Enhancement of Mammary Carcinogenesis by a High-Fat Diet in Fischer, Long-Evans, and Sprague-Dawley Rats

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ABSTRACT

The relationship between dietary fat and mammary carcinogenesis was examined by feeding a high-fat or a low-fat purified diet, differing only in fat and carbohydrate content, to Fischer, Long-Evans, and Sprague-Dawley rats. An additional group of rats from each strain was fed a nonpurified laboratory diet for comparison. The rats were given an i.v. dose of N-nitrosomethylurea (50 mg/kg body weight) at 50 days of age. The results showed that (a) a high-fat purified diet significantly enhanced mammary carcinogenesis; (b) a nonpurified laboratory diet retarded mammary carcinogenesis compared to a low-fat purified diet; (c) rats on three different diets had a similar body weight gain; (d) the susceptibility of N-nitrosomethylurea-induced mammary carcinogenesis in the three strains of rats was in the order of Sprague-Dawley > Fischer > Long-Evans; and (e) Fischer rats appeared to be better suited for further systematic studies of dietary fat and mammary carcinogenesis.

INTRODUCTION

Although human epidemiological studies implicate dietary fat as a causative factor in the rising incidence of breast cancer in certain populations (2-4, 9, 11), there has been no evidence that unequivocally supports this implication of a causal relationship in the pathogenesis of breast cancer. In experimental studies in the rodent species, the data suggest that consumption of high levels of dietary fat enhances mammary tumorigenesis (4-7, 13, 19). However, in these experiments, the animals usually consumed food different not only the amount of fat but also in the quantity of other food ingredients, such as protein, minerals, vitamins, and fiber. As a result, it is difficult to ascertain whether the effects on mammary carcinogenesis were due to dietary fat or other dietary components (17).

The objectives of this investigation are: (a) to study the effect of dietary fat on mammary carcinogenesis in rats fed special diets differing only in fat and carbohydrate content; (b) to compare the effects of a purified and nonpurified diet4 on mammary carcinogenesis; and (c) to examine the relationship between dietary fat and mammary carcinogenesis in 3 strains of rats and to determine which strain is best suited for future systematic studies of diet and mammary carcinogenesis.

MATERIALS AND METHODS

Female Fischer, Long-Evans, and Sprague-Dawley rats were used in the present experiments. They were 21 to 27 days old at the time of purchase from the Charles River Breeding Laboratories, Inc., Wilmington, Mass. Each strain of rat was divided into 3 experimental groups: rats in Group 1 were fed a HF4 diet; those in Group 2 were fed a LF diet; and those in Group 3 were given a NP diet ad libitum. The purified diets were prepared according to the method of Newberne et al. (17) except that dextrose was substituted for starch. This was necessary as dextrose absorbs oil more readily than starch and the food thus prepared stays in solid form. The diets were prepared weekly and kept at 4°C. Fresh food was fed to the rats at 5 p.m. daily. The formulas of the 2 purified diets are shown in Table 1. It should be noted that the proportion of each food ingredient in the HF diet has been adjusted so that the caloric density in 75.1 g of the HF diet is identical to that in 100 g of the LF diet, assuming 4.1 kcal/g for dextrose and casein and 9.2 kcal/g for corn oil. Since rats adjust their food intake by calories, the amount of food consumed by the rats on the HF diet will be 24.9% less than that consumed by the rats on the LF diet. Thus, at any given caloric intake, the rats on the 2 diets will consume an identical quantity of every food ingredient except fat and carbohydrate. The NP diet (Teklad Mouse/Rat Diet) contained 25.5% crude protein, 4.5% fat, 4.6% fiber, 9.9% ash, 10.2% moisture, 45.2% carbohydrate, and vitamins. All animals received one single i.v. dose (50 mg/kg body weight) of NMU (Ash Stevens, Inc., Detroit, Mich.) at 50 days of age. NMU was dissolved in water (10 mg/ml), pH 5.4, acidified with 3% acetic acid (10). We chose NMU as the carcinogen for these experiments rather than DMBA because NMU is water soluble and is not absorbed in the mammary fat pad. Furthermore, NMU is a direct carcinogen that does not require metabolic activation (16).

The animals were examined weekly for mammary tumors after carcinogen administration. The location and size of the palpable tumors were recorded. The body weight of the rats was monitored biweekly. All animals were killed at 6 months after NMU treatment, and the mammary tumors were dissected out and prepared for histopathological studies.

