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Longevity, Body Weight, and Neoplasia in *Ad Libitum*-Fed and Diet-Restricted C57BL6 Mice Fed NIH-31 Open Formula Diet

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ABSTRACT

Groups of C57BL6 mice of each sex were assigned to one of 2 dietary regimens, *ad libitum* (AL) or dietary restriction (DR), to study effects of food restriction on body weight, survival, and neoplasia. The AL and DR groups were subdivided into a scheduled sacrifice group for examination at 6-mo intervals, and a lifetime group to provide longevity data. Necropsies and microscopic examinations were conducted on 911 animals. In the lifetime group food consumption averaged 33.6 and 34.4 g per week by AL males and AL females, respectively; the DR counterparts were given 40% less. The diet contained 4.35 kcal/g. The average lifetime body weights were 34.8, 26.8, 22.6, and 21.6 g for AL males, AL females, DR males, and DR females, respectively, and their age at 50% survival was 27.5, 26.9, 31.7, and 33.5 mo. Maximal lifespan was increased 18% in DR males and females. Lifetime incidence of tumor-bearing mice was 89% and 86% for AL males and females, versus 64% for each sex of DR mice. Dramatic reduction occurred in female DR mice in lymphoma (9% vs 29%), pituitary neoplasms (1% vs 37%), and thyroid neoplasms (0.4% vs 8%). In males, hepatocellular tumors were reduced to 1% from 10% by DR. In contrast, the incidence of histiocytic sarcoma was increased in DR females and unaffected in DR males. Tumor onset was delayed in DR animals; 87% of all neoplasms in males and 95% in females had occurred in the AL mice by 24 mo, whereas the DR animals had only 52% and 39% of their lifetime incidence, respectively, by that age. This study provided comparative AL and DR data from C57BL6 mice examined randomly at 6-mo intervals (cross-sectional group) in parallel with data from animals in similar cohort that was unsampled and allowed to succumb naturally (longevity group). Dietary restriction reduced the lifetime percentage of tumor-bearing animals and the number of tumors per animal, and delayed the age at onset of most neoplasms.

Keywords. Caloric restriction; diet restriction; lifespan; rodent; tumor incidence; histiocytic sarcoma; pituitary neoplasia; lymphoma

INTRODUCTION

The Food and Drug Administration's National Center for Toxicological Research (NCTR) and the National Institute on Aging (NIA) have collaborated on studies of nutrition and aging, in which the effects of 40% restriction of feed was compared with *ad libitum* feeding (11, 14, 18, 24). Several genotypes of rodents were studied. The objectives of these studies include establishment of biomarkers of aging for subsequent use to study the effect of caloric restriction, and of biomarkers of toxicity to determine effects of nutrition on toxicity. When such biomarkers are identified and validated in rodents, they may prove to be applicable to man, providing for extrapolation of data from laboratory animals to man. Development of age-specific pathology profiles for restricted- and *ad libitum*-fed animals of each genotype was a necessary component of the studies. A number of descriptions of age-related

TABLE I.—Distribution of mice among the treatment groups.

	Males				Females			
	Ad libitum		Restricted		*Ad libitum*		Restricted	
	Number allocated	Number examined	Number allocated	Number examined	Number allocated	Number examined	Number allocated	Number examined
LDM	56	50	56	55	56	37	56	56
SS	210	60	210	75	210	57	210	75
SDM	0	111	0	130	0	80	0	125
Total	266	221	266	260	266	174	266	256

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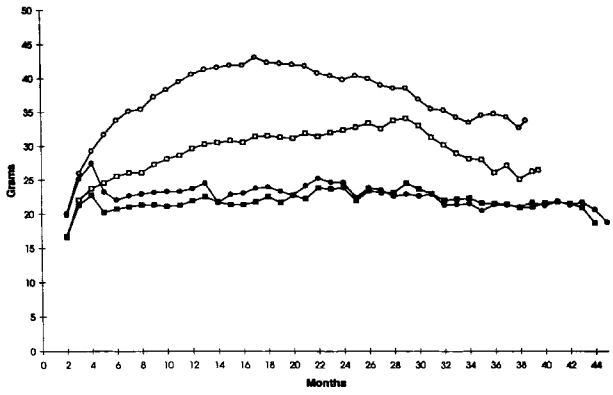

FIG. 1.—Body weights of C57BL6 mice fed NIH-31 diet. Treatment groups are: □ = AL female; ■ = DR female; ○ = AL male; ● = DR male.

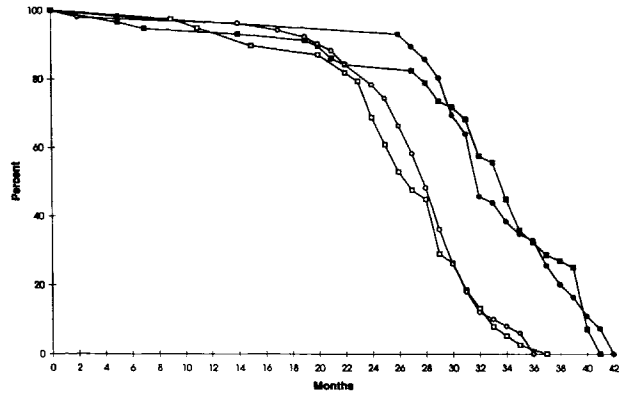

FIG. 2.—Survival curve of C57BL6 mice fed NIH-31 diet. Treatment groups are: □ = AL female; ■ = DR female; ○ = AL male; ● = DR male.

changes in C57BL6 mice have been reported (2, 8, 9), including accounts of longevity and neoplasia (16, 25) and effects of dietary restriction (3, 6, 22, 23). However, environmental factors such as temperature, illumination, noise, and proximity of other animals cause each colony of rodents to age differently (12, 19, 21, 22, 23, 25).

This report describes the longevity and neoplasia in NCTR's C57BL6 mice that became moribund or died naturally as part of the dietary studies, as well as the occurrence of neoplasia in cohorts that were killed for that purpose at intervals throughout their lifespan. A subset of these data is included in a summary submitted elsewhere (13). The effects of dietary restriction on incidence and severity of non-neoplastic conditions will be reported separately.

MATERIALS AND METHODS

Experiment Design. One thousand sixty-four mice were used (Table I). The animals were weaned at 3 wk of age and placed in the study at 4 wk, assigned to one of 2 dietary groups, *ad libitum* (AL) or dietary restriction (DR).

