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Diet, Overfeeding, and Moderate Dietary Restriction in Control Sprague-Dawley Rats: II. Effects on Age-Related Proliferative and Degenerative Lesions*

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ABSTRACT

This study compared the effects of *ad libitum* (AL) overfeeding and moderate dietary restriction (DR) of 2 different diets on Sprague-Dawley (SD) rat survival and spontaneous, age-related proliferative and degenerative lesions. SD rats were fed Purina Rodent Chow 5002 or a modified Rodent Chow 5002-9 containing lower protein, fat, metabolizable energy, and increased fiber by AL or by DR at 65% of the AL amount by measurement or time (6.5 hr). At 106 wk, rats fed the 5002-9 diet AL did not have significantly improved survival over rats fed the 5002 diet AL. The 5002 diet fed DR by time (6.5 hr) improved survival for males but not females. Only DR by measurement of both diets resulted in lower mortality for both sexes. By 106 wk rats fed either diet by AL had the same brain weights as DR fed rats, but AL fed rats had greater body weight, body fat content, and increased heart, lung, kidney, liver, adrenal, thyroid, and pituitary weights that correlated with an increased incidence and severity of degenerative and/or proliferative lesions in these organs. Moderate DR delayed the progression of chronic nephropathy by delaying the early development of glomerular hypertrophy that initiates the development of glomerular sclerosis and nephron loss in AL overfed rats. Moderate DR lowered the incidence, severity, and progression of cardiomyopathy and other degenerative, age-related lesions and appeared to delay the development of reproductive senescence in SD females. The conclusion from this study is that moderate DR delayed onset and progression of degenerative lesions, and death due to cardiovascular or renal disease, and thus potentially improves the bioassay to detect compound-specific chronic toxicity.

Keywords. Caloric restriction; degenerative disease; nephropathy; cardiomyopathy; aging; reproductive senescence

INTRODUCTION

The survival of the commonly used rats in 2-yr carcinogenicity studies (Sprague-Dawley [SD], Wistar, and the Fischer rat [F-344]) has been declining over the past 3 decades throughout the pharmaceutical and chemical industry (4, 20, 23, 24, 26, 27, 30–32, 44, 49, 53, 56, 57). The decline in survival questions the adequacy of exposure of rats to the test article in carcinogenicity studies that result in less than 50% survival at the end of the 2-yr period (4, 23, 32). We have observed a significant correlation between average daily food consumption and 2-yr survival in 58 control groups of *ad libitum* (AL) fed SD rats (25–27). While both genetic and environmental factors are involved, rat survival can be improved by simple dietary restriction (9, 10, 25–27, 38, 39, 41, 42, 56, 57, 72, 73).

In a companion paper we report that moderate dietary restriction (DR) improves SD rat survival and delays death from spontaneous degenerative disease and tumors of the pituitary and mammary gland (27). Moderate DR has this action on these endocrine-related tumors by delaying the time of

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onset, but not the progression of these tumors when measured by age-adjusted incidence, growth time, doubling time, or time between initial detection and death (27). This paper presents data showing the association between AL overfeeding and the early onset of serious and potentially fatal renal and cardiovascular degenerative disease and the beneficial effects of moderate DR while still feeding amounts within the range of customary AL feeding (25–27). The results of this study indicate that moderate DR delays the onset, incidence, severity, and progression of these degenerative, age-related changes in the SD rat.

MATERIALS AND METHODS

Animals

Three hundred fifty male and 350 female SD rats (Crl:CD®(SD)BR) were obtained from Charles River Laboratories, Raleigh, NC. The animals 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. The animals were identified by tattoos and assigned to the 5 different treatment groups described below using a balanced random allocation scheme. Rats selected for the 52-wk interim necropsy and from the 106-wk terminal necropsy for organ weights and minipump implantation were assigned by a stratified random allocation procedure as follows: For each sex and diet group the rats were ordered by body weight from the lowest to the highest weight. They were then divided into 10 strata by body weight and 1 rat from each strata was randomly chosen for the given necropsy with a second backup animal selected in the event the primary animal did not survive until the necropsy date. This procedure was chosen to optimize the probability that a truly representative sample of animals from each dietary regimen would be sampled.

Diet and Dietary Regimen

The experimental groups contained 70 rats/sex/group and were designed to compare 2 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 (approximately 24 g/day for females and 33 g/day for males). This diet contains approximately 21.4% protein, 5.7% fat, 4.1% crude fiber, and has a calculated metabolizable energy value of 3.07 kcal/g.

b) Certified Rodent Chow 5002 fed AL for approximately 6.5 hr day during the light cycle (5002 6.5 hr DR).

c) Purina Certified Rodent Chow given in measured amounts daily (5002 DR) at approximately 65% of our 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, 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 every week over 3 nights for the 5002 AL group and the 5002 6.5 hr DR group, 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 rats were observed daily for clinical signs and mortality and were weighed pretest, once during week 1, twice weekly through week 13, and once weekly thereafter. Ophthalmoscopic examinations were conducted on all animals pretest and at weeks 51 and 103. Hematology, clinical biochemistry, and urinalyses were conducted in weeks 52, 78, and 103 and will be reported separately. Vaginal lavages were performed on all surviving females for 15 consecutive days between 1:00 and 3:00 pm starting in week 95 (approximately 99 wk of age). Individual cell types in the vaginal lavage samples were recorded as epithelial cells, cornified epithelial cells, and/or leukocytes. The percentage of females with estrous cycles was characterized by vaginal cytology as regular 4–5-day cycles, cycles of irregular length, cycles with persistent vaginal cornified epithelial cells (PVC), and cycles with predominant diestrus lavages by routine methods.

Osmotic Minipump Implantation

One week prior to the 52-wk interim necropsy and the 106-wk terminal 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 via a small dorsal mid-

line 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 (12, 25).

Necropsy and Histopathology

All rats dying spontaneously, killed moribund, or surviving until scheduled necropsy underwent a complete necropsy examination. All rats, including those selected for BrdU minipump implantation, special tissue biochemistry, and carcass analysis were weighed, deeply anesthetized by either inhalation, and killed by exsanguination. The minipumps were removed, terminal body weights 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. Organ weights were expressed as absolute values (grams) and as relative values (% of body weight and % of brain weight). Organs containing masses were not weighed. Each animal underwent a complete gross necropsy and numerous tissues were sampled, including all gross lesions. The tissue samples were routinely fixed in 10% neutral buffered formalin (testes were fixed in Bouin's solution) and routine histological sections of paraplast-embedded tissues were stained with hematoxylin and eosin from all rats and included 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, Harderian gland, and any gross lesions. In addition, selected target tissues were sectioned for stereologic analysis and/or stained for BrdU immunohistochemistry and included kidneys, liver, pituitary, thyroid and parathyroid, adrenals, pancreas, and small intestine (12, 25).

