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The Effects of Overfeeding and Dietary Restriction on Sprague-Dawley Rat Survival and Early Pathology Biomarkers of Aging*

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ABSTRACT

A significant correlation exists between average daily food consumption and 2-yr survival in control *ad libitum* (AL)–fed Sprague-Dawley (SD) rats. SD rats were fed Purina Rodent Chow 5002 or a modified chow, 5002-9, with lower protein, fat, metabolizable energy and increased fiber AL or by dietary restriction (DR) to 65% of the AL amount by measurement or time (6.5 hr). At 52 wk, food consumption and key pathology biomarkers correlated with 106-wk survival. The modified chow, 5002-9 fed AL, did not significantly improve survival. SD rats fed either diet AL consumed the greatest amount of feed and kcal/rat but consumed the same amount of feed per gram body weight as DR-fed rats. At 52 wk, AL rats fed either diet had the same brain weights as DR rats, but the AL-fed rats had greater body weight and body fat content and increased heart, lung, kidney, liver, adrenal, thyroid, and pituitary weights as well as an increased incidence and severity of degenerative and/or proliferative lesions in these organs. This study demonstrates that overfeeding best correlates with low 2-yr survival in SD rats and that simple DR by caloric restriction modifies key pathology biomarkers in the pituitary, mammary gland, kidney, and heart of SD rats at 52 wk that are predictive of 106-wk survival.

Keywords. Caloric restriction; cell proliferation; bromodeoxyuridine labeling; pituitary hyperplasia; neoplasia; nephropathy; cardiomyopathy

INTRODUCTION

The Sprague-Dawley (SD) rat is the principal stock used by the pharmaceutical industry for toxicity and carcinogenicity studies in the United States. Two-year laboratory rat survival has been declining over the past 30 yr throughout the pharmaceutical and chemical industry (3, 15, 18, 20, 21, 23, 26–28, 45, 49, 56). This decline has been seen in all rat strains, including the relatively long-lived Fischer-344 (F-344) rat (15, 27, 54). The National Toxicology Program (NTP) has reported F-344 rat 2-yr survival as low as 30% in males and 50% in females (Dr. G. Boorman, personal communication). Thus, the survival of all commercially available rat strains and stocks is decreasing, and the specific reasons for this change are complex, involving an interaction of the rat's genome with its environment. The declining survival has caused some regulatory agencies to question the adequacy of rat carcinogenicity studies submitted with less than 50% survival or 25 animals alive per group at the end of a 24-mo study.

Between the publication of the National Cancer Institute's 1976 recommendations and the NTP's 1984 recommendations, an international consensus developed that the duration of a rat carcinogenicity study should be 24 mo (15, 18, 20, 50). The group size should be at least 50 rats/sex/group, and a 50% survival should be achieved at the terminal necropsy. This guideline is stated in the Food and Drug Administration's "Redbook" and is widely accepted internationally.

The scientific motivation for requiring 50% survival at the end of a rat carcinogenicity study is 2-fold. First, a substantial number of the rats should be exposed to the test compound for the full 2 yr of the bioassay. Second, the ability to detect a treatment effect is the working definition of adequate

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statistical sensitivity or power of the bioassay. For example, a decrease in survival from 50 to 20% does cause a decrease in statistical sensitivity, especially for late-onset tumors. An increase in the total sample size from 50 to 75 rats/sex/group offsets some of this decrease in sensitivity for most tumor types if survival does not decline further, but at a 50% increase in animal use, manpower, and time to complete the study. An attempt to solve the problem of declining survival and potential loss of statistical sensitivity by simply increasing the number of animals in the group has other disadvantages beyond the increased time and expense of the study. Even 75 rats/sex/group is insufficient to offset the loss of statistical power if the 24-mo survival declines near or below 10%. Moreover, potential problems arising from treatment-related mortality is exacerbated in a study with low control group survival. The preferable solution to the potential loss of statistical power is to increase the 2-yr survival to near or above 50%, because the total time the animals are exposed to the test compound and the sensitivity of the bioassay to distinguish true treatment effects from concurrent controls are both increased.

While both genetic and environmental factors are involved, laboratory rat survival can be improved by simple dietary caloric restriction (9, 41, 56, 70). Dietary restriction (DR), or food restriction, is a well-established method of extending the life-span of rodents. It has been known for several decades that the common practice of *ad libitum* (AL) feeding of rodents nutritionally rich, high-energy diets has many negative effects on physiological and toxicological endpoints and results in poor survival when compared to the beneficial effects of simple caloric restriction (8, 9, 34, 35, 39, 41, 70, 72). The beneficial effects of DR have been well documented in studies of aging and senescence in invertebrates, rodents, and nonrodent vertebrates, such as fish, birds, and other mammals, including humans (8, 9, 70). This paper reviews data showing the association between overfeeding and poor survival in SD rats and presents the design of our long-term studies of dietary caloric restriction with the SD rat, the survival results obtained after 106 wk of study, and the key pathologic biomarkers observed at a 52-wk interim necropsy that proved predictive of the final survival outcome of the 106-wk study. A preliminary report of some of these data, the results of the carcinogenicity study, and the effect of these diets and treatments on chronic disease are presented separately (11, 20–23).

Materials and Methods

Animals. Two-yr survival and average food consumption data were obtained on control groups of SD (Crl:CD®BR) rats from 58 carcinogenicity studies containing approximately 50/sex/group that had been initiated between 1978 and 1989 and conducted in different contract and toxicology laboratories, including our own. The studies used VAF® SD rats obtained from Charles River Laboratories, Inc., production sites at Portage, MI, Kingston, NY, Lakeview, NJ, Montreal, Canada, and Raleigh, NC. While conditions varied between laboratories, the rats in these studies were singly housed in wire-mesh cages, maintained on a 12-hr light/dark cycle, and provided water and Certified Purina Rodent Chow 5002 as meal or pellets AL. Additional data were provided by Dr. Patricia L. Lang, consulting toxicologist, Charles River Laboratories; Dr. Gary Wolfe, Hazleton Laboratories, Vienna, VA; and Dr. Charles E. Cover, E. I. DuPont DeNemours and Co., Newark, DE.

For the 106-wk carcinogenicity study, 350 male and 350 female SD rats (Crl:CD®(SD)BR) were obtained from Charles River Laboratories, Raleigh, NC. The rats were 36 days of age at the initiation of the study, with the males weighing 115–175 g and the females weighing 91–156 g. The rats were individually housed in stainless-steel wire cages in environmentally controlled clean air rooms with a 12-hr light/dark cycle.

Assignment to Treatment Groups and 52-Wk Interim Necropsy. The rats were identified by tattoos and assigned to the 5 different diet groups described below using a balanced random allocation scheme. Rats to be selected for the 52-wk interim necropsy described in this report were assigned by a stratified randomization allocation procedure as follows. For each sex and dietary group, the rats were ordered by body weight (BW) from the lowest to the highest weight. They were then divided into 10 strata by BW, and 1 rat from each stratum was randomly chosen for the interim necropsy with a second backup animal selected in the event the primary animal in the stratum did not survive to the necropsy date. This procedure was to optimize the probability that a truly representative sample of animals from each dietary regimen would be examined at the 52-week interim necropsy.

