Effects of High Dietary Fat on the Growth and Development of Ovarian-independent Carcinogen-induced Mammary Tumors in Rats

Paul W. Sylvester, Clement Ip, and Margot M. Ip

ABSTRACT

This study examined the influence of high dietary fat intake on the development of ovarian-independent mammary tumors in both vehicle-treated controls and rats made deficient in estrogen and prolactin during tumor induction. The majority of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors in rats are dependent on estrogen and prolactin for growth, and suppression of prolactin and estrogen at the time of tumor initiation causes a reduction in tumor incidence and increase in tumor latency. However, the majority of mammary tumors which do develop in these animals exhibit ovarian-independent growth. Sprague-Dawley rats were given 7.5 mg DMBA p.o. at 57 days of age. Starting 1 day prior to and continuing for 7 days after DMBA administration, rats were given daily injection of vehicle or the combination of tamoxifen (20 μg/rat) plus bromocryptine (5 mg/kg). At the end of drug treatment, rats in each treatment group were equally divided and placed on normal fat (5% corn oil) or high fat (20% corn oil) diets for the duration of the experiment. Vehicle-treated rats were ovariectomized 27 wk and drug-treated rats 47 wk after DMBA administration to determine tumor ovarian dependency. Vehicle-treated rats fed high fat diets showed significant increases in mammary tumor incidence and number as compared to similarly treated rats fed a normal fat diet, with approximately 80% of the tumors in each group being ovarian dependent. Likewise, tamoxifen-bromocryptine-treated rats fed a high fat diet showed a significant enhancement in mammary tumor number, although not incidence, as compared to similarly treated rats fed a normal diet. Tumors in these drug-treated groups displayed essentially the same incidence of ovarian independence (23%). Tamoxifen-bromocryptine-treated groups displayed a 2-fold increase in latency of tumor appearance as compared to vehicle-treated controls; however, this long latency was not reduced when these rats were fed a high fat diet. These results demonstrate that high dietary fat stimulates ovarian-dependent and -independent mammary tumorigenesis in rats but does not influence the hormonal responsiveness of these tumors.

INTRODUCTION

Previous studies have established that diets high in fat significantly enhance mammary tumorigenesis in rats (1-3). However, the mechanism(s) by which high fat diets stimulate mammary tumor development has not been fully established. Development and growth of mammary tumors in rats are dependent on the presence of hormones, particularly estrogen and prolactin (4, 5).

Recent studies have demonstrated that high fat diets do not stimulate estrogen and/or prolactin secretion (6, 7) or appear to enhance target tissue responsiveness to these hormones (8, 9). It is unlikely, therefore, that high dietary fat intake stimulation of mammary tumorigenesis is mediated through a direct endocrine mechanism.

Studies have also shown that suppression of estrogen and prolactin by tamoxifen-bromocryptine treatment during the first week after DMBA administration results in a significant reduction in mammary tumor incidence and number (10). The tumors developing in these hormonally suppressed animals, however, display ovarian-independent growth (10). These findings demonstrate that the hormonal milieu in rats at the time of initiation influences subsequent mammary tumor hormone responsiveness. The purpose of this study was to determine whether high dietary fat intake affects the development and growth of ovarian-dependent versus ovarian-independent mammary tumors in both control rats and rats made deficient in estrogen and prolactin during the first week after DMBA administration. In this way mammary tumors were allowed to develop in the presence of normal cyclic surges of prolactin and estrogen throughout the subsequent weeks following tumor induction.

MATERIALS AND METHODS

Tumor Induction and Drug Treatment. Fifty-seven-day-old virgin female Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were given a single i.g. intubation of 7.5 mg DMBA (Sigma Chemical Co., St. Louis, MO) (11). All animals were maintained on rat chow (Teklad, Madison, WI) and were housed in suspended metal cages in a temperature-regulated (22 ± 0.5°C) and light-controlled (14-h light and 10-h dark) room. Food and water were available ad libitum. Beginning 1 day prior to and 7 days after DMBA administration, one-third of the rats were given a daily 0.1-ml s.c. injection of ethanol-saline vehicle (0.3% ethanol and 0.87% NaCl solution), while the remaining two-thirds received the combination of tamoxifen (20 μg/rat; ICI, Rotterdam, The Netherlands) and bromocryptine (5 mg/kg; Sandoz, Ltd., Basel, Switzerland) s.c. injection, suspended in 0.1 ml ethanol-saline solution. Tamoxifen, an estrogen receptor antagonist, was given to inhibit the action of estrogen; while bromocryptine, an ergot drug and dopamine receptor agonist, was given to inhibit pituitary release of prolactin during the treatment period. All injections were given between 9 and 11 a.m. for the 8-day treatment period.

