

Total Calories, Body Weight, and Tumor Incidence in Mice

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ABSTRACT

The relation between total caloric intake, body weight, and tumorigenesis, as well as the independence of these effects from those of dietary fat, were evaluated using data from 82 published experiments involving several tumor sites in mice. Comparing experimental (calorie restricted) to control (*ad libitum*) groups showed that the former consumed 29% fewer calories (experimental groups consumed fewer calories than control groups in all but a few isocaloric experiments), 50% less total fat, 11% less protein, and weighed 25% less than control animals. Adult body weight was highly correlated to caloric intake in both males ($r = 0.85$) and females ($r = 0.74$), although this correlation decreased with increasing caloric intake. Cumulative tumor incidence was, on average, 42% lower in the restricted groups. Multivariate regression analyses revealed that, regardless of the level of dietary fat, tumor incidence increased with increasing caloric intake and body weight over a wide range of intakes, including moderate caloric restriction (*i.e.*, 7-20%). These data indicate that total caloric intake is an important determinant of tumorigenesis in mice, and that body weight may be a more sensitive indicator for this effect than is caloric intake alone.

INTRODUCTION

A large number of laboratory studies have demonstrated that tumor incidence in rodents is dependent upon daily caloric intake and body weight (1-14). Based on several experiments in mice, Tannenbaum suggested that the relation between caloric intake and tumor incidence was approximately linear (9). However, no further quantitative assessment of the effect of calories or body weight in these experiments (1-14) has been published. In addition, even though most of the above investigations controlled and measured several dietary components, few specifically assessed the degree to which the effects of total calories and body weight on tumorigenesis were independent of fat intake (3, 5, 11, 12). Regarding this issue, in 1982 the Committee on Diet, Nutrition and Cancer of the National Research Council concluded that a clear interpretation of the unique effect of caloric intake on carcinogenesis in most of these studies was not possible, primarily because of the potential confounding influence of dietary fat (15). However, recent experiments by Kritchevsky, *et al.* (16, 17), which are supported by previous work (5, 11), demonstrate independent tumor promotion for both fat and total calories, with greater tumor reduction resulting from caloric (rather than fat) restriction. The present analyses, which combine data from a large number of previously published experiments, were undertaken in order to evaluate the quantitative relationship between total caloric intake, body weight, and tumorigenesis, as well as the independence of these effects from dietary fat intake.

MATERIALS AND METHODS

Published experimental data were reviewed to identify investigations of the effects of dietary macronutrients and body weight on tumorigen-

esis which provided adequate information concerning the following experimental factors: dietary composition (daily total calories, fat, carbohydrate, and protein); adult body weight; cumulative tumor incidence; and other study factors such as species, strain, and sex of the animals, age at institution of experimental diets, and carcinogens used (if a chemically induced tumor model). These analyses were restricted to 82 experiments involving approximately 5000 mice reported in 14 separate publications (1-14). The experiments are summarized in Table 1. Only murine studies were included since the majority of eligible experiments were conducted using this species, and cross-species comparisons of daily food intake, body weight, and tumor incidence would be difficult.

Each experiment compared an experimental or "restricted" group (*i.e.*, lower caloric intake) to a control or "*ad libitum*" group (*i.e.*, higher and usually *ad libitum* caloric intake), and dietary regimens were fixed for the duration of each experiment. In the earliest series of experiments (1), restriction was achieved by feeding less of the same dietary mixture (hence, "underfeeding" studies), while in subsequent experiments both the quantity and composition (especially with respect to carbohydrate, and to a lesser degree fat) were manipulated to obtain differences in daily caloric intake. The types of fat, protein, and essential vitamins were the same within each experiment, and except for the "underfeeding" experiments which resulted in reduced vitamin intake among calorie restricted animals (1), equivalent essential vitamin intake was generally maintained and reported. Although information concerning specific essential fatty acids was not usually provided, there was no reported evidence of any dietary deficiencies.

