Comparative Effects of Different Animal and Vegetable Fats Fed before and during Carcinogen Administration on Mammary Tumorigenesis, Sexual Maturation, and Endocrine Function in Rats

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ABSTRACT

The purpose of this investigation was to determine whether diets high in animal or vegetable fat affected mammary tumorigenesis when fed to rats only prior to and during the initiation phase of carcinogenesis. Weanling 21-day-old female Sprague-Dawley rats were divided into different dietary treatment groups and were allowed to feed ad libitum on one of the following diets: 5% (normal fat) corn oil; 20% (high fat) corn oil; 20% palm oil; 20% beef tallow; or 20% lard. At 52 days of age, all rats were given p.o. 7.5 mg 7,12-dimethylbenz(a)anthracene (DMBA). One week following DMBA administration, all rats were switched to the 5% corn oil control diet and were maintained on this diet for the duration of the experiment. Rats fed a 20% lard diet during the treatment period showed a significant increase in mammary tumor incidence and number 19 weeks after DMBA administration, the 5% com oil control diet and were maintained on this diet for the duration of the experiment. Rats fed a 20% lard diet during this same time period also demonstrated enhanced mammary tumor development, during the 10- to 19-week time period after DMBA. Mammary tumor development in rats fed 20% corn oil or palm oil diets during this treatment period was similar to that of normal fat controls.

Estrogens are potent stimulators of mammary tumor growth and development in rats. Because mammary tumorigenesis was enhanced in rats fed high animal, but not vegetable fat diets, it was possible that estrogens present in animal fat might be responsible for this stimulation. Further studies demonstrated however, that increased mammary tumorigenesis in rats fed diets high in animal fat could not be explained on the basis of endocrine stimulation. Average day of vaginal opening for all groups fed 20% fat diets was similar and occurred earlier than in normal fat controls. In addition, 50- to 65-day-old rats in the different dietary treatment groups showed no differences in basal or surge levels of serum prolactin, luteinizing hormone, or estradiol. Rat diestrus uterine weight also showed no significant differences among dietary treatment groups. Thus diets containing high levels of animal fat caused little if any increased estrogenic activity in rats. In conclusion, high dietary intake of lard and beef tallow, but not vegetable fat, fed from weaning until only 1 week after DMBA administration, significantly enhances mammary tumorigenesis in rats. The mechanism(s) by which animal fat induces this stimulation is not clear, but it does not appear to result from endogenous or exogenous endocrine stimulation.

INTRODUCTION

High dietary fat intake significantly enhances mammary tumorigenesis in rats (1-3). The mechanism(s) by which high fat diets bring about this stimulation is not fully understood. However, evidence suggests that dietary fat influences tumor growth during the promotional phase of mammary carcinogenesis. In general rodents display increased mammary tumorigenesis when high saturated or unsaturated fat diets are maintained after carcinogen administration, while little effect is observed if diets are given before tumor induction (2, 4, 5). Mammary gland transplants exposed to DMBA in culture display enhanced neoplastic growth in host rats fed high fat diets as compared to normal fat diets, while the dietary fat intake of the donor had little influence on subsequent tumor growth (6). In addition high dietary fat intake has also been shown to stimulate growth of transplantable murine mammary tumors (7). These results demonstrate that high fat diets act as potent promoters of murine mammary tumor development and growth.

While the role of dietary fat during the initiation phase of mammary carcinogenesis is less apparent, it cannot be ruled out. Rogers et al. (8) have recently reported that rats fed a high lard diet for only 5 weeks prior to and until the day of carcinogen administration, displayed a significant increase in mammary tumor development. These investigators did not determine however, whether this stimulatory effect was unique to lard itself, or if other saturated animal or vegetable fats also induced the same effect. In addition it has not been resolved whether estrogens present in animal fat were absorbed from the gut during digestion, entered the circulation, and thereby provided exogenous endocrine stimulation of mammary tumor development and growth. The purpose of this study therefore was to compare the effects of different animal and vegetable high fat diets fed to rats only from weaning until 1 week after DMBA administration, on subsequent mammary tumorigenesis, sexual maturation, and endocrine function.

MATERIALS AND METHODS

Animals and Diets. Weanling female Sprague-Dawley [Crl: CD(SD)BR] rats, 21 days old, were purchased from Charles River Breeding Laboratories (Wilmington, MA). They were immediately divided into different treatment groups and were allowed to feed ad libitum on one of the following diets: 5% (normal fat) corn oil, 20% (high fat) corn oil; 20% palm oil; 20% beef tallow; or 20% lard. The fatty acid composition of these fats is shown in Table 1. The 20% beef tallow diet

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Received 3/25/85; revised 8/27/85; accepted 10/10/85.

