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Survival, Body Weight, and Spontaneous Neoplasms in *Ad Libitum*-Fed and Food-Restricted Fischer-344 Rats

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ABSTRACT

Ad libitum-fed (AL) and food-restricted (FR) Fischer-344 male and female rats were monitored for survival, body weight, and spontaneous neoplasms. Mean and maximal lifespans for each group were inversely related to mean body weights. AL males were the shortest lived (mean lifespan 101 wk) followed by AL females (118 wk), FR males (125 wk), and FR females (132 wk). Gross and microscopic examinations were performed on 851 rats from cross-sectional and longevity components of the study. In FR groups, the incidence of mammary gland fibroadenomas, testicular interstitial cell tumors, and pituitary neoplasms was decreased while the latency of these neoplasms was increased. In longevity components, most FR groups had a higher incidence of leukemia than AL cohorts, but all FR groups had a higher mean age at death for the rats with leukemia. Higher leukemia rate in the FR groups was thought to be a result of their extended mean lifespan.

Keywords. Caloric restriction; diet restriction; leukemia; pituitary neoplasia; neoplasia; longevity; sequential sacrifice

INTRODUCTION

Regulatory agencies generally expect a minimum of 50% survival in all treatment groups in 2-yr (chronic) bioassays (5). However, life expectancy of common strains/stocks of rats used in carcinogenicity testing has been declining over the past 2 decades (3–5, 10). This trend has progressed to the point that continued use of Sprague-Dawley (SD) and Fischer-344 (F-344) rats for chronic bioassays is threatened. This decrease in lifespan coincides with a trend to heavier body weights.

Food restriction is a manipulation of the standard *ad libitum* feeding regimen that may provide one means to continue use of these strains in chronic bioassays. Restriction of energy intake contributes to increased lifespan through decreased incidence of neoplasms, extended time-to-neoplasia, and reduced incidence and severity of nonneoplastic diseases (6, 7, 9). The use of food restriction in toxicity tests is, however, not without complication in that it can also decrease the induction of carcinogenicity by toxicants (1).

In this report, we present data on survival, body weight, and spontaneous neoplasms in *ad libitum*-fed (AL) or food-restricted (FR) F-344 rats at the National Center for Toxicological Research (NCTR). This study is part of an ongoing, integrated project to examine the effects of food restriction on toxicity and to understand its potential use in long-term toxicity testing. Data on survival and body weight were collected from 208 rats in a longevity (lifetime) component of the study. The incidence of spontaneous neoplasia was obtained from complete gross and microscopic examination of 851 rats in longevity or cross-sectional components of the study.

MATERIAL AND METHODS

Animal Husbandry and Dietary Procedures. Male and female F-344 rats were obtained from the NCTR Specific Pathogen Free (SPF) barrier-maintained breeding colony. The animals were maintained in the barrier facility throughout their lives as part of the project on food restriction at the NCTR as described previously (14). The animals were weaned at 3 wk and allocated to study at 4 wk of age. Once on study, rats were housed singly in polycarbonate cages.

The AL rats in this study were fed NIH-31 open formula diet (Purina Mills, Inc., Richmond, IN). The diet fed to the FR rats was the NIH-31 modified to contain fat-soluble and B complex vitamin supplementation at approximately 1.67 times that of the *ad libitum* formulation. Although there is inherent variability in vitamin concentrations of nat-

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TABLE I.—Allocation of F-344 rats to treatment groups.[a]

Treatment group	Allocated	Dead and moribund Necropsied	Cross-sectional Sacrifice
AL male	54		0
FR male	54		0
AL female	54		0
FR female	54		0
AL male	162	100	45
FR male	180	115	57
AL female	162	112	48
FR female	180	102	64

[a]Longevity 1, ; Longevity 2, ; Cross-sectional,

TABLE II.—Age in weeks at 90, 50, 10% and maximum survival in F-344 rats (Longevity 1).

Treatment group	n	Survival 90%	Survival 50%	Survival 10%	Maximum survival age
AL male	49	85	102	120	128
AL female	54	92	115	143	154
FR male	53	94	125	151	175
FR female	52	91	132	159	185

ural ingredient diets, the FR diet was fortified with vitamins in an attempt to provide a similar quantity of vitamins per rat, when fed at the 40% restriction level, as that received in the AL group. Upon receipt at our facility, individual pellets in the FR formulation were automatically sorted by discrete target weights (2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g). The sorted pellets were then combined in various arrays to satisfy the designated daily quantities of diet for the individual FR rats.