The experimental outcome was cumulative incidence of tumors (irrespective of number per animal) for each experimental and control group. Included were 36 experiments of skin tumors (all chemically induced), 34 of mammary neoplasias (all "spontaneous"; *i.e.*, without induction), eight involving all sites (noninduced), and four of induced s.c. sarcomas, shown in Table 1. Forty-six experiments involved female mice, 29 involved males, and seven used groups of mixed sex (but matched between experimental and control groups). Most of the experiments ($N = 50$) were begun with mature mice 10-12 weeks old, while 18 involved younger weaned animals, and 14 used older animals up to 60 weeks of age; the average age was 12.9 weeks. Thus, observed differences in body weight for the majority of studies reflected differences in nondevelopmental, mature weight only. Up to 30 animals were used per group in 21 experiments, 52 experiments used 35-52 animals, and nine used 60 or more animals per group.

The quantitative relation between study dietary factor (*e.g.*, total calories) and body weight levels, and tumor incidence was evaluated in these analyses. Multiple linear regression based on least-squares means (weighted by experimental group sample size) was used to estimate cumulative incidence of tumors among the experimental groups for several levels of caloric intake, body weight, and calories and fat. Two methods were used to maintain analytic validity by controlling for intraexperimental factors which might have affected tumor incidence (*e.g.*, strain or carcinogen dosage). In the first, adjustment was made for cumulative tumor incidence in the control groups when calculating incidence in the experimental groups by including this factor in the regression model (Figs. 2 and 3 and Table 3). Alternatively, the ratio of the experimental to control group incidence (or calories) was analyzed, thereby maintaining pair-wise comparisons and yielding estimates of relative reductions (Table 4). In addition, other potential modifying factors such as age, sex, tumor site, or other dietary components were also evaluated through inclusion in the regression models. Analyses were conducted using the Statistical Analytic System, SAS (18). Because only mean group dietary, body weight, and tumor incidence values were available from the original experiments analyzed (*i.e.*, neither individual animal values nor standard deviations were reported), the estimates of error generated in the present study (standard

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TOTAL CALORIES, BODY WEIGHT, AND TUMOR INCIDENCE IN MICE

Table 1 Summary of 82 murine experiments

Reference	Experiment no.	Mouse strain	Tumor site	Sex	Age (weeks) ^a	No. of animals/group	
Tannenbaum (1)	1	ABC	Skin	F	19	50	
	2	ABC	Skin	M, F	60	50	
	3	ABC	Skin	M, F	60	50	
	4	C57BL	s.c. sarcoma	M, F	24	100	
	5	Swiss	Skin	M	9	45	
	6	DBA	Mammary	F	38	44	
	7	DBA	Mammary	F	23	50	
Tannenbaum (2)	8	DBA	Skin	M	40	50	
Tannenbaum (3)	9	C57BL	s.c. sarcoma	F	11	50	
	10	DBA	Mammary	F	38	44	
	11	DBA	Mammary	F	24	50	
	12	Swiss	Skin	M	10	45	
	13	C57BL	Skin	M	10	50	
Visscher <i>et al.</i> (4)	14	DBA	Skin	M	10	50	
	15	Swiss	s.c. sarcoma	F	10	40	
	16	ABC	s.c. sarcoma	F	9	40	
	17	C3H	All sites	F	4	50	
	Lavik and Baumann (5)	18	MCA	Skin	M, F	10	30
		19	MCA	Skin	M, F	10	30
		20	MCA	Skin	M, F	10	30
Tannenbaum (6)	21	DBA	Skin	M	10	50	
White <i>et al.</i> (7)	22	C3H	Mammary	F	6	10	
	23	C3H	Mammary	F	16	12	
	24	C3H	All sites	M, F	6	20	
	25	DBA	Mammary	F	10	50	
Tannenbaum (8)	26	DBA	Mammary	F	10	50	
	27	DBA	Mammary	F	23	30	
	28	DBA	Mammary	F	23	30	
	29	DBA	Mammary	F	23	25	
	30	DBA	Mammary	F	23	25	
	31	DBA	Skin	M	11	40	
	32	DBA	Skin	M	11	40	
	33	DBA	Skin	M	11	40	
	34	DBA	Skin	M	11	40	
	35	DBA	Skin	M	11	40	
	36	DBA	Skin	M	11	35	
	37	DBA	Skin	M	11	35	
	Tannenbaum (9)	38	DBA	Skin	M	8	50
		39	DBA	Skin	M	8	50
40		DBA	Mammary	F	10	50	
41		DBA	Mammary	F	10	50	
42		DBA	Mammary	F	10	50	
43		C3H	Skin	M	11	50	
44		C3H	Skin	M	11	50	
45		C3H	Skin	M	11	50	
46		C3H	Skin	M	11	50	
47		C3H	Skin	M	11	50	
48		C3H	Skin	M	11	50	
49		C3H	Mammary	F	10	30	
50		C3H	Mammary	F	10	30	
51		C3H	Mammary	F	10	30	
52		C3H	Mammary	F	10	30	
53		C3H	Mammary	F	10	30	
54		C3H	Mammary	F	10	30	
55	C3H	Mammary	F	10	30		
56	C3H	Mammary	F	10	30		
57	C3H	Mammary	F	10	30		
58	C3H	Mammary	F	10	30		
59	C3H	Mammary	F	10	30		
Tannenbaum & Silverstone (10)	60	C3H	Skin	M	9	50	
	61	C3H	Skin	M	9	50	
	62	C3H	Skin	M	9	50	
	63	C3H	Skin	M	9	50	
	64	C3H	Skin	M	9	50	
Boutwell <i>et al.</i> (11)	65	Rockland	Skin	F	10	48	
	66	Rockland	Skin	F	10	48	
	67	Rockland	Skin	F	10	48	
	68	Rockland	Skin	F	10	48	
Silverstone & Tannenbaum (12)	69	C3H	Mammary	F	11	52	
	70	C3H	Mammary	F	11	52	
	71	C3H	Mammary	F	11	52	
	72	C3H	Mammary	F	11	52	
	73	DBA	Mammary	F	12	60	
	74	DBA	Mammary	F	12	60	
	75	DBA	Mammary	F	12	60	
	76	DBA	Mammary	F	12	60	
Tucker (13)	77	Swiss albino	All sites	M	4	50	
	78	Swiss albino	All sites	F	4	50	
Conybeare (14)	79	Swiss	All sites	M	4	80	
	80	Swiss	All sites	M	4	80	
	81	Swiss	All sites	F	4	80	
	82	Swiss	All sites	F	4	80	