Diet and Dietary Regimens. The experimental groups contained 70 rats/sex/group and were designed to compare two different diets as well as moderate DR. The diets and DRs were as follows:

a. Purina Certified Rodent Chow 5002 fed AL (5002 AL) as pellets (this diet contains approximately 21.4% protein, 5.7% fat, and 4.1% crude fiber and has a calculated metabolizable energy value of 3.07 kcal/g).
b. Certified Rodent Chow 5002 fed AL for approx-

imately 6.5 hr/day during the light cycle (5002 DR 6.5 hr).

c. Purina Certified Rodent Chow given in measured amounts daily (5002 DR) at approximately 65% of adult SD rat AL food consumption (approximately 16 g/day for females and 21.5 g/day for males).
d. Purina Certified Rodent Chow 5002-9 fed AL (5002-9 AL) as extruded pellets (this diet contains approximately 13.6% protein, 4.6% fat, and 15.7% crude fiber and has a calculated metabolizable energy value of 2.36 kcal/g).
e. Purina Certified Rodent Chow 5002-9 fed in measured amounts (5002-9 DR) to provide approximately the same caloric intake as animals fed under Regimen c (approximately 20.8 g/day for females and 28.8 g/day for males).

Food consumption was measured over 3 nights for the 5002 AL and the 5002 DR 6.5 hr groups, over 2 nights for the 5002-9 AL group, and over 1 night for the 5002 DR and the 5002-9 DR groups. An estimation of food wastage was made on weeks 32, 35, 74, and 94 by weight for all groups.

Clinical Evaluations. All animals were observed daily for clinical signs and mortality and were weighed pretest, once during week 1, twice through weeks 13, and once weekly thereafter. Ophthalmoscopic examinations were conducted on all animals pretest and at 51 and 103 wk. Hematology, clinical biochemistry, and urinalyses were conducted in weeks 52, 78 and 103 and will be reported separately.

Osmotic Minipump Implantation. One wk prior to the 52-wk interim necropsy, rats selected by the stratified random allocation scheme were implanted with osmotic minipumps (Model #2ML1,2ML, Alza Corp., Palo Alto, CA) for the continuous 7-day delivery of 5-bromo-2′-deoxyuridine (BrdU; Sigma Chemical Co., St. Louis, MO). Prior to implantation, the minipumps were loaded with BrdU at a concentration of 50 mg/ml in a 0.5 N sodium bicarbonate solution. The loaded minipumps were surgically implanted subcutaneously in rats under ether inhalation anesthesia. The minipumps were implanted via a small dorsal midline skin incision with the opening of the minipump facing caudally. The incisions were closed with surgical staples, and the rats were returned to their cages until scheduled necropsy (66).

Necropsy and Histopathology. Rats selected for the 52-wk interim necropsy were weighed, deeply anesthetized by ether inhalation, and sacrificed by exsanguination. The minipumps were removed, terminal BWs were taken, and the following organs were weighed when present: adrenals, ovaries, brain, pituitary, heart, prostate, kidneys, liver, testes, lung, thymus, uterus, and thyroids with parathyroids. The organ weights were expressed as absolute values (grams) and as relative values (percentage of BW and percentage of brain weight). Each animal underwent complete gross necropsy with numerous tissues sampled, including all gross lesions. The tissue samples were routinely fixed in 10% neutral-buffered formalin (testes fixed in Bouin's solution), and routine histologic sections of paraplast-embedded tissues were stained with hematoxylin and eosin from all rats, including salivary gland, stomach, small intestine, large intestine, liver, pancreas, adrenals, pituitary, thyroid and parathyroid, kidneys, urinary bladder, ovaries, uterus, testes and epididymides, prostate, skin, mammary gland, heart, lung, spleen, lymph nodes, thymus, bone and bone marrow, skeletal muscle, brain, spinal cord, sciatic nerve, eyes with optic nerve, and any gross lesions. In addition, selected target tissues were sectioned and stained for BrdU immunohistochemistry and included kidneys, liver, pituitary, thyroid and parathyroid, adrenals, pancreas, and small intestine (66).

Cell Proliferation Studies. The pituitary cell cumulative DNA synthesis measured by BrdU nuclear labeling over 1 wk was expressed as the percent labeling index (% LI). In each pituitary section, 2,000 random pituitary cells in the anterior lobe were scored for BrdU labeling. In addition, any pituitary hyperplasias or adenomas were separately scored for percentage of BrdU labeling.

The hepatocyte cumulative DNA synthesis measured by BrdU nuclear labeling was determined by scoring 2,000 random hepatocyte nuclei per liver for BrdU labeling. The total number of BrdU-labeled hepatocyte nuclei per liver was determined by multiplying the individual % LI by the total hepatocyte nuclei per liver as determined stereologically (7, 55, 58, 66). Liver volume was determined by direct measurement with corrections for process shrinkage by planimetry. Hepatocyte nuclei per cubic centimeter and total hepatocyte nuclei per liver were determined by stereologic evaluation of the paraplast sections with standard methods (7, 58). Hepatocyte nuclei from all hepatic zones were included in the sampling to allow for zonal differences in hepatocyte size.

Tissue Biochemistry and Carcass Analysis. Whole liver glutathione content was estimated by the nonprotein sulfhydryl method, and liver malondialdehyde content was measured using the thiobarbituric (TBA) acid reaction on 5 rats/sex/group. Both methods were adapted from Rush et al (59).

After complete necropsies were performed on all animals, the remaining organs and carcasses were frozen for carcass analysis. These tissues were com-

pletely ground through a #3 (5/32 in.) screen and remixed, and 3-g samples were taken from each animal for the determination of protein by the method of Kjedahl, body fat by ether extract, and moisture and ash content.

Statistics. Mortality curves over the 106-wk study were summarized with life-tables as described by Kaplan and Meier (19) and were compared using the log-rank test (52). Tumor incidences were compared using the method of Peto et al (53), including adjustment for age and context of tumor observation (observed prior to death, observed at necropsy and deemed a cause of death, observed at necropsy but not deemed a cause of death).

Organ weights and terminal BWs from the 52-wk interim necropsy rats were analyzed by linear models, specifically the analysis of variance with the Student-Newman-Keuls (SNK) procedure (48) to identify statistically significant differences among treatment groups. Data were analyzed using a logarithmic or rankit scale (14) when appropriate to satisfy assumptions required for linear models, including normality and variance homogeneity. Cell proliferation and stereology data for the liver and pituitary were analyzed in a logarithmic scale for organ weight, density of nuclei, total labeled nuclei per organ, and labeling index.

Summary statistics on survival and average food consumption in 2-yr studies from multiple laboratories were analyzed using linear models.

Results

Correlation of Daily Food Consumption with 2-Yr Survival

Data were correlated and analyzed by linear regression on 58 SD rat control groups from 2-yr carcinogenicity studies begun between 1978 and 1989 and fed AL with Purina Certified Rodent Chow 5002 (Figs. 1 and 2). There was a statistically significant relationship between the percentage of survival and the average daily food consumption in Charles River SD rats. However, there was considerable variability in survival and food consumption among laboratories and within the same laboratory. Survival ranged from 7.0 to 73.0% for males and 29.0 to 68.0% for females. Average daily food consumption (grams/day) ranged from 21.7 to 32.3 g/day for males and from 16.1 to 24.9 g/day for females. SD rats of both sexes from studies conducted in our laboratory had a statistically significant higher average food consumption compared to SD rats in other laboratories ($p \leq 0.05$). This variability in daily food consumption was likely due to interlaboratory differences in feeders, caging, and husbandry methods.

In males, the average decrease was 4.9 percentage points in survival for each gram increase in average daily food consumption (overall $R^2 = 0.42$). After adjusting for differences in survival among laboratories, the partial correlation between food consumption and survival was $r = -0.48$ ($p < 0.001$).