The AL and DR groups were each subdivided into a lifetime group to determine longevity data and a scheduled sacrifice group for evaluation at specific ages. Animals in the lifetime group were removed from the study only when dead or moribund and were designated LDM (Lifetime: Dead and Moribund). The scheduled sacrifice group was designated SS (Scheduled Sacrifice). The sacrifice schedule began at 12 mo in the study and continued at 6-mo intervals thereafter. Those mice in the SS groups that were removed when moribund or dead between scheduled sacrifices were designated SDM (Scheduled: Dead and Moribund). The number of mice in each subgroup is depicted in Table I. Of the 1,064 animals initially assigned to the study, 911 were available for pathological evaluation. The largest group lost to examination, 134 animals, was removed intermittently between 268 and 956 days because of spontaneous ulcerative dermatitis that affected all subgroups but was most prevalent in AL females (see discussion). These animals were deleted from the study. The remaining 19 animals were unavailable for pathological evaluation for various reasons.

Animals and Diet. C57BL6 mice of both sexes, produced by the NCTR Specific Pathogen Free breeding colony, were allocated to this study as weanlings. They were housed individually in polycarbonate cages fitted with filter tops and bedded with hardwood chips. NIH-31 open formula diet with average energy content of 4.35 kcal/g, (Purina Mills, Inc., Richmond, IN) was fed to both groups. The mice on the restricted diet received 60% of the food consumed by their AL cohorts. Their food allowance began as 90% of AL at 14 wk of age, and

TABLE II.—Age in days at 50% survival[a] and maximal lifespan[b] in the lifetime group (months given in parentheses).

	Males		Females	
	Ad libitum	Restricted	*Ad libitum*	Restricted
50% Survival	836 (27.5)	965 (31.7)	817 (26.9)	1,017 (33.5)
Maximal lifespan	1,056 (34.8)	1,247 (41.0)	1,035 (34.1)	1,221 (40.2)
Oldest survivor	1,078 (35.5)	1,259 (41.4)	1,102 (36.3)	1,231 (40.5)

[a] Median survival (first week of 50% mortality).
[b] Average age of oldest 10% in each group.

TABLE III.—Cause of death[a] and number affected (% given in parentheses).

	Males		Females	
	Ad libitum	Restricted	*Ad libitum*	Restricted
Lymphoma	97 (60%)	97 (52%)	67 (57%)	102 (56%)
Hemangioma/hemangiosarcoma	8 (5%)	2 (1%)	3 (3%)	6 (3%)
Nephropathy	10 (6%)	3 (2%)	3 (3%)	8 (4%)
Liver neoplasm	10 (6%)	1 (0.5%)	2 (2%)	1 (0.5%)
Inflammation	9 (6%)	11 (6%)	6 (5%)	2 (1%)
Pituitary adenoma	—	—	10 (9%)	1 (0.5%)
Heart thrombus	4 (3%)	—	3 (3%)	—
Other[b]	7 (4%)	11 (6%)	12 (10%)	24 (13%)
Unknown	16 (10%)	60 (32%)	11 (9%)	37 (20%)
Total animals	161	185	117	181

[a] Longevity group animals plus those removed as dead or moribund from the serial sacrifice group.
[b] Other = mammary carcinoma, granulocytic leukemia, duodenal polyp, lung carcinoma, uterus dilatation, hemorrhage, paraganglioma, pericarditis, pheochromocytoma, skin trichoepithelioma.

was stepped down to 75% at 15 wk and to 60% at 16 wk. The restricted diet was supplemented with vitamins to provide the same amount available to the AL mice. The room temperature was maintained at 21 ± 3°C and the relative humidity was 50 ± 10%. The room light cycle was 12 hr on and 12 hr off (24). All aspects of the study were conducted in compliance with applicable animal welfare guidelines and regulations (5).

Pathology. Mice were removed from the study either dead, moribund, or scheduled for sacrifice. They were euthanatized for necropsy by carbon dioxide inhalation. Approximately 45 tissues or organs and all gross lesions were collected for microscopic examination. After fixation in 10% neutral buffered formalin, the tissues were processed routinely and stained with hematoxylin–eosin for histopathological examination.

Data Tabulation. The types and numbers of lesions that occurred at each scheduled sacrifice period (age) can be compared directly between the AL and DR SS subgroups. To compare age-related neoplasia in dead and moribund mice from the longevity group with those in SS animals that died or became moribund spontaneously between sacrifices, the data from all animals removed as dead or moribund were tabulated for 6-mo intervals, with each interval centering on a sacrifice date. In this way, for example, SDM and LDM animals that were removed between 639 and 821 days could be compared with each other as well as with the SS animals sacrificed at 24 mo (730 days). Therefore, all references to a particular time point (e.g., 24 mo) in the LDM and SDM animals refer to that time point ± 3 mo. In this report, 1 mo contains 30.4 days.

To compare occurrences of neoplasms among the groups, data in Tables IV–VII were divided into 4 intervals: animals examined at 0–24 mo, 30 mo, 36 mo, and 42 mo. (There were relatively few dead or moribund animals at the 12- and 18-mo periods.) The percentage of animals with neoplasms (benign or malignant) in each group is summarized in Table IV. The average number of tumors per tumor-bearing mouse is presented by group in Table V (some animals had multiple tumors). Table VI lists by sex, diet group, and age the average number of mouse-days in the study per tumor produced. The respective incidence of the 10 most prevalent neoplasms is summarized by diet group, sex, and age in Table VII. In Table VIII, the overall incidence of specific neoplasms is listed by sex and diet group. Tables IX and X (females and males, respectively) list all

TABLE IV.—Number of neoplasm-bearing mice/number of mice examined (% given in parentheses).