Through 13 wk of age, all rats were fed *ad libitum*. At 14 wk of age, a ramping procedure was initiated for the restricted animals to obtain the desired level of food restriction (40%) over a 2-wk period. During week 14, these animals were placed on 10% food restriction; that is, they received 90% of the diet consumed by the AL group. During week 15, the restriction was increased to 25% and finally, at 16 wk, the rats were restricted to the targeted 40% restriction level. To establish the restricted feeding regimen, an initial cohort of AL rats were loaded on study 2 wk before their pair-fed FR cohorts. Total food consumption of AL rats was measured weekly and these data were used to calculate the feeding regimen for the cohort and subsequent FR groups. Once the AL group contained 10 or less survivors, the quantity of food provided to the remaining FR cohorts remained constant.

Experimental Design. This study contained 900 F-344 rats in 8 treatment groups (Table I). These

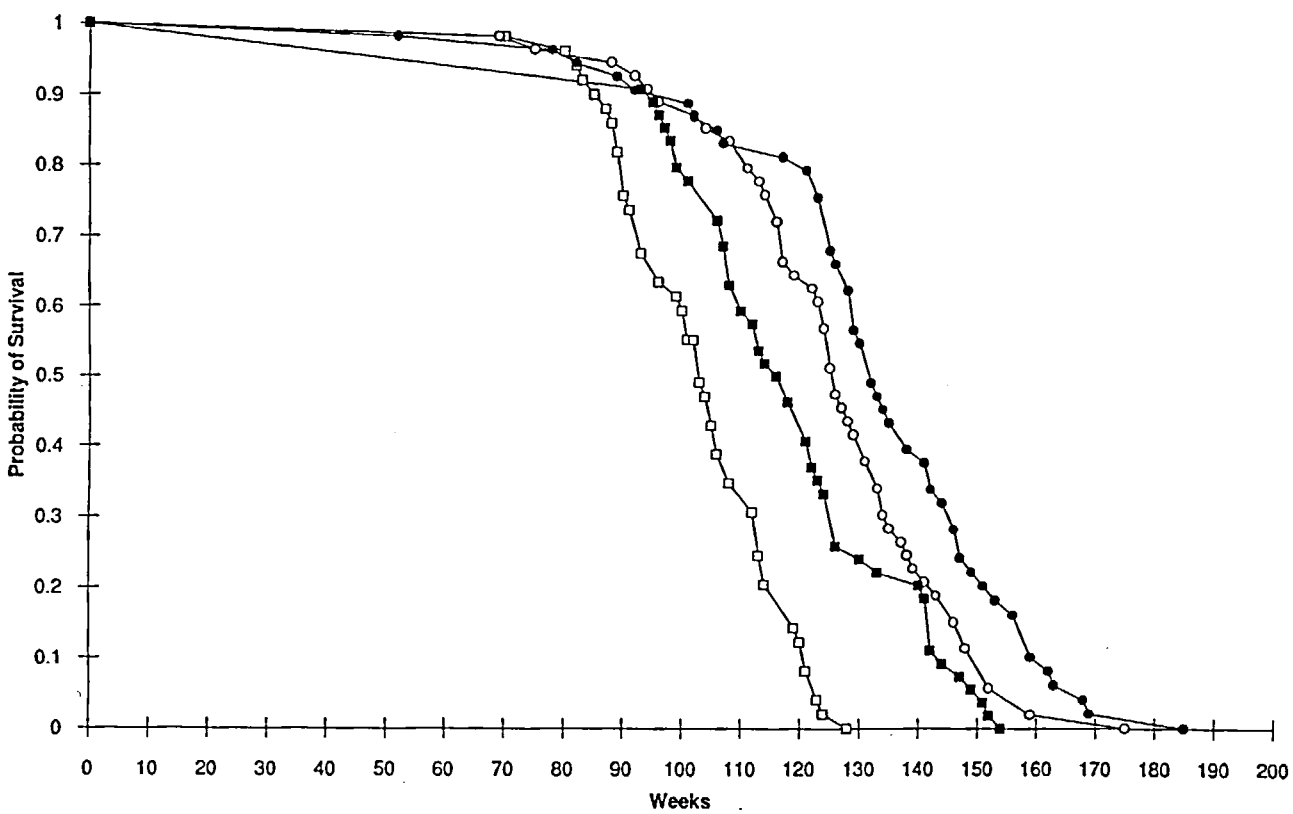

FIG. 1.—F344-rat survival curve. Treatment groups are as follows: □ = male AL; ○ = male FR; ■ = female AL; ● = female FR.

