Dietary Manipulation of Mammary Tumor Development in Adult C3H/Bi Mice (43041)

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Abstract. **Chronic energy intake restriction (CEIR) in virgin female mice is one of the most effective ways of reducing significantly mammary adenocarcinoma in C3H/Bi mice, a strain which develops mammary adenocarcinoma associated with the murine mammary tumor virus spontaneously and at high incidence. In this study, the influence of chronic energy intake restriction imposed on fully mature (4- to 5-month-old), breeding female C3H/Bi mice was addressed, and the influence of energy intake where energy was derived largely from fat versus diets in which energy was derived largely from carbohydrates on tumor development and survival rate was investigated. The results show that chronic energy intake restriction can be delayed until full maturation and successful reproduction and still reduce significantly the incidence of mammary tumor development in this relatively short-lived strain of mice. Our findings demonstrate that the overriding dietary factor controlling mammary tumor development in these experiments in C3H/Bi mice was the level of energy intake, regardless of the primary source of energy (fat or carbohydrates).** [P.S.E.B.M. 1990, Vol 193]

Dietary restriction has been shown to prevent numerous forms of cancer, including the development of murine mammary adenocarcinoma associated with the murine mammary tumor virus (MuMTV) or chemically induced murine mammary tumors (1–4). Decreased incidence of MuMTV-associated mammary adenocarcinoma has been achieved by dietary restriction initiated in virgin mice of the C3H/Bi strain (5–7). A high fat intake has been suggested as the dietary factor responsible for promoting mammary carcinogenesis in numerous analyses (8–10). However, more recent studies have supported the early interpretations of Tannenbaum (4), which indicate that energy intake may play a more crucial role in cancer development than does the intake of fat (11–13). Although the net energy effect of dietary fat has been revealed as crucial in a number of rat models of chemically induced mammary tumors (14–16), it is not known whether this action of energy restriction in suppressing tumor development applies to the model of spontaneous MuMTV-associated mammary adenocarcinoma. Additionally, it was not clear if dietary restriction could be initiated following successful reproduction and lactation and still prevent mammary adenocarcinoma in C3H/Bi mice.

In this study C3H/Bi mice were used as a model of MuMTV-associated mammary adenocarcinoma to investigate (i) the role of dietary fat composition or net energy intake on spontaneous tumorigenesis and (ii) the influence of chronic energy intake restriction (CEIR) imposed following successful breeding and associated lactation.

Materials and Methods

Animals. Sixty 4- to 5-month-old female mice of the C3H/Bi strain from our colonies at the University of South Florida/St. Petersburg were used in our study. This strain of mice was originally derived from the University of Minnesota mouse colony in 1982. The mice were routinely fed laboratory chow and bred. For this study 4- to 5-month-old mice were selected after successful breeding and lactation with at least one litter. Mice were divided into four groups and were randomly assigned to the four dietary regimens described below.

Dietary Manipulations. To compare the influence of carbohydrate versus fat consumption on mammary tumor development, two types of diet were used: a diet relatively high in carbohydrate, A1 or B1 (63% of total

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energy), and very low in fat (4.5% of total energy) versus a diet relatively high in fat, A2 or B2 (68.2% of total energy), and almost free of carbohydrate except for the glycerol in the fat. The mice were fed either *ad libitum* or were chronically restricted in energy consumption (CEIR) by approximately 40%. Thus, the mice were divided into four groups according to their dietary regimen as follows: 1—A1: *Ad libitum* consumption of Diet A1, which is high in carbohydrate and low in fat; 2—B1: CEIR consumption of Diet B1 to provide 60% of the energy consumed by mice in the A1 group; 3—A2: consumption of Diet A2 in an amount to provide the same energy level as was consumed by the mice in Group A1; and 4—B2: CEIR consumption of Diet B2 in an amount to provide the same energy level as was consumed by the mice in group B1.

Composition of the various diets was as described previously (17) and is the same amount of vitamins and minerals and the same protein to calorie ratio (Table I). Thus, the fundamental difference in the various dietary regimens was in the main source of energy (either lard or sucrose) and in the level of energy intake. Mice in the B1 and B2 groups were individually caged because of food restriction, whereas the mice in the A1 and A2 groups were housed two per cage. Mice were fed biweekly and the average daily consumption was approximately 15 kcal for the A1 and A2 mice and 9 kcal for the B1 and B2 mice. The twice weekly feeding schedule used with these mice has been shown to achieve the same life and health-prolonging influence as does a daily feeding.