Months (days)	Females						Males					
	Ad libitum			Restricted			*Ad libitum*			Restricted		
	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
0–24	19/43	40/56	16/19	8/44	22/40	4/10	5/43	41/61	17/22	6/44	15/26	2/6
(1–821)	(44.2)	(71.4)	(84.2)	(18.2)	(55.0)	(40.0)	(11.6)	(67.2)	(77.3)	(13.6)	(57.7)	(33.3)
30	14/14	22/24	15/16	5/15	28/35	11/15	7/14	45/50	21/23	8/15	25/48	17/26
(822–1,003)	(100.0)	(91.7)	(93.8)	(33.3)	(80.0)	(73.3)	(50.0)	(90.0)	(91.3)	(53.3)	(52.1)	(65.4)
36	—	—	2/2	8/15	34/41	13/19	2/3	—	5/5	9/15	31/47	9/14
(1,004–1,186			(100.0)	(53.3)	(82.9)	(68.4)	(66.7)		(100.0)	(60.0)	(66.0)	(64.3)
42–48	—	—	—	1/1	5/9	8/12	—	—	—	0/1	8/9	7/9
(1,117–9,999)				(100.0)	(55.6)	(66.7)				—	(88.9)	(77.8)
Total	33/57	62/80	33/37	22/75	89/125	35/56	14/60	86/111	43/50	23/75	79/130	35/55
(%)	(57.9)	(77.5)	(89.2)	(29.3)	(71.2)	(64.3)	(23.3)	(74.5)	(86.0)	(30.7)	(60.8)	(63.6)

TABLE V.—Mouse-days at risk per neoplasm[a] in lifetime group mice.

Month (days)	Females AL (60/37)[b]	Females DR (43/56)	Males AL (56/50)	Males DR (42/55)
0–24 (1–821)	531	945	742	2,023
30 (822–1,003)	442	1,146	710	1,145
36 (1,004–1,186)	714	1,201	755	1,707
42 (1,187–1,366)	—	1,612	—	1,106
Overall	499	1,271	727	1,330

[a] Aggregate number of mouse-days survived per time period/total number of tumors in these animals.
[b] Overall number of tumors/number of animals examined.

neoplasms diagnosed, within diet group and time period.

Statistical Evaluation. Fisher's Exact Test was used to compare diet groups for the incidence of each neoplasm in the LDM subset, and also in the combined LDM and SDM subsets, within time intervals. The results are included in Table VII.

RESULTS

Body Weights (LDM Animals)

The weekly mean body weight for each treatment group, derived from the LDM animals surviving to the respective time points, is plotted in Fig. 1. Of the 4 treatment groups, DR females maintained the most consistent body weight over time; they peaked at 29 mo (22 g) then gradually declined. DR males reached their peak weight (27.5 g) at 4 mo immediately before onset of full diet restriction, had minor fluctuation in body weight until 22 mo, and gradually declined thereafter. The average lifetime bodyweight was 21.6 g for DR females and 22.6 g for DR males.

The weight of AL females reached 33 g at 29 mo and decreased thereafter. AL males reached 42 g at 17 mo then gradually declined. The weight loss was more abrupt in the females than in the males. The average lifetime body weight was 26.8 g for AL females and 34.8 g for AL males.

Longevity (LDM Animals)

The survival curves for the lifetime groups (LDM) are based on the 198 animals terminated by natural death; they include 38 females and 50 males in the AL groups and 56 females and 55 males in the DR groups (Fig. 2). These were the same animals from which the body weight data were obtained. In Table II, the age of each group at 50% survival and maximal survival (mean age of the oldest 10%) are summarized, and the age of the oldest survivor in each group is listed.

The causes of death as determined microscopically are listed in Table III. The animals represented in this table are those that succumbed or were removed moribund from the lifetime group, plus those similarly removed from the SS group between scheduled sacrifices. For both sexes, ages at 50% survival and at maximal lifespan were greater in DR animals. Dietary restriction increased maximal lifespan by 18% in both sexes.

Neoplasia

As depicted in Table IV, the overall proportion of mice with neoplasia in AL females was 73.6% (128/174) versus 57.4% (147/256) in DR females. The difference was less in males, with 64.7% of AL males (143/221) having tumors versus 52.7% (137/260) in the DR males. However, these overall rates combine serially sacrificed animals with those removed only when they were moribund or dead. The sacrificed animals necessarily included young animals and individuals that were well, yielding a lower tumor burden for that group; removal of these animals on schedule obviously prevented them from developing neoplasms later. Thus the SS/SDM groups have lower incidence than the LDM group.

TABLE VI.—Number of neoplasms/number of tumor-bearing mice (neoplasms/mouse).

Months (days)	Females *Ad libitum* SS	Females *Ad libitum* SDM	Females *Ad libitum* LDM	Females Restricted SS	Females Restricted SDM	Females Restricted LDM	Males *Ad libitum* SS	Males *Ad libitum* SDM	Males *Ad libitum* LDM	Males Restricted SS	Males Restricted SDM	Males Restricted LDM
0–24	22/19	54/40	24/16	9/8	25/22	5/4	7/5	47/41	20/17	7/6	17/15	2/2
(1–821)	(1.2)	(1.4)	(1.5)	(1.1)	(1.1)	(1.3)	(1.4)	(1.1)	(1.2)	(1.2)	(1.1)	(1.0)
30	29/14	48/22	33/15	5/5	33/28	12/11	10/7	61/45	29/21	8/8	32/25	21/17
(822–1,003)	(2.1)	(2.2)	(2.2)	(1.0)	(1.2)	(1.1)	(1.4)	(1.4)	(1.4)	(1.0)	(1.3)	(1.2)
36	—	—	3/2	10/8	42/34	17/13	2/2	—	7/5	10/9	34/31	9/9
(1,004–1,186)			(1.5)	(1.2)	(1.2)	(1.3)	(1.0)		(1.4)	(1.1)	(1.1)	(1.0)
42–48	—	—	—	2/1	6/5	9/8	—	—	—	—	9/8	10/7
(1,187–9,999)				(2.0)	(1.2)	(1.1)					(1.1)	(1.4)
Total	51/33	102/62	60/33	26/22	106/89	43/36	19/14	108/86	56/43	25/23	92/79	42/35
	(1.5)	(1.6)	(1.8)	(1.2)	(1.2)	(1.2)	(1.4)	(1.3)	(1.3)	(1.1)	(1.2)	(1.2)

TABLE VII.—Prevalence of major neoplasms given as number of neoplasm-bearing mice/number of mice examined (% given in parentheses).