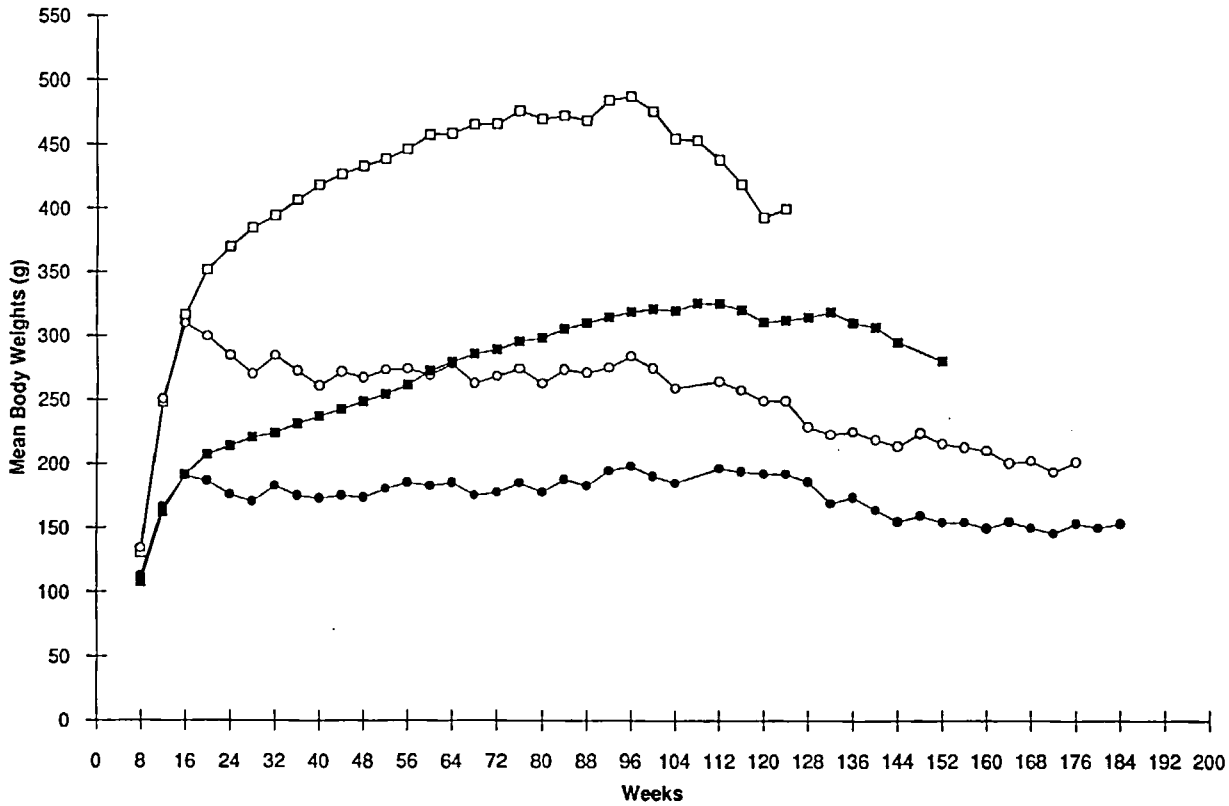

FIG. 2.—F-344 rat mean body weights. Treatment groups are as follows: □ = male AL; ○ = male FR; ■ = female AL; ● = female FR.

treatment groups were arranged into 3 components of the study: Longevity 1, Longevity 2, and Cross-sectional. Longevity 1 consisted of the first 4 treatment groups, which were allocated 54 rats each. The groups were AL males, FR males, AL females, and FR females. The rats were removed from the study for necropsy when they died or became moribund. Four additional treatment groups allocated as 162 AL males, 180 FR males, 162 AL females, and 180 FR females comprised the remaining 2 study components. The Cross-sectional component consisted of 12 rats from each of these latter 4 groups randomly selected for necropsy at 12 mo of age and at subsequent 6-mo intervals. Some of the late time-points had less than 12 survivors in the treatment group available for sacrifice. Rats, from these 4 groups containing the sacrifice animals, that died or became moribund due to spontaneous disease were designated as Longevity 2. Seven of the 8 treatment groups lost rats for various reasons other than scheduled sacrifice or spontaneous disease. This accounts for the difference between the number of rats allocated and the number necropsied. Forty-nine rats were removed from the study for the following reasons: *Pseudomonas* species present in cage waste of 34 rats, 9 were utilized for another study, and 6 for miscellaneous reasons. Survival and body weight data are taken from the 208 rats necropsied in Longevity 1. Data on spontaneous neoplasms was from 851 necropsied rats and is presented for each of the 3 study components.

