Dieta rica em gordura versus dieta pobre em calorias na iniciação e progressão do câncer de mama induzido em camundongo

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Abstract

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Three experiments were performed to study this relationship in 7,12-dimethyl-benz(a)anthracene (DMBA)-treated female Sprague-Dawley rats:

(a) high - or low-fat isocaloric diets were fed in a crossover design;

(b) low-fat, high-calorie and high-fat, low-calorie diets were fed in a crossover design;

(c) pair-fed rats were restricted to 60% of the calories of controls with ad libitum access to food beginning 10 days after DMBA administration.

The pair-fed rats received daily 60% of calories, the same level of fiber, and 115% more fat than did rats fed ad libitum. Tumor yield but not tumor incidence was greater in rats fed high-fat rather than low-fat isocaloric diets prior to initiation of tumorigenesis. **A low-fat, high-calorie diet led to more tumor incidence** and yield than was associated with feeding of a high-fat, low-calorie diet.

Caloric restriction (although with concomitant intake of more fat) led to complete inhibition of tumor formation.

These results indicate that both high-fat and high-calorie diets exhibit cocarginogenic, not merely promotional, properties**.**

Caloric intake may be a greater determinant than dietary fat of a tumor-enhancing regimen.

Finally, restriction of caloric intake during promotion markedly suppresses tumor formation, despite the increased fat content of the restricted diet, suggesting a permissive role for calories in tumor formation. The possibility remains that alterations in levels of other dietary components could also have contributed to the observed effects. PMID: 6430545

Dietary Fat versus Caloric Content in Initiation and Promotion of 7,12-Dimethylbenz(a)anthracene-induced Mammary Tumorigenesis in Rats1

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ABSTRACT

Enhancement of mammary tumor formation by dietary fat may be mediated via increased caloric intake. Three experiments were performed to study this relationship in 7,12-dimethylbenz(a)anthracene (DMBA)-treated female Sprague-Dawley rats: (a) high- or low-fat isocaloric diets were fed in a crossover design; *(b) low-fat, high-calorie and high-fat, low-calorie diets were fed* in a crossover design; (c) pair-fed rats were restricted to 60% of the calories of controls with ad libitum access to food beginning 10 days after DMBA administration. The pair-fed rats received daily 60% of calories, the same level of fiber, and 115% more fat than did rats fed ad libitum. Tumor yield but not tumor incidence was greater in rats fed high-fat rather than low-fat isocaloric diets prior to initiation of tumorigenesis. A low-fat, highcalorie diet led to more tumor incidence and yield than was associated with feeding of a high-fat, low-calorie diet. Caloric restriction (although with concomitant intake of more fat) led to complete inhibition of tumor formation. These results indicate that both high-fat and high-calorie diets exhibit cocarginogenic, not merely promotional, properties. Caloric intake may be a greater determinant than dietary fat of a tumor-enhancing regimen. Finally, restriction of caloric intake during promotion markedly suppresses tumor formation, despite the increased fat content of the restricted diet, suggesting a permissive role for calories in tumor formation. The possibility remains that alterations in levels of other dietary components could also have contributed to the observed effects.

INTRODUCTION

Epidemiological and experimental investigations have attempted to identify those aspects of diet that may play a role in the initiation or promotion of cancer. Breast cancer has been linked to both fat availability and total caloric intake (1, 5) by some studies but not by others (13). Since fat provides more than twice the calories of any other food component, it is possible that the enhancing effects of fat on carcinogenesis might be mediated via increased caloric intake. Both "overnutrition" and body mass, rather than obesity itself, have been identified as factors positively related to breast cancer (11, 30). Obesity appears to correlate with a poor prognosis in women with

diagnosed breast cancer (12, 23). Evaluation of the available data by a committee of the National Research Council led to the conclusion that there was little evidence permitting a clear inter-

pretation of the specific effect of caloric intake on the risk of cancer (9).