^a Age of mice at start of experiment.

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errors of the means) are conservative and represent the lower limits of variability.

RESULTS

Mean values of several study factors for the control and experimental (*i.e.*, calorie restricted) groups appear in Table 2. Compared to the control groups, experimental groups consumed (daily) on average 29% fewer calories, 50% less fat, 11% less protein, 27% less carbohydrate, and derived approximately 30% fewer calories from dietary fat. In contrast, percentage of calories derived from carbohydrate was equivalent, and protein calories were 25% higher among experimental groups. Both final body weight and tumor incidence were substantially lower among experimental groups (by 25 and 42%, respectively). Each of the experimental-control differences was highly statistically significant ($P \leq 0.001$).

Mature body weight and daily caloric intake were highly correlated in these experiments for both male and female animals (Fig. 1). The Spearman correlation coefficients corresponding to the two plots were 0.85 and 0.74 for males and females, respectively. Of note was the reduced variability of body weight at lower levels (≤ 11) of caloric intake ($r = 0.90$

and 0.88 for male and female experimental groups, respectively), compared to that at higher intakes ($r = 0.21$ and 0.39 for males and females, respectively). In contrast to these findings for total calories, the correlations between body weight and fat or protein were much lower ($r = 0.07$ and 0.41, respectively) for all experiments combined, with little difference between sexes.

The relation of total calories to tumor incidence among the experimental groups is shown in Fig. 2. Cumulative tumor incidence increased in the experimental groups with increasing caloric intake (shown by quartiles) in a nearly dose-response fashion. This relation was observed for both major tumor sites studied: chemically induced skin and "spontaneous" mammary tumors. However, a more striking linear relation was evident for caloric restriction in mammary tumorigenesis, including lower incidence among the most restricted animals. The regression model R^2 value for all experiments was 0.70. Fig. 3 shows that cumulative incidence was also linearly related to mature

Table 2 Dietary intake, body weight, and tumor incidence from experimental and control groups of 82 experiments in mice^a