1 This work was supported by Grants CA-33240 and CA-24538 from the National Cancer Institute, NIH.
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CANCER RESEARCH VOL. 46 FEBRUARY 1986
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was taken, animals were sacrificed and diestrus uterine weights were recorded. Serum was separated by centrifugation and stored at -20°C until assayed for prolactin, LH, and estradiol.

Tumor Measurements and Classification. Tumor measurements and body weight were recorded at weekly intervals from the beginning until termination of experiment 1. The procedures for tumor palpation and autopsy examination have been described previously (5). Latency period was calculated as the average time of first tumor appearance in each rat for each group. Approximately 15 to 30% of the animals in each treatment group had to be sacrificed before termination of the experiment, when they became moribund from increased tumor burden. Tumors from these rats, as well as tumors that had been palpable for at least 5 successive weeks and then regressed, continued to be included in the tumor data. Upon termination of the experiment, 19 weeks after DMBA administration, all remaining rats were killed, tumors were removed, fixed in buffered formalin and later embedded, sectioned, and stained with hematoxylin and eosin for routine histological examination. Mammary tumor pathology was confirmed according to the criteria of Young and Hallowes (13). Only adenocarcinomas are reported in "Results." The number of tumors showing only a fibroadenoma pathology in at least two sections was: one in the 20% com oil group, two in the 20% lard group, and one in the 20% palm group. Tumors showing mixed pathology (adenocarcinoma plus fibroadenoma) were considered adenocarcinomas for the purpose of this analysis.

Radioimmunoassays. Serum prolactin and LH were assayed by standard radioimmunoassay procedure with NIADDK kits, using the double antibody method of Niswender et al. (14, 15). Serum prolactin and LH values are expressed as ng/ml in terms of NIADDK rat prolactin-RP-3 and NIADDK rat LH-RP-1, respectively. Serum estradiol was assayed using the method of Gambel (16) with antiestriadiol-6-bovine serum albumin (GDN 244), kindly provided by Dr. G. D. Niswender of Colorado State University, and "RP-3"(2,4,6,7-3H)estradiol purchased from New England Nuclear (Boston, Mass.).

Statistical Analyses. Differences in palpable tumor incidence curves were analyzed using the computer program BMDP1L (17) which is set up to perform a 2 x 2 contingency table of the tumor incidence for each week of the experiment. The Mantel-Cox statistic generated at the end is a generalized Savage test which compares observed events with expected events. Tumor incidence at the final time point (consisting of palpable plus nonpalpable tumors or only palpable tumors) was analyzed by χ² analysis. Tumor multiplicity data were analyzed by the Kruskal-Wallis nonparametric analysis of variance with multiple group comparisons. Statistical differences in serum prolactin, LH, and estradiol levels were determined by analysis of variance, and the Student-Newman-Keuls test was used for multiple comparisons among groups. Differences were considered to be significant if P < 0.05 as compared to rats fed 5% com oil diets for the entire experiment.

RESULTS

Experiment 1: Effects of Different Animal and Vegetable Fat Diets on Carcinogen-Induced Mammary Tumorigenesis in Rats. The effects of the various dietary treatments on mammary tumor development 19 weeks after DMBA administration and 18 weeks after the return of all animals to the 5% com oil control diets, are summarized in Table 2. Rats fed a 20% lard diet during the treatment period showed a significant enhancement in mammary tumor incidence and number when compared to all other dietary treatment groups. Rats fed a 20% beef tallow diet during the same period also demonstrated enhanced tumor development as compared to rats fed the 5% com oil diet for the entire experiment, although these increases barely missed statistical significance. Mammary tumor development in rats fed 20% corn oil or palm oil diets did not differ from that of 5% com

### Table 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>% of total fatty acids</th>
<th>C12:0</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1</th>
<th>Lard⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>0.2</td>
<td>1.0</td>
<td>1.5</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palm oil</td>
<td>4.3</td>
<td>23.7</td>
<td>24.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard</td>
<td>1.9</td>
<td>18.3</td>
<td>18.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>24.9</td>
<td>39.5</td>
<td>40.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All others</td>
<td>1.3</td>
<td>1.2</td>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These are actual analytical values determined by gas-liquid chromatography.

Com oil and palm oil were analyzed by Procter & Gamble Co., Cincinnati, OH, and lard and beef tallow by Best Foods, Englewood Cliffs, NJ.

Supplied by Best Foods.

Supplied by Procter & Gamble Co.

Purchased from Teklad, Madison, WI. Both lard and beef tallow were extracted by steam rendering.