Preparation of Tissues. At each sacrifice time, rats for the cross-sectional component were randomly selected, then weighed, anesthetized by CO_2 inhalation for blood sample collection, and euthanatized by CO_2. The rats were examined for gross lesions. The brain, kidneys, liver, spleen, and thymus were weighed and then abdominal skin, adrenal glands, ears, esophagus, eyes, exorbital lacrimal glands, Harderian glands, heart, gastrointestinal tract, lung, lymph nodes, mammary glands, pancreas, parathyroid glands, peripheral nerve, pituitary gland, salivary glands, skeletal muscle, spinal cord, sternum, thyroid gland, tongue, trachea, turbinates, urinary bladder, and Zymbal's glands were examined and fixed immediately in 10% neutral-buffered formalin. Additionally, in males the testes, epididymides, prostate, coagulating glands, seminal vesicles, penis, and preputial glands and in females the vagina, clitoral glands, uterus and ovaries were examined and formalin-fixed. Any other tissue or organ in which lesions were observed by gross examination was excised and fixed. Fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for histopathological examination. Rats presented for necropsy as dead

TABLE III.—Cross-sectional component: F-344 rat.

Time	Treatment group	Leukemia	Pituitary neoplasms[a]	Mammary fibro-adenoma[b]	Interstitial cell tumor	Islet cell neoplasms[a]	Other neoplasms[c]
12 Mo	AL male	0/12	3/12	0/9	0/12	0/12	—
	FR male	0/12	0/12	0/9	0/12	0/12	—
	AL female	1/12	1/11	0/11	—	0/12	TFC
	FR female	0/12	0/12	0/11	—	0/12	
18 Mo	AL male	0/12	6/10	0/9	5/12	0/12	ACA, HCA, K
	FR male	0/12	2/12	0/8	0/12	0/12	—
	AL female	0/12	3/10	1/12	—	0/12	—
	FR female	0/12	0/10	0/12	—	0/12	MH, PB
24 Mo	AL male	6/12	9/11	0/11	10/12	1/12	AMTB, BA, ENCN, PC
	FR male	7/12	3/12	0/8	2/12	1/12	KA, PB (2)
	AL female	1/12	11/12	4/12	—	0/12	CCA, CGA (4), HCA, MGA, MGAC (3), USP
	FR female	4/12	4/12	0/12	—	0/12	CCA, USP
30 Mo	AL male	5/9	9/9	2/9	9/9	5/8	CCA, CCC, HCA, PB (4), PM, M
	FR male	6/12	7/12	0/11	2/12	2/12	PB(2), PTA, TC, TP
	AL female	7/12	11/12	6/12	—	2/12	CCA, CGA (2), L, PB (3), UESS, USP
	FR female	3/12	4/12	1/11	—	0/12	DC, ELNCN, LU, UAC
36 Mo	AL male	—	—	—	—	—	—
	FR male	6/9	7/9	0/8	4/9	1/9	HCC, PB (2)
	AL female	—	—	—	—	—	—
	FR female	5/12	5/11	0/12	—	0/12	ACA, BGCT, CCA, LAC, PB, USP
42 Mo	AL male	—	—	—	—	—	—
	FR male	—	—	—	—	—	—
	AL female	—	—	—	—	—	—
	FR female	3/4	2/4	0/2	—	0/4	CCC, CGA, EBNCN, PB (2)

Abbreviations: ACA = adrenal cortical adenoma; AMTB = adrenal mixed tumor benign; BA = brain astrocytoma; BGCT = brain granular cell tumor; CCA = C-cell adenoma; CCC = C-cell carcinoma; CGA = clitoral gland adenoma; DC = duodenal carcinoma; EBNCN = eyeball neural crest neoplasm; ELNCN = eyelid neural crest neoplasm; ENCN = ear neural crest neoplasm; HCA = hepatocellular adenoma; HCC = hepatocellular carcinoma; K = keratoacanthoma; KA = kidney adenoma; L = lipoma; LAC = lung alveolar/bronchal carcinoma; LU = luteoma; M = mesothelioma; MGA = mammary gland adenoma; MGAC = mammary gland adenocarcinoma; MH = mesothelioma heart; PB = pheochromocytoma benign; PC = preputial gland carcinoma; PM = pheochromocytoma malignant; PTA = parathyroid adenoma; TC = turbinate chondroma; TFC = thyroid follicular cell carcinoma; TP = tongue squamous papilloma; UAC = uterus adenocarcinoma; UESS = uterus endometrial stromal sarcoma; USP = uterus stromal polyp.

[a] Pituitary and islet cell neoplasm columns contain both adenomas and carcinomas.

[b] Two rats have both MGAC and mammary gland fibroadenoma (1 as separate tumors, 1 as both in same mass).

[c] EBNCN, ELNCN, and ENCN have been reported as amelanotic melanomas.

or moribund (Longevity 1 and 2) were prepared in the same manner, but organ weights and blood were not taken.