Factor	Control groups (N = 82)	Experimental groups (N = 82)
Absolute intake		
Calories (kcal/day)	13.3 ± 0.3	9.5 ± 0.3
Total fat (g/day)	0.26 ± 0.03	0.13 ± 0.01
Protein (g/day)	0.70 ± 0.03	0.62 ± 0.02
Carbohydrate (g/day)	2.04 ± 0.08	1.48 ± 0.06
Relative intake (% of calories)		
Total fat	18.0 ± 0.2	12.6 ± 1.2
Protein	21.1 ± 0.7	26.4 ± 0.8
Carbohydrate	60.5 ± 2.1	60.7 ± 1.6
Body weight (g)^b		
	33.0 ± 0.6	25.4 ± 0.8
Tumor incidence (% of animals)		
	67.8 ± 2.6	39.0 ± 3.3

^a Mean values ± SE. Controls differed significantly from experimental groups ($P \leq 0.001$; unpaired *t* test) for all factors shown.

^b N = 79.

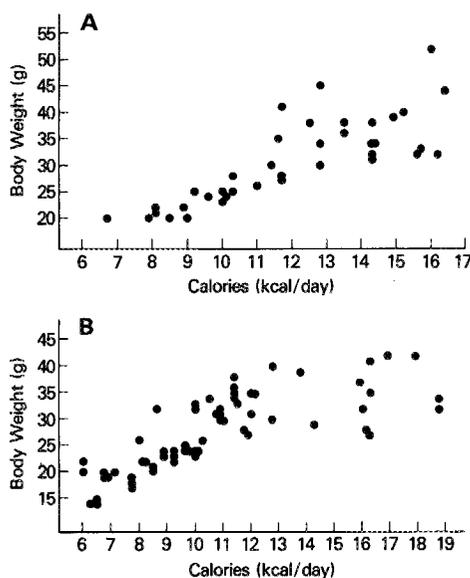


Fig. 1. Relation between adult body weight and daily caloric intake in mice from 82 experiments. A, males; B, females. Lifetime, fixed daily calorie diets were fed and body weight measured at the end of each experiment.

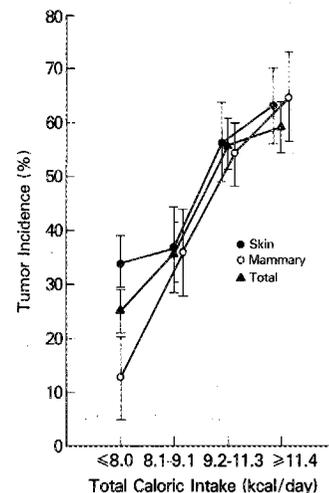


Fig. 2. Relation between cumulative tumor incidence and caloric intake in mice from 82 experiments. All tumor sites (\blacktriangle); skin tumors (\bullet); mammary tumors (\circ). Tumor incidence adjusted for incidence in control groups, site, and sex, and weighted by number of animals per experimental group. Percentage of animals developing one or more tumors by the end of the experiment. Caloric intake categorized by quartiles. Points, mean incidence; bars, SE.

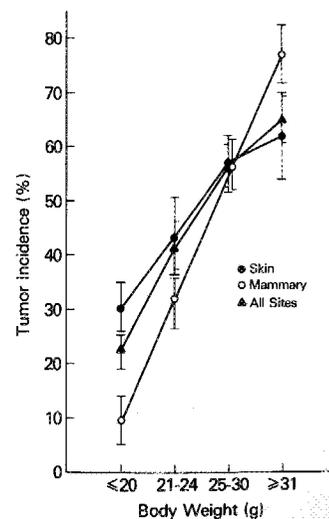


Fig. 3. Relation between cumulative tumor incidence and adult body weight in mice from 82 experiments. All tumor sites (\blacktriangle); skin tumors (\bullet); mammary tumors (\circ). Tumor incidence adjusted for incidence in control groups, site, and sex, and weighted by number of animals per experimental group. Percentage of animals developing one or more tumors by the end of the experiment. Body weight categorized by quartiles. Points, mean incidence; bars, SE.

body weight. As was the case for incidence and caloric intake, a greater range of tumor response to body weight differences was evident in the mammary experiments. The model R^2 for body weight and tumor incidence was 0.77. Adjustment in the regression analyses for animal age, or dietary fat or protein intake, did not materially alter any of the above findings, and neither fat nor protein were significantly related to tumor incidence.

