Influence of Caloric Restriction and Exercise on Tumorigenesis in Rats (42986)

DAVID KRITCHEVSKY
The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

Abstract. **Underfeeding or caloric restriction have been shown to inhibit the growth of spontaneous, transplanted, or chemically induced tumors in rats and mice. At 40% caloric restriction, growth of 7,12-dimethylbenz(*a*)anthracene-induced mammary and 1,2-dimethylhydrazine-induced colonic tumors is inhibited significantly even when the restricted diet contains twice as much fat as the control diet. Some inhibitory effects become evident even at 10% caloric restriction. In studies involving high fat diets, we find that rats receiving 20% fat *ad libitum* exhibit significantly higher 7,12-dimethylbenz(*a*)anthracene-induced mammary tumor incidence, multiplicity, and weight than rats ingesting the same amount of fat daily, but in a diet containing 25% fewer calories. In a study of intermittent *ad libitum* and restrictive feedings, chemically induced tumorigenicity varies inversely with feed efficiency. Exercise has also been shown to inhibit tumor growth. Sedentary rats fed *ad libitum* have a 108% higher incidence of 1,2-dimethylhydrazine-induced colon tumors than rats fed *ad libitum* but subjected to vigorous treadmill exercise. Caloric flux (either reduced intake or increased outflow) appears to reduce tumorigenicity in rodents.** [P.S.E.B.M. 1990, Vol 193]

Restriction of energy intake may be achieved in two ways. The easiest way is simply to underfeed the animals under study. However, if *ad libitum* intake provides limiting amounts of micronutrients such as vitamins or minerals, the underfed animals may be deprived of essential nutrients. The other means of restricting energy entails provision of a diet which, when fed at a level necessary to reduce energy intake, will still contain all necessary micronutrients. Both approaches have been used in studies of the effects of caloric restriction on carcinogenesis.

The first report of effects of underfeeding on carcinogenesis was by Moreschi (1) who found that a sarcoma transplanted into mice grew increasingly slowly as less food was offered (Table I). The growth of spontaneous tumors in mice was shown by Rous (2) and Tannenbaum (3) to be inhibited by underfeeding. In a later study, Tannenbaum (4) examined the effects of caloric restriction on both spontaneous and chemically induced tumors in four strains of mouse and consistently observed inhibition of tumor growth. Lavik and Baumann (5) found that the incidence of methylcholanthrene-induced skin tumors in mice fed a low fat, high caloric diet was twice that seen in mice fed a high fat, low calorie diet (Table II).

0377-9727/90/1931-0035$2.00/0

We (6, 7) examined the effects of caloric restriction on 7,12-dimethylbenz(*a*)anthracene (DMBA) mammary tumors and 1,2-dimethylhydrazine (DMH)-induced colon tumors in rats. An example of the diets fed is given in Table III. The effects of 40% caloric restriction on promotion of mammary and colon tumors are presented in Table IV. It is evident that 40% caloric restriction inhibited tumorigenesis even when the restricted rats are ingesting twice as much fat as the *ad libitum*-fed controls. The previously observed differences in tumor promotion of saturated and unsaturated fat (8) were confirmed.

We next investigated the effects of stepwise caloric restriction on DMBA-induced mammary tumors (9) with results summarized in Table V. It is seen that at a 10% reduction in caloric intake, tumor burden is reduced by 47% and multiplicity by 36%. Analysis of carcass composition showed that reduction of calories by 10, 20, 30, or 40% reduced carcass fat by 16, 43, 63, and 72%, respectively. Our early studies were carried out using diets relatively low in fat (4–8%). To investigate the effects of high fat diets, we established DMBA-induced mammary tumors in rats fed 5, 15, or 20% corn oil *ad libitum* or 20 or 26.7% corn oil in rats whose caloric intake was reduced by 25% (10). Thus, rats fed 20% fat in a 25% calorie-restricted diet ingested exactly as much corn oil daily as those fed 20% corn oil *ad libitum*; the same holds true for the *ad libitum* group fed 20% fat and the restricted group fed 26.7% fat. Table VI details the results. Rats fed 26.7% fat but

restricted in calories by 25% exhibited 54% lower tumor incidence, 21% lower tumor multiplicity, 30% lower average tumor weight, and 45% lower tumor burden than rats fed 5% fat *ad libitum*. Retroperitoneal fat pads of the rats fed the calorie-restricted diets weighed about 70% less than matched controls ingesting 15% fat and 62% less than those ingesting 20% fat.