At the end of the drug treatment period, rats in both the vehicle- and drug-treated groups were equally divided and placed on either a normal fat (5% corn oil) or a high fat (20% corn oil) diet for the duration of the experiment. Animals were placed on the synthetic diets at this time in order to avoid the possibility of a dietary fat effect during the "critical period" of mammary tumor induction (12, 13), while allowing examination of dietary fat effects on subsequent mammary tumor development and...
ovarian dependency during the promotional phase of tumorigenesis. The composition of the normal and high fat diets is given in Table 1 and was formulated according to the method of Newberne et al. (14) on the assumption that rats will consume an equal number of calories. Mazola corn oil was kindly provided by Best Foods (Englewood Cliffs, NJ), dextrose was purchased from Federal Bakers Supply (Buffalo, NY), casein was purchased from Teklad (Madison, WI), and all other nutrients used in formulation of the diets were purchased from ICN Pharmaceuticals (Cleveland, OH).

**Tumor Measurements and Classification.** Tumor measurements and body weights were recorded at weekly intervals from the beginning until termination of the experiment. Rats which survived until tumors appeared in their cohorts were included, while rats which died before tumors appeared in their particular treatment group were not included in calculations of mammary tumor incidence. Average tumor diameter for each palpable tumor was determined by using the mean of the 2 largest perpendicular diameters as measured by vernier calipers. A tumor that decreased by 5 mm or more in average tumor diameter was classified as regressing. A tumor that increased by 5 mm or more in average tumor diameter was classified as growing, and a tumor that changed less than 5 mm or more in average tumor diameter was considered stable 4 wk after ovariectomy. Latency period was calculated by averaging the week of tumor appearance for all palpable tumors within a group. Approximately 15–20% of the animals initiated into this study had to be sacrificed or died before termination of the experiment, when they became moribund. The majority of the tumors from these rats were weighed and sectioned for histological analysis, and they are included in the tumor data. Upon termination of the experiment, the remaining rats were sacrificed and tumors were removed, fixed in buffered formalin, and later embedded, sectioned, and stained with hematoxylin-eosin for routine histological examination.

**Evaluation of Ovarian Dependency of Mammary Tumors.** Twenty-seven wk after DMBA administration, all vehicle-treated rats fed normal or high fat diets were bilaterally ovariectomized under ether anesthesia to determine ovarian dependency of the mammary tumors. Ovariectomy removes the primary source of estrogen and significantly reduces prolactin secretion by the pituitary (15). This period of time after DMBA administration was chosen for ovariectomy of vehicle-treated groups to avoid further loss of experimental animals from progressive tumor burden. Animals which received tamoxifen plus bromocrypine during initiation exhibited a low mammary tumor incidence 27 wk after DMBA administration and appeared in good health. Therefore, ovariectomy was not performed in these rats until 47 wk after DMBA administration when mammary tumor incidence approached levels similar to those of vehicle-treated groups at 27 wk after DMBA administration. Mammary tumor growth was followed in both vehicle- and tamoxifen-bromocrypine-treated groups for 4 wk following ovariectomy.

**Blood Collection and Hormone Assay.** Blood was collected under light ether anesthesia by orbital sinus puncture at 3 different time periods during this study. The first blood sample was taken on the last day of vehicle or drug treatment, 1 h after injection (7 days after DMBA administration). The second blood sample was collected prior to ovariectomy (27 wk after DMBA administration for vehicle-treated groups and 47 wk after DMBA administration for tamoxifen-bromocrypine-treated groups). The last blood sample was taken 4 wk after ovariectomy (31 wk after DMBA administration for vehicle-treated groups and 51 wk after DMBA administration for tamoxifen-bromocrypine-treated groups). At each of the 3 different blood sampling periods, blood was collected between 9 and 11 a.m. when serum prolactin levels in female rats are approximately equal during all stages of the estrous cycle (16). This method of blood collection is mildly stressful and slightly stimulates serum prolactin levels above that seen in blood sampled by an indwelling cannula in conscious, free-moving, undisturbed rats. Serum was separated by centrifugation and stored at −20°C until assayed for prolactin by standard radioimmunoassay procedure with a NIADDK kit, using the double antibody method of Niswender et al. (17). Serum prolactin values are expressed as ng/ml in terms of NIADDK rat prolactin RP-RP-3. All serum samples were assayed for prolactin at the same time in duplicates of 20 μl.