The peroxide values were 2.8 and 2.0 meq/kg, respectively. It should be noted that rancidity occurs when the peroxide value is >20 meq/kg.

It should be noted that rancidity occurs when the peroxide value is >20 meq/kg.
DIETARY FATS AND BREAST CANCER

Table 2
Effect of different levels of animal and vegetable fats fed before and during carcinogen administration on DMBA-induced mammary carcinogenesis

Rats were fed the different diets from weaning until 1 week after DMBA administration at 52 days of age. They were then switched to the 5% corn oil control diet, which was continued until the end of the experiment, 19 weeks after DMBA administration. Tumor incidence curves for rats fed a 20% lard diet during the treatment period also showed high mammary tumor incidence; however, this was found to be significantly different (P < 0.05) from all other dietary treatment groups. The 20% beef tallow group was significantly different (P < 0.05) as compared to 5% corn oil controls between weeks 10 and 19.

![Graph with data points and trend lines showing the effect of different dietary fats on mammary tumor incidence.](cancerres.aacjrournals.org)
Table 3
Effects of the various dietary treatments on sexual maturation of rats*

<table>
<thead>
<tr>
<th>Dietary treatment group</th>
<th>5% corn oil</th>
<th>20% corn oil</th>
<th>20% beef tallow</th>
<th>20% lard</th>
<th>20% palm oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days) of vaginal opening</td>
<td>38.1 ± 1.2</td>
<td>34.4 ± 0.6</td>
<td>35.9 ± 0.6</td>
<td>34.1 ± 0.4</td>
<td>35.6 ± 0.5</td>
</tr>
<tr>
<td>Body wt (g) at first estrus</td>
<td>119 ± 5</td>
<td>108 ± 4</td>
<td>115 ± 4</td>
<td>108 ± 3</td>
<td>110 ± 4</td>
</tr>
</tbody>
</table>

* Treatment diets started at weaning (21 days of age).

** Mean ± SE of 15-16 animals.

** P < 0.05 as compared to 5% corn oil controls.

The effects of the various diets on serum prolactin levels in 50-to 60-day-old rats are shown in Fig. 3. Animals in all dietary groups had similar proestrous morning (basal) levels and proestrous afternoon (surge) levels of serum prolactin. Similarly, rats fed the various diets showed no significant differences in basal or surge levels of LH (Fig. 4) or estradiol (Fig. 5). Diestrous uterine weights of 55- to 65-day-old rats in the various dietary treatment groups are shown in Table 4. No significant differences in diestrous uterine weights were found among the groups, whether compared as whole uteri or on a 100-g body weight basis.

DISCUSSION

The results in the present study demonstrate that rats fed diets high in animal but not in vegetable fat, during the initiation phase of carcinogenesis, display enhanced mammary tumor development, even though these animals consumed normal fat diets throughout the subsequent promotional phase. Rats fed a 20% lard diet from weaning until 1 week after DMBA administration showed a significant increase in mammary tumor development when compared to all other dietary treatment groups.
Similarly, rats fed a 20% beef tallow diet during this dietary treatment period also showed greater mammary tumor incidence and number as compared to rats fed a normal fat diet for the entire experiment. Although all parameters which displayed increased mammary tumor development in the 20% beef tallow dietary treatment group did not reach statistical significance, these increases are believed to be physiologically significant. In contrast, rats fed 20% corn oil or palm oil diets during the dietary treatment period showed no differences in mammary tumor development from that of normal fat controls.

These findings are of particular interest since previous studies have shown that the level of dietary fat consumed after carcinogen administration is more important in influencing rodent mammary tumorigenesis than is the level of dietary fat consumed before exposure to carcinogen (2, 4). Rats fed high fat diets starting as late as 20 weeks after DMBA administration still display enhanced mammary tumor development, as compared to rats fed normal fat diets (5). These and other previous studies (18) have strongly suggested that high dietary fat intake influences primarily the promotional phase of mammary carcinogenesis.

The type of fat consumed has also been shown to be an important factor in determining the magnitude by which high dietary fat intake stimulates murine mammary tumorigenesis. In general, high fat diets containing primarily polyunsaturated fatty acids enhance mammary tumor growth to a greater extent than do diets containing saturated fatty acids, and fats with the highest concentrations of C18:2 fatty acids (linoleic) are the most potent (18–20). Thus high corn oil (60% linoleic) diets have been shown to stimulate mammary tumorigenesis to a greater extent than do equally high lard (10% linoleic), palm oil (10% linoleic), beef tallow (3% linoleic), or coconut oil (2% linoleic) diets, when fed to rats during the promotional phase of carcinogenesis (19, 21–24).