In females, the average decrease was 3.7 percentage points in survival for each gram increase in average daily food consumption (overall $R^2 = 0.41$). The adjusted partial correlation between food consumption and survival was $r = -0.47$ ($p < 0.001$).

The correlation of food consumption and survival data was highly significant. Each regression model included separate intercepts for each laboratory and a common slope for food consumption. Partial correlation coefficients were adjusted for interlaboratory differences. These data show that daily food consumption and survival are tightly correlated although variable among laboratories. The greater the daily food consumption, the greater the likelihood of poor survival by 104 wk. Therefore, it is the amount of feed provided per day that appears critical in the long-term survival of SD rats fed the same feed.

Effects of Diet and DR on 104-Wk Survival

The results of the 106-wk carcinogenicity study used the Kaplan-Meier survival curves to estimate survival through study week 104. Mortality from all causes was considered, except for the approximately 10 rats/sex/group selected for interim necropsy in study week 52. One-sided log rank statistics were used to test for increased survival compared with the 5002 AL groups. In addition, the earliest week that statistical significance was reached and retained through terminal sacrifice was determined and is indicated in Table I.

There was no difference in mortality among any of the dietary groups during the first 52 wk of the study. However, over the course of the second year very obvious differences in survival became apparent. In general, the best survival was seen in dietary-restricted groups. The 5002-9 diet fed AL with reduced protein, fat, and energy content and increased fiber content did not improve 104-wk survival to the 50% level for either sex. Restricting feeding time of the 5002 diet to 6.5 hr per day improved survival for males ($p < 0.001$), but not for females ($p > 0.10$).

DR of either diet, by providing measured amounts of food daily, was associated with a great improvement in survival in both males and females at 104 wk. The difference in survival between the 5002 AL group and the 5002 DR and 5002-9 DR groups reached statistical significance ($p < 0.05$) in week 51 for males and in weeks 65 and 69 for females.

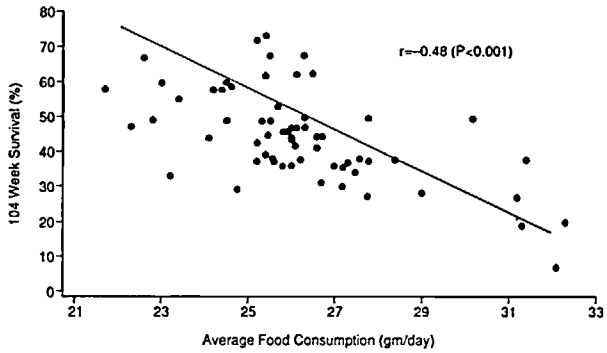

FIG. 1.—The correlation of male SD rat 104-wk survival and average daily food consumption.

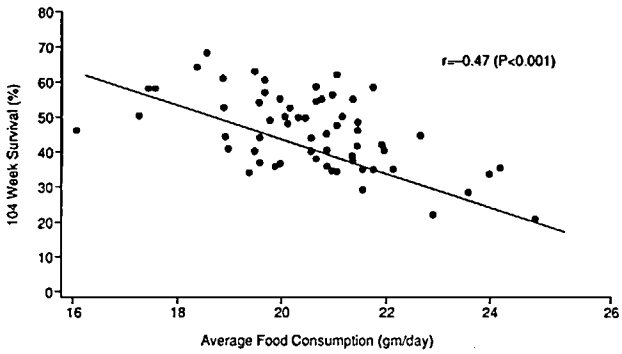

FIG. 2.—The correlation of female SD rat 104-wk survival and average daily food consumption.

The median survival (first week of 50% mortality) for the 5002 AL rats was 85 wk for males and 90 wk for females compared to a median survival of over 104 wk for the 5002 DR rats and the 5002-9 DR rats of both sexes. This comparison clearly shows that moderate DR of the same diet by 30–35% of AL amounts results in dramatic differences in long-term survival.

Average survival for each treatment group was calculated after excluding the 52-wk interim necropsy animals. Average survival (time to natural death or terminal necropsy) is equal to the average number of weeks of exposure in a 2-yr rat carcinogenicity study. The average survival expressed as total weeks of survival per rat and their significance relative to the mortality in the 5002 AL group are shown in Table I.

These data show that DR can be expected to substantially increase the average weeks of survival and, thus, the average duration of exposure in rat carcinogenicity studies compared with AL feeding. For example, the average time on study for 5002 AL males was 80.1 wk, whereas the 5002 DR males were on study for an average of 99.6 wk. This difference was highly significant ($p < 0.001$) and would mean that the 5002 DR males would have 19.5 wk (5 mo) of additional exposure to a test compound. This increased survival and, thus, increased exposure would increase the statistical sensitivity of the bioassay to detect a treatment-related event and distinguish it from controls.

The tumor incidence data from the 106-wk carcinogenicity study will be presented separately (22, 23). However, the most common cause of death in rats fed either diet AL or DR was pituitary tumors. This was followed by mammary gland tumors in females and renal and cardiovascular disease in the AL-fed males. The largest number of undetermined deaths was seen in the 5002 AL males. The only unusual cause of death in SD rats was seen in the 5002-9-fed animals. Six male and 3 female 5002-9 AL rats and 1 5002-9 DR female died or were sacrificed due to the effects of the high fiber content of the 5002-9 diet that resulted in colonic impaction, colonic dilatation, and chronic colitis. Otherwise, the AL rats of both sexes, fed either diet had an earlier onset and more severe lesions and tumors than their DR-fed counterparts (22, 23).

Effects of Diet and DR on 52-Wk BW, Body Fat, Food Consumption, and Organ Weights

Anticipated changes in the rate of BW gain were associated with the restriction of caloric intake and included significant decrements in all groups relative to the 5002 AL group. These changes were apparent after the first week, and the rate of change in weight gain was most dramatic between weeks 1 and 10. After 10 wk, BW gain began to level off.

The typical relationship observed between BW and food consumption for the different dietary regimens is shown in Table II during week 50 prior to the implantation of minipumps and the 52-wk interim necropsy. In general, the 5002 AL and 5002-9 AL groups of both sexes were the largest animals and had the greatest variability in BW as seen by the range.

The measured mean daily food consumption corrected for wastage in grams/day shows that the 5002-9 AL animals eat 25–30% more feed than the 5002 AL groups. However, the measured food consumption is calculated on a gram of feed consumed per gram of BW basis, the amount of food consumed per gram of BW is remarkably similar for the AL and DR regimens for a given diet. When the daily kcal consumed per animal is calculated from the corrected food consumption, it is seen that the 5002 AL and the 5002-9 AL rats consume very similar amounts of kcal per day. Likewise, the DR rats fed either diet consume relatively similar kcal per day. When these figures are converted to kcal per gram

TABLE I.—SD rat survival from all causes.[a]

Group	Males			Females		
	% Survival[b]	Week[c] ($p < 0.05$)	Average weeks of survival	% Survival	Week[c] ($p < 0.05$)	Average weeks of survival
5002 AL	7	—	80.1	36	—	86.4
5002 DR 6.5 hr	57***	57	95.7***	42 ns	—	88.3 ns
5002 DR	74***	51	99.6***	62***	65	98.3***
5002-9 AL	32***	91	86.6***	24 ns	—	89.5 ns
5002-9 DR	75***	51	99.6***	81***	69	98.3***

[a] Except interim necropsy at week 52.
[b] Percentage of survival to end of week 104.
[c] First week $p \leq 0.05$ and remained < 0.05.
*** $p \leq 0.001$. ns = $p > 0.10$ compared with the 5002 group.

of BW, it is evident that all of the groups fed either AL or under a DR consume a similar amount of kcal per gram of BW. Therefore, rats fed either diet AL consume the greatest number of grams and kcal per rat, but approximately the same number of grams and kcal per gram of BW as rats fed DR at 65–70% of AL food consumption.