Cell Proliferation and Stereology Studies

Liver. 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 percent labeling index (% LI) by the total hepatocyte nuclei per liver as determined stereologically (8, 54, 59, 69). 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 by standard methods (8, 59). Hepatocyte nuclei from all hepatic zones were included in the sample to allow for zonal differences in hepatocyte size.

Kidney. Four indices were used to quantify chronic renal disease (CRD). These were glomerular area (GA), glomerular sclerotic index (GSI), tubulointerstitial index (TII), and tubular % LI. Kidneys for stereological evaluation were sectioned at 2–3 μm, stained with hematoxylin and eosin, Masson Goldner Foot modified trichrome, and periodic acid-Schiff (PAS), and evaluated qualitatively and quantitatively as described below. BrdU immunohistochemistry was performed on adjacent sections cut at the same thickness (12, 17). The glomerular area (μm^2) was determined from the glomerular circumference using computerized planimetry on each animal (17, 75). At least 100 randomly chosen glomeruli were traced on the inside circumference of the basement membrane of Bowman's capsule. This method avoids overestimation of the size of the glomeruli with thickened basement membranes. A best estimate of circumference was made at regions of the vascular and urinary poles as well as areas of tuft adhesion to Bowman's capsule. Arithmetic mean areas were calculated for each animal. The GSI was adapted from the method of Raij et al (17, 51, 75) based on grading glomeruli. PAS-stained kidney sections were examined and each glomerulus (approximately 150–250 glomeruli per section) were assigned a grade of 0–4. Grading was based on pathologic changes present, including the thickening of basement membranes of glomerular capillaries, proliferation, and metaplasia of the epithelium of Bowman's capsule, adhesion between the tuft and Bowman's capsule, focal or diffuse mesangial cell hyperplasia, and increased mesangial matrix or sclerosis. A grade of 0 was given to unremarkable glomeruli and grades of 1–4 were based on the relative percentage of each glomerulus affected, from 25% for grade 1 to 100% for grade 4 (17). To avoid skewing the data, tangential sections incorporating less than 30% of a tuft were disregarded. The final GSI score for each kidney was calculated as previously described (17). The TII was modified from the method of Tapp et al (70). Point counting was performed using an 81-point grid visible through a side arm attached to an Olympus microscope. Ten random fields were counted at 200× throughout the cortex and the outer stripe of the medulla. Points on capillaries or other vessels or spaces, glomeruli, metaplastic epithelium of Bowman's space, or tubular lumina were not included. The index for each rat was calculated as the % of interstitial points per total number of points counted. The tubular BrdU % LI was modified from the method described by Short et al (63) with at least 1,000 random nuclei of the tubular epithelial cells counted at 400×

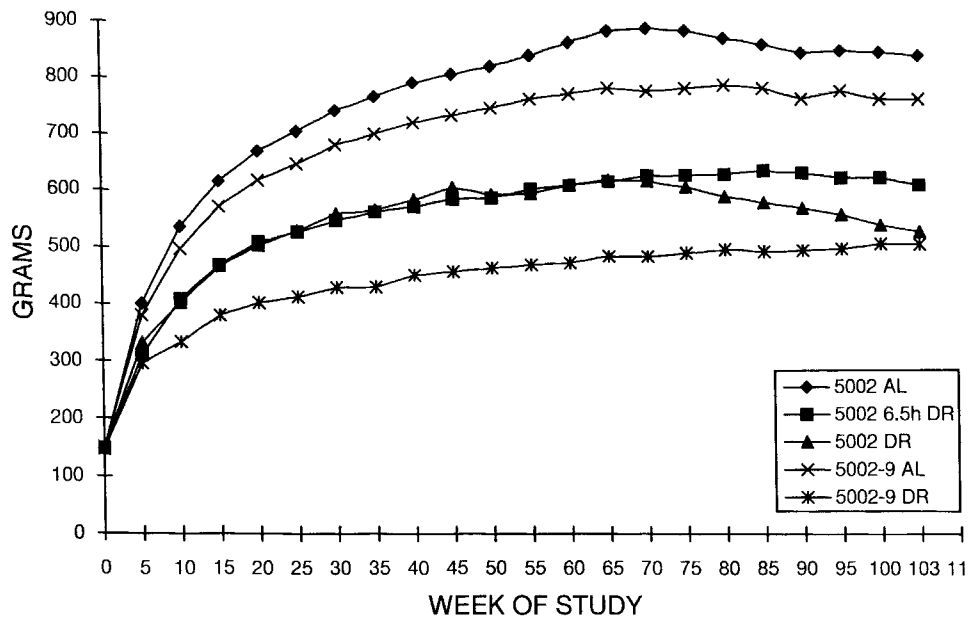

FIG. 1.—The mean body weights for male SD rats.

throughout the cortex and outer stripe of the medulla. For each rat, the % tubular nuclei labeled with BrdU was determined for the % LI.

Tissue Biochemistry and Carcass Analysis

Liver and kidney glutathione (GSH) content were estimated by the nonprotein sulfhydryl method and liver and kidney malondialdehyde (MDA) content were measured using the thiobarbituric (TBA) acid reaction on approximately 5 rats/sex/group. Both methods were adapted from Rush et al (60). After complete necropsies were performed on all animals, the remaining organs and carcasses were frozen for carcass analysis. The tissues were completely ground through a #3 (5/32″) screen, 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

Organ weights and terminal body weights were analyzed by linear models, specifically the analysis of variance with the Student–Newman–Keuls (SNK) procedure (47) to identify statistically significant differences among treatment groups. Data were analyzed using a logarithmic or Rankit scale (19) when appropriate to satisfy assumptions required for linear models, including normality and variance homogeneity. Cell proliferation and stereology data for the liver were analyzed in a logarithmic scale for organ weight, density of nuclei, total labeled nuclei per liver, and % LI. Cell proliferation and stereology data for the kidney were analyzed using the analysis of variance for each index followed by the SNK procedure. A log transformation was used to stabilize the variance. The group averages were summarized by the geometric mean, and the variation between animals in the same treatment group by the coefficient of variation (standard deviation as a percentage of the mean) (19, 47, 65).

RESULTS

Effects on Body Weight, Body Fat, Food Consumption, and Organ Weights

Changes associated with the restriction of caloric intake included significant decrements in the rate of body weight gain in all groups relative to the 5002 AL groups (Figs. 1 and 2). In general, the smallest decrease in body weight gain was seen in the 5002-9 AL groups, which were approximately 92–93% of the 5002 AL groups. The largest decrease in body weight gain was in the 5002-9 DR rats that were approximately 63–68% of the 5002 AL groups. For the 5002 6.5 hr DR and 5002 DR groups, the rate of body weight gain was approximately 73–78% of those observed in the 5002 AL group. After 53 wk, mean body weights of the 5002 6.5 hr DR and the 5002 DR groups were approximately 63–73% of the 5002 AL animals, while the average weights of the 5002-9 AL group were 86–91% of the 5002 AL animals and the 5002-9 DR group were approximately 57% of the 5002 AL animals (Figs. 1 and 2).