**Statistical Analysis.** Statistical differences in serum prolactin levels, mammary tumor size, weights, number, and latency were determined by analysis of variance, and the Student-Newman-Keuls test was used for multiple comparisons among groups (18). Statistical differences in tumor incidence between treatment groups were determined by χ² test with Yates’ correction (19). Differences were considered to be significant if P < 0.05 as compared to vehicle-treated rats fed a normal fat diet or otherwise indicated in the figure and table legends.

### RESULTS

The effects of high dietary fat on mammary tumorigenesis in vehicle- and tamoxifen-bromocrypine-treated rats 27 wk after DMBA administration are shown in Table 2. Tumor incidence (including both palpable and nonpalpable mammary tumors) in vehicle-treated rats fed a normal fat diet was 60%, with an average of 1.07 tumors per tumor-bearing rat and an average tumor diameter of 1.20 cm. Average latency of tumor appearance in these animals was 21.2 wk, and none of the mammary tumors in this group regressed spontaneously. Vehicle-treated rats fed a high fat diet had a 66% tumor incidence and showed a significant increase in the number of tumors per tumor-bearing rat (2.19) and a significant decrease in average latency (14.9 wk) as compared to vehicle-treated rats fed a normal fat diet. Two tumors in the vehicle-treated high fat diet group spontaneously regressed during the 27-wk period following DMBA administration. As Table 2 indicates, rats treated with tamoxifen-bromocrypine for the period around DMBA administration displayed significant suppression in mammary tumor development regardless of dietary fat intake. Tumor incidences (palpable mammary tumors only) in the tamoxifen-bromocrypine-treated rats 27 wk after DMBA administration were 13 and 4% in the normal fat- and high-fat-fed groups, respectively, and did not differ statistically.

During the 27-wk period following DMBA administration, 3 vehicle-treated rats fed a normal fat and 4 vehicle-treated rats fed a high fat diet died or were sacrificed because of large tumor burden. These rats were not included in calculations of mammary tumor ovarian dependence of their respective groups. During this same period, 3 tamoxifen-bromocrypine-treated rats fed a normal fat and 2 similarly treated rats fed a high fat diet died or were sacrificed. Average body weights of rats in the various treatment groups were not significantly different at any time period during the first 27 wk of the experiment (data not shown).

### Table 1

<table>
<thead>
<tr>
<th>Diet ingredient</th>
<th>% by wt</th>
<th>Normal fat diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin-free casein</td>
<td>20.0</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>D-Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>65.0</td>
<td>44.8</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Alphacalcidol</td>
<td>5.0</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>AIN-76 vitamin mix (with modification)</td>
<td>1.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>AIN-76 salt mix</td>
<td>3.5</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>
EFFECTS OF HIGH FAT DIETS ON TUMOR GROWTH

Table 2
Effect of dietary fat on mammary tumor development 27 wk after DMBA administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>No. of rats with tumors</th>
<th>%</th>
<th>Total no. of tumors</th>
<th>No. of tumors/tumor-bearing rat</th>
<th>Av. tumor diameter (cm)</th>
<th>Latency period (wk)</th>
<th>No. of spontaneously regressing tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal fat</td>
<td>25</td>
<td>15</td>
<td>60</td>
<td>16</td>
<td>1.07 ± 0.07</td>
<td>1.20 ± 0.23</td>
<td>21.24 ± 1.41</td>
<td>0</td>
</tr>
<tr>
<td>High fat</td>
<td>24</td>
<td>16</td>
<td>66</td>
<td>35</td>
<td>2.19 ± 0.46</td>
<td>1.31 ± 0.15</td>
<td>14.93 ± 0.86</td>
<td>2</td>
</tr>
<tr>
<td>Normal fat + CB-154</td>
<td>47</td>
<td>6</td>
<td>13</td>
<td>6</td>
<td>1.00 ± 0.00</td>
<td>2.43 ± 1.03</td>
<td>20.51 ± 3.20</td>
<td>0</td>
</tr>
<tr>
<td>High fat + CB-154</td>
<td>48</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1.00 ± 0.00</td>
<td>2.05 ± 0.15</td>
<td>25.00 ± 1.00</td>
<td>0</td>
</tr>
</tbody>
</table>

a Includes palpable and nonpalpable tumors discovered at autopsy.

b Mean ± SE.

c p < 0.05 as compared to normal fat diet, vehicle-treated rats.
d Includes only palpable tumors.

e TAM, tamoxifen; CB-154, bromocryptine.