Table 3 demonstrates mean experimental dietary levels and tumor incidence (the latter adjusted for control group incidence) for four dietary categories, as defined by the median intakes of fat and calories. The lowest incidence was observed in the two low calorie (and low weight) categories, while the high calorie categories exhibited significantly higher (and equivalent) incidence. Thus, the effect of caloric intake was independent of the level of dietary fat intake. In contrast, no effect (or a small inverse effect) on incidence was observed for the two levels of dietary fat. Greater stratification of fat and calories (*e.g.*, by tertiles) yielded similar results, although based on smaller numbers of experiments in each category.

Tumor incidence reduction (*i.e.*, experimental compared to control groups) generally increased with the degree of caloric restriction in these experiments; that is, relative tumor incidence decreased with increasing restriction (Table 4). Despite reduced fat intake, the nine isocaloric experiments (eight of which involved mammary tumors in females) resulted in increased relative tumor incidence, although the 95% confidence interval overlapped zero, and the unadjusted mean showed a 5% tumor reduction. Proportionately greater tumor reduction was obtained through caloric restriction in the 7–40% range compared to more severe restriction, and the data from 18 experiments demonstrate efficacy at even the lower levels of restriction tested (*i.e.*, 7–20%). Although not shown, the data also suggest that somewhat greater tumor reduction resulted among the "spontaneous" mammary tumor experiments compared to other sites.

DISCUSSION

The results of these analyses indicate that the incidence of chemically induced and "spontaneous" tumors in mice is proportional to the level of caloric intake and resulting body weight over a wide range of caloric, as well as other macronutrient, intake in a variety of experimental settings. Although most abundantly demonstrated for skin and mammary tumors (with evidence for greater efficacy in the mammary models), this effect was also observed for other sites including all-sites incidence. (Efficacy at other sites, *e.g.*, hepatoma (10), lung adenoma (1, 19), and leukemia (20), has also been shown in experiments which could not be included in these analyses either because they were included for another tumor site or provided insufficient experimental data.) Furthermore, the relation is not due to the confounding effects of dietary fat intake, which was only minimally related to tumor incidence in these

experiments. Lower tumor yield, delayed tumor onset, and increased longevity were also observed among the calorie restricted animals in most studies. However, since these data were not uniformly reported they were not analyzed here.

Tannenbaum first suggested that an approximate dose-response relation existed between caloric intake and cumulative tumor incidence in mice based on his early experiments (9). The findings presented here are supportive of this hypothesis, while also demonstrating a nearly linear relationship between incidence and body weight. The data confirm that under controlled, experimental conditions where basal metabolic and physical activity levels are relatively similar, body weight is directly proportional to caloric intake. This has also been shown in the laboratory for humans (21), in contrast to some epidemiological studies which have failed to demonstrate that obese individuals have greater caloric intake than the nonobese (22). One possible explanation for this is suggested by the present finding of a linear relation between calories and body weight at lower, more restricted intake levels, while body weight varies more at any given intake over the higher and *ad libitum* intake range.

Body weight accounted for a somewhat greater proportion of the variability in incidence than did total calories *per se* in the regression models (R^2 of 0.77 versus 0.70, respectively), suggesting that it may be a more sensitive marker for tumor or cancer risk than is caloric intake alone. This is plausible since adult body weight represents a summary index of the net energy balance of caloric intake on the one hand, and basal metabolic and physical activity requirements on the other. The importance of animal body weight with respect to tumorigenesis has similarly been emphasized in studies of rats (23, 24). In addition, studies have shown that experimental animals exposed to thyroid hormones (25), low environmental temperature (26), or high levels of physical activity (27), all of which resulted in lower body weight despite increased caloric intake, experienced reduced tumor incidence. These data suggest that caloric intake in excess of energy requirements, as evidenced by increased body weight, is of primary importance to the carcinogenic effects observed.

Adult body weight or mass is composed of both lean tissue (the primary metabolic body compartment) and adipose tissue. Unfortunately, body composition was not measured in these experiments, so that the relative contribution of lean and fat mass to tumorigenesis cannot be assessed. However, two studies in rats providing such data demonstrate a positive body weight-tumor relation (27, 28); in one (27), both lean mass and percentage body fat were greater in the high tumor group than in the low tumor groups, while in the other (28), percentage body fat was less important than lean body tissue alone. Although these experiments are based on relatively small numbers of animals (6–11 per group for body composition), they suggest that the total calorie effects observed may be partially due to differences in lean body mass, and point out the need for body composition information in future studies of this hypothesis.