After 53 wk, females in the 5002 AL group continued to gain weight, while the body weight gain in the 5002-9 AL group remained the same from this period onward (Fig. 2). In addition, although food consumption and, therefore, caloric intake were

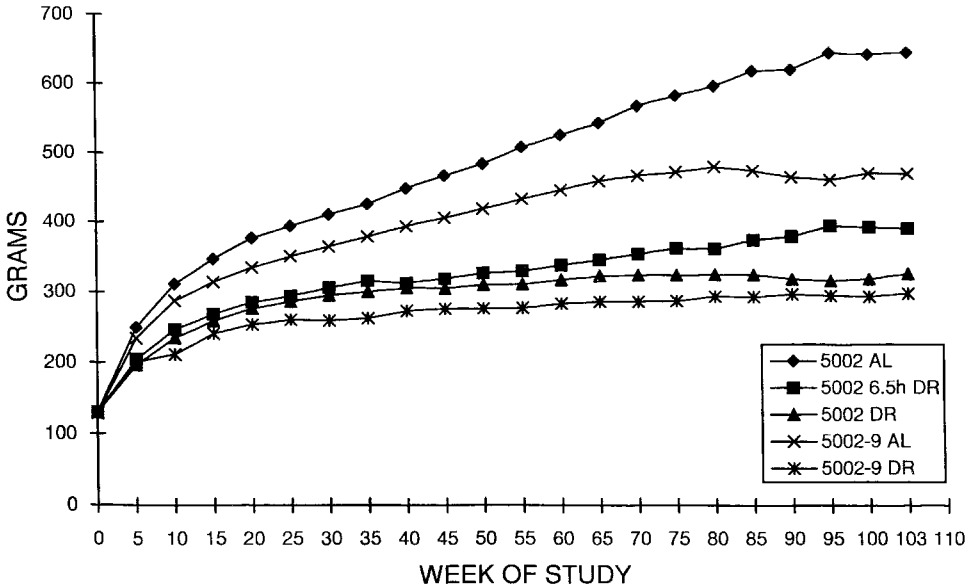

FIG. 2.—The mean body weights for female SD rats.

similar, 5002 6.5 hr DR females continued to gain weight, while 5002 DR females reached a plateau after 25 wk, which was maintained for the study duration (Fig. 2). In the male groups, the body weight relationships that were present at 52 wk remained essentially the same for the remainder of the study (Fig. 1).

The mean daily food consumption (Table I) shows that the 5002-9 AL rats consumed approximately 30% more feed than the 5002 AL animals. However, when corrected for wastage and calculated as kcal per gram of body weight, the amount of kcal (and food) per gram of body weight is similar for all of the AL and DR regimens for each diet. Therefore, rats fed either diet AL consumed the greatest number of grams and kcal per rat but approximately the same number of grams and kcal per gram of body weight as rats fed DR (24–26).

Carcass analysis from the animals sampled at the terminal necropsy demonstrate anticipated responses as shown by the percentage of body fat in Table II. All of the restricted groups were leaner with a significantly lower body fat content and a higher carcass protein than their AL counterparts. All of the AL females had a higher body fat content than their DR female counterparts. The DR females had a lower body fat content than their DR male counterparts. The 5002 6.5 hr DR females had a higher body fat content than the 5002 DR females in spite of a similar calculated metabolizable energy intake. However, it was noted over the course of the study that the 5002 6.5 hr DR animals had a more erratic pattern of food consumption (data not shown) and this variability could, in part, be responsible for the differences seen in body weight gain, body fat, and survival between the 5002 6.5 hr DR and the 5002 DR female groups.

As reported in the 52-wk interim necropsy (25), the smaller body weights of both sexes of DR groups, compared to their AL fed counterparts reflected body fat composition. Minimal differences in absolute brain weights between any group, indicating no effect of DR on brain development (Table II). These data indicate that the percentage of brain weight is the most appropriate relative comparison when

TABLE I.—SD rat food consumption and caloric intake over 2 yr (weeks 1–103).

	Mean food consumption (g/day)	Per rat		Per gram body weight[c]	
		g/day[a]	Kcal/day[b]	g/day	kcal/day
Females					
5002 AL	24.2	24.3	76.3	0.038	0.119
5002 6.5 hr DR	15.9	18.7	58.7	0.048	0.150
5002 DR	16.2	14.6	45.8	0.046	0.144
5002-9 AL	35.1	25.5	58.9	0.054	0.125
5002-9 DR	21.3	17.7	40.9	0.059	0.136
Males					
5002 AL	32.0	27.5	86.4	0.033	0.103
5002 6.5 hr DR	20.2	21.6	67.8	0.034	0.107
5002 DR	22.5	18.9	59.3	0.035	0.109
5002-9 AL	46.5	35.8	82.7	0.046	0.106
5002-9 DR	28.6	25.5	58.9	0.049	0.113

[a] Week 103 food consumption (g/day) corrected for wastage (5002, 12%; 5002-9, 14%).
[b] 5002 diet has 3.07 kcal/g and 5002-9 diet has 2.36 kcal/g metabolizable energy.
[c] Calculated from week 103 average food consumption (g/day) and average body weights.

TABLE II.—Average body weight, body fat, and organ weights.

	5002 AL	5002 6.5 hr DR	5002 DR	5002-9 AL	5002-9 DR
Females					
Body weight (g)	641	391	317	469	299
Body fat (%)	42.1	17.9	7.2	30.3	8.5
Organ weights (g)					
Brain	2.11	2.13^{ns}	2.12^{ns}	2.13^{ns}	2.06^{ns}
Spleen	1.22	0.62^{s}	0.51^{s}	0.62^{s}	0.42^{s}
Heart	1.62	1.17^{s}	1.04^{s}	1.43^{ns}	1.01^{s}
Kidneys	3.54	2.67^{s}	2.46^{s}	3.30^{ns}	2.32^{s}
Liver	16.79	11.31^{s}	9.14^{s}	12.55^{s}	7.69^{s}
Adrenals	0.120	0.090^{s}	0.081^{s}	0.112^{ns}	0.084^{s}
Lungs	1.57	1.38^{s}	1.32^{s}	1.44^{ns}	1.20^{s}
Thyroid	0.034	0.025^{s}	0.021^{s}	0.031^{ns}	0.023^{s}
Ovaries	0.104	0.084^{ns}	0.084^{s}	0.073^{s}	0.059^{s}
Uterus	0.68	0.84^{ns}	0.95^{ns}	0.88^{ns}	0.82^{ns}
Pituitary	0.030	0.020^{ns}	0.022^{ns}	0.026^{ns}	0.025^{ns}
Males					
Body weight (g)	846	616	534	769	512
Body fat (%)	29.6	19.5	16.0	30.5	16.3
Organ weights (g)					
Brain	2.32	2.29^{ns}	2.27^{ns}	2.30^{ns}	2.30^{ns}
Spleen	1.42	0.92^{s}	0.86^{s}	1.35^{ns}	0.81^{s}
Heart	2.13	1.65^{s}	1.54^{s}	2.31^{ns}	1.54^{s}
Kidneys	5.43	4.26^{s}	4.25^{ns}	4.94^{ns}	3.55^{s}
Liver	22.10	14.38^{s}	13.27^{s}	18.24^{s}	11.48^{s}
Lungs	2.54	1.94^{s}	1.86^{s}	2.22^{ns}	1.72^{s}
Thyroid	0.044	0.037^{ns}	0.036^{ns}	0.044^{ns}	0.032^{s}
Pituitary	0.045	0.020^{s}	0.019^{s}	0.022^{s}	0.024^{ns}
Testes	3.61	3.82^{ns}	3.14^{ns}	3.44^{ns}	3.48^{ns}
Prostate	0.60	0.49^{ns}	0.44^{ns}	0.51^{ns}	0.44^{s}