RESULTS AND DISCUSSION

Survival

The survival curves from Longevity 1 are based on 208 rats necropsied (Fig. 1). Maximum survival age and the 90, 50, and 10% survival ages of this study component are shown in Table II. The FR females were the longest lived group followed by the FR males, AL females, and AL males. The mean lifespans were as follows: FR female 132 wk, FR male 125 wk, AL female 118 wk, and AL male 101 wk. Similarly, the maximal lifespan (the average age at death of the longest lived 10% of the group) was FR female 169 wk, FR male 159 wk, AL female 150 wk, and AL male 123 wk. The maximum lifespan for each group also follows the same pattern as mean and maximal lifespans. Food restriction tends to narrow the difference between survival curves of FR males and females compared to AL males and females.

Body Weights and Food Consumption

The mean body weight curves for Longevity 1 (Fig. 2) are from the same rats used for survival data. For practical purposes, mean body weights peaked at about 16 wk of age in FR females and males, whereas AL males and females peaked at 96 and 112 wk, respectively. Peak mean body weights were AL males 486 g, FR males 309 g, AL females 325 g, and FR females 198 g. FR males, after reaching their peak weight at implementation of food restriction, had a steady, gradual mean weight loss over time. The AL males and females had gradual weight gains to their peak and then experienced a decline in mean body weight in the terminal week of survival. This decline was much more abrupt in the AL males. Of the 4 treatment groups, FR females

TABLE IV.—Spontaneous neoplasms in F-344 male rats: longevity (dead and moribund) components.

	Longevity 1						Longevity 2					
	AL			FR			AL			FR		
Neoplasm	# Exam	# Neo	%	# Exam	# Neo	%	# Exam	# Neo	%	# Exam	# Neo	%
Hematopoietic												
Leukemia	49	20	41	53	30	57	100	59	59	115	75	65
Lymphoma	49	0	0	53	0	0	100	0	0	115	1	1
Endocrine												
Pituitary gland neo	46	29	63	47	23	49	82	63	77	105	32	30
Adrenal cortical ad	49	0	0	51	0	0	93	0	0	109	2	2
Pheochromocytoma bgn	49	6	12	51	2	4	93	5	5	109	5	5
Pheochromocytoma mal	49	3	6	51	0	0	93	1	1	109	2	2
Adrenal mixed mal	49	0	0	51	0	0	93	1	1	109	2	2
Islet ad	47	6	13	52	4	8	89	7	8	103	6	6
Islet ca	47	3	6	52	1	2	89	3	3	103	3	3
C-Cell ad	47	1	2	51	2	4	85	1	1	108	3	3
C-Cell ca	47	1	2	51	0	0	85	1	1	108	0	0
Parathyroid ad	44	2	5	51	1	2	93	2	2	107	0	0
Skin												
Fibroma	48	0	0	52	0	0	96	5	5	113	0	0
Sarcoma	48	1	2	52	0	0	96	2	2	113	0	0
Keratoacanthoma	48	1	2	52	0	0	96	3	3	113	0	0
Ear Neural Crest neo[a]	49	0	0	53	1	2	98	0	0	114	1	1
Mammary												
Fibroadenoma	35	1	3	32	0	0	71	1	1	73	0	0
CNS												
Astrocytoma	49	0	0	53	0	0	100	1	1	115	5	4
Oligodendroglioma	49	0	0	53	0	0	100	1	1	115	1	1
Ependymoma	49	0	0	53	0	0	100	1	1	115	0	0
Ependymoblastoma	49	0	0	53	1	2	100	0	0	115	0	0
Granular cell tumor	49	0	0	53	0	0	100	0	0	115	1	1
Respiratory												
Alveolar/bronchiolar ad	49	0	0	53	0	0	99	1	1	115	1	1
Alveolar/bronchiolar ca	49	0	0	53	0	0	99	0	0	115	1	1
Digestive												
Duodenum ca	41	0	0	49	1	2	71	0	0	85	0	0
Jejunum leiomyoma	38	0	0	44	0	0	68	0	0	76	1	1
Hepatocellular ad	49	0	0	53	1	2	100	1	1	114	1	1
Reproductive												
Interstitial cell ad	49	24	49	52	10	19	98	59	60	112	26	23
Preputial gland ad	48	0	0	48	0	0	96	2	2	110	0	0
Preputial gland ca	48	2	4	48	1	2	96	1	1	110	1	1
Urinary												
Papilloma u bladder	48	0	0	52	1	2	91	1	1	107	1	1
Kidney												
Liposarcoma	49	0	0	52	0	0	95	1	1	109	0	0
Transitional epithelial ca	49	1	2	52	0	0	95	0	0	109	0	0
Mesenchymal tumor	49	0	0	52	0	0	95	1	1	109	0	0
Miscellaneous												
Heart schwannoma	49	1	2	53	0	0	99	1	1	115	0	0
Zymbal's gland ad	45	0	0	43	0	0	93	1	1	91	0	0
Zymbal's gland ca	45	1	2	43	1	2	93	1	1	91	0	0
Osteosarcoma	49	1	2	53	0	0	100	0	0	115	1	1
Thymoma bgn	45	0	0	30	0	0	81	2	2	83	0	0
Mesothelioma	49	4	8	53	1	2	100	0	0	115	5	4
Hemangiosarcoma	49	0	0	53	0	0	100	1	1	115	0	0
Primary site not identified												
Adenocarcinoma	49	0	0	53	0	0	100	1	1	115	0	0
Sarcoma	49	0	0	53	0	0	100	1	1	115	1	1
Abd cavity liposarcoma	49	0	0	53	1	2	100	0	0	115	0	0