Carcass analysis from 52-wk interim necropsy animals reflected anticipated responses as shown by the percentage of body fat in Table II. In general, all of the restricted groups were leaner with a significantly lower body fat content and a higher carcass protein than their AL counterparts. The AL females had a higher body fat content, while the DR females had lower body fat content than their male counterparts. The carcass fat of the 5002-9 DR animals was less than either the 5002 DR 6.5 hr and 5002 DR rats, despite a similar calculated metabolizable energy intake.

The 52-wk interim necropsy mean terminal BWs and mean organ weights are shown in Tables III–VI. Differences in terminal BWs of the 52-wk interim necropsy animals reflected the changes in BW gains observed in the entire groups to this time point. In both sexes, the 5002 AL-fed animals were the heaviest, followed by the 5002-9 AL groups. All of the DR groups were smaller. These differences appear to reflect body fat composition. In contrast, the differences in absolute brain weight was minimal (0–3% of 5002 AL) between the different diets and the DR regimens for both sexes. This observation, coupled with the carcass analysis, indicates that DR did not interfere with brain development and that in comparing various organ weights between groups the percentage of brain weight is the most appropriate relative comparison.

Absolute and relative (percentage of brain weight) weights of spleen, heart, kidneys, liver, adrenals, lungs, thyroids, pituitary, or uterus were generally smaller in the DR groups compared to the 5002 AL group. Absolute and relative weights of testes, prostates, or ovaries were not different in the DR groups compared to the AL groups. In most cases, the lower organ weights seen in the different DR groups correlated with a decrease in lesion incidence or severity, as discussed later.

TABLE II.—Fifty-wk BW, body fat,[a] and food consumption.

Group	BW (g)		Mean food consumption			
	Mean (Range)	% Body fat	g/day[b]	g/g BW[c]	Kcal/day[d]	Kcal/g BW[e]
			Males			
5002 AL	831 (642–1,073)	26.5	27.3	0.033	83.8	0.10
5002 DR 6.5 hr	597 (464–785)	15.8	15.8	0.027	48.5	0.08
5002 DR	603 (543–707)	16.1	21.1	0.035	64.8	0.11
5002-9 AL	758 (529–1,003)	24.0	35.5	0.047	83.8	0.11
5002-9 DR	472 (336–562)	9.3	23.6	0.050	55.7	0.12
			Females			
5002 AL	436 (348–733)	35.1	22.7	0.047	69.7	0.14
5002 DR 6.5 hr	329 (260–499)	13.5	13.9	0.042	42.7	0.13
5002 DR	312 (271–344)	11.7	16.7	0.054	51.3	0.16
5002-9 AL	421 (311–642)	27.1	28.5	0.068	67.3	0.16
5002-9 DR	278 (240–315)	6.6	18.6	0.067	43.9	0.16

[a] Body fat determined at 52-wk interim necropsy (approximately 10/sex/group).
[b] Corrected for food wastage of 8.4% for 5002 diet and 14.5% for 5002-9 diet.
[c] Corrected for food wastage and expressed as grams/gram of mean BW.
[d] Calculated from corrected food consumption with 3.07 Kcal/g for 5002 diet and 2.36 Kcal/g for 5002-9 diet.
[e] Kcal consumed/gram BW.

TABLE III.—Fifty-two-wk interim necropsy mean BW and organ weights.

Group	5002 AL	5002 DR 6.5 hr	5002 DR	5002-9 AL	5002-9 DR
		Males			
Number	10	10	10	7	10
BW (g)	734.0	559.0[s]	570.0[s]	640.0	431.0[s]
Brain (g)	2.320	2.240	2.320	2.300	2.280
Spleen (g)	1.040	1.030	0.770[s]	0.870	0.640[s]
Lungs (g)	2.010	1.760[s]	1.790	1.900	1.500[s]
Thyroids (g)	0.035	0.030	0.025[s]	0.034	0.024[s]
Testes (g)	3.240	3.520	3.740	3.670	3.590
Prostate (g)	0.650	0.650	0.510	0.740	0.510
		Females			
Number	10	9	10	8	12
BW (g)	456.0	309.0[s]	286.0[s]	380.0[s]	244.0[s]
Brain (g)	2.090	2.140	2.120	2.090	2.050
Spleen (g)	0.770	0.540[s]	0.530	0.520[s]	0.490[s]
Lungs (g)	1.480	1.290[s]	1.240[s]	1.360[s]	1.110[s]
Thyroids (g)	0.025	0.020[s]	0.020[s]	0.033	0.019[s]
Ovaries (g)	0.068	0.067	0.069	0.078	0.062
Uterus (g)	1.080	0.930	0.840	0.750[s]	0.670[s]

[s] Statistically significant ($p \leq 0.05$) compared to the 5002 AL group. Unmarked values were not statistically significant compared to the 5002 AL group.

Effects of Diet and DR on Pituitary and Mammary Glands up to 52 Wk

Table IV shows the incidence of anterior pituitary focal hyperplasia and adenoma, the mean pituitary weight, and the mean 7-day cumulative BrdU % LI of the normal anterior pituitary. The highest incidence and severity of focal hyperplasia and/or adenoma was seen in the 5002 AL rats followed by the 5002-9 AL rats of both sexes. All DR groups had a lower incidence of hyperplasia. However, the 5002 DR 6.5 hr females had an increased incidence of adenoma and hyperplasia compared to the other female DR groups.

The 5002 AL males had the largest pituitary glands. The female pituitary gland weights were similar among the 5002 AL, the 5002 DR 6.5 hr, and the 5002-9 AL groups. Compared to the 5002 AL group, the females in the 5002 DR and the 5002-9 DR groups had significantly smaller pituitaries.

Compared to the 5002 AL females, the 7-day cumulative BrdU % LI was statistically significantly lower in the 5002-9 DR group. It was also lower, although not significantly, in the 5002 DR group. This general pattern was repeated for the males, but none of the male groups were statistically significantly different ($p > 0.05$).

A total of 12 females and 10 males had pituitary

TABLE IV.—Fifty-two-wk pituitary and mammary gland changes.

Group	5002 AL	5002 DR 6.5 hr	5002 DR	5002-9 AL	5002-9 DR
		Males			
Number[a]	13	10	10	12	10
Anterior pituitary					
Adenoma[b]	2	—	—	—	—
Hyperplasia[b]	4	1	1	3	—
Weight (g)[c]	0.020	0.015[s]	0.016[s]	0.015[s]	0.013[s]
% LI[d]	1.420	1.310	1.130	1.140	1.020
		Females			
Number[a]	12	11	10	10	15
Anterior pituitary					
Adenoma[b]	3	1	—	2	—
Hyperplasia[b]	1	3	1	2	—
Weight (g)[c]	0.028	0.026	0.020[s]	0.022	0.016[s]
% LI[d]	1.530	1.120	0.980	1.160	0.710[s]
Mammary glands					
Fibroadenoma[b]	2.000	—	—	1.000	—
Galactocele[b]	6.000	1.000	—	1.000	2.000

[a] Number examined including 52-wk necropsy and early deaths.
[b] Number per group with the diagnosis up to 52 wk (early deaths and interim necropsy).
[c] Fifty-two-wk interim necropsy rats only.
[d] Anterior pituitary BrdU percent labeling index in normal tissue of interim necropsy.
[s] Statistically significant ($p \leq 0.05$) compared to the 5002 AL group.

