Effects on C3H Mouse Mammary Cancer of Changing from a High Fat to a Low Fat Diet before, at, or after Puberty

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ABSTRACT

A low fat/low calorie (LF/LC) diet is a relative inhibitor of murine mammary cancer. Because the mammary gland may be most sensitive to the action of a carcinogen at or near the age of puberty, we studied whether beginning a LF/LC diet before puberty would decrease C3H/OuJ mouse mammary cancer incidence more than if such a diet was begun at or after puberty. We also studied whether the advancement of the age of puberty by a high fat/high calorie (HF/HC) diet would in itself be a mammary cancer risk factor and whether dietary fat levels would affect mammary cancer metastases.

In the first study, mice were changed from a HF/HC diet to a LF/LC diet at 12 days of age, puberty, or 60 days of age. Other groups were always HF/HC or always LF/LC. In a second experiment, mice were fed a high fat diet for the same length of time, i.e., from 21 to 75 days of age (before and through puberty) or 75 to 129 days of age (well after puberty).

In one aspect of the first study, puberty was advanced by feeding a HF/HC diet until the first day of puberty. However, tumor latency, incidence, and multiplicity were not statistically different from those of the LF/LC control. Other results of the first study indicated, in general, that mice consuming a LF/HC diet beginning at or before puberty had a longer tumor latency and a lower tumor incidence and multiplicity than mice either beginning a LF/LC diet at 60 days of age or continuously fed a HF/HC diet. Lung metastases were greater in mice fed a HF/HC diet continuously compared to LF/LC continuously. In the second study, beginning the high fat diet before or after puberty did not result in statistically significant differences in tumor latency, incidence, or multiplicity.

It was concluded that the longer a LF/LC diet was fed, the lower was the mammary cancer risk. An early puberty in itself was not a mammary cancer risk factor and mouse puberty had no particular significance as an age before which a LF/LC diet should begin.

INTRODUCTION

Many studies, epidemiological in humans (1–5) and experimental in mice (6–10) and rats (11–17), have strongly suggested that there is a mammary tumor promoting effect of diets high in total fat (e.g., approximately 20% fat by weight, which is similar to the American diet), as compared to diets low in total fat (approximately 5% fat). Other studies, again epidemiologically in humans (18–20) and experimental in rats (21–25), suggest that the mammary gland may be most sensitive to the tumor initiating action of a carcinogen at or near the age of puberty.

There is currently no information as to whether there is an optimal age at which to start feeding a LF diet. We hypothesized that if the mammary gland is most sensitive to the action of a carcinogen near the age of puberty, by changing from a HF to LF diet before puberty, we would decrease the subsequent tumor incidence more than if the change were made after puberty.

Epidemiological studies have also indicated that the risk of premenopausal and/or postmenopausal mammary cancer is greater in women who have an early menarche as compared to those with a late menarche (26–28). We wished to determine if the advancement of puberty in the mouse by high levels of dietary fat was, in itself, a mammary cancer risk factor.

Finally, by using a mouse model, we wished to determine if lung metastases of a mouse mammary adenocarcinoma could be affected by differing levels of dietary fat.

MATERIALS AND METHODS

Experiment 1. The study was performed in two experiments. In the first, 120 pregnant C3H/OuJ mice carrying the mammary tumor virus were obtained commercially (The Jackson Laboratory, Bar Harbor, ME). The virus is subsequently transmitted to their offspring when they suckle. Upon arrival at approximately 14 days of gestation, they were randomly assigned to either a HF or LF diet (Table 1), with one of every five dams being placed in the LF group. The dams were individually housed. When the pups were born, they were placed in one of five dietary groups (Table 2). Those pups whose dams were initially fed a LF diet were kept in the LF diet group. Other litters were randomly placed into one of the four following groups: Group 2 was changed from a HF to a LF diet at 12 days of age. This was 2 days past the age when baby mice begin to pick at solid food. Group 3 was changed from a HF to a LF diet at puberty. Group 4 was changed from a HF to a LF diet at 60 days of age (which is well past puberty) and the final group was fed a HF diet throughout their lives. As the number of animals born per litter differed, the number of animals per group also differed.

Pups were kept with their dams until they were 18 days of age. At that time male pups were also separated from females, although they were kept in the same room.

Puberty (first vaginal estrus) was determined by daily vaginal lavage using a small pipet inserted just inside the vagina. The collected cells were stained with new methylene blue and examined microscopically for the degree of cornification and other standard signs of estrus.

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Mice were sacrificed by carbon dioxide overdosage when moribund or when they reached 332 days of age (the age when approximately 20% of the animals were still surviving). A necropsy was performed and the lungs were inflated with 10% buffered formalin. In addition to grossly visible tumors, one section each of lung, liver, spleen, and kidney was examined histopathologically for possible metastases. The number of grossly visible lung metastases per animal was not counted; thus animals are reported as having or not having lung metastases.