Abbreviations: abd = abdominal; ad = adenoma; bgn = benign; ca = carcinoma; mal = malignant; neo = neoplasm; u = urinary.
[a] Ear neural crest neoplasm has been reported as amelanotic melanoma.

TABLE V.—Spontaneous neoplasms in F-344 female rats: longevity (dead and moribund) components.

	Longevity 1						Longevity 2					
	AL			FR			AL			FR		
Neoplasm	# Exam	# Neo	%	# Exam	# Neo	%	# Exam	# Neo	%	# Exam	# Neo	%
Hematopoietic												
Leukemia	54	28	52	52	34	65	112	48	43	102	42	41
Lymphoma	54	1	2	52	0	0	112	0	0	102	3	3
Endocrine												
Pituitary gland neo	53	42	79	47	23	49	107	76	71	90	37	41
Pheochromocytoma bgn	53	3	6	51	2	4	109	8	7	101	4	4
Pheochromocytoma mal	53	1	2	51	0	0	109	0	0	101	0	0
Islet ad	54	0	0	52	1	2	106	1	1	98	5	5
Islet ca	54	3	6	52	0	0	106	0	0	98	0	0
C-cell ad	52	3	6	52	2	4	105	5	5	97	4	4
C-cell ca	52	2	4	52	1	2	105	1	1	97	1	1
Thyroid follicular ad	52	1	2	52	0	0	105	0	0	97	0	0
Parathyroid ad	49	0	0	48	0	0	103	0	0	94	0	0
Mammary												
Fibroadenoma	53	19	36	48	1	2	107	37	35	88	1	1
Adenoma	53	2	4	48	0	0	107	1	1	88	1	1
Adenocarcinoma	53	4	8	48	1	2	107	5	5	88	0	0
Skin												
Fibroma	53	1	2	52	1	2	111	3	3	101	3	3
Basal cell ca	53	0	0	52	0	0	111	2	2	101	0	0
Carcinoma	53	0	0	52	0	0	111	0	0	101	1	1
Hemangiosarcoma	53	0	0	52	0	0	111	0	0	101	1	1
Keratoacanthoma	53	0	0	52	0	0	111	2	2	101	0	0
Lipoma	53	0	0	52	0	0	111	1	1	101	0	0
Sarcoma	53	0	0	52	0	0	111	1	1	101	0	0
Papilloma	53	0	0	52	0	0	111	1	1	101	0	0
CNS												
Astrocytoma	54	0	0	52	0	0	112	0	0	102	1	1
Granular cell tumor	54	0	0	52	2	4	112	1	1	102	0	0
Respiratory												
Alveolar/bronchiolar ca	54	0	0	52	0	0	112	1	1	102	1	1
Digestive												
Rectum polyp	50	0	0	49	0	0	92	0	0	80	1	1
Parotid gland ca	54	0	0	52	0	0	112	1	1	102	0	0
Tongue sq cell ca	41	0	0	47	0	0	91	2	2	87	0	0
Reproductive												
Clitoral gland ad	45	4	9	51	3	6	96	12	13	96	1	1
Clitoral gland ca	45	2	4	51	1	2	96	3	3	96	2	2
Ov granulosa cell mal	53	0	0	49	0	0	112	1	1	100	0	0
Endometrial stromal sar	54	0	0	50	0	0	112	2	2	101	1	1
Endometrial stromal polyp	54	7	13	50	0	0	112	18	16	101	0	0
Vagina sq papilloma	53	0	0	51	0	0	111	0	0	97	1	1
Urinary												
U bladder papilloma	52	0	0	50	0	0	112	1	1	98	0	0
Miscellaneous												
Heart schwannoma	54	1	2	52	0	0	112	1	1	102	0	0
Zymbal's gland ad	48	0	0	37	0	0	97	1	1	81	0	0
Zymbal's gland ca	48	1	2	37	1	3	97	1	1	81	1	1
Osteosarcoma	54	1	2	52	0	0	112	0	0	102	0	0
Primary site not identified												
Carcinoma	54	1	2	52	0	0	112	0	0	102	0	0
Neoplasm mal	54	0	0	52	0	0	112	1	1	102	0	0
Sarcoma	54	0	0	52	0	0	112	0	0	102	1	1

Abbreviations: ad = adenoma; bgn = benign; ca = carcinoma; mal = malignant; neo = neoplasm; ov = ovary; sar = sarcoma; sq = squamous; u = urinary.

