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. Weaning 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, 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 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 [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 appears 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.

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

The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; LH, luteinizing hormone.
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Table 1
Fatty acid composition of fats*%

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Corn oil</th>
<th>Palm oil</th>
<th>Lard</th>
<th>Beef tallow</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>0.2</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C16:0</td>
<td>10.4</td>
<td>43.8</td>
<td>39.5</td>
<td>24.2</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.1</td>
<td>4.8</td>
<td>9.9</td>
<td>1.3</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.9</td>
<td>3.3</td>
<td>39.5</td>
<td>40.8</td>
</tr>
<tr>
<td>C18:1</td>
<td>24.9</td>
<td>18.3</td>
<td>9.2</td>
<td>3.2</td>
</tr>
<tr>
<td>C18:2</td>
<td>60.8</td>
<td>10.2</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.3</td>
<td>0.4</td>
<td>0.4</td>
<td>3.2</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td>3.2</td>
</tr>
<tr>
<td>All others</td>
<td>2.0</td>
<td>3.2</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* These are actual analytical values determined by gas-liquid chromatography. Corn oil and palm oil were analyzed by Procter & Gamble Co., Cincinnati, OH, and were obtained from Teklad, Madison, WI. Lard and beef tallow were analysis by steam rendering. The peroxide values were 2.8 and 2.0 meq/kg, respectively.

RESULTS

Experiment 1: Effects of Different Animal and Vegetable Fats on Carcinogen-Induced Mammary Tumorigenesis in Rats. The effects of the various dietary treatments on mammary tumor development were studied in rats administered DMBA. The experiments were performed with rats fed 5% corn oil diets for 20 weeks after DMBA administration. The results showed that the dietary fat treatments had a significant effect on mammary tumor incidence and number. The rats fed the 20% com oil diet had a significantly lower tumor incidence and number compared to the rats fed the 5% corn oil diet. The rats fed the 20% lard diet also had a lower tumor incidence and number compared to the rats fed the 5% corn oil diet. The results also showed that the rats fed the 20% palm oil diet had a higher tumor incidence and number compared to the rats fed the 5% corn oil diet.

Radioimmunoassays. Serum prolactin and LH were measured by standard radioimmunoassay procedure with NIAIDK 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 NIAIDK rat prolactin-RP-3 and NIAIDK rat LH-RP-1, respectively. Serum estradiol was assayed using the method of Gambel (16) with antiestriol-6-bovine serum albumin (GDN 244), kindly provided by Dr. G. D. Niswender of Colorado State University, and 17β(2,6,7)-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 of the program is a generalized Savage test which compares observed events with expected events. Tumor incidence at the final time point (consisting of palpable and nonpalpable tumors or only palpable tumors) was analyzed by chi-squared 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% corn oil diets for the entire experiment.
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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 following 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.

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Final tumor incidence</th>
<th>Latency period (wk)</th>
<th>Initial body wt (g)</th>
<th>Carcass wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% corn oil</td>
<td>15/32 (46.9%)</td>
<td>11.9 ± 1.3</td>
<td>202 ± 2</td>
<td>339 ± 6</td>
</tr>
<tr>
<td>20% corn oil</td>
<td>16/32 (56.3%)</td>
<td>10.3 ± 2.6</td>
<td>200 ± 2</td>
<td>327 ± 4</td>
</tr>
<tr>
<td>20% palm oil</td>
<td>16/32 (50.0%)</td>
<td>9.6 ± 0.7</td>
<td>205 ± 2</td>
<td>338 ± 7</td>
</tr>
<tr>
<td>20% lard</td>
<td>17/32 (53.1%)</td>
<td>9.8 ± 0.7</td>
<td>203 ± 2</td>
<td>325 ± 8</td>
</tr>
<tr>
<td>20% beef tallow</td>
<td>21/32 (65.6%)</td>
<td>11.4 ± 0.6</td>
<td>201 ± 2</td>
<td>335 ± 5</td>
</tr>
</tbody>
</table>

* Includes both palpable and nonpalpable adenocarcinomas.
* When the incidence of only palpable tumors is considered, the only change is in the 5% corn oil group, which had a palpable tumor incidence of 13 of 32 or 40.6%. The statistical significance of the incidence data remains the same.
* This value is derived considering both tumor-bearing and non-tumor-bearing rats. The statistical significance of the data does not change when only palpable tumors are considered.
* The 20% lard group is significantly different from the 5% com oil and 20% palm oil groups (P < 0.05).

Fig. 1. Tumor incidence in rats fed a 20% lard diet during the treatment period also showed high mammary tumor incidence; however this was found to be significantly different from 5% corn oil controls only between 10 and 19 weeks after DMBA administration. Tumor incidence curves for rats fed a 20% com oil or palm oil diet did not differ statistically from that of the 5% corn oil controls, although there was a trend toward a higher incidence from 10–19 weeks after DMBA administration for the former.

The number of palpable mammary tumors as they appeared in the various dietary treatment groups is shown in Fig. 2. Throughout the course of the experiment the tumor number curves for rats fed the 20% lard and beef tallow diets during the treatment period had a greater slope than all other dietary treatment groups. Tumor number curves of the other dietary treatment groups, showed little differences from that of the 5% corn oil controls.

Experiment 2: Effects of the Different Animal and Vegetable Fat Diets on Sexual Maturation and Endocrine Function in Rats. The average day of vaginal opening for rats fed the various dietary treatments is shown in Table 3. First estrus occurred at 38.2 ± 1.2 days in rats fed a 5% corn oil diet. Vaginal opening occurred significantly earlier in rats fed 20% corn oil, lard, or palm oil diets as compared to 5% corn oil controls. Rats fed a 20% beef tallow diet also displayed an earlier age of vaginal...
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Table 3

Effects of the various dietary treatments on sexual maturation of rats

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

Fig. 4. Effects of the various dietary treatments on serum LH levels in 50- to 60-day-old rats at 0900, 1000, 1100, 1500, 1600, and 1700 h on proestrus. Bars, mean ± SE of 10-11 animals.

Fig. 5. Effects of the various dietary treatments on serum estradiol levels at 12 noon diestrus (basal levels), and proestrus (surge levels) in 55- to 65-day-old rats. Bars, mean ± SE of 10-11 animals.

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 prolactin 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, dichlorodiphenyldichloroethylene, 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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