Tumor free survival times (the time to the appearance of the first tumor) were evaluated by the Gehan-Wilcoxon test for product limit survival estimate (29). Tumor incidence was evaluated by the Bonferroni simultaneous confidence interval (30) and the average number of tumors per tumor bearing mouse by the Student-Newman-Keuls test (31). Presence or absence of metastases was evaluated by the analysis of variance and the χ² test.

Experiment 2. Twenty-five C3H/OuJ mice were fed commercial laboratory rodent pellets and placed on a HF diet at 21 days of age (approximately 1 week before puberty) and continued on that diet for 54 days. A second group that was fed a LF diet was placed on a HF fat diet at 75 days of age (well past puberty) and kept on that diet for 54 days. They were then given a LF diet. The study continued until the animals were 310 days of age or were sacrificed due to their moribund state. They were then given a LF/LC diet. The study continued until the day of first estrus and was then placed on a LF/LC diet. Other groups fell between these two extremes.

Effect of Feeding a HF/HC Diet for Differing Lengths of Time. Table 2 shows that the groups that consumed the HF/HC diet for no longer than the day of puberty (Groups 1, 2, and 3) also had the longest tumor latency, and there were no significant differences between them (P > 0.05). Group 4 consumed the HF/HC diet until 60 days of age and had a significantly shorter latency period than did the previous three groups, and Group 5, which consumed the HF/HC diet throughout the study, had the shortest latency period. The tumor incidence data in Table 2 show that the groups that consumed the HF/HC diet for no longer than the first day of puberty (Groups 1, 2, and 3) also had the lowest percentage of tumor bearing animals, and again they were not statistically different. The groups that consumed the HF/HC diet for the longest periods of time (until 60 days of age or continuously) had the highest percentage of tumor bearing animals and also were not statistically different from each other. Interestingly, Group 1, which consumed a LF/LC diet throughout the study, was not statistically different from the group that ate the HF/HC diet until 60 days of age.

When the number of tumors per tumor bearing mouse was studied, a similar pattern emerged. Those mice on the HF/HC diet for no longer than the initiation of puberty had the fewest average number of tumors per animal, and there were no statistical differences between these three groups (Table 2). The remaining two groups were not statistically different, having more tumors per mouse than the other groups.

Lastly, we found that there were no statistical differences between the groups of mice with metastases to the lungs when examined by an analysis of variance (Table 3). This is described below in more detail.

From these results it can be concluded that if a HF/HC diet is begun at an early age and continued beyond the age of puberty, there is, in general, an enhancement of carcinogenesis in the C3H/OuJ mouse. As a corollary, the data also imply that initiating the feeding of a LF/LC diet at or before the age of puberty and continuing it through the animal's life may have a relative inhibitory effect on subsequent tumor development.

Effect of Length of Time for Which a HF/HC Diet Is Fed versus Age at Which a HF/HC Diet Is Begun. In Experiment 1 there was no obvious stepwise change in the parameters studied relative to the length of time the diet was fed which would have indicated that the length of time that the diet was fed was an important factor in its ability to promote mammary carcinogenesis. Therefore, another possibility to consider is that there is a tumor promoting effect on the developing mammary gland when a HF/HC diet is fed during puberty. Experiment 2 helped clarify this issue. In Experiment 2 a HF diet was fed to two groups of mice for the same length of time (54 days), but one group was fed the diet before, during, and after puberty, while the other group consumed the diet only well after puberty. Table 4 indicates that there were no statistical differences between the two groups in tumor latency, the percentage of mice with tumors, or the number of tumors per tumor bearing mouse. This suggests that the length of time a mouse is on a HF/HC diet is more important for the enhancement of mammary carcinogenesis than is the feeding of the diet during the age of puberty.

Effect of Diets on Tumor Metastases (Table 3). A total of 282 mice were examined histopathologically of which 216 were suitable for data reporting. As anticipated, metastases that were seen occurred only in the lungs, with no evidence of metastases to the kidney, liver, or spleen (33). Frequently, the only evidence of pulmonary metastasis was tumor emboli. All tumors ap...
time during which the developing mammary gland would be exposed to the action of the viral carcinogen as well as increased estrogen levels. Thus an early puberty (even in the absence of high levels of dietary fat fed throughout puberty) might result in the enhancement of mammary carcinogenesis (41–43).

That carcinogenesis was not enhanced may indicate that the viral inducing agent of the mouse, even when coupled with a HF/HC diet, does not have the tumor initiating ability of certain chemical and physical carcinogens. Alternately, it may be that in this model tumor initiation by the virus does not occur until later in life. This is unlikely for mitotic and \([\text{H}]\)-thymidine indices remain elevated well past puberty in mice fed a HF diet (36, 38). It is also possible that an early puberty may be accompanied by the differentiation of the mouse mammary gland at an earlier chronological age. Therefore, although the final differentiation of the gland may not occur until the birth of a litter, sufficient differentiation may occur to simply shift the risk period rather than lengthen it.