We have attempted to address the question of fat intake *versus caloric intake using 3 separate dietary approaches to* altering fat and caloric intake in DMBAMreated rats. The 3 dietary regimens were: (a) isocaloric diets containing high or low fat; (b) comparison of low-fat, high-calorie and high-fat, lowcalorie diets; and (c) restriction of food intake in pair-fed animals. In the first 2 studies, manipulations of caloric content in ad libitum diets required the use of widely variable amounts of fiber. While we are unaware of experimental evidence that dietary fiber affects the course of breast cancer, we deemed it important to compare caloric effects in diets with similar levels of fiber. The first 2 studies examined dietary influence during initiation and promotion of mammary tumors, while the last set of diets was instituted 10 days after carcinogen administration. Evidence is presented that high caloric intake enhances tumorigenesis, a high-fat diet affects initiation as well as promotion of mammary tumorigenesis in this model, and caloric restriction suppresses tumor formation during the promotion period, even if more fat is consumed.

MATERIALS AND METHODS

Female Sprague-Dawley rats were received at 22 days of age (Charles River Breeding Laboratories, Kingston, NY) and housed 3/cage in an airconditioned room maintained at 21°with a 12-hr light-dark cycle. DMBA (Eastman Kodak, Rochester, NY) was dissolved in corn oil, and 5 mg were administered in 0.5 ml by gastric intubation to each rat at 50 days of age. Food consumption, body weight, and palpable tumors were checked weekly.

Three approaches to alteration of caloric intake were studied. The first experiment (Groups A to D) used semipurified isocaloric diets (3.51 kcal/ g) which contained either 3.9 or 19.4% fat (Table 1) in a crossover design for which the switch time was determined by DMBA administration (Chart 1). All diets were pelleted and prepared to our specifications by Dyets, Inc., Bethlehem, PA. The second study (Groups E to H) used a low-fathigh-calorie (3.73 kcal/g) diet and a high-fat-low-calorie (2.68 kcal/g) diet, also in a crossover pattern. In both studies, the rats were allowed ad *libitum access to food. From 2 days prior until 2 days after dosing with* DMBA, all rats were fed a standard rodent diet (Wayne Lab-Blox; Allied Mills, Chicago, IL) so there would be no differences in dietary effect on absorption of the carcinogen. In theses 2 studies, there were 15 rats/ treatment group, and the animals were killed 120 days after administra tion of DMBA. The third experiment (Groups I and J) examined the effects of 60% food restriction in pair-fed animals. The diets used were

isocaloric and were formulated so that rats on the restricted diet con sumed the same amounts of fiber and minerals as did the ad libitum-ied counterparts yet received more than twice the fat (8.4 versus 3.9%). There were 24 rats/group housed individually. This study was terminated

3The abbreviation used is: DMBA, 7.12-dimethylbenz(a)anthracene.

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Chart 1. Design of 3 feeeding experiments. Rats were received from supplier at 22 days of age and were given DMBA at 50 days of age. â€¢periods of feeding standard rodent diet.

134 days after DMBA. All diets contained 1% com oil to ensureadequate intake of linoleic acid.

Rats were killed with an i.p. injection of sodium pentobarbital. Follow ing midline ventral incision of the skin, blunt dissection was used to reflect the dermis. Position of all tumors was noted, and the tumors were measured in 3 perpendicular dimensions, weighed, and fixed in 10% buffered formalin. Sections were cut at 5 Mmand stained with hematoxylin and eosin. All tumors were evaluated histologically.

RESULTS

Isocaloric Diets. Growth of rats in the first experiment was
similar among all 4 groups [average terminal body weight, 323 Â±9 (S.E.) g]. No significant differences were noted in consump tion of food when measured by weight or calories. However, the rats consuming the high-fat diet averaged 3.64 Â±0.18 g of fat/ day, while the animals fed the low-fat diet ingested only 0.67 Â± 0.01 g of fat/day.