Neoplasm	Months (days)	Females						Males					
		Ad libitum			Restricted			*Ad libitum*			Restricted		
		SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
Histiocytic sarcoma	0–24 (1–821)	1/43 (2.3)	14/56 (25.0)	5/19 (26.3)	3/44 (6.8)	16/40 (40.0)	4/10 (40.0)	1/43 (2.3)	25/61 (41.0)	9/22 (40.9)	1/44 (2.3)	8/26 (30.8)	1/6 (16.7)
	30 (822–1,003)	5/14 (35.7)	11/24 (45.8)	7/16 (43.8)	3/15 (20.0)	19/35 (54.3)	8/15 (53.3)	4/14 (28.6)	35/50 (70.0)	19/23 (82.6)	5/15 (33.3)	17/48 (35.4)	14/26 (53.8)
	36 (1,004–1,186)	—	—	0/2 —	3/15 (20.0)	27/41 (65.9)	10/19 (52.3)	0/3 —	—	1/5 (20.0)	5/15 (33.3)	26/47 (55.3)	8/14 (57.1)
	42–48 (1,187–9,999)	—	—	—	1/1 (100.0)	1/9 (11.1)	6/12 (50.0)	—	—	—	0/1 —	7/9 (77.8)	7/9 (77.8)
Total		6/57 (10.5)	25/80 (31.3)	12/37 (32.4)	10/75 (13.3)	63/125 (50.4)	28/56[a] (50.0)	5/60 (8.3)	60/111 (54.1)	29/50 (58.0)	11/75 (14.7)	58/130 (44.6)	30/55 (54.5)
Lymphoma	0–24 (1–821)	11/43 (25.6)	17/56 (30.4)	7/19 (36.8)	3/44 (6.8)	4/40 (10.0)	0/10 —	2/43 (4.7)	6/61 (9.8)	3/22 (13.6)	2/44 (4.5)	5/26 (19.2)	1/6 (16.7)
	30 (822–1,003)	5/14 (35.7)	7/24 (29.2)	3/16 (18.8)	0/15 —	6/35 (17.1)	3/15 (20.0)	2/14 (14.3)	2/50 (4.0)	1/23 (4.3)	0/15 —	1/48 (2.1)	2/26 (7.7)
	36 (1,004–1,186)	—	—	0/2 —	4/15 (26.7)	2/41 (4.9)	2/19 (10.5)	0/3 —	—	0/5 —	1/15 (6.7)	1/47 (2.1)	1/14 (7.1)
	42–48 (1,187–9,999)	—	—	—	0/1 —	0/9 —	0/12 —	—	—	—	0/1 —	0/9 —	0/9 —
Total	16/57 (28.1)	24/80 (30.0)	10/37 (27.0)	7/75 (9.3)	12/125[a] (9.6)	5/56[a,b] (8.9)	4/60 (6.7)	8/111 (7.2)	4/50 (8.0)	3/75 (4.0)	7/130 (5.4)	4/55 (7.3)	
Pituitary neoplasms	0–24 (1–821)	6/43 (14.0)	10/56 (17.9)	7/19 (36.8)	0/44 —	0/40 —	0/10 —	0/43 —	0/61 —	0/22 —	0/44 —	0/26 —	0/6 —
	30 (822–1,003)	9/14 (64.3)	18/24 (75.0)	13/16 (81.2)	0/15 —	1/35 (2.9)	1/15 (6.7)	0/14 —	0/50 —	0/23 —	0/15 —	0/48 —	0/26 —
	36 (1,004–1,186)	—	—	1/2 (50.0)	0/15 —	1/41 (2.4)	0/19 —	1/3 (33.3)	—	0/5 —	1/15 (6.7)	0/47 —	0/14 —
	42–48 (1,187–9,999)	—	—	—	0/1 —	0/9 —	0/12 —	—	—	—	0/1 —	0/9 —	0/9 —
Total		15/57 (26.3)	28/80 (35.0)	21/37 (56.8)	0/75 —	2/125 (1.6)	1/56 (1.8)	1/60 (1.7)	0/111 —	0/50 —	1/75 (1.3)	0/130 —	0/55 —
Pulmonary neoplasms	0–24 (1–821)	1/43 (2.3)	2/56 (3.6)	0/19 —	1/44 (2.3)	1/40 (2.5)	0/10 —	3/43 (7.0)	1/61 (1.6)	2/22 (9.1)	2/44 (4.6)	0/26 —	0/6 —
	30 (822–1,003)	0/14 —	2/24 (8.3)	1/16 (6.2)	0/15 —	1/35 (2.9)	0/15 —	2/14 (14.3)	3/50 (6.0)	1/23 (4.3)	1/15 (6.7)	5/48 (10.4)	3/26 (11.5)
	36 (1,004–1,186)	—	—	0/2 —	1/15 (6.7)	0/41 —	0/19 —	0/3 —	—	0/5 —	0/15 —	1/47 (2.1)	0/14 —
	42–48 (1,187–9,999)	—	—	—	0/1 —	1/9 (11.1)	1/12 (8.3)	—	—	—	0/1 —	0/9 —	0/9 —
Total		1/57 (1.8)	4/80 (5.5)	1/37 (2.7)	2/75 (2.7)	3/125 (2.4)	1/56 (1.8)	5/60 (8.3)	4/111 (3.6)	3/50 (6.0)	3/75 (4.0)	6/130 (4.6)	3/55 (5.5)
Liver neoplasms	0–24 (1–821)	0/43 —	2/56 (3.6)	1/19 (5.3)	0/44 —	1/40 (2.5)	0/10 —	1/43 (2.3)	8/61 (13.1)	2/22 (9.1)	0/44 —	1/26 (3.8)	0/6 —
	30	1/14	1/24	0/16	0/15	0/35	0/15	1/14	6/50	2/23	0/15	1/48	0/26

TABLE VII.—Continued.