Mammary Tumor Incidence. The mice were weighed and palpated for tumors at least once a week. The estimated size of palpable tumors was approximately 0.5 cm when first detected. Nontumor-associated deaths were recorded separately from those of tumor-bearing mice. Tumor incidence was calculated based on the number of mice reaching the age of tumor development (approximately 11–12 months of age) taken as number of mice at risk, or on the total number of mice that did not die from mammary tumor-related causes.

Data Analysis. Tumor incidence was calculated as described above, and mortality rate was calculated for tumor-unrelated causes and for total deaths. The Chi-square test was used to compare the death rate or tumor incidence between the various groups. P values <0.05 were considered significant.

Results

Influence of Diet on Body Weight. The average body weights of the mice were recorded weekly during the 40 weeks of experimental feeding (Fig. 1). The average body weight of the CEIR mice (B1 and B 2) was approximately 30% lower than that of their counterparts consuming 40% more energy (A1 and A2). However, at equivalent energy consumption, the mice fed the high fat diet gained more weight than those fed a high carbohydrate and low fat diet. Thus, as calculated, apparent energy intake did not determine precisely the body weight for diets based on different sources of energy (fat or carbohydrates).

Influence of Diet on Mammary Tumor Incidence. The occurrence of deaths unrelated to tumors and the appearance of mammary tumors were recorded in Figure 2. A number of mice died from tumor-unrelated causes during the feeding period, before as well as after detection of the first mammary tumors, which occurred at 11 months of age (or after the seventh month of feeding). Table II shows the mortality distribution of the mice into tumor-related deaths and tumor-unrelated deaths. The number of mice at risk for tumor development was calculated by either censoring the number of tumor-unrelated deaths before tumor development age or by censoring the total number of tumor-unrelated deaths during the experimental period as follows:

1. Considering that there was no tumor development before 11 months of age, the number of live mice at that age was taken as mice at risk to calculate mammary tumor incidence. Mice in Groups A1 and A2 consuming the highest energy level showed significantly higher tumor incidence as compared with their counterparts which consumed 40% less energy (69% A1 versus 16.7% B1, corresponding to 52.3% reduction: $P < 0.01$ and 71.5% A2 versus 21.5% B2, corresponding to 50% reduction: $P < 0.05$). By contrast, no difference was observed in mammary tumor incidence between mice consuming equivalent energy levels (A1 versus A2 and B1 versus B2) (Table III).

2. Alternatively, since a large number of tumor-unrelated deaths occurred before 11 months of age, these could be censored from the number of mice at risk. In this case, higher tumor incidence values were obtained for each group with significant reduction in tumor incidence associated with CEIR (90% A1 versus 20% B1, corresponding to 70% reduction: $P < 0.01$ and 83.3% A2 versus 37.5% B2, corresponding to 45% reduction: $P < 0.05$) (Table IV). Both analyses showed significant reduction in tumor incidence.

Influence of Diet on Mortality. As described above, a number of mammary tumor-unrelated deaths occurred during the feeding period (33.3% in A1, 33.3% in B1, 60% in A2, and 46.7% in B2 mice). Although there were apparent differences in the proportion of dead mice between the various groups (Table II), analysis by chi-square test showed that these differences were not significant. Thus, tumor-unrelated deaths were not influenced by diet. Combined mortality from all causes showed the highest death rate in the A1 and A2 mice consuming the highest energy level (93.3%) and a lower rate in the CEIR mice (46.6% B1 and 66.6% B2).

Influence of Diet on Survival Rate. At the end of the experiment, only one mouse remained in the A1 or

Table I. Composition of Diets

Constituent	A1		B1		A2		B2	
	g	kcal	g	kcal	g	kcal	g	kcal
Casein	29.4	117.6	17.64	70.56	29.4	117.6	17.64	70.56
Methionine	0.6	2.4	0.36	1.44	0.6	2.4	0.36	1.44
Safflower oil	2.0	18.0	2.0	18.0	2.0	18.0	2.0	18.0
AIN Vitamin mix	1.0	3.95	1.0	3.95	1.0	3.95	1.0	3.95
AIN Mineral mix	3.5	1.65	3.5	1.65	3.5	1.65	3.5	1.65
Inositol	0.05	0.2	0.05	0.2	0.05	0.2	0.05	0.2
Choline bitartrate	0.2	0.8	0.2	0.8	0.2	0.8	0.2	0.8
Sucrose	47.25	189.0	26.51	106.05	—	—	—	—
Glycerol	16.0	64.0	8.98	35.91	—	—	—	—
Lard	—	—	—	—	28.1	252.9	15.77	141.93
Total	100.0	397.6	60.2	238.5	64.8	397.5	40.5	238.5

