0.99 ± 0.02c</td>
<td>7.7 ± 0.4c</td>
<td>3.6 ± 0.1c</td>
</tr>
<tr>
<td>30%</td>
<td>10</td>
<td>358 ± 3f</td>
<td>0.92 ± 0.01c</td>
<td>2.5 ± 0.3d</td>
<td>1.7 ± 0.1c</td>
</tr>
<tr>
<td>Meal fed</td>
<td>9</td>
<td>432 ± 5g</td>
<td>0.95 ± 0.02c</td>
<td>8.8 ± 0.3c</td>
<td>4.1 ± 0.2c</td>
</tr>
</tbody>
</table>

- Rats were autopsied at 4 months postinitiation. Data are presented as mean ± SE. For each column of data, values with the same superscript are not statistically different at P = 0.05 (analysis of variance followed by Newman-Keuls multiple comparison test).

and have not been related to the development of cancers of the pancreas; therefore, they will not be discussed further.

The observed acidophilic focal data (Table 3) must be transformed by stereological methods before statistics can be applied (16); the transformed data are presented in Fig. 3. Both the focal number per cm³ (Fig. 3A) and the mean focal diameter (Fig. 3B) of the acidophilic foci decreased with calorie restriction (P < 0.05, linear regression). The small but significant decrease in each of these two parameters results in a large and obvious effect of calorie restriction on volume % (P < 0.0001, linear regression) as illustrated in Fig. 3C. On the basis of the volume %, the meal-fed group was most similar to the 15 or 20% calorie restriction. As mentioned previously, the meal-fed group exhibited a 12% restriction in food intake.

Focal volume % is the most robust morphometric parameter for study of the modulation of focal growth (2). Correlation (Pearson product moment correlation coefficient) of this parameter with the body weight was high (r = 0.676, P < 0.01). Equally strong, volume % correlated with perirenal (r = 0.637, P < 0.01) and epididymal fat pads weights (Fig. 3; Table 2). Body compositional analyses were undertaken for the meal-fed and ad libitum-fed groups and correlation of focal volume % (i.e., burden of foci) with carcass fat content (g) was significant (r = 0.498, P < 0.05). Fat pad weights were correlated with total body fat from body compositional analysis; the correlation was 0.735 (P < 0.01) for perirenal fat pads and 0.784 (P < 0.01) for epididymal fat pads. In summary, pancreatic focal burden as expressed by volume % correlated with total body weight, the weights of each of the fat pads, and total carcass fat content.

Intervention in Pancreatic Carcinogenesis by Meal Feeding.

The protocol for this second experiment is given in Fig. 1. Calorie-restricted rats eat their limited food as a meal (see above and Refs. 8 and 9). In this second experiment, we fed rats meals (ad libitum food for 5 to 6 h/day) and achieved a 10% restriction in food intake during the first 2 months of the postinitiation phase; during the second 2 months we achieved a 13% restriction. This closely matched the 12% reduction in food intake achieved over a full 4 months by meal feeding during the first 2 months of the postinitiation phase: during the second 2 months we achieved a 10% restriction. This closely matched the 12% restriction, the meal-fed group was most similar to the 15 or 20% calorie restriction. As mentioned previously, the meal-fed group exhibited a 12% restriction in food intake.

The observed acidophilic focal data (Table 3) must be transformed by stereological methods before statistics can be applied (16); the transformed data are presented in Fig. 3. Both the focal number per cm³ (Fig. 3A) and the mean focal diameter (Fig. 3B) of the acidophilic foci decreased with calorie restriction (P < 0.05, linear regression). The small but significant decrease in each of these two parameters results in a large and obvious effect of calorie restriction on volume % (P < 0.0001, linear regression) as illustrated in Fig. 3C. On the basis of the volume %, the meal-fed group was most similar to the 15 or 20% calorie restriction. As mentioned previously, the meal-fed group exhibited a 12% restriction in food intake.

Focal volume % is the most robust morphometric parameter for study of the modulation of focal growth (2). Correlation (Pearson product moment correlation coefficient) of this parameter with the body weight was high (r = 0.676, P < 0.01). Equally strong, volume % correlated with perirenal (r = 0.637, P < 0.01) and epididymal fat pads weights (Fig. 3; Table 2). Body compositional analyses were undertaken for the meal-fed and ad libitum-fed groups and correlation of focal volume % (i.e., burden of foci) with carcass fat content (g) was significant (r = 0.498, P < 0.05). Fat pad weights were correlated with total body fat from body compositional analysis; the correlation was 0.735 (P < 0.01) for perirenal fat pads and 0.784 (P < 0.01) for epididymal fat pads. In summary, pancreatic focal burden as expressed by volume % correlated with total body weight, the weights of each of the fat pads, and total carcass fat content.