Neoplasm	Months (days)	Females						Males					
		Ad libitum			Restricted			*Ad libitum*			Restricted		
		SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
	(822–1,003)	(7.1)	(4.2)	—	—	—	—	(7.1)	(12.0)	(8.7)	—	(2.1)	—
	36	—	—	0/2	0/15	2/41	0/19	1/3	—	2/5	0/15	1/47	0/14
	(1,004–1,186)			—	—	(4.9)	—	(33.3)		(40.0)	—	(2.1)	—
	42–48	—	—	—	0/1	0/9	0/12	—	—	—	0/1	0/9	0/9
	(1,187–9,999)				—	—	—				—	—	—
Total		1/57	3/80	1/37	0/75	3/125	0/56	3/60	14/111	6/50	0/75	3/130[a]	0/55[a,b]
		(1.8)	(3.8)	(2.7)	—	(2.4)	—	(5.0)	(12.6)	(12.0)	—	(2.3)	—
Vascular neoplasms	0–24	0/43	3/56	0/19	0/44	0/40	0/10	0/43	3/61	1/22	0/44	0/26	0/6
	(1–821)	—	(5.4)	—	—	—	—	—	(4.9)	(4.5)	—	—	—
	30	0/14	0/24	0/16	0/15	1/35	0/15	1/14	3/50	2/23	1/15	2/48	0/26
	(822–1,003)	—	—	—	—	(2.9)	—	(7.1)	(6.0)	(8.7)	(6.7)	(4.2)	—
	36	—	—	0/2	0/15	2/41	3/19	0/3	—	1/5	0/15	2/47	0/14
	(1,004–1,186)			—	—	(4.9)	(15.8)	—		(20.0)	—	(4.3)	—
	42–48	—	—	—	0/1	1/9	0/12	—	—	—	0/1	0/9	0/9
	(1,187–9,999)				—	(11.1)	—				—	—	—
Total		0/57	3/80	0/37	0/75	4/125	3/56	1/60	6/111	4/50	1/75	4/130	0/55
		—	(3.8)	—	—	(3.2)	(5.4)	(1.7)	(5.4)	(8.0)	(1.3)	(3.1)	—
Harderian neoplasms	0–24	0/43	0/56	1/19	0/44	0/40	0/10	0/43	1/61	1/22	2/44	0/26	0/6
	(1–821)	—	—	(5.3)	—	—	—	—	(1.6)	(4.5)	(4.5)	—	—
	30	1/14	0/24	0/16	0/15	0/35	0/15	0/14	2/50	2/23	0/15	4/48	0/26
	(822–1,003)	(7.1)	—	—	—	—	—	—	(4.0)	(8.7)	—	(8.3)	—
	36	—	—	0/2	0/15	2/41	1/19	0/3	—	1/5	2/15	2/47	0/14
	(1,004–1,186)			—	—	(4.9)	(5.3)	—		(20.0)	(13.3)	(4.3)	—
	42–48	—	—	—	0/1	0/9	0/12	—	—	—	0/1	0/9	1/9
	(1,187–9,999)	—			—	—	—				—	—	(11.1)
Total		1/57	0/80	1/37	0/75	2/125	1/56	0/60	3/111	4/50	4/75	6/130	1/55
		(1.8)	—	(2.7)	—	(1.6)	(1.8)	—	(2.7)	(8.0)	(5.3)	(4.6)	(1.8)
Thyroid gland neoplasms	0–24	0/43	2/56	1/19	0/44	1/40	0/10	0/43	0/61	0/22	0/44	0/26	0/6
	(1–821)	—	(3.6)	(5.3)	—	(2.5)	—	—	—	—	—	—	—
	30	4/14	3/24	4/16	0/15	0/35	0/15	0/14	0/50	0/23	0/15	0/48	0/26
	(822–1,003)	(28.6)	(12.5)	(25.0)	—	—	—	—	—	—	—	—	—
	36	—	—	0/2	0/15	0/41	0/19	0/3	—	1/5	0/15	0/47	0/14
	(1,004–1,186)			—	—	—	—	—		(20.0)	—	—	—
	42–48	—	—	—	0/1	0/9	0/12	—	—	—	0/1	0/9	0/9
	(1,187–9,999)				—	—	—				—	—	—
Total		4/57	5/80	5/37	0/75	1/125[a]	0/56[a,b]	0/60	0/111	1/50	0/75	0/130	0/55
		(7.0)	(6.2)	(13.5)	—	(0.8)	—	—	—	(2.0)	—	—	—
Small intestine neoplasms	0–24	0/43	0/56	0/19	0/44	0/40	0/10	0/43	0/61	0/22	0/44	0/26	0/6
	(1–821)	—	—	—	—	—	—	—	—	—	—	—	—
	30	0/14	0/24	0/16	0/15	0/35	0/15	0/14	0/50	0/23	0/15	0/48	0/26
	(822–1,003)	—	—	—	—	—	—	—	—	—	—	—	—
	36	—	—	0/2	2/15	1/41	0/19	0/3	—	0/5	1/15	0/47	0/14

TABLE VII.—Continued.

Neoplasm	Months (days)	Females						Males					
		Ad libitum			Restricted			*Ad libitum*			Restricted		
		SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
	(1,004–1,186)			—	(13.3)	(2.4)	—	—		—	(6.7)	—	—
	42–48	—	—	—	0/1	1/9	1/12	—	—	—	0/1	1/9	0/9
	(1,187–9,999)				—	(11.1)	(8.3)				—	(11.1)	—
Total		0/57	0/80	0/37	2/75	2/125	1/56	0/60	0/111	0/50	1/75	1/130	0/55
		—	—	—	(2.7)	(1.6)	(1.8)	—	—	—	(1.3)	(0.8)	—
Uterine neoplasm	0–24	2/43	2/56	1/19	0/44	0/40	0/10						
	(1–821)	(4.7)	(3.6)	(5.3)	—	—	—						
	30	2/14	2/24	1/16	1/15	0/35	0/15						
	(822–1,003)	(14.3)	(8.3)	(6.2)	(6.7)	—	—						
	36	—	—	0/2	0/15	0/41	0/19						
	(1,004–1,186)			—	—	—	—						
	42–48	—	—	—	0/1	0/9	0/12						
	(1,187–9,999)				—	—	—						
Total		4/57	4/80	2/37	1/75	0/125	0/56						
		(7.0)	(5.0)	(5.4)	(1.3)	—	—						

[a] Incidence in (LDM + SDM) differs significantly between AL and DR, $p \leq 0.05$.
[b] Incidence in LDM differs significantly between AL and DR, $p \leq 0.05$.

The LDM groups, therefore, reflect the true lifetime tumor incidence: 89.2% versus 64.3% in the AL and DR females, respectively; and 86.0% versus 63.6% in AL and DR males. The DR regimen reduced the overall lifetime incidence of a number of tumors, and it is also clear that most neoplasms were delayed in onset in DR animals. At the end of the 30-mo period, for example, only 60% (15/25) of the DR females had tumors diagnosed microscopically, versus 88.6% (31/35) of the AL females. Likewise, 59.4% (19/32) of the DR males were affected, versus 84.4% (38/45) of the AL males.