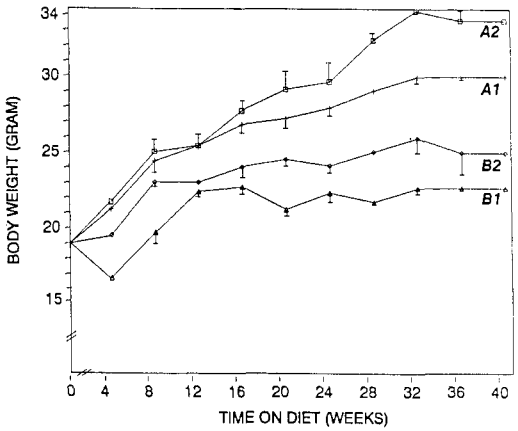

Figure 1. Influence of diet on body weight. Average body weights of C3H/Bi mice on various dietary regimens; A1 and A2: equal energy consumption equivalent to an *ad libitum* feeding of diet A1. B2 and B2: equal energy consumption equivalent to 40% reduction in energy consumption as compared with the A1 and A2 groups. The main source of energy is sucrose in A1 and B1 and lard in A2 and B2.

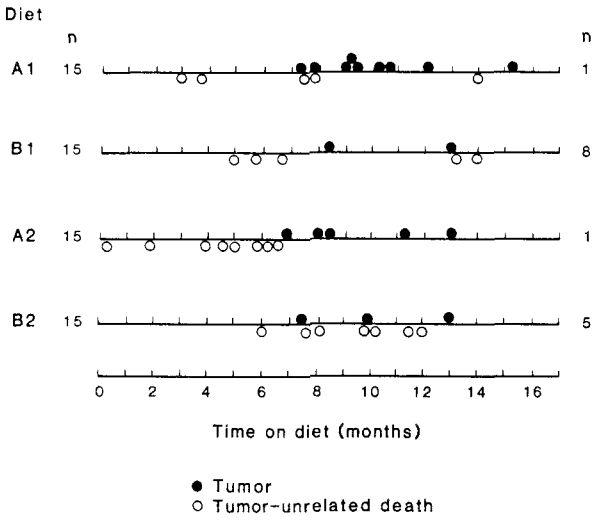

Figure 2. Influence of diet on tumor development. Tumor-unrelated deaths of the mice and mammary tumor appearance were recorded as they occurred. Tumors were first detected in the A2 mice, but occurred shortly thereafter in the other groups.

A2 group (6.4%), whereas eight mice remained in the B1 (53.3%) and five in the B2 group (33.3%). Thus, the lowest survival rates occurred in the A1 and A2 mice, which consumed the highest energy level. Since there was no significant difference in the tumor-unrelated deaths between the various dietary groups, any difference in survival rate or combined mortality rate reflects the influence of energy consumption on tumor incidence.

Discussion

Previous studies have shown that CEIR initiated in virgin C3H/Bi female mice inhibits development of MuMTV-associated proliferative minimal alveolar lesions and mammary adenocarcinoma, and reduces formation of both type A and B viral particles (5–7). Many investigators have suggested that dietary fat intake is the critical variable in mammary tumorigenesis (6–10). However, the early work of Tannenbaum (4), which

Table II. Mortality of C3H/Bi Mice[a]

Diet	No. of mice	Diet periods			
		7 Months		7–17 Months	
		Nontumor	Tumor	Nontumor	Tumor
A1	15	2	0	3	9
B1	15	3	0	2	2
A2	15	8	0	1	5
B2	15	1	0	6	3

[a] Cause of death was classified as mammary tumor-associated death (tumor) or mammary tumor-unrelated death (nontumor). Mammary tumors did not appear until the seventh month of feeding or 11 months of age. After 7 months, more deaths unrelated to tumor development occurred. The experiment was ended after 17 months of feeding. The number of survivors was: A1, one; B1, eight, A2, one; and B2, five.

Table III. Mammary Tumor Incidence Based on Number of Mice Reaching Tumor Development Age

No. of mice at risk[a]		Tumor development		Nontumor death (*n*)	Survivors (*n*)
Diet	*n*	*n*	%		
A1	13	9	69	3	1
B1	12	2	16.7	2	8
A2	7	5	71.5	1	1
B2	14	3	21.5	6	5

[a] Number of mice at risk represents the number of mice reaching mammary tumor age, i.e., 11 months of age. The mice that died before 11 months of age from tumor-unrelated causes were censored from the analysis of tumor incidence. Chi-square test: A1 versus B1, $P < 0.01$; A2 vs B2, $P < 0.05$.