Table 3
Effect of dietary fat on mammary tumor development 47 wk after DMBA administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>No. of rats with tumors</th>
<th>%</th>
<th>Total no. of tumors</th>
<th>No. of tumors/tumor-bearing rat</th>
<th>Av. tumor diameter (cm)</th>
<th>Latency period (wk)</th>
<th>No. of spontaneously regressing tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal fat + CB-154</td>
<td>47</td>
<td>24</td>
<td>51</td>
<td>30</td>
<td>1.25 ± 0.20</td>
<td>2.19 ± 0.38</td>
<td>35.74 ± 2.41</td>
<td>1</td>
</tr>
<tr>
<td>High fat + CB-154</td>
<td>48</td>
<td>29</td>
<td>60</td>
<td>63</td>
<td>2.17 ± 0.34</td>
<td>1.86 ± 0.16</td>
<td>40.33 ± 0.85</td>
<td>2</td>
</tr>
</tbody>
</table>

a Includes palpable and nonpalpable tumors discovered at autopsy.
b Mean ± SE.
c TAM, tamoxifen; CB-154, bromocryptine.
d p < 0.05 as compared to normal fat diet, tamoxifen-bromocryptine-treated rats.

e TAM, tamoxifen; CB-154, bromocryptine.

At sacrifice at 27 wk, average body weights of the 2 vehicle-treated groups were 335 ± 11 and 373 ± 21 g for the normal and high fat groups, respectively.

The effects of dietary fat on mammary tumor development in tamoxifen-bromocryptine-treated rats 47 wk after DMBA administration are shown in Table 3. Mammary tumor incidence (including palpable and nonpalpable tumors) in these rats fed a normal fat diet was 51%, with an average of 1.25 tumors per tumor-bearing rat, an average tumor diameter of 2.19 cm, and an average latency of tumor appearance of 35.7 wk. One tumor in this group spontaneously regressed during the 47 wk following DMBA administration. Tamoxifen-bromocryptine-treated rats fed a high fat diet showed a high fat diet showed a significant increase in the number of tumors per tumor-bearing rat (2.17) and a slight but not significant increase in mammary tumor incidence (60%) as compared to similarly treated rats fed a normal fat diet (51%). By 47 wk after DMBA administration, a total of 13 tamoxifen-bromocryptine-treated rats fed a normal fat and 11 similarly treated rats fed a high fat diet had to be sacrificed because of excess tumor burden or died. These rats were not included in calculations of mammary tumor ovarian dependence of their respective groups but were included in all other tumor calculations. No significant differences were found between average body weight of drug-treated rats fed normal or high fat diets at any time during the experiment (data not shown). At sacrifice at 47 wk, the average body weights of the drug-treated groups were 363 ± 14 and 402 ± 26 g for the normal and high fat groups, respectively.

Mammary tumor incidence throughout the course of the experiment in the various drug and diet treatment groups is shown in Fig. 1. Mammary tumors first appeared in vehicle-treated rats fed a normal fat diet approximately 10 wk after DMBA administration. Tumor incidence remained under 10% in these animals until almost 18 wk after exposure to DMBA. Mammary tumors in vehicle-treated rats fed high fat diets first appeared approximately 9 wk after DMBA administration, and the incidence of tumors found in these rats increased sharply over the next few weeks. Between 11 and 18 wk after DMBA administration, vehicle-treated rats fed a high fat diet showed a significantly higher mammary tumor incidence when compared to similarly treated rats fed a normal fat diet. Beyond 18 wk after DMBA administration, however, there was no significant difference in mammary tumor incidence in vehicle-treated groups.

One mammary tumor was found in a tamoxifen-bromocryptine-treated rat fed a normal fat diet at 10 wk after DMBA administration, and by 27 wk after DMBA, only 6 rats in this group had palpable tumors. This was found to be significantly different from vehicle-treated rats fed a normal fat diet. Mammary tumors in tamoxifen-bromocryptine-treated rats fed a high fat diet did not first appear until 24 wk after DMBA administration, and only 2 rats in this group had palpable tumors 27 wk after DMBA. This was also found to be significantly different from vehicle-treated rats fed a normal fat diet. Beyond 27 wk after DMBA administration, mammary tumor incidence increased gradually in the tamoxifen-bromocryptine-treated rats fed either a normal or high fat diet, and at 47 wk after DMBA, tumor incidence in these animals reached levels similar to those seen in vehicle-treated rats 27 wk after DMBA administration.