Another perspective of the interaction between incidence and delayed onset can be obtained by calculating the aggregate number of mouse-days at risk per tumor produced, within each time period. This compilation is summarized in Table V. Here it is clear that there was a modest decrease in lifetime tumor risk overall between DR and AL mice, and that approximately twice as many days were required on average for tumors to develop in the DR animals.

One other characteristic of interest was tumor multiplicity, depicted in Table VI as number of tumors per tumor-bearing animal. Table VI reveals that the 60 tumors produced by AL LDM females occurred in 33 animals, that is, an average of 1.8 separate neoplasms per tumor-bearing animal, in comparison to 1.2 (43 tumors in 36 animals) in the DR counterparts. Among males, diet had less effect, with 56 tumors in 43 mice (1.3 per animal) for AL and 42 tumors in 35 mice (1.2 per animal) in DR mice.

DISCUSSION

Restriction of caloric intake in rodents to levels below approximately 85% of AL has been associated consistently with increased lifespan, delay in onset of neoplasia and nonneoplastic degenerative diseases, and with reduction in age-specific incidence of neoplasia (23). The overall findings reported here are compatible with those in the literature.

A major objective of this study was to use substantial dietary restriction (40%) to promote longevity in a search for biomarkers of aging, comparing cohorts of mice fed *ad libitum*. The relative merit of dietary restriction in murine toxicity studies was not investigated in this work. It is clear that 40% restriction in this study, as well as more moderate restriction (23), reduced both early deaths and the background of spontaneous lesions, suggesting benefit in interpretation of toxicity studies. Dietary restriction does modify the rodent response to xenobiotics, in comparison to *ad libitum*-fed cohorts (7, 20). The authors believe that *ad libitum* feeding as practiced currently produces overnutrition in ro-

TABLE VIII.—Overall incidence of specific neoplasms.

Neoplasm	Females				Males			
	Ad libitum		Restricted		*Ad libitum*		Restricted	
	No.	%	No.	%	No.	%	No.	%
Histiocytic sarcoma	43	24.7	101	39.5	94	42.5	99	38.1
Lymphoma	50	29	24	9.4	16	7.2	14	5.4
Pituitary	64	36.8	3	1.2	1	0.5	1	0.4
Alveolar–bronchiolar (lung)	6	3.4	6	2.3	12	5.4	12	4.6
Hepatocellular	5	2.9	3	1.2	23	10.4	3	1.2
Vascular	3	1.7	7	2.7	11	5.0	5	1.9
Harderian gland	2	1.1	3	1.2	7	3.2	11	4.2
Thyroid follicular cell	14	8.0	1	0.4	1	0.5	—	—
Uterine	10	5.7	1	0.4	—	—	—	—
Urinary	—	—	—	—	4	1.8	4	1.5
Skin and subcutaneous	6	3.4	3	1.2	—	—	—	—
Small intestine	—	—	5	2.0	—	—	2	0.8
All others	9	5.2	18	7.0	14	6.3	8	3.1
Total neoplasms	212	121.9	175	68.5	183	82.8	159	61.2

dents, but the level of restriction that may be most beneficial in toxicity bioassays has not been established and requires validation. The precise degree of restriction of current diet formulations to achieve the ideal balance will probably differ among species and genotypes, and each level of restriction may affect metabolism of different classes of xenobiotics differently.

The statistical outcome of our study was adversely affected by selective loss of female AL animals to a debilitating skin condition; the animals were removed for humanitarian reasons and deleted from the study. As a result, only 37 AL females remained in the lifetime group. When the incidence of various neoplasms in this group was compared to that in their DR counterparts (56 animals), significant differences in incidence could not always be demonstrated despite large percentage differences. However, Table VII reveals that for virtually every neoplasm, the incidence in the individual subsets (SS, SDM, LDM) within each diet group was consistent, suggesting that the LDM outcome was not a random effect of small group size. Further, when the LDM group was combined with the SDM group, the differences in incidence for several neoplasms could be demonstrated statistically (although the combined result underestimates the true incidence, because some animals were removed periodically from both the AL and DR SDM cohorts).

The skin condition that affected our C57BL6 colony was similar to that reported some time ago in C57BL-related mice (17) and recently as an immune-mediated disease (1). These diseases are primarily ulcerative; a proliferative dermatitis in C57BL mice was also described recently, as the result of a spontaneous mutation (10). The 2 recent reports (1, 10) describe both sexes being affected equally, whereas our disease affected females predominantly. Whether or not the condition experienced by our colony was also the manifestation of a genetic aberration, the degree to which diet affected its expression was notable: it occurred in 69 (26%) and 36 (13.5%) of AL females and males, respectively, versus 5 (2%) and 1 (0.3%) of the DR counterparts. We have observed a comparable inhibitory effect of DR on expression of a hereditary ocular degeneration in DBA/2NNia mice. That condition included development of glaucoma and was more severe in females (15).

The mean lifespan (age at 50% survival) of our AL mice was consistent with values reported for C57BL mice by other investigators, despite inherent differences in animal sources, husbandry, and dates of the studies. The mean lifespan of our C57BL6 mice was 27.5 mo for AL males and 26.9 mo for AL females. Zurcher et al (25) reported 24.0 and 22.2 mo, respectively, for their C57BL animals in 1982, Weindruch and Walford (22) reported 24.9 mo for male C7BL/6J mice in 1982, and Storer (16) reported 22.7 and 22.2 mo, respectively, for male and female C57BL/6J mice in 1966. Dietary restriction provided a 15% increase in mean lifespan in our males and 25% increase in our females. Weindruch and Walford (22) achieved a 20% increase in their males, with dietary restriction of approximately 45% beginning at 12–13 mo of age. Our animals were fed 40% less than AL, beginning at 15 wk of age. The smaller relative increase in longevity of our DR males may relate to the longer lifespan of our AL males.

Because of different diagnostic criteria, it is difficult to compare published incidences of specific lymphoreticular neoplasms, but the report by Zurcher et al (25) included a 38% total prevalence in 105 males and 56% in 44 females. The mean age of the group was 23 mo (6–34) for males and 20 mo

TABLE IX.—Neoplasms in female C57BL6 mice (number examined given in parentheses).