The possibility that body fat may play a role in our findings prompted us to carry out a study in a strain of genetically obese rats (LA/N-Corp) and their lean littermates (11). Corpulent female rats were fed *ad libitum* or placed on a diet restricted in calories by 40%. They and their lean littermates were treated with DMBA to induce mammary tumors. Table VII shows that caloric restriction resulted in a significant reduction in tumorigenicity but not in percentage of carcass fat.

Table I. Underfeeding and Growth of Sarcoma 7 in Mice[a]

Group	Number	Food intake (g/day)	Weight change (g)	Tumor weight (g)
1	13	1.0	−4.2	1.3 ± 0.2
2	7	1.5	−1.9	3.6 ± 0.5
3	8	2.0	−2.4	5.2 ± 0.5
4	8	*Ad libitum*	+1.8	7.6 ± 0.8

[a] After Moreschi (1).

Table II. Influence of Fat and Calories on Methylcholanthrene-Induced Skin Tumors in Mice[a]

Regimen		Tumor Incidence (%)
Calories	Fat	
High	High	66
High	Low	54
Low	High	28
Low	Low	0

[a] After Lavik and Baumann (5).

Table III. Composition of Diets

Ingredient	*Ad libitum*	Restricted[a]	
		As formulated	As fed
Sucrose	58.0	47.3	37.8
Casein	21.6	27.0	21.6
DL-Methionine	0.3	0.4	0.3
Fat	5.0	6.3	5.0
Cellulose	10.1	12.6	10.1
Mineral mix	3.8	4.8	3.8
Vitamin mix	1.0	1.3	1.0
Choline dihydrogen citrate	0.2	0.3	0.2
kcal/g	3.65	3.55	2.83

[a] 20% restricted.

Table IV. Influence of 40% Caloric Restriction on Mammary and Colon Tumors

Group[a]	% Fat	Incidence (%)
DMBA-induced mammary tumors		
AL	3.9[b]	58
R	8.4	0
AL	3.9[c]	80
R	8.4	20
DMH-induced colon tumors		
AL	3.9[d]	85
R	8.4	35
AL	3.9[c]	100
R	8.4	53

[a] AL, *ad libitum*; R, restricted.
[b] Coconut oil + 1% corn oil.
[c] Corn oil.
[d] Butter oil + 1% corn oil.

Table V. Effect of Graded Caloric Restriction on DMBA-Induced Mammary Tumorigenesis in Female Sprague-Dawley Rats

Diet	Incidence (%)	Multiplicity[a]	Tumor weight (g)	Tumor burden (g)
Ad libitum	60	4.7 ± 1.3	2.0 ± 0.8	10.1 ± 3.3
10% Restricted	60	3.0 ± 0.8	1.8 ± 0.5	5.4 ± 3.0
20% Restricted	40	2.8 ± 0.7	1.9 ± 0.7	4.7 ± 1.9
30% Restricted	35	1.3 ± 0.3	0.7 ± 0.6	0.9 ± 0.8
40% Restricted	5	1.0	—	—
	$P < 0.005$	NS	NS	$P < 0.05$

[a] Tumors per tumor-bearing rat.

Table VI. Effect of Caloric Restriction (25%) and Fat Level on DMBA-Induced Mammary Tumors in Rats

Regimen	Incidence (%)	Multiplicity	Tumor weight (g)	Tumor burden (g)
Ad libitum				
5% corn oil	65	1.9 ± 0.3	2.0 ± 0.7	4.2 ± 1.9
15% corn oil	85	3.0 ± 0.6	2.3 ± 0.7	6.6 ± 2.7
20% corn oil	80	4.1 ± 0.6	2.9 ± 0.5	11.8 ± 3.2
Restricted				
20% corn oil	60	1.9 ± 0.4	0.8 ± 0.2	1.5 ± 0.5
26.7% corn oil	30	1.5 ± 0.3	1.4 ± 1.0	2.3 ± 1.6
	$P < 0.005$	$P < 0.0001$	$P < 0.0001$	$P < 0.05$

Table VII. Effect of Caloric Restriction (40%) on Mammary Tumors in Obese and Lean LA/N-Corp Rats Given DMBA

Regimen	Corpulent *ad libitum*	Corpulent restricted	Lean restricted
Incidence (%)	100	27	21
Multiplicity	4.1 ± 0.6	1.5 ± 0.4	1.0 ± 0.0
Tumor weight (g)	5.3 ± 1.4	6.4 ± 3.2	1.7 ± 0.4
Tumor burden (g)	22.0 ± 5.0	9.6 ± 4.0	1.7 ± 0.4
% Carcass fat	51 ± 2	48 ± 2	7 ± 1

In general, caloric restriction has been found to inhibit spontaneous, transplanted, or induced tumors in rats and mice. Albanes (12) summarized the results of 82 published studies on caloric restriction and carcinogenesis in mice. As the level of caloric restriction increased, tumor yield decreased. For all 82 studies, the restricted mice ingested 29% fewer calories, 50% less fat, weighed 23% less, and exhibited 42% reduced tumor incidence.