RESULTS

Effect of the Experimental Diets on Growth Rate in 3 Strains of Rats. The body weights of the 3 strains of rats fed the purified and NP diets are shown in Chart 1. The data clearly show that there is no difference in weight gain among rats of different strains fed a HF, LF, or NP diet. All 3 strains of rats

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2 To whom requests for reprints should be addressed.

3 A purified diet is composed primarily of refined ingredients and a nonpurified diet predominantly of unrefined plant and animal material (1).

4 The abbreviations used are: HF, high-fat, low-carbohydrate purified diet; LF, low-fat, high-carbohydrate purified diet; NP, nonpurified laboratory diet; NMU, N-nitrosomethylurea; DMBA, 7,12-dimethylbenz[a]anthracene.

5 The vitamins contained in each kg of the nonpurified diet are: vitamin A, 11931.00 IU; carotene, 3.52 mg; vitamin D3, 1540.00 IU; vitamin E, 12.27 IU; vitamin B12, 0.0109 mg; choline, 1710.00 mg; folic acid, 0.43 mg; menadione, 12.14 mg; niacin, 38.51 mg; pantothenic acid, 12.36 mg; pyridoxine, 5.47 mg; riboflavin, 7.44 mg; and thiamin, 4.36 mg.
Table 1
Composition of the purified diets

<table>
<thead>
<tr>
<th>Diet ingredient</th>
<th>LF (g)</th>
<th>HF (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Dextrose</td>
<td>64.5</td>
<td>19.6</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin mix (AIN-76)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mix (AIN-76)</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Water</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>75.1</td>
</tr>
</tbody>
</table>

* Purchased from ICN Nutritional Biochemicals Division, Cleveland, Ohio.

Chart 1. Body weight of rats on a HF (O), LF (O), or NP (x) diet. Fischer rats; Long-Evans rats; Sprague-Dawley rats. Bars, S.E.

receiving a HF diet had a lower average body weight at the beginning of the experiment, and this difference persisted throughout the experimental period. It should be noted that the growth rate of Fischer rats is significantly lower than that of either the Sprague-Dawley or Long-Evans rats. In Fischer rats, the body weight appears to plateau at about the age of 60 days; whereas in the Sprague-Dawley and Long-Evans rats, the body weight shows a continued increase until the age of 150 days. This difference in growth rate, however, is not related to the diet they consume.

Effect of Diet on Mammary Carcinogenesis in 3 Strains of Rats. The results presented in Table 2 summarize the mammary cancer incidence and number of adenocarcinomas observed at the time of autopsy in the rats fed different dietary formulas. The cumulative palpable mammary tumor incidences are shown in Charts 2 to 4. The data disclosed that the mammary tumor incidence varied depending on the diet. The incidence was highest in rats fed a HF diet, intermediate in rats fed a LF diet, and lowest in rats fed a NP diet.

Table 2
Mammary cancer incidence in 3 strains of rats fed 3 different types of diets

<table>
<thead>
<tr>
<th>Rat</th>
<th>Diet</th>
<th>No. of rats at risk</th>
<th>% of mammary cancer incidence</th>
<th>No. of adenocarcinomas/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fischer</td>
<td>HF</td>
<td>25</td>
<td>80%</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>LF</td>
<td>27</td>
<td>33%</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>46</td>
<td>34%</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Long-Evans</td>
<td>HF</td>
<td>29</td>
<td>59%</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>LF</td>
<td>29</td>
<td>31%</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>51</td>
<td>20%</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>HF</td>
<td>16</td>
<td>100%</td>
<td>9.6 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>LF</td>
<td>16</td>
<td>88%</td>
<td>6.4 ± 0.8†</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>41</td>
<td>70%</td>
<td>3.3 ± 0.5†</td>
</tr>
</tbody>
</table>

* Including nonpalpable mammary tumors discovered at autopsy.
† HF versus LF, p < 0.05 (χ² test).
‡ Mean ± S.E.
§ HF versus LF, p < 0.05 (t test).
* LF versus NP, p < 0.05 (t test).