Abbreviations: ns = not significant ($p > 0.05$) compared to 5002 AL group; s = significant ($p \leq 0.05$) compared to 5002 AL group.

comparing various organ weights between groups (relative data not shown).

Absolute and relative (% of brain weight) weights of spleen, heart, kidneys, liver, adrenals, lungs, thyroids, ovaries, pituitaries, and uteri were generally smaller in all of the DR groups compared to the 5002 AL group (Table II). Absolute and relative weights of testes and prostates were generally not different in the DR male groups compared to the AL male groups. In most cases, the lower absolute and relative (% of brain weight) organ weights seen in the different DR groups correlated with the decrease in lesion incidence or severity as discussed below.

Effects on Liver

A summary of the quantitative measurements of hepatocellular proliferation and stereology are shown in Table III. The density of hepatocyte nuclei (nuclei per cm^3) showed no statistical difference among groups, so the results for total hepatocyte nuclei per liver are quite similar to those for liver weight. The 5002 AL animals of both sexes had the largest livers and, therefore, the largest number of nuclei per liver. The smallest livers and those with the least nuclei per liver were seen in the 5002-9 DR groups of both sexes. Percent LI and total number of BrdU-labeled nuclei per liver were not statistically different among the different dietary regimens or by comparison to the 5002 AL group. However, the 5002-9 DR group did show in males and females an increase in the average % LI and the total labeled nuclei per liver in individual animals. Because of the large variability among the animals in this group, these differences were not statistically significant.

At 106 wk no differences were seen in liver MDA content or liver GSH content (data not shown).

While no differences were seen in liver tumor incidence between the different dietary groups compared to the 5002 AL group (27), degenerative changes, such as hepatocellular periportal vacuolation and telangiectasis were most evident and severe in the 5002 AL groups compared to the DR groups, although most of the DR animals lived for a significantly longer time than their AL counterparts. In animals with hepatocellular periportal vacuolation, particularly females, there was an increased BrdU nuclear labeling of hepatocytes in that region. Other proliferative changes, such as bile duct hyperplasia, occurred at a similar incidence in the AL and DR fed rats but were of greater severity in the AL fed animals. Basophilic and eosinophilic foci of cellular alteration or altered hepatocellular foci

TABLE III.—Geometric means of liver stereology and proliferation.

Diet group[a]	Liver weight (g)	Nuclei × 10^6/cm^3	Nuclei × 10^6/liver	Hepatocyte % LI	Labeled nuclei × 10^6/liver
Male					
5002 AL	21.96[cdef]	105.02	1,891.01[cdef]	1.16	22.03
5002 6.5 hr DR	14.28[bef]	99.26	1,162.66[b]	1.20	13.92
5002 DR	13.16[bef]	99.55	1,074.34[bc]	1.75	18.77
5002-9 AL	18.16[bdef]	94.63	1,409.71[bef]	2.04	28.72
5002-9 DR	11.45[bcde]	109.19	1,025.31[bc]	4.90	50.19
Female					
5002 AL	16.45[cdef]	100.44	1,354.83[cdef]	1.26	17.09
5002 6.5 hr DR	11.07[bf]	90.02	817.06[bf]	2.63	21.54
5002 DR	9.09[bc]	103.04	768.52[bf]	2.50	19.18
5002-9 AL	12.01[bef]	92.12	907.13[bf]	1.61	14.59
5002-9 DR	7.42[bcd]	94.44	574.59[bcde]	2.35	13.49

[a] Each group included 9–10 rats (except $n = 4$ for 5002 AL males).
[b] Statistically different from 5002 AL group by SNK test ($p = < 0.05$).
[c] Statistically different from 5002-9 AL group by SNK test ($p = < 0.05$).
[d] Statistically different from 5002 6.5 hr DR group by SNK test ($p = < 0.05$).
[e] Statistically different from 5002 DR group by SNK test ($p = < 0.05$).
[f] Statistically different from 5002-9 DR group by SNK test ($p = < 0.05$).

were seen in all groups with a similar incidence and severity.

Effects on Kidney

All groups, regardless of sex or diet, had more severe nephropathy at 106 wk than those reported at 52 wk (17). Male rats generally had more severe lesions than females. End-stage kidneys were seen in individual animals from all groups, particularly males. Glomerular changes were similar at 106 wk, but the extent of damage was more severe compared to 52 wk (17). The basement membrane of the capillary loops and Bowman's capsule were thicker at this time point, and a greater accumulation of mesangial matrix was present. Adhesions between the glomerular tuft and Bowman's capsule were more extensive than observed at 52 wk (17). BrdU labeling of the various cellular components of the glomerular tuft and metaplastic epithelium lining the Bowman's capsule were higher at 106 wk than reported at 52 wk (17).

Tubular and interstitial lesions were also more advanced at 106 wk with focal areas of tubular basement membrane thickening and infiltrates of inflammatory cells in the interstitium. Multifocal interstitial fibrosis and mixed cellular infiltrates occurred prominently throughout the cortex in all rats regardless of dietary group. BrdU-labeled cells occurred in the tubular epithelial cells as well as the mononuclear cells infiltrating the interstitium. BrdU labeling of the various cellular elements was more diffusely scattered throughout the cortex and outer stripe of the medulla.

The results of the stereological measurements at 106 wk of GA, the GSI, TII, and tubular BrdU % LI are shown in Table IV. The glomerular area was the largest for males of both AL groups. The 5002-9 DR males had a significantly lower glomerular area than other male groups. For female rats, the 5002 AL, 5002-9 AL, and 5002 6.5 hr DR groups had larger glomerular areas than the 5002-9 DR and 5002 DR groups, respectively. The 5002 AL males had the highest GSI that was statistically significantly different from the other groups. The 5002 6.5 hr DR males had the lowest GSI. The female 5002 AL, 5002-9 AL, and 5002 6.5 hr DR groups had the largest GSIs, and these were statistically greater than the 5002-9 DR and 5002 DR females. The TII for male rats of both AL groups were statistically greater than all of the restricted male groups, but for females, only the 5002 AL group differed from the 5002-9 DR group. Both AL groups, regardless of sex or diet, had larger tubular BrdU % LI than the restricted groups. Significant differences in BrdU % LI for males were noted between the 5002 AL and the 5002 DR, 5002 6.5 hr DR, and 5002-9 DR groups, whereas for females, the 5002 AL group was only different from the 5002 DR and 5002-9 DR groups (Table IV).