SS				SDM				LDM			
Ad libitum		Diet restricted		*Ad libitum*		Diet restricted		*Ad libitum*		Diet restricted	
12 mo (1-456 days)											
(14)		(14)		(9)		(7)		(3)		(4)	
Lymphoma, malignant	2	Osteosarcoma	1	Lymphoma, malignant	2	None		Histiocytic sarcoma	1	None	
		Nose schwannoma	1					Lymphoma, malignant	1		
18 mo (457-638 days)											
(14)		(15)		(19)		(7)		(1)		(4)	
Pituitary adenoma	1	None		Histiocytic sarcoma	9	Lymphoma, malignant	1	Liver carcinoma	1	Histiocytic sarcoma	2
Uterus polyp	1			Lymphoma, malignant	3	Histiocytic sarcoma	1				
Vagina carcinoma	1			Vascular neoplasm	1						
				Liver carcinoma	1						
				Thyroid follicular cell adenoma	1						
24 mo (639-821 days)											
(15)		(15)		(28)		(26)		(15)		(2)	
Lymphoma, malignant	9	Lymphoma, malignant	3	Lymphoma, malignant	12	Histiocytic sarcoma	15	Pituitary adenoma	7	Histiocytic sarcoma	2
Pituitary adenoma	5	Histiocytic sarcoma	3	Pituitary adenoma	10	Lymphoma, malignant	3	Histiocytic sarcoma	4	Eye schwannoma	1
Histiocytic sarcoma	1	Alveolar/bronchiolar adenoma	1	Histiocytic sarcoma	5	Liver carcinoma	1	Lymphoma, malignant	6		
Uterus polyp	1			Vascular neoplasm	2	Adrenal neoplasm NOS	1	Thyroid follicular cell adenoma	1		
Alveolar/bronchiolar adenoma	1			Liver adenoma	1	Thyroid follicular cell carcinoma	1	Mammary carcinoma	1		
				Thyroid follicular cell carcinoma	1	Peripheral nerve schwannoma	1	Harderian adenoma	1		
				Uterus adenoma	1	Alveolar/bronchiolar adenoma	1	Uterus polyp	1		
				Uterus polyp	1						
				Mammary carcinoma	1						
				Mammary mix tumor	1						
				Alveolar/bronchiolar adenoma	1						
				Alveolar/bronchiolar carcinoma	1						
30 mo (822-1,003 days)											
(14)		(15)		(24)		(35)		(16)		(15)	
Pituitary adenoma	9	Histiocytic sarcoma	3	Pituitary adenoma	18	Histiocytic sarcoma	19	Pituitary adenoma	13	Histiocytic sarcoma	8
Lymphoma, malignant	5	Adrenal pheochromocytoma	1	Histiocytic sarcoma	11	Lymphoma, malignant	6	Histiocytic sarcoma	7	Lymphoma, malignant	3
Histiocytic sarcoma	5	Uterus polyp	1	Lymphoma, malignant	7	Vascular neoplasm	1	Thyroid follicular cell adenoma	4	Pituitary adenoma	1
Thyroid follicular cell adenoma	4			Adrenal pheochromocytoma	2	Adrenal pheochromocytoma	1	Lymphoma, malignant	3		
Uterus polyp	2			Uterus polyp	2	Pituitary adenoma	1	Parathyroid adenoma	2		
Gall bladder papilloma	1			Mammary carcinoma	2	Neoplasm NOS	1	Ovary adenoma	1		
Liver adenoma	1			Liver adenoma	1	Mammary carcinoma	1	Uterus adenoma	1		
Parathyroid adenoma	1			Thyroid follicular cell adenoma	2	Alveolar/bronchiolar adenoma	1	Mammary mixed cell tumor	1		
Harderian adenoma	1			Thyroid follicular cell carcinoma	1	Nose osteosarcoma	1	Alveolar/bronchiolar carcinoma	1		
				Alveolar/bronchiolar carcinoma	2	Nose schwannoma	1				

TABLE IX.—Continued.

SS		SDM		LDM	
Ad libitum	Diet restricted	*Ad libitum*	Diet restricted	*Ad libitum*	Diet restricted
		36 mo (1,004-1,186 days)			
(0)	(15)	(0)	(41)	(2)	(19)
	Histiocytic sarcoma 3		Histiocytic sarcoma 27	Adrenal pheochromocytoma 1	Histiocytic sarcoma 10
	Lymphoma, malignant 4		Vascular neoplasm 2	Pituitary adenoma 1	Vascular neoplasm 3
	Duodenum polyp 2		Lymphoma, malignant 2	Leukemia, granulocytic 1	Lymphoma, malignant 2
	Alveolar/bronchiolar adenoma 1		Liver adenoma 2		Cranium osteoma 1
			Harderian adenoma 2		Harderian adenoma 1
			Duodenum polyp 1		
			Neoplasm NOS 1		
			Pituitary adenoma 1		
			Vagina granular cell tumor 1		
			Skin trichoepithelioma 1		
			Skin fibrosarcoma 1		
			Nose osteosarcoma 1		
		42 mo (1,187-1,368 days)			
(0)	(1)	(0)	(9)	(0)	(12)
	Adrenal pheochromocytoma 1		Vascular neoplasm 1		Histiocytic sarcoma 6
	Histiocytic sarcoma 1		Histiocytic sarcoma 1		Duodenum polyp 1
			Ileum polyp 1		Ovary adenoma 1
			Adrenal pheochromocytoma 1		Alveolar/bronchiolar carcinoma 1
			Ovary adenoma 1		
			Alveolar/bronchiolar adenoma 1		

TABLE X.—Neoplasms in male C57BL6 mice (number examined given in parentheses).