Table 3 Effect of caloric restriction and meal feeding on acidophilic foci

<table>
<thead>
<tr>
<th>Treatment (% of restriction)</th>
<th>No/cm²</th>
<th>Mean area (100 x mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>16.9 ± 1.1b</td>
<td>13.4 ± 1.7</td>
</tr>
<tr>
<td>10%</td>
<td>18.7 ± 1.8</td>
<td>9.5 ± 0.7</td>
</tr>
<tr>
<td>15%</td>
<td>12.7 ± 1.4</td>
<td>8.6 ± 1.1</td>
</tr>
<tr>
<td>20%</td>
<td>11.7 ± 1.3</td>
<td>8.8 ± 0.6</td>
</tr>
<tr>
<td>30%</td>
<td>7.6 ± 0.9</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>Meal fed</td>
<td>15.4 ± 1.8</td>
<td>7.6 ± 0.6</td>
</tr>
</tbody>
</table>

- A single transection from the splenic section of the pancreas, representing approximately 150 mm² of tissue, was examined from each rat.
- Mean ± SE.

At 2 months postinitiation, meal-fed rats had significantly decreased body weights, pancreatic weights, and fat depots as compared to ad libitum-fed rats (Table 4). Comparing ad libitum- and meal-fed rats at 4 months, the same trends were observed, and the magnitude of the differences were greater than at 2 months. Evaluated at 4 months into the postinitiation phase, intervention by meal feeding during the first 2 months had no significant effect on the weight of the rats or on the organ weights when compared to ad libitum feeding. In other words, rats restricted during the first part of the postinitiation phase and fed ad libitum thereafter “caught up” to rats fed ad libitum for the entire 4 months with respect to body weight, pancreatic weight, and fat depots. However, if ad libitum-fed rats were meal fed during the second half of the 4-month postinitiation phase, these parameters were generally similar to rats meal fed for the entire 4 months. These same relationships between groups were observed when comparing these parameters at 14 months or at 4 months. To summarize for parameters...
The observed acidophilic focal data are presented in Table 5. Basophilic foci were small in number and size and will not be discussed. Acidophilic foci of the pancreas were observed in the saline-treated rats examined at 4 and 14 months postinitiation. The number and focal transectional size of acidophilic foci increased from 4 to 14 months, but as will be discussed below, the acidophilic foci in azaserine-treated rats were so numerous as to preclude the counting and sizing of them at 14 months. In saline-treated rats, there were few foci. The volume % of acidophilic foci for the saline-treated rats was 0.011 ± 0.006 at 4 months and is 100-fold smaller than the focal burden in the azaserine-treated rats. By 14 months in saline-treated rats, the acidophilic foci had grown in size, yet their volume % was 0.711 ± 0.354 and still was smaller than it was for the azaserine-treated rats at 4 months.

Fig. 5 presents the resultant acidophilic focal data from the quantitative stereological transformation of the observed data of Table 5. Effects of meal feeding on the acidophilic foci were observed as early as 2 months with a significantly smaller number of foci per cm² of pancreas. Continuous meal feeding for 4 months postinitiation, there was a significant decrease in the number and volume % of acidophilic foci as compared to ad libitum-fed rats. Continuous meal feeding resulted in a smaller mean focal size of acidophilic foci; however, this reduction in size was not statistically significant. Compared to the ad libitum-fed group, meal feeding either in the first 2 months (“meal/ad lib” group) or in the second months (“ad lib/meal” group) of the 4-month postinitiation period resulted in a significant reduction in the number and volume % of acidophilic foci. There was no effect on the mean size of the foci. Intervention had the same effect on focal development irrespective of whether it was initiated early or late in the postinitiation phase. This is in contrast to the lack of a significant effect of early meal feeding on the growth of the rats (Table 4).

As compared to the ad libitum group, food restriction by meal feeding significantly reduced the incidence of both adenomas and carcinomas of the pancreas (Table 6). Importantly, there were no carcinomas in the meal-fed group. The mean number of adenomas per tumor-bearing rat (i.e., multiplicity of neoplasms) was reduced 8-fold in the meal-fed group as compared to the ad libitum-fed group and this decrease was significant.