Tumor incidence was reduced slightly in Group B (low fat switched to high fat) compared with the other 3 groups (Table 2). A significant difference was observed in the total tumor yield. Groups C and D, fed the high-fat diet prior to carcinogen admin istration, exhibited twice as many tumors as did Group A or B. The number of tumors/tumor-bearing rat reflect this, although no statistically significant difference was found for this parameter. These observations suggest that a high-fat diet may affect initiation and/or be cocarcinogenic in DMBA-induced mammary tumorigenesis.

Low-Fat, High-Calorie and High-Fat, Low-Calorie Diets.
Maintenance of rats on the high-fat, low-calorie diet resulted in a significantly smaller weight gain for Groups F and H from 110 days of age onward (Chart 2). These 2 groups attempted to compensate for the lower caloric density of their diet by increas ing food consumption to 23 Â±1 g/day versus 15 Â±1 g/day for the rats consuming the low-fat, high-calorie diet (p < 0.001). Also, Groups F and H ingested 4.3 times as much fat as did the other 2 groups (3.35 Â±0.16 g/day versus 0.77 Â±0.02 g/day). Despite the markedly higher fat intake of the rats fed the highfat, low-calorie diet, tumor incidence was lower (33 and 40%)

Table 2
Effects of dietary treatments on DMBA-inducedmammarytumorigenesis

Chart 2. Mean body weights of rats in studies of low-fat-high-calorie versus

high-fat-low-calorie diets (Groups E to H) and ad libitum feedings versus 60% restriction (Groups I and J). 'Groups F and H were significantly lighter than were groups E and G from 110 days onward; fGroup J was significantly lighter than was Group I from 67 days onward. Dietary regimens are described in Chart 1.

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than in the rats fed the low-fat, high-caloric regimen (67 and 50%). Tumor yield was enhanced significantly only in the group fed the low-fat, high-calorie diet both before and after carcinogen administration (Table 2).

Pair-fed Diets. This experiment was performed to examine the effects of caloric restriction during the period of tumor promotion only. Feeding of the diets was begun 10 days after DMBA administration; within 1 week, the growth of rats fed the restricted diet was significantly lower than that of the ad libitumfed rats (Chart 2). Total inhibition of mammary tumorigenesis was observed during the study period in the rats subjected to 60% dietary restriction (Table 2). This was observed despite a fat intake by the calorie-restricted rats of more than double that of controls.

In all 3 experiments, more than 95% of tumors were classified histologically as well-differentiated adenocarcinomas. No differences in tumor type were observed among dietary groups. In general, palpable tumors reflected total tumor yields in all groups.

DISCUSSION

The data from 3 separate studies altering caloric and fat intake support the conclusions that a high-fat diet influences initiation as well as promotion of DMBA-induced mammary tumorigenesis, that high caloric intake increases tumor yield, and that restriction of caloric intake (even concomitant with the provision of twice as much dietary fat) inhibits tumor formation during the promotion period.

Dao and Chan (10) found that A/-nitroso-A/-methylurea-induced mammary tumorigenesis was influenced by a high-fat diet during both the initiation and promotion phases. This observation was made in rats fed corn oil (5 or 25%) as the only dietary fat. Carroll and Khor (6) reported that DMBA-treated rats fed saturated fat exhibited fewer tumors than did rats fed unsaturated fats. In order to avoid the very high tumor yields associated with polyunsaturated fat, the present study used coconut oil, a highly saturated fat, as the major dietary fat, with enough corn oil to provide equal amounts of essential fatty acids to all groups. It has been reported that lard, a relatively saturated fat, exerted an influence on initiation of DMBA-induced mammary carcinogenesis but that corn oil acted only at the promotional stage (19, 28). However, Visek and Clinton (25) reported recently that DMBA-induced tumors in rats increased proportionally to the amount of corn oil in the diet, whether the diets were fed prior to initiation or during the promotion period.