No differences in kidney GSH and MDA content at 106 wk were seen (data not shown).

The histological changes observed in the kidneys were more severe in all groups of rats at 106 wk compared to those reported at 52 wk (17), and all morphologic, stereological indices were increased at this time. The GSI and GA were best able to distinguish among the dietary groups. Both AL groups, regardless of diet and sex, had the greatest GSI and GA and the most severe histological changes. A cor-

TABLE IV.—Geometric means of the renal indices for male and female rat dietary groups at 106 wk.

Diet group[a]	GA ($\mu m^2 \times 10^4$)	GSI	TII	Tubular LI (%)
Male				
5002 AL	29.3[f]	140.7[cdef]	21.6[def]	5.1[def]
5002 6.5 hr DR	26.8[f]	66.2[bcef]	12.7[bc]	1.4[b]
5002 DR	26.2[f]	87.9[bd]	11.5[bc]	1.1[bc]
5002-9 AL	28.9[f]	102.0[bdf]	18.9[def]	4.1[e]
5002-9 DR	20.9[bcde]	80.4[bcd]	9.3[bc]	1.3[b]
Coefficient of variation	11%	20%	39%	94%
Female				
5002 AL	24.2[def]	104.3[ef]	16.5[f]	3.6[ef]
5002 6.5 hr DR	21.0[bef]	91.2[ef]	12.6	1.8
5002 DR	18.1[bcd]	72.4[bcd]	11.8	1.0[b]
5002-9 AL	22.9[ef]	102.4[ef]	11.5	2.1
5002-9 DR	19.1[bcd]	74.0[bcd]	10.5[b]	1.1[b]
Coefficient of variation	10%	18%	32%	77%

[a] Each group included 9–10 rats (except $n = 4$ for 5002 AL males).
[b] Statistically different from 5002 AL group by SNK test ($p = < 0.05$).
[c] Statistically different from 5002-9 AL group by SNK test ($p = < 0.05$).
[d] Statistically different from 5002 6.5 hr DR group by SNK test ($p = < 0.05$).
[e] Statistically different from 5002 DR group by SNK test ($p = < 0.05$).
[f] Statistically different from 5002-9 DR group by SNK test ($p = < 0.05$).

relation was made between GSI and GA, which showed that the AL groups of both sexes had the highest indices at this time point. The overall incidence and number of rats whose death was due to chronic nephropathy are presented in Table I of the companion paper of this study (27). The average histologic grades (0–5) of the rats examined from 53 wk onward for the 5002 AL, 5002 6.5 hr DR, 5002 DR, 5002-9 AL, and 5002-9 DR groups were 3.0, 1.3, 0.5, 2.1, and 0.4 for males, and 1.1, 0.4, 0.1, 1.4, and 0.2 for females, respectively. The average histological grades for chronic nephropathy were consistent with the quantitative indices shown in Table IV.

Effects on Estrous Cyclicity and Female Reproductive Tract

Table V illustrates the percentages of 99-wk-old females with estrous cycles characterized by vaginal cytology as regular 4–5-day cycles, cycles of irregular length, constant estrous cycles with persistent vaginal cornified cells (PVC), and cycles with predominant diestrous lavages (persistent diestrus).

At 99–100 wk of age, none of the females examined by vaginal lavage appeared to be anestrous. Regular 4–5-day cycles were seen in 1 of 30 females in the 6.5 hr DR group and 1 of 45 females in the 5002 DR group. In all groups, except the 5002-9 DR group, the majority of the females exhibited irregular estrous cycles or cycles with persistent diestrous lavages. In the 5002-9 DR group, constant estrous cycles of PVC were seen in 20 of 49 females. Constant estrous cycles were also seen in 5 of 30 females in the 5002 6.5 hr DR group, 1 of the 45 females in the 5002 DR group and 1 of the 24 females in the 5002-9 AL group. The overall results of the study suggest that females in the 5002 AL and 5002-9 AL groups were in later stages of reproductive senescence compared to the females in the 5002 6.5 hr DR, 5002 DR, or 5002-9 DR groups.

The indication that the AL fed females were in later stages of reproductive senescence was supported by the morphologic observations of the onset and incidence of degenerative lesions in the ovaries and uteri. While the AL females did not differ from the DR females in their incidence of ovarian or uterine tumors (27), the AL females did have a earlier onset of degenerative lesions, such as follicular cysts, ovarian atrophy, corpora lutea retention, and endometrial cystic hyperplasia and fibrosis, which was consistent with an earlier onset of reproductive senescence in the AL females.

Effects on Heart, Pancreas, Adrenals, and Other Organs

The incidence and severity of chronic cardiomyopathy seen morphologically as cellular infiltration, myocardial degeneration, and myocardial fibrosis was greater in the 5002 AL animals and a contributing factor to the mortality of the 5002 AL males (27). The average histologic grades (0–5) of the rats examined from 53 wk onward for chronic cardiomyopathy for the 5002 AL, 5002 6.5 hr DR, 5002 DR, 5002-9 AL, and 5002-9 DR groups were 1.3, 0.8, 0.3, 0.8, and 0.2 for males, and 0.3, 0.4, 0.1, 0.5, and 0.1 for females, respectively. In general, the grade of chronic cardiomyopathy correlated well with the increased heart and lung weights seen in the 5002 AL and 5002-9 AL groups compared to their DR counterparts (Table II).

TABLE V.—Estrous cycle patterns in 99-wk-old female rats.

Group	*n*	Regular estrus (%)	Irregular estrus (%)	Constant estrus (%)	Persistent diestrus (%)
5002 AL	27	0 (0)	11 (40.7)	0 (0)	16 (59.3)
5002 6.5 hr DR	30	1 (3.3)	12 (40.0)	5 (16.7)	12 (40.0)
5002 DR	45	1 (2.2)	24 (53.3)	1 (2.2)	19 (42.2)
5002-9 Al	24	0 (0)	8 (33.3)	1 (4.2)	15 (62.5)
5002-9 DR	49	0 (0)	18 (36.7)	20 (40.8)	1 (22.5)

The AL fed rats of both sexes had the highest incidence and severity of proliferative and degenerative changes in their pancreas, particularly islet fibrosis and acinar atrophy. In contrast, the overall incidence of islet cell tumors was similar between the AL and DR fed rats. However, the age-adjusted incidence of islet cell carcinoma was significantly higher in the 5002 AL males (27).

