

Inhibition of Chemically Induced Mammary and Colon Tumor Promotion by Caloric Restriction in Rats Fed Increased Dietary Fat¹

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ABSTRACT

Tumor promotion associated with increased dietary fat may be inhibited by reduction in total caloric intake. This hypothesis was tested in rats given either 7,12-dimethylbenz(a)anthracene to induce mammary tumors or 1,2-dimethylhydrazine to induce colon tumors. One week after dosage with either carcinogen, the rats were fed semipurified diets that provided 4% fat with *ad libitum* calories or 13.1% fat with a reduction of calories by 40% from *ad libitum* intake. Rats treated with 7,12-dimethylbenz(a)anthracene and subjected to caloric restriction weighed 40% less than those fed *ad libitum*; rats treated with 1,2-dimethylhydrazine were heavier at the onset of caloric restriction and lost weight and weighed approximately 40% less than animals fed *ad libitum*. At 20 weeks after 7,12-dimethylbenz(a)anthracene administration, rats fed *ad libitum* had 80% tumor incidence while in those fed restricted calories, 20% had tumors ($P < 0.001$). All other measures of mammary tumor growth were significantly reduced in rats given restricted calories. Six months after 1,2-dimethylhydrazine administration, colon tumor incidence was 100% in rats fed *ad libitum* and 53% in those fed the calorie-restricted diet ($P < 0.001$). This reduction of colonic carcinogenesis was seen despite a significant increase in mucosal labeling index following [³H]thymidine autoradiography. This paradoxical finding may be due to the increased fat content of the calorie-restricted diet. These data demonstrate that the tumor-promoting effects of dietary fat can be more than offset by a reduction in total caloric intake and that the promoting effect of fat may be due, at least in part, to its greater caloric density.

INTRODUCTION

Dietary fat has been reported to increase the risk of both breast and colon cancer in epidemiological (1, 2) and experimental (3, 4) studies. Some recent studies have also linked intake of total calories with these diseases (5, 6). Willett and Stampfer (7) pointed out that differences in interindividual caloric intake are confounding factors in assessing the role of fat consumption in epidemiological studies of diet and disease. In addition, increased body weight (as either body mass or obesity) has been associated with greater risk of both breast and colon cancer (8, 9).

A previous study from our laboratory demonstrated that caloric restriction by 40% resulted in complete inhibition of the appearance of DMBA³-induced mammary tumors while 58% of the rats fed *ad libitum* had mammary tumors at necropsy (10); that study used diets whose fat was 1.0% corn oil and the remainder coconut oil (2.9% in the *ad libitum* diet and 13.0% in the restricted diet). Since that work was performed, evidence has been published that the essential fatty acid requirement of DMBA-induced mammary tumors is greater than that of the rat for normal growth and approximates one-fifth of the total dietary fat (11). For this reason, it was deemed essential to

utilize diets that provided more than adequate essential fatty acids in this model. Therefore, the diets for the present study were designed to contain only corn oil which is a good source of linoleic acid. In addition, our first study used a diet for the calorie-restricted rats whose ingredients were adjusted to provide identical daily nutrient intakes except for carbohydrate (which was reduced to decrease calories) and protein. The present study was designed to address the points concerning adequate essential fatty acid, equal intake of protein, and to extend our observations to a chemically induced colon cancer model. Results of the present study indicate that both chemically induced mammary and colon cancer are inhibited significantly by caloric restriction during the promotion period even when the percentage of dietary fat is more than tripled.

MATERIALS AND METHODS

Female Sprague-Dawley rats for the mammary tumor study were purchased from Charles River (Kingston, NY) and received at 43 days of age. Male Fischer 344 rats for the colon tumor study were also from Charles River and were received at 35 days of age. Animals were housed three per polycarbonate cage on corn cob bedding during administration of the carcinogens. 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. DMH (Aldrich Chemical Co., Milwaukee, WI) was dissolved in 0.9% saline and buffered to neutral pH with sodium bicarbonate; the equivalent of 30 mg/kg of the dihydrochloride salt was given by gavage for six consecutive weeks. All carcinogen handling was in accordance with NIH and institutional guidelines.