The effects of drug and dietary treatment on cumulative average tumor diameter following DMBA administration are shown in Fig. 2. Vehicle-treated rats fed a high fat diet showed a significantly larger cumulative average tumor diameter compared to similarly treated rats fed a normal fat diet 27 wk after DMBA administration. In contrast, tamoxifen-bromocryptine-treated rats fed either a normal or high fat diet showed significantly lower cumulative average tumor diameters compared to vehicle-treated rats fed a normal fat diet. Beyond 27 wk after DMBA
Effects of high fat diets on tumor growth

The effect of ovariectomy 47 wk after DMBA administration on mammary tumor regression in tamoxifen-bromocryptine-treated rats is shown in Table 5. In contrast to vehicle-treated rats, incidence of mammary tumor regression after ovariectomy in tamoxifen-bromocryptine-treated rats was only 23.8% in rats fed a normal fat diet and 23.2% in rats fed a high fat diet. In addition, tamoxifen-bromocryptine-treated rats fed either a normal or high fat diet showed greater than a 5-fold increase in the incidence of tumors which continued to grow after ovariectomy as compared to vehicle-treated rats.

The percentage of change in average tumor diameter in the 4-wk period following ovariectomy is shown in Fig. 3. Ovariectomy caused a significant reduction in average tumor diameter in vehicle-treated rats fed either a normal or high fat diet compared to preovariectomy values. No differences were found between vehicle-treated rats fed a normal or high fat diet. Average tumor weight at autopsy (31 wk after DMBA administration and 4 wk postovariectomy) was 1.26 ± 1.03 g in vehicle-treated rats fed a normal fat diet and 1.72 ± 0.63 g in vehicle-treated rats fed a high fat diet. This was not a statistically significant difference. Average tumor burden at autopsy (31 wk after DMBA administration and 4 wk postovariectomy) was 7.71 ± 1.03 g in vehicle-treated rats fed a normal fat diet and 7.43 ± 1.52 g in similarly treated rats fed a high fat diet. This was not statistically different. Average tumor burden at
EFFECTS OF HIGH FAT DIETS ON TUMOR GROWTH

Fig. 3. Percentage of change in average tumor diameter in the respective treatment groups during the 4-wk period following ovariectomy. Bars, SE. TAM, tamoxifen; CB-154, bromocryptine. +, P < 0.05 compared to initial values prior to ovariectomy.

Fig. 4. Serum prolactin levels for each treatment group at different time periods during the course of the experiment. One wk after DMBA administration, blood was collected on the last day of vehicle or drug treatment 7 days after DMBA administration and 1 day prior to the start of dietary treatment. Serum prolactin levels were significantly lower in rats treated with tamoxifen plus bromocryptine as compared to vehicle-treated rats. The second blood sample was collected 1 day prior to ovariectomy, 27 wk after DMBA administration for vehicle-treated rats and 47 wk after DMBA administration for tamoxifen-bromocryptine-treated rats. At this time, all animals had long since been removed from vehicle or drug treatment, and no differences in serum prolactin levels were found among the treatment groups. The last blood sample was collected 4 wk after ovariectomy, 31 wk after DMBA administration for vehicle-treated rats and 51 wk after DMBA administration for tamoxifen-bromocryptine-treated rats. At this time, rats in all the treatment groups showed reduced prolactin levels, but no differences were found among groups.

Histological classification of mammary tumors removed from rats at autopsy and their response to ovariectomy are shown in Table 6. Mammary tumors were classified as either adenocarcinomas, which contained characteristic columns of epithelial cells many cell layers thick, or fibroadenomas, which contained overgrowth of fibrous connective tissue, and were almost entirely lacking epithelial tissue. Mammary tumors which contained overgrowth of both epithelial and fibrous tissue ("mixed tumors") were included in the group classified as adenocarcinomas. Vehicle-treated rats, 31 wk after DMBA administration and 4 wk postovariectomy, fed a normal fat diet had 92.9% adenocarcinomas and only 7.1% fibroadenomas. Tumors in vehicle-treated rats fed a high fat diet showed no differences in mammary tumor histology when compared with rats fed a normal fat diet. Tamoxifen-bromocryptine-treated rats fed a normal fat diet had 81.0% mammary tumors classified as adenocarcinomas and 19.0% fibroadenomas, 51 wk after DMBA administration and 4 wk postovariectomy. Similar results were found in tamoxifen-bromocryptine-treated rats fed a high fat diet.