Compared to the ad libitum group, if rats were meal-fed for only the first 2 months of the 14-month postinitiation phase and fed ad libitum

---

Table 4 Effects of intervention by meal feeding during postinitiation on size of rats and organ weights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Body wt (g)</th>
<th>Pancreas wt (g)</th>
<th>Perirenal fat pads (g)</th>
<th>Epididymal fat pads (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mo, azaserine b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>10</td>
<td>340 ± 7 ü</td>
<td>0.83 ± 0.03 ü</td>
<td>9.6 ± 0.5 ü</td>
<td>3.8 ± 0.2 ü</td>
</tr>
<tr>
<td>Meal</td>
<td>10</td>
<td>296 ± 6 ü</td>
<td>0.71 ± 0.02 ü</td>
<td>5.8 ± 0.4 ü</td>
<td>3.0 ± 0.1 ü</td>
</tr>
<tr>
<td>4 mo, azaserine c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>10</td>
<td>501 ± 12 ü</td>
<td>0.89 ± 0.03 ü</td>
<td>23.4 ± 1.1 ü</td>
<td>10.0 ± 0.6 ü</td>
</tr>
<tr>
<td>Meal/ad lib</td>
<td>10</td>
<td>482 ± 5 ü</td>
<td>0.85 ± 0.02 ü</td>
<td>22.6 ± 0.5 ü</td>
<td>9.0 ± 0.3 ü</td>
</tr>
<tr>
<td>Ad lib/meal</td>
<td>10</td>
<td>432 ± 7 b</td>
<td>0.93 ± 0.02 b</td>
<td>12.8 ± 0.8 b</td>
<td>5.7 ± 0.3 b</td>
</tr>
<tr>
<td>Meal</td>
<td>10</td>
<td>433 ± 5 b</td>
<td>0.77 ± 0.03 b</td>
<td>14.5 ± 0.4 b</td>
<td>6.4 ± 0.2 b</td>
</tr>
<tr>
<td>4 mo, saline d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>19</td>
<td>488 ± 8 ü</td>
<td>0.86 ± 0.02 ü</td>
<td>22.3 ± 0.9 ü</td>
<td>9.5 ± 0.4 ü</td>
</tr>
<tr>
<td>14 mo, azaserine e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>23</td>
<td>809 ± 16 ü</td>
<td>2.07 ± 0.14 ü</td>
<td>48.6 ± 1.7 ü</td>
<td>16.7 ± 0.7 ü</td>
</tr>
<tr>
<td>Meal/ad lib</td>
<td>23</td>
<td>813 ± 13 ü</td>
<td>1.84 ± 0.05 ü</td>
<td>46.8 ± 1.7 ü</td>
<td>16.6 ± 0.3 ü</td>
</tr>
<tr>
<td>Ad lib/meal</td>
<td>24</td>
<td>583 ± 8 ü</td>
<td>1.32 ± 0.07 ü</td>
<td>23.3 ± 0.7 ü</td>
<td>10.7 ± 0.3 ü</td>
</tr>
<tr>
<td>Meal</td>
<td>22</td>
<td>575 ± 6 ü</td>
<td>1.25 ± 0.05 ü</td>
<td>22.8 ± 0.6 ü</td>
<td>10.3 ± 0.3 ü</td>
</tr>
<tr>
<td>14 mo, saline d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>24</td>
<td>807 ± 11 ü</td>
<td>1.35 ± 0.02 ü</td>
<td>47.5 ± 1.3 ü</td>
<td>17.0 ± 0.4 ü</td>
</tr>
</tbody>
</table>

a Mean ± SE. Values with the same superscript are not statistically different at P = 0.05.
b Two month ad libitum and meal values were compared by t test.
c Four- and 14-month azaserine groups were analyzed by analysis of variance followed by a Newman-Keuls multiple comparison test.
d Four and 14-month saline ad libitum groups were compared to their respective (same age) azaserine ad libitum groups by t test.
Therefore, one can conclude that caloric restriction during the pos-
initiation phase of pancreatic carcinogenesis is inhibitory for that
major factor that decreased when food was restricted (see Table 1).

Caloric restriction by meal feeding of control diet for 5 to 6 h/day.

The relationship of focal volume % to the degree of caloric restric-
tion is interesting in that pancreatic carcinogenesis is remark-
ably sensitive to restriction (Fig. 3C). Even mild degrees of caloric restric-
tion such as 10% have significant effects on the focal volume %, yet
only modest and variable effects on the growth of the rat (Fig. 2; Table
2). Previously, we reported that food restriction of about 10% inhib-
ited pancreatic cancer in long term studies (8, 9).