One week after the final dose of each carcinogen, the rats were randomized into groups of 20 for the mammary tumor study and 19 for the colon tumor study, housed individually, and fed one of two semipurified diets (Table 1). The diets were formulated to provide equal daily nutrient intake by rats fed either diet except for fat and carbohydrate. The percentage of fat was more than tripled in the calorie-restricted diet and carbohydrate was reduced proportionately. Diets were prepared in pellet form by Dyets, Inc. (Bethlehem, PA). Thirty grams of food was given to each rat fed *ad libitum* daily (which is more than any rat consumes in 24 h). Food consumption was measured daily and fresh food given. Rats in the calorically restricted groups were pair fed to those in the group fed *ad libitum* and given 60% of the weight of food that the *ad libitum*-fed rats had consumed the day before. Rats were weighed and checked for palpable mammary tumors weekly; in the colon tumor study, rats were examined at least twice a week for evidence of tumors. Animals that exhibited at least two objective signs of illness including anorexia, weight loss, anemia, ruffled fur, or fecal staining around the anus were euthanized and necropsied; these animals are included in the statistical analyses. Surviving animals were killed in the DMBA study after 20 weeks of feeding the semipurified diets and in the DMH study after 28 weeks.

Five rats from each dietary treatment group that had been given DMH were given i.p. injections of [³H]thymidine (New England Nuclear Corp., Boston, MA), 1 μ Ci/g body weight, 6.7 Ci/mmol, 1 h prior to killing. All injections were given between 0900 and 1000 h following an overnight fast. Samples were taken from the midcolon, processed to paraffin, and sectioned at 3 μ m. Autoradiographs were prepared by the dipping method using Kodak NTB2 emulsion:water (1:1), followed by development with D-19, acid fixation, and staining with hematoxylin and eosin. Fifty crypt columns per animal were counted.

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³The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; DMH, 1,2-dimethylhydrazine.

INHIBITION OF MAMMARY AND COLON TUMOR PROMOTION

Table 1 Semipurified diet composition

Ingredient	Ad libitum	Restricted
Sucrose	59.0	25.0
Casein, edible	21.6	36.2
DL-methionine	0.3	0.5
Corn oil ^a	4.0	13.1
Cellulose	10.1	16.8
Mineral mix ^b	3.8	6.4
Vitamin mix ^c	1.0	1.7
Choline dihydrogen citrate	0.2	0.3

^a Additive-free corn oil from Welch, Clark, & Holmes.

^b Bernhart-Tomarelli mix (12).

^c AIN-76A mix (13).

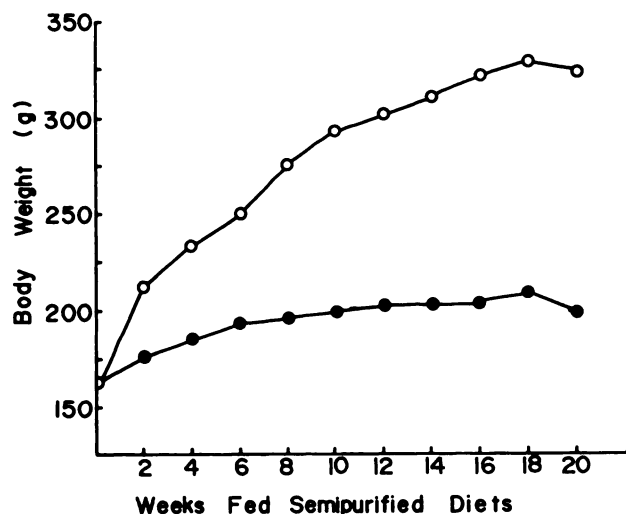


Fig. 1. Body weights of female Sprague-Dawley rats given DMBA and fed *ad libitum* (○) or a 40% calorie-restricted diet (●). Animals were 57 days of age at 0 weeks.