The present study confirms and extends some of these earlier investigations and establishes the important stimulatory effects of diets high in animal fat (lard and beef tallow) on mammary tumorigenesis, when fed to rats around the time of carcinogen administration (initiation). Rogers et al. (8) have reported similar findings in rats fed high lard diets. However, the current study suggests that this stimulation may not be unique to lard or is an effect which can be induced by all high saturated fat diets. Therefore the role of dietary fat in the development and growth of mammary cancer appears to depend not only on the type of fat consumed, but also on the phase of carcinogenesis when that particular fat is ingested.

It has been established that mammary tumors in rats are dependent on hormones for development and growth (25). An increase (or decrease) in circulating estrogen and/or proestrus levels around the time of carcinogen administration results in a corresponding increase (or decrease) in rat mammary tumorigenesis (26, 27). Since mammary tumorigenesis was enhanced only in the groups given diets high in animal fat (lard and beef tallow), it was possible that estrogens present in animal fat might be responsible for this stimulation in mammary tumorigenesis. Results from experiment 2, however, do not support this hypothesis.

Conditions of chronic hyperprolactinemia have been shown to significantly advance the onset of puberty in rats (28), and estrogens are a potent stimulus of prolactin secretion from the pituitary (29). It does not appear however, that the reduction in the age of sexual maturation in rats fed diets high in fat is mediated through endocrine stimulation. No correlation was observed between the time of first estrus and subsequent mammary tumorigenesis in rats fed the various dietary treatments. Animals fed high fat diets during the prepubertal period reached sexual maturity earlier than did normal fat controls, regardless of the type of fat in the diet. Thus advancing the age of sexual maturation in rats is dependent on the level rather than on the type of fat consumed.

The exact mechanism by which high fat diets advance the onset of puberty in rats is not fully understood (30). Increased body mass has been positively correlated with a reduction in the age of sexual maturation (31). However, no differences in mean body weight were found among the various dietary treatment groups throughout the experiment. Furthermore, rats fed high fat diets weighed slightly less than did normal fat controls at the time of first estrus, reflecting the younger age of these animals.

Clearly if estrogens present in animal fat diets were absorbed during digestion and entered the circulation in sufficient concentrations to stimulate mammary tumorigenesis, these estrogens should also affect normal endocrine function. However, no effects were observed in estrous cycle regularity in rats fed the different normal and high fat diets. In addition basal and surge levels of serum prolactin, LH, and estradiol were similar, as were diestrus uterine weights of rats fed the various diets. It appears therefore that diets containing high levels of animal fat cause little or no expressed increases in estrogenic activity in the rat. Subsequent analysis of the lard and beef tallow failed to show any estrogenic activity.

In summary, a high dietary intake of lard and beef tallow during early development and until 1 week after carcinogen administration results in significant enhancement in mammary tumorigenesis in rats. In contrast, diets high in vegetable fat containing either polyunsaturated (corn oil) or saturated (palm oil) fatty acids had no effect on subsequent mammary tumorigenesis when fed to rats during this time period. The mechanism(s) by which animal fat diets bring about this stimulated tumor development is unknown; however, it does not appear to result from exogenous or endogenous endocrine stimulation. It has been demonstrated that fat type can influence other factors including carcinogen metabolism (32), host immune function (18, 32), or cell to cell communication (18), which indirectly affect mammary carcinogenesis, as well as exert a direct effect on the mammary gland (20). The first of these, carcinogen activation, could be easily examined by repeating the experiment using a direct acting carcinogen such as N-nitrosomethylurea. However, the specific effects of animal fat fed during the initiation phase of carcinogenesis on the other factors has not yet been clarified. Finally there exists the disturbing possibility that animal fats might contain materials with carcinogenic or cocarcinogenic activity, as the result of livestock consumption of lipid soluble food contaminants (e.g., pesticides) or additives (e.g., antibiotics). While the total pesticide (dieldrin, dichlorodiphenyl dichloroethylene, dichlorodiphenytrichloroethane, and metabolites) level of the lard (36 ppb) and tallow (52 ppb) used in this study were below the "allowable" level of 300 ppb, the safety of daily consumption of low levels of these compounds has not been established and further studies are clearly warranted.
ACKNOWLEDGMENTS

We are very grateful to Best Foods (Englewood Cliffs, NJ) for their determination of the fatty acid composition, peroxide value, pesticide content, and estrogenic activity of the lard and beef tallow used in this study, and to Procter & Gamble Co. (Cincinnati, OH) for the fatty acid analysis of the corn and palm oils.

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