The adrenal glands of the 5002 AL females had the greatest severity of cortical cystic degeneration, although the incidence of this lesion was similar between the AL and DR groups. These degenerative changes tended to correlate with the increased adrenal weights noted in the AL animals (Table II). Both AL and DR groups had a similar incidence of adrenal cortical and medullary tumors, although the 5002 DR animals had slightly more tumors than their AL counterparts (27). However, these tumors did not differ in their age-adjusted incidence statistically. The adrenal tumors were usually incidental findings at necropsy, and the slightly higher incidence of tumors in the 5002 DR animals reflects their greater survival and, thus, their increased likelihood of developing these late-onset incidental proliferative changes.

The remaining tumors, proliferative lesions, and degenerative changes observed in the other organ sites in this study generally indicated that the 5002 AL animals had a higher incidence and/or severity of these changes. However, the overall and age-adjusted incidence of benign and malignant neoplasia was remarkably similar between the AL and DR groups (27).

DISCUSSION

Dietary factors are known to be causative determinants of chronic degenerative disease and cancer through studies of human pathology and epidemiology and have been shown to be the second major nongenetic external factors that contribute to human death in the United States, after tobacco use (15, 35, 43, 48, 58). Likewise, overfed, sedentary laboratory rodents also suffer early deaths from an early onset of diet-related degenerative disease and tumors (9, 10, 24–27, 38, 39, 41, 42, 56–58, 72, 73).

In contrast to the similar incidence of spontaneous neoplasia observed in this study between the AL and DR groups (27), there were clear differences in the onset, incidence, severity, and progression of renal changes observed in this study. The 5002 AL males had the earliest onset, highest incidence, and most severe chronic renal disease (CRD) and this was a common cause of death in this group (27). The DR rats fed either diet (5002 or 5002-9) had a lower incidence and severity of most of the morphologic changes, and severe CRD was not a significant contributing factor to early death in the DR fed animals (27).

These qualitative observations were stereologically quantified and the determination of GA, the GSI, the TII and the BrdU % LI support our recently proposed hypothesis for the pathogenesis of spontaneous CRD (17). Two hypotheses have been proposed by Brenner et al (3) and Fogo and Ichikawa (11) concerning the pathogenesis of CRD in rats. We proposed a modified hypothesis in which AL feeding leads to hemodynamic changes and hyperfiltration at the level of the glomerulus that result in damage and subsequent proliferation of mesangial, epithelial, and endothelial glomerular cells. This results in glomerular hypertrophy with continued accumulation of mesangial matrix, thickening of the glomerular capillary basement membrane, and endothelial cell damage. Eventually, glomerular sclerosis and functional loss of the entire nephron occurs. The nephron loss, in turn, stimulates a compensatory feedback mechanism that causes similar hemodynamic changes in another population of glomeruli. This cycle of hypertrophy, sclerosis, and nephron loss continues until it culminates in end-stage renal disease. Our hypothesis that glomerular hypertrophy leads to CRD was supported by the quantitative renal indices obtained in kidneys from the 52-wk interim necropsy (17). At 52 wk the GA was the most useful index to distinguish among the different dietary groups and indicated that the increase in glomerular size (glomerular hypertrophy) was the initiating event in the pathogenesis of CRD (17).

Evaluation of the glomerular indices at 106 wk indicated that the GA for males distinguished only the 5002-9 DR group, whereas GA for females distinguished all of the DR groups from their AL coun-

terparts. At 106 wk the AL groups of both sexes had the greatest GSI and the most severe glomerular histological changes. The renal lesions were much less severe in the females than in the males. Similar findings have been reported in experimental and spontaneous CRD models. In a renal ablation study, greater GSI were present at 21 wk postablation in AL fed rats than in DR groups (51). In spontaneous CRD, male rats fed for 6.5 hr during daylight had less severe glomerular sclerosis at 12 and 24 mo than AL fed rats (64). In F-344 rats studied until the time of spontaneous death, animals fed 60% of AL had no or minimal CRD at the time of death compared to that seen in AL fed rats (62).

A correlation was made between the GA and the GSI for all of the dietary groups at 52 and 106 wk. The 2 AL fed groups had the highest indices at both time points for both sexes. The results obtained at 106 wk indicate there is a progression of glomerular disease from an initial glomerular hypertrophy observed at 52 wk to greater proliferation, increased GA, and increased sclerosis (GSI). A similar progression of hypertrophic glomerular changes over time for CRD have been observed by others (6, 50). Their findings support the idea that there is a progression of glomerular hypertrophy to sclerosis and that animals with smaller glomerular areas at both 12 and 24 mo have less advanced renal disease (6, 50).

The progression from glomerular hypertrophy to glomerular sclerosis has also been shown in renal ablation models (13, 71, 76). These rapidly progressive renal ablation models of glomerular sclerosis have a negative correlation between glomerular size and the severity of glomerular sclerosis, with the severely sclerosed glomeruli having much smaller glomerular areas (76). This is probably due to the chronic compensatory nature of the spontaneous CRD that allows unaffected glomeruli to hypertrophy in compensation for those glomeruli that have sclerosed and decreased in area. Thus, the results obtained in studies of spontaneous CRD (6, 17, 50) describe a chronic compensatory disease that is slowly progressive as compared to the rapidly progressive changes observed in acute ablation models (13, 71, 76).

The degree of tubular and interstitial damage as indicated by the TII and the tubular LI also indicate more advanced CRD in the AL fed males at 106 wk consistent with results reported by others of spontaneous rat CRD (37, 64). For female rats in our study, the interstitial and tubular damage was increased only in the 5002 AL and the 5002-9 AL groups.

The low protein content of the 5002-9 diet (13.6%) may have provided a slight sparing effect on CRD, since the indices from the 5002-9 AL and DR groups were slightly lower than those obtained from the groups fed the 5002 standard diet. However, the differences between the renal indices of the 5002 AL and the 5002-9 AL groups at 106 wk were generally slight and only statistically significantly different for the GSI of male rats. The greatest benefit appears to be gained from caloric restriction and not protein restriction per se, since the modified 5002-9 diet was only associated with significant decrease in the qualitative and quantitative renal indices when fed in restricted manner. Other studies have reported similar findings. In a renal ablation model, end-stage renal disease after ablation was prevented by food restriction regardless of the protein content of the diet (70). In a study of spontaneous CRD in F-344 rats, animals that were protein restricted but not calorie restricted developed similar renal lesions as AL fed rats (37). Similarly, rats fed 20% protein diet in a time-restricted fashion developed less CRD than AL fed rats (37). The most convincing study demonstrating the relative importance of DR versus protein restriction was by Masoro et al (40) in which AL fed F-344 rats developed more severe CRD than DR groups even though the dietary protein intake per unit of body mass of the DR group was approximately 1.7 times that of the AL group. While several studies have shown that protein intake will affect the severity of CRD in rats (21, 52), the exact role of dietary protein in the pathogenesis of rat CRD remains unknown. A recent study of human patients with chronic renal disease demonstrated that a very-low-protein diet did not significantly slow the progression of renal disease (28).