Chart 2. Cumulative palpable mammary tumor incidence in Fischer rats fed 3 dietary regimens: HF; LF; NP. The rats were given NMU i.v. at 50 days of age. The incidence at 24 weeks included nonpalpable mammary tumors discovered at autopsy.
Statistically, mammary tumor incidence was significantly higher in both Fischer and Long-Evans rats fed a HF diet than in those fed a LF diet. Although the tumor incidence was also higher in Sprague-Dawley rats fed a HF diet, the difference was not significant. In all 3 strains of rats, the number of tumors per rat was lower among those fed a LF diet than it was among their counterparts fed a HF diet.

It is interesting to observe that rats fed a NP diet had a lower mammary tumor incidence than those fed a LF diet, even though the fat content of the 2 diets was almost identical. This difference is particularly evident in the Long-Evans rats \( p < 0.05 \) (Chart 3). In addition, the mammary tumors appeared later and in smaller numbers in all 3 strains of rats receiving the NP diet. This observation led to the suggestion that the NP diet contains factor(s) which may retard mammary carcinogenesis.

**Susceptibility to Mammary Tumor Induction by NMU.** A comparison of mammary tumor incidence and tumor numbers in the 3 strains of rats showed that there was a difference in susceptibility to NMU in these 3 strains of rat. Sprague-Dawley rats appeared to be the most susceptible, whereas Long-Evans rats appeared to be the least susceptible. The difference in sensitivity was observed regardless of whether the rats were fed a HF, LF, or NP diet.

**DISCUSSION**

We observed that rats fed 3 different types of diet had similar body weight gain (Chart 1) in spite of the differences in the caloric density of the diets. We measured the food intake of 120-day-old Fischer rats on the HF and the LF diet and found that the daily average calorie intake per rat was 43 kcal in both groups of rats. Due to the difference in caloric density, rats consumed 8.3 g of the HF diet and 11.0 g of the LF diet. Our findings support the contention of Newberne et al. (17) that rats, in general, regulate their body weight by adjusting their food intake so that similar energy intake is maintained.

In order to ascertain the effects of dietary fat on mammary carcinogenesis, the experimental animals must consume the same amount of all food ingredients except fat and carbohydrate. For this reason, Newberne et al. (17) formulated 2 diets with which to study the effects of dietary fat. By using these 2 diets, we have now demonstrated convincingly that a relationship exists between dietary fat and mammary carcinogenesis. A high-fat, low-carbohydrate intake enhances, whereas a low-fat, high-carbohydrate intake inhibits, mammary carcinogenesis. These results obviously do not distinguish whether the enhancing effect in mammary carcinogenesis is due to the fat or carbohydrate content of the diet.

The mechanism underlying the modulatory effect of dietary fat on mammary carcinogenesis is not known. Dietary fat may exert a direct effect on mammary cell growth (20). It may modulate the endocrine system (6, 7, 13, 14) or the immune system (15). However, these studies were conducted under dietary regimens different from those used in the present studies. Experiments are being conducted in our laboratory to study how dietary fat modulates mammary carcinogenesis.
Our data also demonstrated that, in 3 strains of rats, the mammary tumor incidence was lower in rats fed a NP diet (Group 3) than it was in rats fed a LF diet, in spite of the fact that the fat levels in the 2 diets were similar. As the source of each food ingredient in the NP diet is a proprietary secret of the manufacturer, the factors in the NP diet which retard mammary carcinogenesis are not fully known. The type of fat, protein, and carbohydrate contained in the cereal-based NP diet may play a role since saturated fat (4), protease inhibitor (18), and starch (12) are known to have an inhibitory effect on mammary carcinogenesis.

The DMBA-induced mammary tumor in Sprague-Dawley rats has been used widely as a model for breast cancer studies. Because DMBA is fat soluble, sequestered in the mammary fat pad, and released slowly (8), high dietary fat may exert profound effects on the distribution and metabolism of this carcinogen. The use of NMU in the present studies has the advantage over DMBA, since the possible effects of dietary fat on the distribution and localization of the carcinogen may be avoided. It is known that NMU is water soluble, has a short half-life (less than 60 min at neutral pH), and is a direct-acting carcinogen (16).

The results of the present study also disclosed that the enhancing effects of dietary fat on mammary carcinogenesis can be best demonstrated in Fischer rats. It appears that Fischer rats may be better suited for studies on the relationship between dietary fat and mammary carcinogenesis. On the other hand, Long-Evans rats can be used to study the inhibition of mammary carcinogenesis by a NP diet.

REFERENCES


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