Few experiments have been undertaken detailing the sensitivity of
other model systems to caloric restriction. In the azoxymethane-F344
rat model of colon cancer and when feeding diets of similar high

<table>
<thead>
<tr>
<th>Treatment (%) of restriction</th>
<th>Observed transectional data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./cm²</td>
</tr>
<tr>
<td>2 mo, azaserine Ad libitum</td>
<td>5.8 ± 1.6</td>
</tr>
<tr>
<td>Meal</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>4 mo, azaserine Ad libitum</td>
<td>19.4 ± 2.1</td>
</tr>
<tr>
<td>Meal/ad lib</td>
<td>12.5 ± 1.8</td>
</tr>
<tr>
<td>Ad lib/meal</td>
<td>10.9 ± 2.1</td>
</tr>
<tr>
<td>4 mo, saline Ad libitum</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>

14 mo, azaserine Ad libitum | 1.9 ± 0.3 | 35.5 ± 12.5 |

a Mean ± SE. A single transection from the splenic section of the pancreas, represent-
ing approximately 140 mm² of tissue, was examined from each rat.

b Foci were too numerous to count in the 14-month azaserine-treated rats.

c Foci in this group were of a size comparable to that of foci in other saline-treated rats
at 14 months of age (5) and one-third the size of the foci of similar azaserine-treated rats
(5).

**DISCUSSION**

In previous experiments (8, 9), we had shown that reduced food
intake (underfeeding) resulted in fewer pancreatic cancers. In our
early experiments, we simply fed less of the same diet as was fed to
the ad libitum group. This method of restriction is subject to the
criticism that by restricting the total quantity of food available, the rats
are simultaneously restricted to not only fewer calories but also fewer
vitamins, minerals, and other nutrients. Thus, the effects of simple
food restriction on carcinogenesis could be the result of the numerous
and simultaneous decreases in nutrients. Diets of our first experiment
reported herein were constructed such that the caloric content was the
major factor that decreased when food was restricted (see Table 1).
Therefore, one can conclude that caloric restriction during the pos-
initiation phase of pancreatic carcinogenesis is inhibitory for that
process. We have made similar observations regarding the similarity
of outcome between food restriction and caloric restriction using this
same model (7). Similar comparisons and conclusions have been
made for food restriction (underfeeding) and caloric restriction ex-
periments on skin, colon, and mammary carcinogenesis as reviewed by
Visek et al. (19, 20). Because of these similarities, the use of meal
feeding where meals represented restricted quantities of daily food
intake as a practical method to institute caloric restriction seems
justified. In both experiments presented here, we achieve 10 to 15%
caloric restriction by meal feeding of control diet for 5 to 6 h/day.

![Fig. 5. Acidophilic foci as affected by intervention by meal feeding. □, data at 2
months; ♦, at 4 months postinitiation.](image)

**Table 6 Effect of intervention by meal feeding on neoplasms at 14 months
postinitiation**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence (%)</th>
<th>Tumors/TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>100(23/23)c</td>
<td>8.04 ± 0.99c</td>
</tr>
<tr>
<td>Meal/ad lib</td>
<td>91(21/23)c</td>
<td>3.52 ± 0.50d</td>
</tr>
<tr>
<td>Ad lib/meal</td>
<td>37 (9/24)d</td>
<td>1.78 ± 0.43d</td>
</tr>
<tr>
<td>Meal</td>
<td>23 (5/22)d</td>
<td>1.00 ± 0.43d</td>
</tr>
<tr>
<td>None</td>
<td>0 (0/22)</td>
<td>None</td>
</tr>
</tbody>
</table>

a Mean ± SE. Values with the same superscript were not statistically different at
P = 0.05. Incidence data were compared by Fisher's exact test. Tumors per tumor-bearing
animal (numbers in parentheses) were compared by analysis of variance followed by a
Bonferroni multiple comparison test.