TABLE V.—Fifty-two-wk liver changes.

Group	5002 AL	5002 DR 6.5 hr	5002 DR	5002-9 AL	5002-9 DR
		Males			
Basophilic AHF[a]	0.30	—	0.20	0.66	0.10
Eosinophilic AHF[a]	0.46	0.20	0.20	0.50	—
Bile duct hyperplasia[a]	0.61	0.10	0.40	0.66	0.60
Periportal vacuolation[a]	1.07	—	0.10	0.25	—
Liver weight (g)	19.38	16.34[s]	14.01[s]	15.51[s]	11.09[s]
Nuclei × 10^6/cm^3	99.00	92.00	106.00	116.00	94.00
Nuclei × 10^6/liver	1,551.00	1,216.00[s]	1,215.00[s]	1,469.00	852.00[s]
Hepatocyte % LI	0.40	0.46	0.62	0.45	2.44[s]
Labeled nuclei × 10^6/liver	6.20	5.60	7.50	6.70	20.80[s]
Mitotic rate/1,000	0.05	0.10	0.10	0.07	0.15
GSH (μg/mg)[b]	19.90	27.50[s]	29.40[s]	22.40	33.00[s]
MDA (μM/mg)[c]	0.55	0.23[s]	0.41[s]	1.16	0.55[s]
		Females			
Basophilic AHF[a]	0.33	0.36	0.50	0.60	0.46
Eosinophilic AHF[a]	0.16	0.27	0.10	—	0.06
Bile duct hyperplasia[a]	0.91	0.27	0.40	0.90	0.80
Periportal vacuolation[a]	1.66	0.27	—	1.10	0.20
Liver weight (g)	12.26	10.06[s]	8.72[s]	10.26[s]	6.88[s]
Nuclei × 10^6/cm^3	104.00	94.00	98.00	95.00	98.00
Nuclei × 10^6/liver	1,028.00	775.00[s]	701.00[s]	795.00[s]	553.00[s]
Hepatocyte % LI	3.60	3.20	3.80	4.30	14.90[s]
Labeled nuclei × 10^6/liver	37.10	25.00	26.50	34.20	82.60[s]
Mitotic rate/1,000	0.25	0.22	0.25	0.38	0.79
GSH (μg/mg)[b]	17.40	21.80	18.90	18.80	18.40
MDA (μM/mg)[c]	1.90	0.85[s]	0.46[s]	2.25	0.74[s]

[a] Average grade (0–5) of histologic lesion.
[b] Glutathione content, μg/mg protein; values are means from 5 rats/group.
[c] Malondialdehyde content, μmoles/mg protein; values are means from 5 rats/group.
[s] Statistically significant ($p \leq 0.05$) compared to 5002 AL.

adenomas and/or focal hyperplasia of the anterior pituitary. BrdU % LIs were similar whether an animal had multiple adenomas or hyperplasias, so these values were averaged so that each animal might contribute equally to the analysis. Too few animals at 52 wk had hyperplasia or adenoma for comparison of all 5 treatment groups. The AL groups were combined for comparison with the DR groups. In each of the 22 animals, the % LI was higher for the adenoma or focal hyperplasia compared with the normal adjacent anterior pituitary (pars distalis) tissue ($p < 0.001$ and $p = 0.002$ for females and males, respectively). Some of the highest pituitary % LIs were observed in the adenomas, but the adenomas also had some of the lowest % LIs.

The females in the 5002 AL and the 5002-9 AL groups were the only rats with fibroadenomas by 52 wk. The 5002 AL females also had the highest incidence of galactoceles (Table IV).

Effects of Diet and DR on Liver

A summary of the liver changes observed at the 52-wk interim necropsy are shown in Table V. Degenerative changes were most evident in the 5002 AL rats. Degenerative changes, such as periportal hepatocellular vacuolation and telangiectasis were more evident and severe in the 5002 AL rats than any other group. Periportal vacuolation and other degenerative changes were less evident in the DR groups on both diets. In rats showing periportal vacuolation, particularly the females, there was an increase in BrdU nuclear labeling of hepatocytes in this region. These and other changes appear to correlate directly with increased malondialdehyde content observed in the 5002 AL and 5002-9 AL groups (Table V).

Bile duct hyperplasia was more frequent and severe in both AL groups and was accompanied by increased BrdU labeling of the bile ductules. Basophilic and eosinophilic altered hepatocellular foci (AHF) were seen in all groups, but clear differences among groups were not evident at 52 wk. The AHF had increased BrdU labeling compared to the surrounding normal hepatocytes.

The mean glutathione content of the male livers was statistically significantly greater in the DR rats than in either AL group ($p < 0.001$). In the females rats, a similar trend was noted but was not statistically significant.

Liver malondialdehyde (MDA) content was higher in both the 5002 AL and the 5002-9 AL groups. In both males and females, the liver MDA was statistically significantly less in the DR groups compared to either AL group.

For both males and females, the 5002 AL group had the heaviest livers, while the 5002-9 DR group

TABLE VI.—Fifty-two-wk degenerative changes of kidney, heart, pancreas, and adrenals.

Group	5002 AL	5002 DR 6.5 hr	5002 DR	5002-9 AL	5002-9 DR
		Males			
Kidneys					
Weight (g)	5.14	3.69[s]	3.74[s]	4.08[s]	3.01[s]
Nephropathy[a]	1.92	0.20	0.20	0.83	0.00
Tubular basophilia[a]	2.31	0.80	1.00	1.75	1.10
Glomerular sclerosis[a]	1.31	0.10	—	0.58	—
Heart					
Weight (g)	1.85	1.58[s]	1.55[s]	1.78	1.36[s]
Cardiomyopathy[a]	0.84	0.50	0.60	0.58	0.10
Pancreas					
Acinar atrophy[a]	0.30	0.20	0.30	0.16	0.20
Islet fibrosis[a]	0.92	0.50	0.60	1.00	0.00
Adrenal					
Weight (g)	0.074	0.067	0.064[s]	0.066	0.054[s]
Cystic degeneration[a]	—[b]	—	—	—	—
		Females			
Kidneys					
Weight (g)	2.87	2.35[s]	2.30[s]	2.60	1.95[s]
Nephropathy[a]	0.58	—	—	—	—
Tubular basophilia[a]	1.50	0.64	0.40	1.30	1.06
Glomerular sclerosis[a]	0.25	—	—	—	—
Heart					
Weight (g)	1.34	1.11[s]	1.01[s]	1.19	0.87[s]
Cardiomyopathy[a]	0.83	0.27	0.10	0.80	0.20
Pancreas					
Acinar atrophy[a]	0.08	0.36	—	—	0.06
Islet fibrosis[a]	0.16	—	—	—	—
Adrenal					
Weight (g)	0.100	0.091	0.072[s]	0.068[s]	0.059[s]
Cystic degeneration[a]	1.330	1.180	0.700	0.400	0.130

[a] Average histological grade (0–5).
[b] One pheochromocytoma in 5002 AL group.
[s] Statistically significant ($p \leq 0.05$) compared to 5002 AL.

had the lightest livers. The geometric means by treatment group of the hepatocellular proliferation and stereologically determined nuclear density are shown in Table V. The density of hepatocyte nuclei (nuclei/cm^3) showed no statistical differences among groups, so the results for total hepatocyte nuclei per liver are quite similar to those for liver weight. The 5002-9 DR group showed an increased average % LI and total labeled nuclei per liver compared with other groups for sexes. In every treatment group, females had higher average % LI and total labeled nuclei per liver compared with the respective male groups.