SS		SDM		LDM	
Ad libitum	Diet restricted	*Ad libitum*	Diet restricted	*Ad libitum*	Diet restricted
12 mo (1-456 days)					
(15)	(15)	(3)	(4)	(2)	(1)
None	Alveolar/bronchiolar adenoma 1	Histiocytic sarcoma 1	None	None	None
18 mo (457-638 days)					
(14)	(14)	(6)	(5)	(4)	(0)
Histiocytic sarcoma 1	Histiocytic sarcoma 1	Histiocytic sarcoma 1	Histiocytic sarcoma 2	Histiocytic sarcoma 1	
Lymphoma, malignant 1	Lymphoma, malignant 1	Liver carcinoma 1	Lymphoma, malignant 1	Lymphoma, malignant 1	
Alveolar/bronchiolar carcinoma 1				Thymoma 1	
24 mo (639-821 days)					
(14)	(15)	(52)	(17)	(16)	(5)
Alveolar/bronchiolar adenoma 2	Harderian adenoma 2	Histiocytic sarcoma 23	Histiocytic sarcoma 6	Histiocytic sarcoma 8	Histiocytic sarcoma 1
Liver adenoma 1	Lymphoma, malignant 1	Lymphoma, malignant 6	Lymphoma, malignant 4	Lymphoma, malignant 2	Lymphoma, malignant 1
Lymphoma, malignant 1	Alveolar/bronchiolar adenoma 1	Liver carcinoma 5	Liver carcinoma 1	Liver adenoma 2	
		Vascular neoplasm 3	Urethra papilloma 1	Alveolar/bronchiolar adenoma 2	
		Liver adenoma 2	Urinary bladder papilloma 1	Vascular neoplasm 1	
		Adrenal adenoma 1	Kidney papilloma 1	Harderian adenoma 1	
		Parathyroid adenoma 1		Kidney neoplasm NOS 1	
		Alveolar/bronchiolar adenoma 1			
		Harderian adenoma 1			
		Kidney adenoma 1			
30 mo (822-1,003 days)					
(14)	(15)	(50)	(48)	(23)	(26)
Histiocytic sarcoma 4	Histiocytic sarcoma 5	Histiocytic sarcoma 35	Histiocytic sarcoma 17	Histiocytic sarcoma 19	Histiocytic sarcoma 14
Lymphoma, malignant 2	Vascular neoplasm 1	Liver carcinoma 4	Alveolar/bronchiolar adenoma 5	Vascular neoplasm 2	Alveolar/bronchiolar adenoma 3
Alveolar/bronchiolar adenoma 2	Adrenal pheochromocytoma 1	Adrenal adenoma 4	Harderian adenoma 4	Harderian adenoma 2	Lymphoma, malignant 2
Vascular neoplasm 1	Alveolar/bronchiolar adenoma 1	Vascular neoplasm 3	Vascular neoplasm 2	Lymphoma, malignant 1	Adrenal pheochromocytoma 1
Liver carcinoma 1		Lymphoma, malignant 2	Lymphoma, malignant 1	Liver adenoma 1	Islet adenoma 1
		Alveolar/bronchiolar adenoma 3	Liver adenoma 1	Ito cell tumor, benign 1	
		Liver adenoma 2	Adrenal adenoma 1	Stomach squamous papilloma 1	
		Adrenal pheochromocytoma 2	Adrenal pheochromocytoma 1	Alveolar/bronchiolar adenoma 1	
		Harderian adenoma 2		Urinary bladder schwannoma 1	
		Stomach squamous papilloma 1			
		Thymoma 1			
		Sinus polyp 1			
		Kidney adenoma 1			
36 mo (1,004-1,186 days)					
(3)	(15)	(0)	(47)	(5)	(14)
Liver carcinoma 1	Histiocytic sarcoma 5		Histiocytic sarcoma 26	Histiocytic sarcoma 1	Histiocytic sarcoma 8

TABLE X.—Continued.

SS			SDM			LDM		
Ad libitum		Diet restricted	*Ad libitum*		Diet restricted	*Ad libitum*		Diet restricted
Pituitary adenoma	1	Harderian adenoma	2		Vascular neoplasm	2	Vascular neoplasm	1	Lymphoma, malignant	1
		Lymphoma, malignant	1		Harderian adenoma	2	Liver carcinoma	1		
		Duodenum polyp	1		Lymphoma, malignant	1	Liver adenoma	1		
		Pituitary adenoma	1		Liver carcinoma	1	Adrenal adenoma	1		
					Adrenal pheochromocytoma		Thyroid follicular cell adenoma	1		
					Alveolar/bronchiolar carcinoma	1	Harderian adenoma	1		
			42 mo (1,187–1,368 days)							
(0)		(1)	(0)		(9)	(0)		(9)		
		None			Histiocytic sarcoma	7			Histiocytic sarcoma	7
					Duodenum polyp	1			Adrenal pheochromocytoma	1
					Parathyroid adenoma	1			Harderian adenoma	1
									Kidney adenoma	1

(8–29) for females. The incidence of combined lymphoreticular neoplasms in our 0–24-mo-old LDM groups was 63% in 19 females and 55% in 22 males. Tucker (19) reported incidences of "composite lymphoma" between 15–34% in 5 different studies of 80–92-wk duration involving 65–156 C57BL/6J mice of each sex per study.

Histiocytic sarcoma was the most prevalent neoplasm in our study, occurring in 337 of the 991 animals. It accounted for more than half the spontaneous deaths, except in the AL females, where the rate was just over 30%. It was one of the 2 neoplasms that occurred more frequently in restricted animals, and was not delayed in onset. The other was intestinal polyp, which was seen only in DR animals after the 30-mo period (only 2 AL animals survived into the 36-mo period). The intestinal polyp in this genotype is apparently related to chronological age.

Lymphomas other than histiocytic sarcoma were the next most common neoplasms, with AL females having the highest incidence, approximately 3-fold greater than DR females. Diet had little effect on the relatively low incidence of lymphoma in males.

The most dramatic effect of DR was its reduction of pituitary neoplasms. In AL females of the LDM group, 21/37 (57%) had this tumor, with 28/80 (35%) in the SDM AL females and 15/57 (26%) in SS; there were none in the 75 SS restricted females, 2/125 (2%) in the restricted SDM group, and 1/56 (2%) in the LDM group. Dietary fat has been shown to increase prolactin production in rats (4). Because mammary gland hyperplasia often occurred in the animals with pituitary neoplasia in this study (Blackwell, unpublished data), prolactin may be a product of the pituitary neoplasms in this genotype as well.

Similar to pituitary tumors, thyroid gland follicular cell tumors were most prevalent in AL females with 4/57 in SS, 5/80 in SDM, and 5/37 in LDM, with only one in the AL males.

Hepatocellular tumors were common in males and were reduced significantly by DR, with 3/260 in DR males versus 23/221 in AL males, overall.

Specific genotypes have been identified with higher or lower incidences of specific diseases. Despite this, longevity and disease incidence in specific genotypes when compared over time, and particularly when on different diets, demonstrate broad variances, suggesting that diet and other environmental factors are more influential than genotype in determining the disease experience of a cohort of animals (12). The work reported here demonstrates that 40% dietary restriction significantly prolongs life and diminishes the incidence and multiplicity of neoplasia in C57BL6 mice. Our yet-unpublished data reveal this to be true of nonneoplastic conditions as well.

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