[a] One animal (AL female) in Longevity 1 is counted as C-cell adenoma and C-cell carcinoma.

TABLE VI.—Mean age at death (days), dead and moribund, F-344 rats.

Study component	Males						Females					
	All rats		Pituitary neoplasm		Leukemia		All rats		Pituitary neoplasm		Leukemia	
	AL	FR	AL	FR	AL	FR	AL	FR	AL	FR	AL	FR
Longevity 1	717	876	690	916	744	892	826	920	839	948	849	946
Longevity 2	713	819	720	825	733	829	763	848	791	895	788	910

maintained the most consistent mean body weights over time.

The adult (>6 mo of age) individual average daily food consumption for AL rats was approximately 17.9 g (77.9 kcal) for males and 14.3 g (62.2 kcal) for females. The FR rats were given approximately 10.7 g (46.5 kcal) and 8.6 g (37.4 kcal), respectively.

Neoplasia

Data from the Cross-sectional component are presented in Table III; Tables IV (male) and V (female) contain the incidence of spontaneous tumors in both longevity groups. The incidence of neoplasms is based on the number of lesions at the specific site relative to the number of animals in which the site was adequately examined histologically. Incidence of neoplasms, such as hemangiosarcomas, that are not considered organ-specific were calculated from the total number of rats examined in the treatment group. In the few instances (e.g., mammary gland, skin neoplasm) where macroscopic detection is essential for histologic diagnosis, the reader may prefer to use the number of animals necropsied in the treatment group for the denominator. The number of animals necropsied can be obtained from Table I.

Neoplasms that occurred with high frequency (>16%), in at least 1 group, were leukemia, mammary fibroadenoma, pituitary neoplasia, and testicular interstitial cell tumor. Tumors of intermediate frequency (5–16%) include adenoma and carcinoma of the mammary gland, C-cell neoplasia, clitoral gland neoplasia, endometrial stromal polyp, fibroma, pancreatic islet cell neoplasia, parathyroid adenoma, pheochromocytoma, and mesothelioma. The remaining neoplasms occurred at such low incidence (<5%) that any FR effect was difficult to assess.

The neoplasms are reported as prevalence at the time of sacrifice or as incidence across the lifespan in the longevity portions of the experiment. Whether a given neoplasm was the cause of death is not addressed in this report. It also should be noted that removal of serially sacrificed animals from a lifetime study (Longevity 2) creates a different distribution of time- to-death of dead or moribund animals than occurs in the cohort lifetime study without periodic sacrifice removals (Longevity 1). The studies with serial sacrifice removals would be expected to have a shorter mean time-to-death for dead or moribund animals than the cohort study without sacrifice removals (Table VI). Removal of serial sacrifice animals decreases the base population at each sacrifice point, causing the earlier deaths to contribute relatively more to the mean time-to-death.

Leukemia/Lymphoma. Leukemia was common in all treatment groups. Most cases were typical of the large granular cell leukemia but all leukemias were grouped together without separation into various types. This is compatible with the National Toxicology Program position that the most meaningful analysis comes from combining all leukemias, rather than analyzing for any one particular type (2). The presence of neoplastic leukocytes in liver sinusoids was the most important criterion for diag-

TABLE VII.—F-344 rats with pituitary neoplasia and leukemia at death, by age and treatment group: Longevity 1.

	Age (days)									
	0–699		0–799		0–899		0–999		0–1,000+	
	AL	FR	AL	FR	AL	FR	AL	FR	AL	FR
Males										
Rats examined microscopically	20	6	39	13	49	31	49	43	49	53
Rats with pituitary neoplasia	17	1	25	3	29	10	29	18	29	23
Rats with leukemia	5	2	15	6	20	19	20	24	20	30
Females										
Rats examined microscopically	11	5	26	9	40	22	48	35	54	52
Rats with pituitary neoplasia	5	1	19	3	30	8	37	14	42	23
Rats with leukemia	3	2	10	3	19	14	26	23	28	34

nosis; evidence of disease in the spleen and lung were important supporting criteria. Lymphoma was diagnosed when focal accumulations of neoplastic lymphocytes were present without evidence of a leukemic component. There were only 5 clear-cut cases conforming to these criteria, although several cases diagnosed as leukemia had substantial focal accumulations of neoplastic lymphocytes with a modest leukemic component.