Table IV. Mammary Tumor Incidence Based on Number of Mice that did not Die from Other Causes during the Entire Feeding Period

No. of live mice[a]		Tumor development	
Diet	*n*	*n*	%
A1	15-5 = 10	9	90
B1	15-5 = 10	2	20
A2	15-9 = 6	5	83.3
B2	15-7 = 8	3	37.5

[a] Number of live mice was calculated by censoring all animals that died from mammary tumor-unrelated causes during the feeding period. A1 versus B1, $P < 0.01$; A2 versus B2, $P < 0.05$ ($\chi^2 = 2.941 > 2.706$).

was somewhat difficult to interpret because of the nature of the diets employed, has been buttressed by recent reports which indicate that energy intake rather than fat composition may be the most crucial dietary influence in carcinogenesis in animal models, including chemically induced mammary tumors in rats (11–16).

The present study, using isocaloric diets which differ only in the main source of calories, was designed to investigate whether energy consumption or dietary fat composition plays a greater role in the spontaneous development of MuMTV-associated mammary adenocarcinoma in mature breeding female mice. In view of the observations that dietary restriction in many strains of rats and mice can be delayed until after midlife and still exert beneficial influence on the health span and life-span of these animals (18–22), in the present study we focused on dietary manipulation after sexual maturation and completion of at least one cycle of successful reproduction and associated lactation in the C3H/Bi mice.

We report herein that in a model of spontaneous MuMTV-associated mammary adenocarcinoma, energy intake rather than fat intake appears to be the crucial variable. Moreover, this is the first report of a decreased incidence of mammary tumors achieved by CEIR initiated in mature female mice after successful reproduction and lactation. Recently, Silverman *et al.* (23) reported that imposition of a low fat/low calorie diet at or before puberty lowered the incidence of mammary adenocarcinoma in C3H/OuJ mice, although the total duration of dietary restriction played the greatest role in reducing mammary cancer risk. Our findings suggest that even when initiated well beyond puberty, a low calorie diet can significantly inhibit development of mammary cancer. It is noteworthy that C3H/Bi mice have a relatively short life-span due to the early onset of mammary adenocarcinoma, which occurs at approximately 11 months of age. Generally, the animals dies within 3 weeks after tumor appearance. Survival curves were not established for the mice used in this experiment due to the small number of mice available for study in each group.

Mice fed the high fat diet (A2 and B2) had higher average body weights than those consuming a high sucrose diet. This difference may be attributable in part to the possibility of a refeeding potential or to differences in scattering potential of the two different diets as a consequence of difference in consistency or in fecal content. However, the addition of glycerol to the high sucrose diet, to avoid a powdery consistency, minimized scattering. Therefore, the difference in body weight between the mice on a high fat diet and those on a high carbohydrate diet, may rather be attributed to a difference in metabolizable energy between lard and sucrose, e.g., fat may be utilized as much as 24% more efficiently than sucrose, as reported by Donato and Hegsted (24). In the present study, however, this potential difference in energy utilization between fat and sucrose did not appear to have a crucial impact on mammary tumor incidence in mice of the full fed or restricted groups.

A relatively high mortality rate occurred among A2 mice prior to tumor development. However, analysis of the total number of mice that died from tumor-unrelated deaths during the experimental period did not indicate a specific influence of diet. Because the number of mice at risk was reduced by these early deaths, we expect that more dramatic results might have been obtained had a larger number of mice been studied. This study, however, does appear to establish that initiation of CEIR in C3H/Bi mice can be delayed until after successful reproduction and still inhibit significantly mammary tumor development. Furthermore, our findings support the growing consensus that energy intake is a more crucial determinant than dietary fat composition in carcinogenesis and also in promoting longer life-span and health span in rodents. Although the mortality rate did not appear to be directly attributable to diet in our study, the strikingly high occurrence in the fat-fed group suggested that further studies are necessary to establish the cause of these deaths.

Specifically, the aspect of toxicity due to lipid peroxidation that might result from a high fat content in the diet should be addressed. Additional studies are also needed to determine the basis for the dramatic influence of CEIR initiated after sexual maturation. Further investigation of the influence of CEIR on host and muMTV gene expression and/or hormonal, immunologic, or biochemical functions may provide greater insight into the mechanisms by which CEIR, imposed later in life, can prevent diseases in numerous animal models.

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