Table 6

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Growth after ovariectomy</th>
<th>Adenocarcinoma</th>
<th>Fibroadenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated rats fed a normal fat diet</td>
<td>Regressed 11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stable 1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grew 1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total 13</td>
<td>91.9</td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td>Vehicle-treated rats fed a high fat diet</td>
<td>Regressed 26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stable 2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grew 2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total 30</td>
<td>93.8</td>
<td>2</td>
<td>6.2</td>
</tr>
<tr>
<td>TAM + CB-154-treated rats fed a normal fat diet</td>
<td>Regressed 5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stable 2</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grew 10</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total 17</td>
<td>81.0</td>
<td>4</td>
<td>19.0</td>
</tr>
<tr>
<td>TAM + CB-154-treated rats fed a high fat diet</td>
<td>Regressed 10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stable 3</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grew 22</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total 35</td>
<td>81.4</td>
<td>8</td>
<td>18.6</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of total number of tumors in the particular group.

** TAM, tamoxifen; CB-154, bromocryptine.
DISCUSSION

The results from this study demonstrate that diets high in unsaturated fat enhance development of both DMBA-induced ovarian-dependent and -independent mammary tumors in rats. These results also show that dietary fat does not influence the subsequent ovarian dependency of these tumors. Vehicle-treated rats fed a high fat diet displayed a significant enhancement of mammary tumorigenesis as compared to similarly treated rats fed a normal fat diet. These vehicle-treated rats, however, showed no differences in the incidence of ovarian-dependent versus ovarian-independent mammary tumors. Mammary tumors in these rats also responded to ovariectomy in essentially the same manner. Likewise, rats fed a high fat diet and treated with the combination of tamoxifen plus bromocryptine one day prior to and 7 days after DMBA administration, a treatment protocol shown to induce the development of predominately ovarian-independent mammary tumors (10), also increased the number of mammary tumors as compared to similarly treated rats fed a normal fat diet. It was also found that tamoxifen-bromocryptine-treated rats fed either a normal or high fat diet had essentially the same incidence of ovarian-independent versus ovarian-dependent mammary tumors, and tumors in these groups displayed similar growth responses following ovariectomy. Thus, while hormonal dependency of DMBA-induced mammary tumors in rats is greatly influenced by the animal’s hormonal environment at the time of initiation, ovarian dependency is not related to subsequent dietary fat intake.

In agreement with previous findings (1–3, 20–23), average latency of tumor appearance in vehicle-treated rats fed a high fat diet was significantly reduced as compared to vehicle-treated rats fed a normal fat diet. Tamoxifen plus bromocryptine treatment in rats at the time of initiation induced nearly a 2-fold increased latency of mammary tumor appearance. In contrast to vehicle-treated rats, however, this long tumor latency in tamoxifen-bromocryptine-treated rats was not reduced when these animals were fed a high fat diet. These results suggest that diets high in fat in some way hasten the development of ovarian-dependent but not ovarian-independent mammary tumors in rats.

The mechanism(s) by which high fat diets stimulate mammary tumorigenesis in rodents is not completely understood. Most evidence suggests that high levels of dietary fat act as a classical tumor promoter to enhance expression of transformed epithelial cells already present within the mammary tissue (24). The promotion effects of high dietary fat on mammary tumor development are not mediated by increased circulating hormone or target tissue receptor levels. This study found no significant differences in serum prolactin levels or tumor hormone responsiveness in similarly treated rats fed normal or high fat diets. These findings are in agreement with recent studies which showed that high fat diets had no effect on circulating estrogen and prolactin levels throughout the estrous cycle in rats (6, 7) when compared to normal fat diets. Others have also found target tissue receptor levels and responsiveness to estrogen and prolactin are little affected by normal or high levels of fat in the diet (6, 9, 25–27). However, it is possible that high dietary fat intake may modulate the endocrine signal through postreceptor mechanisms.