Lavik and Baumann (16) found marked effects of both fat and

caloric intake in methylcholanthrene-induced skin tumors of mice. They reported that, regardless of the amount of fat fed, the incidence of tumors was much higher in mice given high-caloric diets than in those fed low-caloric diets. A number of sponta neous tumors, including those of the mammary gland, pituitary, and skin, were reduced significantly in rats subjected to 20% food restriction for their entire life span (24). More severe (50%) food restriction for 7 weeks after weaning, followed by ad libitum access to food, also resulted in a decrease of tumor incidence in rats (20). Dietary restriction begun as late as 1 year of age in mice has resulted in significantly increased survival time and an inhibition of spontaneous cancers (26). Reduction in tumor inci dence associated with restriction of food intake might have been due to the reduction of some specific nutrient, such as fat. The current study, as well as those of Tannenbaum (22) and Boutwell *et al. (4), were designed so that animals in experimental and* control groups received the same amounts of fat, vitamins, and minerals. Tannenbaum (22) reported that development of both spontaneous mammary tumors and benzopyrene-induced skin tumors were inhibited proportionally to the degree of caloric restriction. Boutwell ef al. (4) showed that the greater net energy value of a 27% fat diet was sufficient to account for all of the tumor-stimulating effect when compared with a 2% fat diet in mice treated topically with benzopyrene.

A recent report concluded that 50% reduction in food intake during a critical period encompassing 1 week prior to 1 week after carcinogen administration was necessary to reduce tumor incidence (81 versus 21%) significantly in DMBA-treated rats (21). No effects were seen when restricted feeding was imposed for a 2-week period beginning either 1 or 2 weeks after DMBA. The results of our restricted feeding study show clearly that dietary restriction begun 10 days following DMBA treatment has a marked inhibitory effect on tumor development. Since DMBA must be activated to its ultimate carcinogenic form, food restric tion prior to carcinogen administration may alter metabolism of the chemical. Therefore, we chose to utilize the approach of food restriction during the period of tumor promotion only. Differences between the results reported here and those reported by Sylves ter ef al. (21) are probably due to differences in patterns of caloric restriction. Sylvester ef al. (21) restricted dietary intake for only 2 weeks, while we maintained the restriction for 20 weeks. Although both fat and calories appear to influence chemically induced tumorigenesis, the mechanism remains elusive (15). Changes in either fat or caloric intake may affect the immune system (14), alter endocrine status (7), increase adrenocorticotropic hormone activity (3), or alter target tissue membrane composition, with subsequent change in local growth rate (29). The possibility exists that high-fat diets fed prior to administration of DMBA could alter metabolism or distribution of the compound. However, similar findings from studies in which W-nitroso-A/-methylurea, a water-soluble and direct-acting carcinogen, was used (10) suggest that the mechanism by which dietary fat

enhances carcinogenesis is independent of metabolic effects on the carcinogen. A recent review of the evidence for a mechanistic link between high levels of dietary fat and enhancement of mammary tumorigenesis found little support for the view that increased secretion of mammotropic hormones was responsible and suggested that changes in membrane lipid composition were the most significant event related to altered dietary fat (27). However, this hypothesis neglects the permissive effects of increased calories on tumorigenesis.

Nutritional intervention in disease processes can be achieved by various approaches to alteration of dietary composition. The 3 studies described in this report have attempted to address the question of the roles of fat intake versus caloric intake in carcinogenesis. Alterations of other dietary components could have contributed to the observed effects. In the second experiment, the rats ingesting the high-fat, low-calorie regimen (Groups F and H) ate 53% more food, which means their intakes of mineral and vitamin mix were increased, and their casein intake per day was 2.05 g compared to 1.78 g for rats on the low-fat, highcalorie diet. It is unlikely that restriction of casein intake in rats of Group J of the pair-feeding study affected tumor formation. Clinton ef al. (8) found enhanced formation of DMBA-induced mammary tumors in rats maintained on diets low in protein. Reduction of dietary protein levels also leads to greater yields of

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DMBA-induced hepatomas in rats (17). Our data and those of others (10, 19, 25, 28) indicate that high fat and high calorie intake are cocarcinogenic, rather than merely tumor promoters. Under the dietary conditions imposed in this study, dietary calories are a stronger determinant of tumor enhancing effects than is fat content alone.

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