Too few mitotic cells were observed for analysis of variance, so a nonparametric test was used. In males, no treatment group differed with the 5002 AL group. Females in the 5002-9 group had more mitotic hepatocytes compared with other groups, but this was not statistically significant. As with the labeling data, the observed average mitotic rate was higher for females than male rats in every treatment group.

Effect of Diet and DR on Kidney, Heart, and Other Organs

There were clear differences in the incidence and severity of the changes observed in the kidneys, heart, pancreas, and adrenals of the 5002 AL group versus the other treatment groups (Table VI). In general, the 5002 AL rats had the largest kidneys with the most severe morphologic changes. The glomerular sclerosis, tubular basophilia, interstitial fibrosis, and cellular infiltrates were individually graded for all animals. When an individual manifested all of these changes, an overall grade was given under the category of chronic nephropathy. The BrdU-labeled slides showed high labeling in areas of tubular basophilia, cellular infiltration, interstitial fibrosis, and glomerular sclerosis. The 5002-9 rats had less severe renal lesions, suggesting a sparing effect of the lower protein diet. However, in all DR groups fed either diet, there was a clear decrease in the incidence and severity of renal lesions compared to either diet fed AL. Quantitative analyses of the morphologic and

proliferative changes have been presented elsewhere (11, 22).

The 5002 AL and 5002-9 AL rats had the largest hearts and their incidence and severity of cardiomyopathy as manifest by myocardial degeneration, focal fibrosis, and cellular infiltration was greater than in their restricted counterparts (Table VI).

The rats fed AL either diet had the highest incidence and severity of degenerative changes in the pancreas, particularly islet fibrosis and acinar degeneration (Table VI).

The 5002 AL females had the highest incidence and severity of adrenal cortical cystic degeneration. The only pheochromocytoma seen by the time of the 52-wk interim necropsy was in a 5002 AL male. The 5002 AL rats had the largest adrenals (Table VI).

Additional changes observed in the aforementioned and other organs, generally indicated the 5002 AL group had the highest incidence and severity of degenerative and proliferative lesions; however, only a slight improvement was seen in the 5002-9 AL group. In general, all of the DR regimens decreased the incidence and severity of lesions in the kidney, liver, pituitary, heart, mammary gland, pancreas, adrenal, and other organs compared to the 5002 AL and 5002-9 AL groups. The 5002-9 diet fed AL appeared to have a slight sparing effect on the severity of renal lesions but did not have an appreciable effect on the overall incidence of lesions observed in other organs or in relative BW gain or carcass composition. DR by measurement or time with both diets appeared to have a sparing effect on the incidence and severity of the common degenerative and proliferative lesions of this stock, with the exception of the 5002 DR 6.5 hr regimen in females that did not prevent the early onset of pituitary hyperplasia and adenoma.

Discussion

The data demonstrate that overfeeding is a major contributor to poor SD rat survival. Moreover, moderate controlled DR that provides feed in amounts that are in the range of current "*ad libitum*" studies (Figs. 1 and 2) will improve survival, lower the incidence and/or severity of chronic diseases associated with overfeeding, and improve the rat as a model in which to test candidate pharmaceuticals (20–23). We have also studied a modified diet with lower protein, fat, and metabolizable energy content and increased fiber content and found no survival benefit if this diet was fed AL. These data indicate that SD rats should be maintained on a standardized diet by dietary caloric restriction by providing approximately 60–65% of their true AL food consumption per day.

Any attempt to modify "*ad libitum*" food consumption must be done with an appreciation of the wide variability seen in daily food consumption and 2-yr survival for the same SD rat stock, fed the same feed in different laboratories (Figs. 1 and 2). In our laboratory, adult SD rats fed AL eat approximately 33.0 or 24.6 g of Purina 5002 diet daily for males or females, respectively. The 65% amounts of these AL amounts (35% DR) are 21.5 and 16.0 g for males and females, respectively. Laboratories that feed less than this amount see an increase in long-term survival, as indicated by the correlations plotted in Figs. 1 and 2. Compared to our daily AL food consumption, many other laboratories are currently practicing a form of food restriction under their feeding conditions. For example, SD male rats given 21.7 g/day are fed at our 35% DR level, and those given 23 g/day would require less than a 10% reduction to reach our 35% DR level of 21.5 g/day. Only laboratory rodents are maintained by AL feeding, whereas other laboratory animals (i.e., dogs and primates) are fed measured amounts of feed, and it is considered a poor veterinary practice to do otherwise.

The most successful DR regimens provide essential nutrients at adequate amounts but restrict caloric intake to 30–70% below the true AL food consumption levels. The beneficial effects on longevity appear to depend primarily on caloric restriction, because the specific restriction of fat, protein, minerals, or other nutritional components without caloric restriction does not increase the overall long-term survival or the maximum species-specific life-span (8, 9, 34, 39, 41, 56, 70). A chronic 30–40% restriction of energy intake without essential nutrient deficiency lowers the incidence and/or delays the onset of most spontaneous and induced tumors; reduces the severity and/or onset of most spontaneous degenerative diseases, such as nephropathy and cardiomyopathy of rats; and extends the average and maximum life-span (11, 20–23, 34, 35, 41, 56, 57, 63, 70, 72).

McCay et al (44) first clearly demonstrated an extension of maximum rat life-span via DR almost 60 yr ago and postulated that food restriction extends life-span by slowing growth and development. Recent studies, however, have shown that DR begun in adult life also increases survival in rats (33, 35, 41, 74). A 40% DR of 6-mo-old F-344 male rats was as effective as a similar degree of food restriction started at 6 wk of age (33, 74). Thus, DR is very effective in mature rats when growth is complete, indicating that DR is not mechanistically linked with a delay in growth and development or early BW gain.

Rats fed either diet by DR had lower body fat

than AL-fed animals (Table II). It was proposed in the 1960s that food restriction retarded aging and extended life-span by reducing body fat content (34, 39). This view is consistent with the general belief that excessive body fat leads to premature death in humans and AL-fed rats have body fat content similar to obese humans. In contrast, DR-fed rats have body fat content in the range of normal adult humans. This simple analogy may be ill advised due to the marked differences between the 2 species in metabolic rate and other physiological processes (8, 34, 70, 72). Moreover, it has been shown that no correlation between body fat content and length of life exists for AL-fed male F-344 rats and, in fact, a positive correlation between body fat content and length of life was observed in dietary-restricted F-344 rats (1, 2, 36). Similar conclusions have been drawn from studies with male Wistar rats (65) and in studies of food restriction with lean and genetically obese mice (13). These data make it highly unlikely that reducing the body fat content per se plays a causative role in extending life-span by DR (1, 2, 36).

The data from this study with 2 diets differing in specific nutrient content and energy content indicate DR increases longevity by restriction of energy rather than restriction of a specific nutrient. While food restriction involves restricting all of the specific nutrients as well as calories, energy intake appears to be the main process improving survival because DR rats fed either diet have the same or slightly higher food intake per gram of BW as AL rats given a specific diet (Table II). Many studies using different diets and rodents have shown food restriction per se extends life (21, 32, 52, 70, 71).