Rats were killed by injections of T-61 euthanasia solution. For the mammary tumor study, all tumors were dissected, weighed, and processed for histological examination. For the colon tumor study, location and size of all tumors were recorded followed by histological examination and classification according to criteria of the International Agency for Research on Cancer (14). Statistical analyses were performed using Statpak software (Northwest Analytical, Portland, OR). Tumor incidence and yield were compared using the χ^2 test. Extracolonic tumors found in the DMH study were compared with Fisher's exact test using a two-sided level of significance. Tumors per tumor-bearing rat, tumor weight and burden, and cytokinetic parameters were all compared using the Mann-Whitney test with two-sided significance levels.

RESULTS

Mammary Tumor Study. Within one week of instituting caloric restriction, the pair-fed female Sprague-Dawley rats gained significantly less weight than the animals allowed *ad libitum* food consumption (Fig. 1). Over the 20-week period, the mean weight gain among rats fed *ad libitum* was 157 g while the rats subjected to 40% caloric restriction gained only 36 g ($P < 0.001$). The feed efficiency (grams of weight gained/1,000 kcal consumed) of the two groups was 18.77 and 7.17 for the *ad libitum* and restricted groups, respectively. Mean daily fat consumption was 0.66 g in the *ad libitum*-fed group and 1.3 g in the calorie-restricted group.

The tumor results are presented in Table 2. Only a single palpable tumor developed in the calorie-restricted group while the cumulative incidence in the rats fed *ad libitum* was 65% at 20 weeks. Final tumor incidence at necropsy was 80% in the rats fed *ad libitum* and 20% in the rats given restricted calories

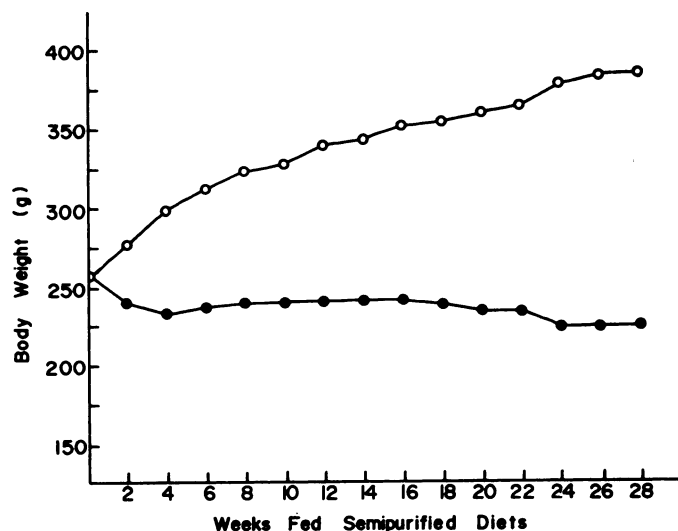


Fig. 2. Body weight of male Fischer 344 rats given DMH and fed *ad libitum* (○) or a 40% calorie-restricted diet (●). Animals were 84 days of age at 0 weeks.

 Table 2 Mammary tumors in rats fed *ad libitum* or restricted calories

	Ad libitum	Restricted	Significance
Tumor incidence	16/20 80%	4/20 20%	$P < 0.001$
Tumors/tumor-bearing rat	4.0 \pm 0.5	1.0 \pm 0.0	$P < 0.001$
Total tumor yield	62	4	$P < 0.001$
Mean tumor weight, g	2.8 \pm 1.1	0.2 \pm 0.1	$P < 0.001$
Mean tumor burden, g ^a	11.1 \pm 4.4	0.2 \pm 0.1	$P < 0.001$

^a Total weight of all tumors per tumor-bearing rat.

 Table 3 Colon tumors in rats fed *ad libitum* or restricted calories

	Ad libitum	Restricted	Significance
Tumor incidence	19/19 100%	10/19 53%	$P < 0.001$
Tumors/tumor-bearing rat	3.5 \pm 0.4	2.1 \pm 0.6	NS ^a
% well-differentiated colonic adenocarcinomas	11%	6%	$P < 0.05$
Extracolonic tumors ^b	32%	11%	$P < 0.05$

^a NS, not significant.