A few other aspects of this research area merit discussion. Giovanella *et al.* (13) showed that caloric restriction (by about 50%) inhibited the growth of human tumors transplanted into nude mice. The level of inhibition ranged from 57 to 81%. Pollard and Luckert (14) found that caloric restriction inhibited the tumorigenicity of methylazoxymethanol, an indirect acting carcinogen, but not of the direct acting carcinogen methylnitrosourea. This finding suggests that caloric restriction may exert its effect on an energy requiring activation step. We (15) have shown metabolic differences between mammary tumor tissues from *ad libitum*-fed or calorie-restricted donors.

The time of institution of a reduced calorie regimen may be of importance. Weindruch and Walford (16) calorically restricted mice at 1 year of age. Spontaneous hepatomas, lymphomas, and lung tumors were inhibited in the calorie-restricted mice by 7, 34, and 50%, respectively. Their average life-span was extended from 33.6 to 37.6 months. We (17) examined the effects of variable times of caloric restriction on DMBA-induced mammary tumors in rats. Table VIII shows that the incidence of tumors was related to the level of weight gain and feed efficiency of the rats.

In their review of causes of cancer death in the United States, Doll and Peto (18) had the following to say in discussing the experimental work on caloric restriction, "More interest might have been aroused, however, if the freely fed mice had been described as obese instead of the mice on the restricted diet being described as small!" A keen observation indeed.

Another means of adjusting caloric flux is vigorous exercise. In 1944, Rusch and Kline (19) examined the effect of exercise (cage rotation) on the growth of a transplanted fibrosarcoma in ABC mice. In two experiments, the mice were placed into cages which were revolved for 16 hr in every 24-hr period for 4 weeks. Weight gain was reduced by 46 and 66% in the two experiments and tumor area by 25 and 34%. Cohen *et al.* (20) showed that voluntary (activity wheel) exercise reduced the incidence of *N*-methylnitrosourea-induced mammary tumors in rats. Oddly, the extent of inhibition was not correlated with the level of exercise. In rats whose running mileages (per day) over a 142-day period were 1.03–1.69, 1.70–2.00, or 2.05–2.87, tumor incidence was 40, 78, and 70%, respectively. We (21) examined effects of exercise with and without caloric restriction on DMH-induced colon tumors in male rats. Incidence of tumors in *ad libitum*-fed exercised rats and in 25% calorie-restricted sedentary rats was similar (Table IX). Exercise did not reduce levels of carcass fat.

The mechanism(s) by which caloric restriction and/or exercise exert their effects are under study. Caloric restriction has been shown to reduce levels of serum insulin (9, 11) and insulin has been shown to be a growth factor for normal and malignant human mammary cells (22). The possibilities that insulin like growth factors and corticoids may be affected by caloric restriction, and may, in turn, influence tumorigenesis, are under investigation.

Table VIII. Feed Efficiency and Tumor Incidence in Rats Subjected to Variable Caloric Restriction

Group	Regimen[a]	Tumor incidence (%)	Weight gain, (g)	Caloric intake	FE × 10^{2a}
A	A-A-A-A	50	156	7508	2.08
B	R-R-R-R	20	76	5624	1.35
C	R-A-A-A	60	152	7401	2.05
D	R-R-A-A	40	126	6746	1.87
E	A-R-R-A	45	126	6691	1.88
F	A-A-R-R	30	99	6958	1.42
Correlation with tumor incidence, *r*			0.96	0.83	0.94

[a] A, *ad libitum*; R, restricted. Each letter = 1 month.
FE: weight gain (g)/caloric intake (kcal).

Table IX. Effects of Exercise and Caloric Restriction on DMH-Induced Colon Tumors in Male F344 Rats

Regimen		Incidence (%)	Multiplicity	Tumor weight (g)
Diet[a]	Exercise			
AL	–	75	2.1 ± 0.4	3.9 ± 0.6
AL	+	36	1.3 ± 0.2	3.8 ± 0.6
CR[b]	–	35	1.3 ± 0.2	7.5 ± 2.8
CR[b]	+	29	1.1 ± 0.1	3.5 ± 0.5
CR[c]	–	21	1.2 ± 0.2	2.3 ± 0.5

[a] AL, exercised; CR, Caloric restriction.
[b] 25%.
[c] 40%.