Finally, it may be that those mice that were fed a HF/HC diet until 12 days of age or until puberty simply were not exposed to the HF/HC diet for a long enough period of time. This would be particularly true in the group that consumed the HF/HC diet until 12 days of age, because the major amount of dietary fat that they consumed came from their dam's milk. Although the dam was also fed a HF/HC diet, preliminary unpublished data from our laboratory suggests that there are no differences in the total milk fat content between HF or LF fed mice.

We also investigated the effect that dietary fat might have on tumor metasizes. When only the HF/HC and LF/LC groups were compared, the former group had significantly more pulmonary metastases than the latter group ($P = 0.008$).

DISCUSSION

We have concluded that the length of time a C3H/OuJ mouse consumes a HF/HC diet is a more significant enhancing factor for mammary carcinogenesis than is the feeding of a HF/HC diet through the age of puberty. This finding has caused us to reject our working hypothesis which was that the age of puberty, in itself, would act as a risk factor for mammary carcinogenesis if a HF/HC diet was fed throughout this period.

The hypothesis was based on studies with chemical and physical carcinogens which indicated that the mammary gland of both humans and experimental animals was most sensitive to the action of a carcinogen near the age of puberty (21–25) and that increased levels of dietary fat can cause proliferations of the mammary ductal epithelium in the rat (34, 35) and increases in mitotic figures in the terminal ducts of the mouse (36). Still other studies demonstrated that the sensitivity of the mammary gland to a carcinogen is dependent on the frequency of cell division at the time the carcinogen acts on the gland (37). It therefore seemed plausible that the sensitivity of the pubertal mouse mammary gland to mammogenic hormones (38) coupled with increased cellular proliferation would provide an appropriate setting for the enhancement of mammary carcinogenesis.

It had previously been shown in rats and mice that a HF diet could accelerate the initiation of puberty (32, 39, 40). We had anticipated that the advancement of the age of puberty in this study by the HF/HC diet would result in a longer period of

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Table 2 Puberty and mammary tumors in mice fed different diets at different ages

<table>
<thead>
<tr>
<th>Group (no. of mice)</th>
<th>Days of age at puberty (SD)</th>
<th>Tumor latency (days [SD])</th>
<th>No. of tumor bearing mice</th>
<th>Av. no. of tumors/tumor bearing mouse</th>
</tr>
</thead>
</table>
| 1. LF/LC always (73) | 32.9 (4.0)$^a$ | 283.9 (38.4)$^a$ | 57 (78%)$^a$ | 1.2$^a$
| 2. HF/HC to LF/LC at 12 days (55) | 30.4 (3.4)$^a$ | 281.5 (41.5)$^a$ | 35 (64%)$^a$ | 1.5$^a$
| 3. HF/HC to LF/LC at puberty (62) | 28.1 (2.8)$^a$ | 280.2 (40.2)$^a$ | 41 (66%)$^a$ | 1.3$^a$
| 4. HF/HC to LF/LC at 60 days (55) | 27.8 (3.5)$^a$ | 240.0 (46.0)$^a$ | 49 (89%)$^a$ | 1.9$^a$
| 5. HF/HC always (60) | 29.0 (2.9)$^a$ | 203.9 (31.8)$^a$ | 57 (95%)$^a$ | 2.1$^a$

$^a$ Same superscript indicates no significant differences ($P > 0.05$).

Table 3 Mammary tumor metastases to the lungs of mice fed different diets at different ages

<table>
<thead>
<tr>
<th>Group (no. of mice)</th>
<th>No. with metastases</th>
<th>No. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. LF/LC always</td>
<td>15</td>
<td>53</td>
</tr>
<tr>
<td>2. HF/HC to LF/LC at 12 days</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>3. HF/HC to LF/LC at puberty</td>
<td>11</td>
<td>36</td>
</tr>
<tr>
<td>4. HF/HC to LF/LC at 60 days</td>
<td>16</td>
<td>46</td>
</tr>
<tr>
<td>5. HF/HC always</td>
<td>27</td>
<td>50</td>
</tr>
</tbody>
</table>

$^a$ No significant differences between groups (analysis of variance); $P > 0.05$.

Table 4 Mammary tumors in mice fed a HF diet for the same length of time either during or well after puberty

<table>
<thead>
<tr>
<th>Group (no. of mice)</th>
<th>Tumor latency (days [SD])</th>
<th>No. of tumor bearing mice (%)</th>
<th>Av. no. of tumors/tumor bearing mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF through puberty (25)</td>
<td>230.5 (37.5)</td>
<td>19 (76)</td>
<td>1.5</td>
</tr>
<tr>
<td>HF after puberty (25)</td>
<td>243.0 (28.0)</td>
<td>18 (72)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

$^a$ No significant differences between groups; $P > 0.05$. 

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ACKNOWLEDGMENTS

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REFERENCES

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