^b Tumors of the duodenum and ear canal.

(in spite of nearly double the fat consumption) ($P < 0.001$). Tumors per tumor-bearing rat and total tumor yield were significantly reduced by restricted caloric consumption, as well. Of great interest is the fact that the size of mammary tumors developing in the rats subjected to caloric restriction was significantly less than that of tumors in animals allowed *ad libitum* food consumption.

Colon Tumor Study. The male F344 rats exhibited an average weight of 257 g 1 week after the final dose of DMH. Rats fed *ad libitum* continued to gain weight and achieved a final mean weight of 372 \pm 6 g. The calorically restricted rats lost weight during the first 4 weeks and then reached a plateau with slight weight loss during the last 6 weeks of the study to a final weight of 216 \pm 4 g ($P < 0.001$) (Fig. 2). These weight gains resulted in feed efficiencies of 11.16 in the rats fed *ad libitum* and -6.63 due to weight loss in the calorie-restricted rats. The feed efficiency of the rats fed *ad libitum* in this second study is lower than that calculated for the DMBA study because the rats were growing more slowly, in part due to older age at start of the study and in part due to strain differences.

One hundred % of the rats fed *ad libitum* developed tumors of the colon while only 53% of the calorie-restricted rats had colon tumors at necropsy ($P < 0.001$) (Table 3). Although there was also a slight reduction in colon tumors/tumor-bearing rat, this difference did not reach statistical significance. There were no differences between the two groups in mean tumor size or

location. Using the stringent criteria for diagnosis of invasive carcinoma, 11% of the colon tumors in the *ad libitum* group and only 6% of the tumors in the calorie-restricted group came under this classification ($P < 0.05$). Extracolonic tumors (in the duodenum or ear canal) were also significantly reduced in the calorie-restricted rats ($P < 0.05$). No tumors were found at any other sites.

Study of mucosal cytokinetics in the two groups (Table 4) revealed that the rats restricted in caloric intake had significantly increased labeling index ($P < 0.0001$) and mean highest labeled cell position ($P = 0.0018$). This paradoxical finding may be due to the increased fat consumption in the calorie-restricted group which should have resulted in greater bile acid concentration in the colon with subsequent enhancement of epithelial turnover. The post hoc observations on cytokinetics were unexpected, especially in light of the significant reduction in tumors among the calorie-restricted rats, so no fecal collection was made.

DISCUSSION

The two experiments described here show that the effect of caloric restriction during the promotion phase of mammary and colonic carcinogenesis is a more dominant factor than increased dietary fat content or absolute fat intake. By use of carefully designed semipurified diets, we have been able to provide more dietary fat to animals fed fewer calories and significantly inhibit promotion of colon and mammary tumors. This confirms our previous observation with the mammary tumor model (10) and extends the effect to colonic carcinogenesis.

Since our previous publication, other laboratories have also reported inhibition of mammary carcinogenesis by caloric restriction. Thompson *et al.* (15) fed rats diets containing either 5 or 20% fat following treatment with methylnitrosourea. Food intake was restricted so that both groups consumed the same amount of net utilizable energy for the first 16 weeks and then the diets were fed *ad libitum* for an additional 16 weeks. During the restricted feeding phase, there was no difference in mammary tumors between the two groups but when the diets were fed *ad libitum*, significantly more tumors developed in rats fed the high fat diet. These authors concluded that the stimulatory effect of dietary fat on tumorigenesis occurs only when consumption was *ad libitum*. Boissonneault *et al.* (16) used DMBA to induce mammary tumors and fed rats 5 or 30% fat diets *ad libitum* plus one group that received the high fat diet at a restricted level of calories equal to those consumed by the rats fed the 5% fat diet *ad libitum*. Tumor incidence in the three groups, respectively, was 43, 73, and 7%. These investigators reported that tumor appearance did not depend on the fat content of the diet but rather on a complex interaction involving energy intake, energy retention, and body size.