b Tumors/TBA, number of adenomas or carcinomas per tumor-bearing animal.
levels of corn oil as in our experiments, Kumar et al. (21) showed that inhibition of colonic adenomas occurred only at 20% restriction and cancers were insensitive to caloric restriction at even 30% restriction. Previously, Reddy et al. (22) had shown that 30% caloric restriction inhibited colonic carcinogenesis significantly with a 30% to 50% reduction in tumor incidence and a 4-fold reduction in multiplicity of tumors. In the DMBA-rat model of mammary cancer, Klurfeld et al. (23) found that caloric restriction significantly inhibited only with 30% and greater caloric restriction. Feeding high fat diets similar to ours, Birt et al. (24) found that a 25% reduction in the caloric intake moderately inhibited pancreatic carcinogenesis of the ductular cells of the hamster pancreas. The effects of the degree of caloric restriction on cellular proliferation of colonic and mammary tissue in non-carcinogen-treated mice have been determined (25). Lok et al. (25) found that at 20% caloric restriction and greater, but not at 10% restriction, cellular proliferation as assessed by \(^{3}H\) thymidine labeling indices was significantly suppressed. It remains to be determined if pancreatic cell proliferation is more sensitive to caloric restriction than mammary or colonic tissue. Interestingly, the exocrine pancreas seems to be more sensitive to dietary modulation of carcinogenesis than the colon, mammary gland, or ductular cells of the pancreas. Intestinal cells release cholecystokinin, a trophic hormone for the pancreas, in response to the quality and quantity of food present. Brand and Morgan (26) have shown that food deprivation leads to a rapid decrease in plasma cholecystokinin and pancreatic DNA synthesis. Upon refeeding, these parameters return to previous values. This sensitive effect may be specific to the exocrine pancreas, but at higher levels of caloric restriction other mechanisms involving energy intake, energy retention, and body size may be operative (27–30).

Because of the long latent period from exposure to a carcinogen to the actual development of cancers, there is opportunity to intervene. In the N-nitroso-N-methylurea-rat model of mammary cancer, Dao and Chan (31) have shown that mammary cancer incidence increased directly with the length of the time that a diet high in unsaturated fat was fed and inversely to the time a diet low in fat was fed. Ip and Ip (32) fed a high fat diet and, at various times up to 6 weeks following initiation with DMBA, groups of rats were transferred to a low fat diet. They found that the extent of inhibition of mammary carcinogenesis increased with earlier transfer to low fat diets. Additionally, Ip (33) showed that the transfer of DMBA-initiated rats fed a low fat diet for 24 weeks to a high fat diet for 20 additional weeks significantly increased the development of mammary tumors as compared to those rats remaining on the low fat diets. In concept, these experiments involving dietary intervention are similar to ours (see Fig. 1) and they indicate that the postcarcinogenic processes are sensitive to dietary interventions.

We have reported other similar intervention experiments using the azaserine-rat model of pancreatic carcinogenesis. As with the experiments reported herein, the postinitiation phase of 4 months was divided into two 2-month segments and focal development was evaluated at the end of 4 months. A switch of dietary fat from 20% to 5% unsaturated fat (34) or a switch from 20% corn oil to 20% menhaden oil (35, 36) resulted in a significant inhibition in the development of pancreatic foci. For example, if during the latter half of the postinitiation phase a switch from corn oil to menhaden oil is made, the development of foci is significantly arrested. We now show that meal feeding (which reduces caloric intake by about 10 to 15%) during the latter half of the postinitiation phase resulted in significant inhibition in focal development. If continued until cancers developed (14 months) in the \textit{ad libitum}-fed rats, intervention by meal feeding resulted in the inhibition of pancreatic cancer.

Of particular interest was the observation that meal feeding during the first 2 months followed by \textit{ad libitum} feeding resulted in a significant inhibition in focal development at 4 months (Fig. 5). There was a significant decrease, as compared to the \textit{ad libitum} group, in the incidence of adenocarcinomas at 14 months if rats were meal fed for only the first 2 months. Importantly, the growth of these rats was not inhibited as with more extensive periods of meal feeding. A reduction in cancer was achieved without inhibiting the ultimate growth of the rats.

ACKNOWLEDGMENTS

We thank Dr. Daniel Freeman, Director of Shared Statistical Services of the Norris Cotton Cancer Center, Dartmouth Medical School, for statistical consultation. We thank Dr. Anthony Tagliaferro of the University of New Hampshire for critical comments. We thank Drs. David H. Bechtel, Mark A. Bieber, and Robert E. Landers of Best Foods, CPC International, Inc., for advice in design and execution of these experiments.

REFERENCES

Caloric Restriction and Intervention in Pancreatic Carcinogenesis in the Rat

B. D. Roebuck, Karen J. Baumgartner and Denise L. MacMillan


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/53/1/46

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.