The results obtained in the present study and our 52-wk data (17) demonstrate the temporal events in CRD. The initial pathologic event, glomerular hypertrophy, is thought to be stimulated by hemodynamic changes within the glomerulus, but as shown in remnant kidney disease models, glomerular hypertension alone is not sufficient to induce sclerotic changes of CRD (1, 13). It has been proposed that cellular damage may cause release of certain growth factors that locally stimulate mesangial, epithelial, and endothelial cell proliferation (11). Such a continuous proliferative response in the glomerulus was demonstrated in our study at both 52 and 106 wk with the observation of BrdU labeling in the various glomerular cell types. Similar proliferative events have been documented and quantitated in cell proliferation studies of glomerular mesangial and endothelial cells, as well as the parietal epithelial cells of Bowman's capsule (71). Such proliferative events have been shown to increase glomerular tuft volume without concomitant increase in visceral epithelial cells. This is thought to produce

an insufficiency in the filtration mechanism of the glomerulus leading to early proteinuria (13). Data from our 52-wk study did demonstrate greater protein excretion in the dietary groups with the greatest glomerular hypertrophy (AL groups) (17). Increased proteinuria is also found in renal ablation models in which the glomeruli are considered to be free of initial disease (1, 71, 77). Glomerular hypertrophy and protein loss are thought to contribute further to mesangial matrix accumulation, proliferation of mesangial cells, a thickening of the basement membrane, and endothelial cell damage. These events result in glomerular sclerosis. This disease progression is consistent with the observations in the present study that the AL groups that had the greatest glomerular hypertrophy at 52 wk developed sequentially the greatest glomerular sclerosis and overall renal disease by 106 wk. In this study the relative incidence of BrdU labeling in the glomerular cells was increased, reflecting continual mesangial and endothelial cell proliferation. In addition, there was greater nephron loss and interstitial damage as indicated by increased TII and tubular LI in the AL groups at 106 wk. As suggested by Remuzzi and Bertani (55), this may be due to tubular damage secondary to increased proteinuria.

These observations support our hypothesis that initial glomerular hypertrophy leads to glomerular sclerosis and, subsequently, tubular and interstitial damage in the progression of CRD. This study provides strong evidence that caloric restriction, not protein restriction, is primarily responsible for causing a decrease in glomerular disease and retards the progression of spontaneous CRD. These data support our hypothesis that caloric restriction is the most important factor in delaying the progression of glomerular sclerosis and nephron loss initiated by the early development of glomerular hypertrophy in AL overfed rats.

After early lethal pituitary tumors and chronic renal disease, the third most significant factor contributing to mortality in the 5002 AL males was chronic cardiomyopathy (27). This condition, which is manifest by various combinations of myofiber degeneration, mononuclear cell infiltration, and myocardial fibrosis, was seen with the earliest onset and highest incidence and severity in the 5002 AL and 5002-9 AL males (27). These same groups also had the largest hearts and lungs at 106 wk. All of the DR groups fed either diet had smaller hearts and lungs and a lower incidence and severity of cardiomyopathy. These results correlated closely with those reported in our 52-wk interim necropsy (25). Cardiomyopathy is common in most strains and stocks of laboratory rats and increases in severity and incidence with age (36). DR in other rat strains and stocks partially protects against cardiomyopathy, although the mechanisms of this protection have not been well studied (7, 66).

While no difference was seen in the age-adjusted incidence of hepatocellular or biliary tumors in any group compared to the 5002 AL group (27), the proliferative and degenerative changes seen in the livers in this study indicate that DR rats fed either diet were better able to withstand the long-term metabolic load and oxidative damage of AL overfeeding. The onset, incidence, and severity of bile duct hyperplasia, periportal hepatocellular vacuolation, telangiectasis, and related degenerative changes were greater in AL fed rats given either diet compared to their DR fed counterparts. In response to the increased total food intake per rat overfed either diet AL, liver size was greater in both AL fed groups expressed as relative (% of brain weight) 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 the hepatocyte BrdU % LI were not statistically different among the AL and DR groups. These data demonstrate that the AL overfed, overweight rats developed a greater liver mass with more evidence of hepatocellular degeneration than their DR counterparts. The only exception to this general observation was the 5002-9 DR rats fed the low protein, low energy, high fiber diet. These animals had the smallest livers with least evidence of damage, but relatively high BrdU % LI and total labeled hepatocytes per liver. This observation was not statistically significantly different from the other groups at this time point; however, a similar observation was made at the 52-wk interim necropsy that was statistically significant (25). These observations indicate that increased hepatocellular DNA synthesis per se or increased liver size per se in the absence of other biochemical or pathological changes do not necessarily indicate an increased risk for hepatocellular carcinogenesis.

Data from SD rats sampled at 52 wk indicate that DR rats are better able to withstand spontaneous oxidative injury than AL rats. At 52 wk, the mean hepatic GSH content of males from all DR groups was higher than that of livers from either AL group (25). This difference was not seen at 106 wk. While a similar difference in GSH content was not observed at 52 wk in females, the hepatic MDA content in DR females of all groups was lower than that of the 5002 AL group by 24–45% for the 5002 DR rats and by 33% versus the 5002-9 AL group levels for the 5002-9 DR rats (25). While these differences were not seen at 106 wk, the changes noted at 1 yr do indicate that the livers of dietary-restricted SD rats are better able to defend against oxidative injury

at this earlier time point and are consistent with studies in F-344 rats under DR (29, 33, 34, 38, 39, 41, 42, 73, 74). These and other observations are consistent with the free radical hypothesis of aging (18, 73). This hypothesis views aging as a result of a continuous oxidative damage inherent from basic metabolic processes and suggests that DR protects the organism from this damage by maintaining the integrity of cellular structure and function into advanced age. In studies of F-344 rats, a 40% DR modulates free radical production, scavenger enzyme activities, free radical damage, and the detoxification of products of free radical injury (18, 29, 33, 34, 67, 74). GSH levels fall with age in AL fed but not DR fed F-344 rats and DR influences the metabolism of MDA, a product of lipid peroxidation from free radical damage (29, 33, 34, 74). These and other mechanisms may result in the ability of DR to prevent the age-associated accumulation of lipofuscin and related substances in the livers of aging animals and reflects the general delay in degenerative changes in the livers of animals maintained by DR (22).

The evaluation of estrous cyclicity in 99–100-wk-old females is consistent with several studies in the literature that suggest reproductive senescence is delayed in moderately dietary-restricted females (5, 16, 46). None of the females in any of the AL or DR groups appeared to be anestrous at 99–100 wk of age by examination of vaginal lavages. The results suggested that females in the 5002 AL and 5002-9 AL groups were in later stages of reproductive senescence compared to females in any of the DR regimens. Rats are a polyestrous species with cycle lengths of approximately 4–5 days. In general, regular estrous cycles are established by 2 mo of age. Between 2 and 12 months most female rats display regular estrous cycles of 4–5 days duration. During this period, interruption in these cycles into a persistent diestrus phase generally occurs only if the rat is in gestation, postpartum lactation, or pseudopregnancy. Decreasing frequency and increasing variability in estrous cycles are characteristic of age and decline of reproductive function in female rats. Although generalizations can be made as to the onset and patterns of the changes accompanying reproductive senescence, it is recognized that there is considerable variation, not only between rodent species but also within the strains and stocks of the same species. There are no published data that address specifically the chronological patterns of reproductive senescence in this stock of SD rat.