Pollard and coworkers (17, 18) have studied intestinal tumorigenesis under conditions of restricted food intake. However, their studies were conducted using natural ingredient diets so the rats subjected to caloric restriction simply ate less of all

nutrients. Therefore, it is impossible to ascribe an effect to calories alone. Nevertheless, these investigators reported that tumors induced by methylazoxymethanol were significantly inhibited by caloric restriction but colon tumors induced by methylnitrosourea were unaffected by dietary restriction. Although it was concluded that dietary restriction influenced only the indirect-acting carcinogen methylazoxymethanol, this is in disagreement with the present findings since dietary restriction was not imposed until 1 week following the final dose of DMH which should have been completely metabolized by that time (19).

A wide variety of spontaneous tumors in rodents has been reported to be responsive to either enforced or self-selected reduction in caloric intake. Ross and associates (20, 21) found that rats that consumed fewer calories and/or had lighter mature weights were significantly less likely to develop tumors of epithelial tissues, especially of the endocrine glands. These researchers concluded that dietary practices relatively early in life had the greatest influence on longevity and susceptibility to tumors. Rats and mice subjected to a restriction of food intake by 20% for their entire lifespans exhibited fewer of the common types of tumors in each species, *i.e.*, liver tumors in mice and pituitary, mammary, and skin tumors in rats (22). Mammary tumor development in C3H mice was significantly reduced by a low calorie diet; this was accompanied by reductions of circulating prolactin, mammary tumor virus expression, and proliferation of mammary alveolar cells (23).

Tumors induced by means other than chemical carcinogens have also been shown to be responsive to reduced caloric intake. These include tumors elicited by X-ray irradiation, inoculation with Rauscher leukemia virus, and transplantation of B16 melanoma cells (24–26).

The many types of tumors responsive to caloric restriction suggests that reduction of circulating gonadal hormones may not be causally related to the reduced appearance of mammary tumors. The present study indicates that colon tumors are equally responsive to caloric restriction as are mammary tumors during the promotion phase of carcinogenesis. Although the mechanism of tumor inhibition by caloric restriction is unknown some potential mediators include immunodulation or alteration of circulating levels or sensitivity to glucocorticoids, insulin, and a variety of other growth factors that are responsive to dietary energy intake and can affect proliferation of both normal and transformed epithelial tissues. Boutwell proposed that a relationship existed among dietary energy, retained carcass energy, and tumor formation that was mediated via corticosteroids (27). Although this relationship appears to be true, it is probably an incomplete explanation of the complex growth regulatory phenomena that occur during tumor promotion.

It is important from a nutritional standpoint to determine if caloric restriction is effective at even higher fat levels than those used here and whether lesser degrees of caloric restriction also inhibit tumor growth. Understanding of the mechanism by which caloric restriction inhibits tumor growth may allow development of pharmacological interventions that mimic the effects of caloric restriction. The data presented here also question the relevance of using rats fed *ad libitum* as controls for carcinogenesis assays. Perhaps the *ad libitum*-fed rat is the rodent equivalent of a morbidly obese human. Grossly overweight individuals are significantly more likely to die of many types of malignancies (28). Doll and Peto (29) have pointed out that more interest in caloric restriction and carcinogenesis might have been aroused if freely fed mice had been described as obese instead of mice on restricted diets being described as

Table 4 Colonic mucosal cytokinetics in rats fed *ad libitum* or restricted calories

	<i>Ad libitum</i>	Restricted	Significance
Crypt column height (cells)	32.6 ± 0.2	33.0 ± 0.2	NS ^a
Mean labeled cell height	8.1 ± 0.2	8.5 ± 0.3	NS
Labeling index (%)	6.9 ± 0.7	9.4 ± 0.6	$P < 0.0001$
Highest labeled cell position	10.3 ± 0.4	12.1 ± 0.4	$P = 0.0018$

^a NS, not significant.

small by investigators who studied this relationship during the 1940s. There is also a need to extend the current observations to other species—the inhibitory effects of caloric restriction on tumor growth have only been demonstrated in rodents.

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