This research was supported, in part, by a Career Research Award (HL-00734) from the National Institutes of Health and by funds from the Commonwealth of Pennsylvania.

1. Moreschi C. Beziehungen zwischen ernahrung und tumorwachstum. Z Immunitatsforsch **2**:651–675, 1909.
2. Rous P. The influence of diet on transplanted and spontaneous tumors. J Exp Med **20**:433–451, 1914.
3. Tannenbaum A. The initiation and growth of tumors. Introduction. I. Effects of underfeeding. Am J Cancer **38**:335–350, 1940.
4. Tannenbaum A. The genesis and growth of tumors. II. Effects of caloric restriction per se. Cancer Res **2**:749–756, 1943.
5. Lavik PS, Baumann CA. Further studies on tumor promoting action of fat. Cancer Res **3**:749–756, 1943.
6. Kritchevsky D, Weber MM, Klurfeld DM. Dietary fat versus caloric content in initiation and promotion of 7,12-dimethylbenz(*a*)anthracene-induced mammary tumorigenesis in rats. Cancer Res **44**:3174–3177, 1984.
7. Klurfeld DM, Weber MM, Kritchevsky D. Inhibition of chemically-induced mammary and colon tumor promotion by caloric restriction in rats fed increased dietary fat. Cancer Res **47**:2759–2762, 1987.
8. Carroll KK, Khor HT. Effect of level and type of dietary fat on incidence of mammary tumors induced in female Sprague-Dawley rats by 7,12-dimethylbenz(*a*)anthracene. Lipids **6**:415–420, 1970.
9. Klurfeld DM, Welch CB, Davis MJ, Kritchevsky D. Determination of degree of energy restriction necessary to reduce DMBA-induced mammary tumorigenesis in rats during the promotion phase. J Nutr **119**:286–291, 1989.
10. Klurfeld DM, Welch CB, Lloyd LM, Kritchevsky D. Inhibition of DMBA-induced mammary tumorigenesis by caloric restriction in rats fed high fat diets. Int J Cancer **43**:922–925, 1989.
11. Klurfeld DM, Lloyd LM, Buck CL, Davis MJ, Tulp OL, Kritchevsky D. Inhibition of mammary tumorigenesis by caloric restriction in LA/N-cp (corpulent) rats. Fed Proc **46**:436, 1987.
12. Albanes D. Total calories, body weight and tumor incidence in mice. Cancer Res **47**:1987–1992, 1987.
13. Giovanella BC, Shepard RC, Stehlin JS, Venditti JM, Abbott BJ. Calorie restriction: Effect on growth of human tumors heterotransplanted in nude mice. J Natl Cancer Inst **68**:249–257, 1982.
14. Pollard M, Luckert PH. Tumorigenic effect of direct- and indirect-acting chemical carcinogens in rats on a restricted diet. J Natl Cancer Inst **74**:1347–1349, 1985.
15. Ruggeri BA, Klurfeld DM, Kritchevsky D. Biochemical alterations in 7,12-dimethylbenz(*a*)anthracene-induced mammary tumors from rats subjected to caloric restriction. Biochim Biophys Acta **929**:239–246, 1987.
16. Weindruch R, Walford RL. Dietary restriction in mice beginning at 1 year of age: Effect on life-span and spontaneous cancer incidence. Science **215**:1415–1418, 1982.
18. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the US today. J Natl Cancer Inst **66**:1191–1308, 1981.
19. Rusch HP, Kline BE. The effect of exercise on the growth of a mouse tumor. Cancer Res **4**:116–118, 1944.
20. Cohen LP, Choi K, Wang CX. Influence of dietary fat, caloric restriction and voluntary exercise on *N*-nitrosomethylurea-induced mammary tumorigenesis in rats. Cancer Res **48**:4276–4283, 1988.
21. Klurfeld DM, Welch CB, Einhorn E, Kritchevsky D. Inhibition of colon tumor promotion by caloric restriction or exercise in rats. FASEB J **2**:A433, 1988.
22. Imagawa W, Tomooka Y, Nandi S. Serum free growth of normal and tumor mouse mammary epithelial cells in primary culture. Proc Natl Acad Sci USA **79**:4074–4077, 1982.