Transition from regular estrous cycles to a final state of anestrous begins about 12 mo of age. At this time the regular 4–5-day cycles gradually lengthen and become interspersed with shorter cycles. Most rats then enter a state of PVC associated with anovulatory polyfollicular ovaries, moderate sustained estrogen secretion, low progesterone levels, and impaired luteinizing hormone surges. PVC or a constant estrous period lasts approximately 1–6 mo and can be followed by a 1–4-mo interval of irregular, repetitive pseudopregnancies (RPP). During RPP, cycles of 10–14 days in length are seen interspersed with cycles of shorter duration. The cycles of 10–14 days duration are characterized by predominant diestrous lavages. Although the causes for resumption of ovulatory activity in aging rats is unknown, the prolonged maintenance of corpora lutea may be the result of high serum prolactin levels produced by prolactin-secreting pituitary tumors (45, 61, 68). The final stage of reproductive senescence, persistent anestrus, marks the cessation of ovarian endocrine activity and is characterized by the presence of sparse numbers of leukocytes and occasional epithelial cells in the vaginal lavages. The data presented in Table V show the number and percentages of females with estrous cycles characterized by vaginal cytology as regular estrus, irregular estrus, constant estrus, and persistent diestrus. Because estrous cycles were monitored for only 15 days starting at 99 wk of age and because of the inherent variability of cycles during aging, it is not possible to distinguish conclusively the stages of reproductive senescence in the females at this time of the study. However, in the 5002-9 DR group the data strongly suggest that senescence was delayed compared to the 5002-9 AL group. At 99 wk of age the females in the 5002-9 DR group had a lower mean body weight, a lower body fat content at 106 wk and a higher survival than their AL counterparts. Forty percent of the 5002-9 DR females appeared to be in an earlier stage of senescence (constant estrus) and 22% exhibited persistent diestrus lavages. At the same age the 5002-9 AL females had higher mean body weights, higher body fat content at 106 wk and a lower survival. Only 4% of the 5002-9 AL females were in constant estrus, while 63% exhibited persistent diestrus. In the 5002 groups, the results were less easy to interpret. However, the data suggest that the 5002 AL females with even larger body size, greater body fat content, and lower survival were further along in the reproductive aging process as compared to the 5002 6.5 hr DR and 5002 DR groups. None of the 5002 AL females had regular 4–5-day cycles and 58% had persistent diestrus lavages compared to 40% and 42% in the 5002 6.5 hr DR and 5002 DR females, respectively.

The delay in reproductive senescence in DR fed females is believed to be the result primarily of re-

tarded aging of the hypothalamic–pituitary axis compared to the AL fed females (2, 5, 14, 16, 45, 46, 61, 68). These data are consistent with the observation of the early onset of prolactin-secreting pituitary tumors in the AL females in this study and the early onset of palpable mammary tumors in the 5002 AL and 5002-9 AL groups compared to their DR counterparts (27). It is also known that overfed obese individuals are associated with reproductive malfunctions, the severity ranging from menstrual irregularities in women (14) correctable by weight reduction, to sterility in some types of obese rodents (2). Hypothalamic and genetic obesities in rodents are usually associated with reproductive impairments, but the underlying cause of these changes is not clear because concomitant metabolic abnormalities occur in these animal models (2). In studies of metabolically intact Wistar rats, AL overfeeding resulted in obesity and an increased brown adipose tissue thermogenesis in association with long estrous cycles accounted for by a long diestrus phase (16). These cyclic irregularities do not appear to result from weight gain per se, but were associated with an increase in endogenous heat production induced by overeating. These data lead to a hypothesis that an optimal level of endogenous heat production is necessary for normal reproductive function and that an increase in dietary thermogenesis induced by overfeeding impairs these functions (16).

Although it is well known that severe food restriction (starvation) of growing animals will delay puberty and sharply impair rat reproduction, the effects of moderate DR on adult animals have been less well studied (46). DR that increases life span by 50% and delays body weight gain will prolong reproductive life by 50% by slowing the loss of oocytes. However, ovulation rates are reduced and litter size is decreased (46). Data from a recent study of adult SD rats that were moderately food restricted indicated that this treatment had no effect on male or female fertility or on the total number of implants per dam and suggest that the SD rat is largely resistant to adverse reproductive changes caused by moderate feed restriction (5). These data and results from our own study indicate that AL overfeeding accelerates reproductive senescence while moderate DR delays reproductive senescence in female SD rats (5, 16, 46).

Whereas both genetic and environmental factors are involved, it is clear that low rat survival is closely correlated with AL overfeeding and can be improved by simple moderate dietary (caloric) restriction as practiced in the above studies. Our laboratory has undertaken studies of dietary (caloric) restriction with the SD rat and has shown that this method will improve survival and lower the incidence and severity of diseases associated with overfeeding. This method does not adversely affect the health, physiology, or metabolic profile of the rat and, thus, improves the animal as a model to test human safety. We have also studied the 5002-9 modified diet and found no survival benefit if this diet was fed AL. These data demonstrate the complex multisystemic mechanisms induced by AL overfeeding and underline the healthful actions of moderate DR on SD rat survival, spontaneous tumors, age-related degenerative disease, and reproductive senescence.

These data are consistent with the observations of others over the past 4 decades that reducing energy intake by caloric restriction will increase the maximum life span, retard age-related senescence and degeneration, and delay or prevent the appearance of age-related diseases and tumors (9, 10, 38, 39, 41, 42, 56, 57, 72, 73). Although the mechanisms underlying these effects are not completely understood, our data support several of the most widely held hypotheses for which there are scientific data supporting the observation that caloric restriction acts by modulating the characteristics, but not the rate, of fuel use in such a fashion as to prevent long-term damage of such fuel use through oxidative damage or glycation (26, 41, 72, 73). It is apparent that moderate caloric restriction is affecting survival primarily through its action on fuel utilization by preserving the protective molecular mechanisms against oxidative damage and glycation resulting from such fuel use (26, 41, 72, 73). Conversely, our present methods of AL feeding (overfeeding) rodents apparently accelerates oxidative and glycation damage as manifested by lower survival and the acceleration of the onset and severity of age-related degenerative diseases and tumors. Our ongoing studies of moderate dietary (caloric) restriction should result in a more appropriate rodent model for long-term toxicity and carcinogenicity studies to assess the human safety of candidate pharmaceuticals.

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