For example, in studies of male F-344 rats, restricting the protein intake by 40% without restricting calories resulted in only a small improvement on longevity, and, except for decreased renal disease, few other aging processes were affected (31, 37). However, a 40% caloric restriction without protein restriction was as effective as caloric restriction with protein restriction in improving F-344 rat survival (15, 31, 37). Our studies of SD rats have led to the same conclusion that protein restriction without caloric restriction is not a major factor in most of the actions of food restriction in improving longevity (11, 22).

In studies restricting dietary fat or minerals in a similar way, no effect on F-344 rat longevity was seen (16, 74). In our studies of SD rats, marked increases in dietary fiber were not effective in improving survival above 50% at 2 yr when fed AL (Table I). While a possible specific role for the carbohydrate component of the diet cannot be ruled out, these and other studies indicate that the ability of DR to extend longevity is due to the restriction of energy intake rather than the restriction of a specific nutrient.

Some have speculated that food restriction increases longevity simply by reducing the intake of toxic contaminants in the diet. This is unlikely considering the variety of feeds used in different food restriction studies (8, 9, 16, 23, 31, 56, 70, 74). That DR-fed rodents eat approximately the same or slightly more food per gram of BW as AL-fed rodents indicates they would be exposed to the same level of contaminants as their AL counterparts on a per gram of BW basis. Also, in many food-restricted studies, vitamins have not been restricted or have been supplemented, showing that a possible contamination from this source is not involved (8, 16, 70, 71). Thus, research from a number of different laboratories strongly indicates that the retardation of aging processes and the increasing longevity that food restriction produces is due to the restriction of energy rather than the restriction of a specific nutrient.

The data from this study rule out hypotheses that DR acts by reducing the intake of calories or other nutrients per unit of body mass, because the amount of grams of feed or kcal consumed per gram of BW is very similar between AL and DR groups. Because AL and DR rats fed either diet consume approximately the same kcal per gram of BW, these data also rule out hypotheses that DR increases survival by decreasing metabolic rate (51, 60). The BW and food consumption data of SD rats fed DR either diet and similar data of F-344 rats restricted by 40% of AL (34, 39, 41) show that caloric-restricted rats rapidly change BW in response to food restriction that results in similar food intake per gram of BW in both DR- and AL-fed rats. Metabolic studies of F-344 rats have shown that 40% DR initially does cause a transient fall in energy expenditure per unit of lean body mass, but within 6 wk the DR- and AL-fed F-344 rats had similar rates of oxygen consumption per unit of lean body mass (42, 43). The reason for these results is that lean body mass is rapidly reduced in the DR animals in proportion to the reduction of energy intake. These data make it unlikely that the effects of DR are due to a reduction in metabolism or a decrease in energy intake or any other nutrient per unit of lean body mass. Rather, these data indicate that the effects of DR on aging and longevity depend not on the rate of food use but on total amount of food consumed (energy intake) by the entire organism. Thus, the antiaging action of dietary restriction is not a reduction in metabolic rate per unit of "metabolic mass," but the reduction in energy intake per animal (34, 39,

42, 43). It appears that this reduction of energy intake per animal modulates aging by altering characteristics of fuel use rather than the rate of use (32, 34, 39, 41, 43).

Data from this study do support 2 widely held theories that caloric restriction acts not by changing the rate but by modulating certain characteristics of fuel use to prevent long-term damage of fuel use from the glycation reaction or oxidative metabolism. Glucose, a reducing sugar, undergoes a nonenzymatic reaction with amino groups of proteins called the glycation reaction. The glycation theory proposes glucose is a mediator for aging by glycation of proteins and nucleic acids (5, 38). The importance of damage from glycation can readily be seen in diabetes mellitus.

In this study, glucose levels at 52 wk presented elsewhere (11) were elevated in the 5002 AL and 5002-9 AL groups relative to their DR counterparts. The lowest levels were seen in the 5002-9 DR males and 5002 DR and 5002-9 DR females (11). Glomerular sclerosis, a component of chronic nephropathy, is a structural change similar to that seen in diabetic nephropathy (10). This change was most evident in the 5002 AL males and females and the 5002-9 males. Similarly, pancreatic islet degeneration and fibrosis was most evident in the same groups (Table VI). In studies of F-344 male rats, 40% DR lowered plasma glucose levels to about 15% of AL-fed counterparts (38). However, the rate of glucose utilization per unit of lean body mass of DR-fed F-344 male rats is as great as that of AL-fed animals (38). These data indicate that glucose effectiveness or insulin sensitivity or both are increased by DR (38, 40). These findings indicate that the effect of food restriction on plasma glucose levels may be a fundamental mechanism, because it results in the more efficient use of an important, but potentially toxic, fuel (glucose) at sustained lower, and presumably less long-term damaging, concentrations (5, 38, 40).

While fuels such as glucose are reactive molecules in their own right, oxygen use in the oxidative metabolism of fuels results in free-radical production and forms the basis of the free-radical theory of aging (12, 71–73). This theory assumes that life-long damage from reactive oxygen species occurs during basic metabolic processes of life and that DR protects the organisms by maintaining protective mechanisms into advanced age (17, 24, 25, 29, 64, 71–73). A 40% DR of male F-344 rats will modulate free-radical production, scavaging enzyme activities, free-radical damage, and the detoxification of products of free-radical damage (17, 24, 25, 29, 64, 71, 72). Lipid peroxidation is inhibited as measured *in vitro* by analysis of the age-related increase in MDA produced by liver microsomal and mitochondrial membranes, and hydroperoxide content of hepatic membranes is reduced by DR (73). Glutathione reductase and catalase change little with age but are maintained at significantly higher levels in DR F-344 rats. Reduced glutathione levels are stable through 18 mo of age but fall by 24 mo of age in the AL-fed but not DR-fed F-344 rats. Mn-superoxide dismutase and glutathione peroxidase changed little with age (17, 24, 25, 29, 64, 71–73). DR influences the metabolism of MDA, a product of lipid peroxidation from free-radical damage. Hepatic mitochondria from AL-fed rats lose the ability to oxidize MDA with age, and this decrease is partially prevented by DR (73). This action may underlie the ability of food restriction to prevent the age-associated accumulation of lipofuscin and related substances in the livers of aging animals (17).

Data from SD rat livers in this study indicate that DR rats are better able to withstand spontaneous oxidative injury than the AL rats. At 52 wk, the mean hepatic glutathione (GSH) content of males from all DR groups was clearly higher than that of livers from either AL group. While a similar difference in GSH was not observed in females, the hepatic malondialdehyde (MDA) content in the DR females (all groups) was lower (24–45% of the 5002 AL group for 5002 DR rats and 33% of the 5002-9 AL group in 5002-9 DR rats). The differences noted at 1 yr indicate that livers of dietary-restricted SD rats are better able to defend against oxidative injury, as has been shown in F-344 rats (17, 24, 25, 29, 71–73).

The proliferative and degenerative changes seen in the livers at 52 wk also indicate that DR-fed SD rats are better able to withstand long-term metabolic load and oxidative damage. The incidence and severity of bile duct hyperplasia, periportal hepatocellular vacuolation, and related degenerative changes were greater in AL-fed rats given either diet compared to their DR-fed counterparts. The changes observed in this study were consistent with those reported in F-344 rats (17).