The majority of spontaneous and carcinogen-induced mammary tumors in rats are dependent on the presence of estrogen and prolactin for continued growth (4, 5). A small percentage of tumors arise, however, which are ovarian independent as indicated by continued growth after ovariectomy. Ovariectomy removes the major source of estrogen and significantly reduces circulating levels of prolactin (15). At present, the mechanism(s) involved in ovarian-dependent mammary tissue transformation into ovarian-independent mammary cancer is not understood. It is known that animals made deficient in estrogen and prolactin at the time of carcinogen administration develop a majority of mammary tumors which display growth independent of these hormones and suggest ovarian independence is determined during early development of these tumors (10).

Previous studies have examined the effects of variable fat diets on the growth of ovarian-independent mammary tumors in rats (25, 28). In these studies, animals were ovariectomized to suppress the development of ovarian-dependent tumors and create an environment allowing for exclusive development of ovarian-independent tumors. In these studies, therefore, it was not possible to compare the effects of variable fat diets on development of both ovarian-dependent and -independent mammary tumors in the presence of normal cyclic surges of estrogen and prolactin. In addition, the present study compares the effects of normal and high fat diets containing adequate levels of essential fatty acids on ovarian-independent tumor growth in rats. In contrast, previous studies have compared ovarian-independent tumor growth in rats fed diets containing adequate levels versus rats fed diets deficient in essential fatty acids (28). Studies utilizing transplantable ovarian-independent mammary tumors, while able to examine the effects of dietary fat on tumor growth, are unable to study the effects of these diets on the development of this ovarian-independent growth (29). Treatment with tamoxifen plus bromocryptine, 1 day prior to and 7 days after DMBA administration, provided an excellent model for the induction of ovarian-independent mammary tumors in rats and, together with vehicle-treated groups, allowed useful comparisons to further clarify the influence of dietary fat on development of mammary tumor hormone dependency.

Histological examination of mammary tumors upon termination of the experiment indicated nearly a 3-fold increase in the incidence of tumors classified as fibroadenomas in tamoxifen-bromocryptine-treated animals as compared to vehicle-treated rats. Greater numbers of fibroadenomas found in tamoxifen-bromocryptine-treated rats most likely reflect the older age of these animals at autopsy. It has been shown that the percentage of rat mammary tumors classified as adenocarcinomas sharply decreases in the months following DMBA administration (30). Vehicle-treated rats were ovariectomized for evaluation of mammary tumor ovarian dependency 27 wk after DMBA administration, in order to avoid further loss of animals from tumor burden. At this same period of time, tamoxifen-bromocryptine-treated rats had a very low incidence of palpable mammary tumors (<10%). Hence, tamoxifen-bromocryptine-treated rats were ovariectomized at 47 wk after DMBA administration when tumor incidence in these animals approached levels similar to those seen in vehicle-treated rats at 27 wk after DMBA. While the incidence of fibroadenomas was higher in tamoxifen-bromocryptine-treated rats 20 wk past the time vehicle-treated rats were examined, no differences in tumor histology were found when similarly treated groups fed normal or high fat diets were compared.

In conclusion, the feeding of a high fat diet to rats treated with
tamoxifen and bromocryptine for an 8-day period at the time of DMBA administration significantly increased mammary tumor number compared to similarly treated rats fed a normal fat diet. Tumor incidence and latency were not affected in these rats. Vehicle-treated control rats fed a high fat diet showed a similar increase in mammary tumor number, but in addition, tumor incidence was increased and latency decreased. Within vehicle- and tamoxifen-bromocryptine-treated groups, no differences were found in the incidence of ovarian-dependent versus ovarian-independent mammary tumor development or in tumor responsiveness to ovarioectomy. These results suggest that a high fat diet stimulates growth of both ovarian-dependent and -independent DMBA-induced mammary tumors in rats but does not influence the ovarian dependency of these tumors. The hormonal environment of the animal during the time of tumor induction appears to be of critical importance in influencing subsequent mammary tumor latency and hormone dependency, while high dietary fat intake appears only to enhance expression of mammary tumorigenesis. The mechanism of the high fat effect is not known; however, it may involve changes in host immune function, cell-to-cell communication and/or cell membrane composition, or a direct effect on the mammary gland resulting in stimulated mammary tumor growth (24).

REFERENCES

Effects of High Dietary Fat on the Growth and Development of Ovarian-independent Carcinogen-induced Mammary Tumors in Rats

Paul W. Sylvester, Clement Ip and Margot M. Ip


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/2/763

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.