Table 3 Effect of dietary total calories and fat, and body weight on tumor incidence in 82 experiments^a

Diet ^b		Number of experiments	Total calories (kcal/day)	Total fat (g/day)	Body weight (g)	Tumor incidence (%) ^c
Calories	Fat					
Low	Low	23	7.1 ± 0.3	0.08 ± 0.02	20.2 ± 1.0	34.4 ± 4.3
High	Low	18	10.8 ± 0.3	0.04 ± 0.02	29.0 ± 1.1	52.4 ± 4.7
Low	High	19	8.1 ± 0.3	0.19 ± 0.02	20.9 ± 1.1	23.1 ± 4.7
High	High	22	12.0 ± 0.2	0.19 ± 0.02	32.6 ± 0.8	54.4 ± 3.4

^a Site-adjusted mean values ± SE, weighted by number of animals per experimental group.

^b Low/high calorie and fat levels defined by the median intakes (9.2 kcal and 0.072 g fat).

^c Mean experimental group incidence also adjusted for incidence in control groups.

Table 4 Level of caloric restriction and resulting tumor incidence reduction^a

Number of experiments	Caloric restriction (%)		Tumor reduction (%)
	Range	Mean (SE)	Mean (SE)
9	0	0 (1.5)	-9.5 (10.2)
18	7-20	15.3 (1.2)	20.2 (8.1)
22	21-30	25.9 (1.1)	49.6 (6.4)
17	31-40	37.0 (1.2)	52.5 (7.8)
16	41-58	52.9 (1.1)	62.2 (7.6)

^a Site- and fat-adjusted means \pm SE, weighted by number of animals per experimental group.

These analyses also add to the growing literature documenting the independence of a total calorie-body weight effect on tumorigenesis from that of dietary fat in rodents (5, 11, 16, 17, 28). The lack of correlation between total caloric and fat intake in the experiments analyzed ($r = 0.04$) offered a unique opportunity to address this issue. Both the stratified analysis and the linear regression of calories on tumor incidence adjusted for fat intake support the hypothesis of independent effects. A surprising finding is the absence of an overall effect (or a minimally negative one) for fat, although it did exert a small, statistically nonsignificant tumor-promoting effect in the few isocaloric experiments included in these analyses (3, 5, 11, 12). However, information concerning specific fat fractions was not reported or evaluated, and only 16 of the experimental groups were fed diets composed of more than 14% fat (range 14-34%), whereas previous investigations of dietary fat and tumorigenesis usually demonstrated promotional effects for diets composed of 20-30% fat (29). In addition, given the presence of caloric restriction in each experiment, the tumor-enhancing effects of fat may have been overshadowed. Although the data are limited in these respects, they do suggest a greatly diminished role for fat as compared to total calories and body weight. This is supported by the work of others who have concluded that the effects of caloric restriction on tumorigenesis are apparently of greater magnitude than are modifications (including large increases or decreases) in fat intake *per se* (5, 11, 16, 17). It has been suggested that the effect observed for fat may be an indirect one acting through increased net energy availability (or metabolic efficiency of utilization) of dietary fat (11, 28). Most recently, questions have also been raised concerning possible confounding by total calories of previous experimental studies of dietary fat (30, 31).

The present findings are significant in several respects. Roe has already pointed out that *in vivo* experimental investigations of tumorigenesis must take into consideration the substantial effects of caloric intake (32). The demonstration of efficacy of caloric restriction for both "spontaneous" and induced tumors is important and encouraging from the standpoint of potential human applications. Epidemiological studies also demonstrate an association between body weight (and, to a lesser degree, caloric intake) and cancer risk (33). Although extrapolation to humans should be cautious, the present findings suggest that any effort to reduce caloric intake, especially among the overweight and obese, and maintain body weight to near desirable levels (as defined at the time of growth cessation), may result in a reduction in cancer incidence. Further research should evaluate several mechanisms potentially involved, including effects on mitotic activity (34), basal metabolism (35), or the immune (36, 37) or endocrine systems (32, 38, 39), as well as investigating possible interactions with other dietary components. Additional testing of the hypothesis in humans is also warranted.

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