In the cross-sectional component, cases of leukemia, with 1 exception, were first observed at the 24-mo timepoint. Approximately one-half of the male AL and FR rats had leukemia at the 24- and 30-mo timepoints. The incidence of leukemia in females at these timepoints was more variable. Within longevity components, the incidence of leukemia in FR groups was higher than or about equal to that in their AL cohorts; however, the mean age at death of rats having leukemia was increased in all FR groups (Table VI).

Since Fischer rat leukemia is a rapidly fatal, age-associated condition (8, 12, 13), the most likely explanation for the increased incidence of leukemia in FR rats is that they live to an older age and, therefore, more are available to develop the disease. This hypothesis is supported by the observation that sacrificed AL and FR groups both have about the same prevalence of leukemia beginning at 24 mo. Also, Table VII shows that at 899 days for males and 999 days for females, dead or moribund AL and FR rats have about the same number of leukemia cases even though there is a marked difference in the number of spontaneous deaths up to these timepoints. A delay in onset of leukemia in the FR rats, similar to that reported in a recent study (11), may be present before 799 days. A detailed analysis of the presence or absence of a delayed onset in leukemia will be presented elsewhere.

Pituitary Neoplasms. Pituitary gland tumors were the most common neoplasms in the AL groups. In the cross-sectional component, there was a 6-mo delay in onset of pituitary neoplasms in FR rats, relative to the AL cohort (Table III). Additionally, there were fewer pituitary neoplasms in FR rats in the Cross-sectional and both longevity components (Tables III–V). The mean age at spontaneous death of male and female rats with pituitary neoplasia is also substantially extended in the FR groups of both longevity components (Table VI). If invasion of the brain by pituitary neoplasms constitutes evidence of malignancy, then 9 cases in the longevity studies would be classified as carcinomas. Only 1 of these was in a FR animal and 8 were in AL animals. The delay in onset, reduction in incidence, and decrease in invasiveness of pituitary neoplasms are probably the most important factors accounting for the increased lifespan of the FR rats. These effects of food restriction eliminate or delay a significant cause of death for many F-344 rats.

Mammary Fibroadenoma. The incidence of fibroadenoma of the mammary gland was markedly decreased in the FR groups of both the Cross-sectional and the longevity components of this study. In females of all 3 study components, there were 67 mammary fibroadenomas in AL rats and only 3 in FR rats. In the Cross-sectional component, fibroadenoma was first observed at 18 mo in the AL group, and the only case in the FR group was present at 30 mo. Interestingly, there were 4 cases in male rats and all occurred in AL groups. While this neoplasm is usually benign, its large size is often a "reason for removal" of rats from chronic bioassays.

Testicular Interstitial Cell Tumors. The Cross-sectional component showed a delay in onset and a reduction in the number of interstitial cell tumors present in the FR group. The AL groups of the longevity components had more than twice the number of rats with interstitial cell tumor as did their FR cohort groups.

Other Neoplasms. While there are some exceptions, most neoplasms occurring at $<17\%$ incidence tended to be more prevalent in the AL than in the FR groups. The uterine stromal polyp is the most obvious example of the FR effect in this frequency range. In the longevity components, there were 25 cases of this neoplasm in AL rats and none in FR cohorts.

CONCLUSION

In this study, survival of F-344 rats was inversely related to body weight. The shortest mean and maximal lifespans for all treatment groups occurred in the AL males, which also had the greatest mean body weight. The longest mean and maximal lifespans were in the FR females, which had the lightest mean body weight. At 111 wk of age, which would approximate the age of rats at the end of a chronic bioassay, survival rates for FR males and FR females in Longevity 1 were 79 and 83%, respectively. Survival to the same age in AL males was only 35% and was 59% in AL females. These survival rates between sexes were closer together for FR rats and were well above the minimal survival ages recommended by regulatory agencies for chronic bioassay studies.

In the cross-sectional and longevity components, food restriction resulted in decreased incidence and delayed onset of pituitary neoplasia, mammary fibroadenoma, and testicular interstitial cell tumor. FR also may have reduced the frequency of leukemia at early timepoints. However, in both longevity components, the overall incidence of leukemia,

which is an age-associated disease, was increased in FR groups compared to AL cohorts. The increase in leukemia in FR groups is attributed to the greater number of rats alive past 2 yr of age in FR groups compared to AL cohorts.

The age-specific incidence of spontaneous tumors is altered significantly by FR; the precise effects may differ by type of neoplasm and among different genotypes of animal. These diverse effects will need to be established by comprehensive studies, such as the one reported here, before food restriction can be incorporated into toxicity/carcinogenicity bioassays.

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