In response to increased total fuel intake per rat from both diets, liver size was greater in AL-fed rats, expressed as relative and absolute weight, total hepatocyte nuclei per liver, or total BrdU-labeled hepatocyte nuclei per liver. The density of hepatocyte nuclei per cm^3 and BrdU % LI were generally the same among the AL and DR groups. These data indicate that AL-fed rats have a greater liver mass at 52 wk, more hepatocellular degeneration, more oxidative damage (less GSH, more MDA), and potentially more hepatocytes in DNA synthesis at risk

of DNA damage from ongoing chronic oxidative metabolism and glycation reactions.

An exception to this general observation was the DR rats fed the 5002-9 low-protein, low-energy, high-fiber diet. These rats had the smallest, least damaged livers but the highest BrdU % LI and total labeled hepatocyte nuclei per liver. This observation indicates that increased hepatocellular DNA synthesis per se, in the absence of other biochemical or pathological changes, is not necessarily indicative of hepatocellular injury.

The most common cause of death in old SD rats in general and in SD rats in this study was pituitary tumors in both sexes and mammary gland tumors in females (3, 20, 22, 23, 44, 50, 68). At 52 wk, the 5002 AL and 5002-9 AL rats of both sexes and the 5002 DR 6.5 hr females had the largest pituitaries and the highest incidence of pituitary adenomas or focal hyperplasias. The anterior pituitary (pars distalis) BrdU % LI of normal cells was lower in the 5002 DR and 5002-9 DR females. Significant differences were seen between the AL- and DR-fed males. However, the BrdU % LI of focal hyperplasias and adenomas of the anterior pituitary were similar for both lesions and did not differ among groups or sexes. These data suggest that both lesions represent a continuum and, once established, have similar proliferative kinetics.

Since increased prolactin secretion has been associated with pituitary adenomas (46, 61, 62, 67, 68), it is not surprising that the 5002 AL and 5002-9 AL females had the only mammary gland fibroadenomas at 52 wk. The 5002 AL females also had the highest incidence of galactoceles at this time. These data indicate that AL feeding accelerates the time of onset of pituitary and mammary gland proliferative lesions, and DR delays their time of onset. The changes observed in these 2 organs at 52 wk were the pathology biomarkers that best correlated with final 2-yr survival and the final incidence of fatal lesions and tumors (20–23).

Spontaneous prolactin-secreting pituitary tumors in aging rats have been well studied, with certain stocks and strains developing a high incidence (4, 46, 47, 49, 61–63, 67, 68). In aging rats, a decrease develops in secretion by hypothalamic neurons of catecholamines, particularly dopamine and norepinephrine, that lowers hypothalamic release of gonadotropin-releasing hormone, growth hormone-releasing hormone and thyrotropin-releasing hormone. These result in reduced pituitary secretion of gonadotropic hormones, growth hormone, thyrotropic hormone, and increased pituitary secretion of prolactin. This age-related decrease in hypothalamic prolactin-inhibitory activities is associated with the development of prolactin-secreting pituitary tumors (4, 46, 47, 49, 62). While DR has been shown to reduce hormone secretion by the hypothalamus, DR also prevents the age-related decrease in hypothalamic dopaminergic activity. DR does not decrease the responsiveness of the pituitary to hypothalamic hormones or the target endocrine glands to pituitary hormones (4, 46, 47). This indicates that DR has a major protective effect on the hypothalamus. In contrast, overfeeding by AL apparently accelerates the reduction of hypothalmic dopaminergic activity that increases pituitary prolactin release leading to the early development of potentially fatal pituitary tumors and mammary gland tumors in the rat (46, 49, 62).

After pituitary tumors, the most common cause of early death in male SD rats was renal disease (11, 20–23). The earliest onset, highest incidence, and most severe chronic nephropathy was seen in the 5002 AL males, followed by the 5002-9 AL males. The rats fed either diet by DR had a low incidence and severity of renal disease that was not a contributing factor to mortality in the DR-fed animals. Interestingly, the low-protein, low-energy, high-fiber 5002-9 diet was of minimal benefit in preventing chronic nephropathy if fed AL but was of some benefit when fed DR. Quantitative data presented elsewhere (11) support these observations and indicate AL feeding of either diet was associated with the early development of glomerular hypertrophy that leads to glomerular sclerosis (11, 22). These and other data suggest that the pathogenesis of chronic nephropathy in the rat is initiated by glomerular hypertrophy and DR prevents the early development of this change (11, 22).

Besides pituitary tumors and renal disease, the third contributing factor to mortality in the 5002 AL males was chronic cardiomyopathy. This lesion, involving combinations of myofiber degeneration, mononuclear cell infiltration, and myocardial fibrosis was seen in the highest incidence and severity in the 5002 AL and 5002-9 AL males. These same groups also had the largest hearts and lungs at 52 wk. All of the DR groups had smaller hearts and lungs and a lower incidence and severity of cardiomyopathy. This lesion is common in most strains of laboratory rats and increases in severity and incidence with age (6, 22, 30, 56, 63). DR in other rat strains and stocks partially protects against cardiomyopathy (6, 30, 56, 63).

While not a significant contributing factor to mortality by 2 yr, the changes seen in the adrenal glands at 52 wk were predictive of the lesion and tumor incidence seen at the end of this study (23). The single pheochromocytoma in the 5002 AL males reflected an earlier onset time of this common adrenal tumor. The greater adrenal weights and se-

verity of adrenal cortical cystic degeneration in the 5002 AL and 5002 DR 6.5 hr females were predictive of the severity of adrenal changes seen by 2 yr and indicate an earlier onset time of these degenerative changes (22).

The mechanisms underlying the action of DR on aging and longevity have proven to be difficult to explore and several of the major hypotheses proposed for the action of DR have been ruled out by recent studies (31, 34, 36, 38–41, 47, 71, 72). However, it is apparent that DR does retard aging processes and extends longevity by allowing the rodent to utilize fuel in less damaging ways than is the case for its AL-fed counterparts. Most of the basic work in dietary-restricted rodents has accumulated evidence on the use of glucose and oxygen, both potentially toxic processes (31, 34, 39–41, 64, 70–73).

The data from this study of SD rats are consistent with the observations of others over the past 4 decades that reducing energy intake by caloric restriction will increase the survival, retard age-related senescence and degeneration, and delay the onset of age-related diseases and tumors (8, 9, 31, 37, 41, 47, 56, 63, 70, 74). Although the mechanisms underlying these effects are not yet completely understood, our data support several of the most widely held hypotheses that caloric restriction acts by modulating the characteristics, but not the rate, of fuel use in a way to prevent long-term damage of such fuel use through oxidative damage or glycation (5, 8, 12, 34, 38–42, 70–73). While other hypotheses should be considered, it is apparent that caloric restriction is affecting survival primarily through its action on fuel utilization. It is likely that primary aging processes are involved in the pathogenesis of many of these different age-associated diseases and that caloric restriction retards the occurrence and progression of these diseases by its ability to slow primary aging processes. Conversely, our present methods of feeding rodents AL apparently accelerates primary aging processes as manifested by lower survival and the acceleration of the onset and severity of age-related degenerative diseases and tumors.

Considering the beneficial effects of moderate DR, it is anticipated that the chronic toxicity would be readily detected in animals maintained by this method. Moreover, the beneficial effects of DR in preventing long-term degenerative disease and delaying the onset of diet-related endocrine tumors would result in the animals being exposed to the test compound for a longer period of time and, thus, allowing a better assessment of chronic toxicity and carcinogenicity. This controlled method of moderate DR, which provides feed in amounts that are within the range of current "*ad libitum*"–fed studies (Figs. 1 and 2), should result in a more appropriate rodent model for long-term carcinogenicity studies to assess the human safety of candidate pharmaceuticals.

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