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ORIGINAL ARTICLES

The effects of different levels of dietary restriction on aging and survival in the Sprague-Dawley rat: Implications for chronic studies

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ABSTRACT. A study was undertaken to determine the effects of incremental levels of dietary restriction (DR) in rats. Survival, growth, reproductive, and dietary intake (DI) variables were monitored in a chronic study in which male Sprague Dawley (SD) rats (NCTR colony) were fed their ration ad libitum (AL), or DR. The main objectives were to determine if low levels of DR could be used to increase the survival rate of SD rats in the chronic bioassay, and to identify the survival characteristics of a long-lived SD rat strain (NCTR colony). The average life span of AL rats was 115 months. At 104 weeks on study (110 weeks of age), the survival rate for the AL and 10%, 25%, and 40% DR groups was 63.4, 87.5, 87.5, and 97.5%, respectively. The largest increase in survival (24.1%) occurred between AL and 10% DR, indicating that very low levels of DR have a significant effect on survival. Whole-body, liver, prostate, and epididymis weights and body length were decreased by DR, whereas brain weight, testicular weight, and skull length were not altered by DR. Rats from the NCTR colony were found to be ideal for chronic studies because they are much longer-lived than other SD stocks. Although the 104-week survival rate for these SD, non-obese AL rats exceeds the FDA's "Redbook" survival guideline (> 50%) for chronic bioassays, the use of DR is advocated because it reduces individual variability in body weight.
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INTRODUCTION

Recently, there has been considerable interest in the scientific community as to how dietary restriction (DR) modulates biological processes related to disease and drug toxicity. Although little is known about the effects of chronic and acute DR in humans, a wide array of rodent studies indicates that reduced energy (calorie) intake alters a number of key biological processes in such ways as to promote good health, decrease disease, and increase longevity (1-7). Numerous studies have shown that DR decreases the carcinogenic capacity of a number of well-studied carcinogens (8, 9). In other studies, the relative toxicity and mortality associated with certain prescription drugs was found to be reduced significantly by DR (10, 11).

The results of recent studies show that 24-month survival of male Sprague-Dawley (SD) rats varies significantly among different studies from as low as 7% in one study (12) to 39% in another (13). The steady decline in survival over several decades is inversely correlated with increased body weight in these species, possibly resulting from genetic drift, selective breeding, and a number of animal husbandry practices such as the level of dietary intake (DI), cage and feeder design, etc. The poor survival rate of standard rodent species (< 50%) in chronic 24-month bioassays has forced some regulatory agencies to scrutinize the validity of carcinogenicity studies. The recommendations of the National Cancer Institute (1976) and the National Toxicology Program (1984) clearly stipulate that rodent carcinogenicity studies

Key words: Aging, chronic, dietary restriction, survival, rat.

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should be 24 months in duration. The Food and Drug Administration's (FDA) official "Redbook" guideline, which is widely accepted among the toxicological community, clearly defines the 24-month survival criterion. In an effort to conform to these guidelines, some pharmaceutical companies, as well as toxicologists from government and academia have roused considerable interest in using DR as an effective tool to solve the problem of declining survival in populations of SD and Fischer 344 laboratory rats, and the B6C3F1 mouse during two-year chronic bioassays (12-17).

The primary purpose of the chronic bioassay is to detect an increase in drug-induced carcinogenesis. Therefore, scientists argue that excessive obesity in control rodent populations causes them to be extremely susceptible to early-onset pathologies such as cardiomyopathy, chronic renal disease, and acute infectious diseases that are unrelated to age-dependent tumors (1). Furthermore, the results obtained from morbidly obese rodents may be difficult to apply to human clinical trials since excessively obese human subjects are rarely used for normal population studies.

To date, most of the DR studies related to aging, disease, and drug toxicity have employed high (40%) to moderate (25%) levels of DR (10-17). Few studies have been conducted to determine the "dose response" relationship between DR and survival using DR feeding regimens as low as 10% (18, 19). Additionally, there is no consensus as to what level of DR is optimal for chronic bioassays. The bewildering array of different DR regimens, species, strains, and dietary compositions that were used in previous studies makes it very difficult to standardize experimental conditions, and to compare the results among different studies and laboratories.

The primary purpose of this study was to determine the long-term survival characteristics of male SD rats that were conditioned to four different levels of DI, comprised of a control group that was fed AL, and three different DR groups (10%, 25%, and 40% reduction in total food intake). Another major objective was to determine if incremental amounts of DR would alter the 24-month survival rate of rats in a linear fashion. The SD rat was chosen because this strain has been routinely used by the pharmaceutical industry for drug testing purposes for decades, and hence a large database has been developed over the course of numerous studies. The NIH-31 cereal-based diet was chosen because previous studies in our own lab have clearly demonstrated that this formulation allows for excellent survival characteristics (10, 11, 19-21), and because it was accepted by the nutrition community as a standardized diet. The results of this study will be used to determine if DR can be appropriately used in chronic studies and, if so, what level of DR is most appropriate. A third objective of this present study was to identify a long-lived subpopulation of rodents that can be used, with or without DR, to meet the survival requirements for chronic bioassay studies.

MATERIALS AND METHODS

Animal husbandry and feeding regimens

The animal husbandry procedures used in this study have been reported previously (20, 21). The original founder stocks of SD rats [CRl:CD®(SD)BR] were obtained from Charles River Laboratory in 1972, and the subsequent breeding colony stock has been bred and maintained in a specific pathogen-free environment at the National Center for Toxicological Research (NCTR).

The male SD rats that were used in this study were maintained at 23°C, and the test subjects were conditioned to a 12-hour light/12-hour dark photoperiod cycle with lights on from 06:00 to 18:00 hrs daily. An AL feeding regimen was used for all animals from the time they were weaned until the time they entered the experimental protocol. All animals were singly housed in standard rat cages with metal lids, fed the NIH-31 diet AL, and given water AL. AL rats were fed the standard formulation of NIH-31 diet, and DR rats were fed a modified formulation that contained 1.67 x more vitamins to ensure adequate vitamin supplementation for the most restricted rats.

At 6 weeks of age, the rats were separated into four groups, consisting of a control group which continued to receive food AL, and three DR groups which were assigned to intakes that were 90%, 75%, and 60% of the AL level of DI. All rats were fed at 10:00 hrs daily, which corresponded to 4 hours after the onset of lights-on. The highest (40%) level of DR was utilized in this experiment so that the results from the present study could be compared with those from previous experiments in which the same level of DR successfully supported extended longevity in rodents (20, 21). The 25% level of DR was chosen because this regimen has been promoted and used by the pharmaceutical industry for chronic bioassay studies (12-14, 22). The 10% level of DR was selected because little is known about the effects of a small reduction in calories on life span.

Four different studies were conducted in which the nutritional groups were maintained on their respective rations for periods of 6, 12, or 24 months prior to sacrifice. The sample sizes for the various experiments and nutritional groups are given in Table 1. One month prior to the end of the various studies, all of the surviving rats were transferred to a separate animal room where they were allowed to adapt to a reversed light-dark cycle. During this time, the rats were conditioned to a 12-

Table 1 - *Male Sprague-Dawley Rat Protocol (NIH-31 Diet).*

Nutritional groups	Duration of dietary regimen Total no. of animals sacrificed at: 6 months	1 year	2 years
AL	40[a]	46	60
10% DR[b]	40	46	58
25% DR	40	44	54
40% DR	40	42	50
			560 animals total

[a]Number in each cell signifies sample size for each group, 10-16 animals from each group were sacrificed at four different circadian stages (3 HAF, 8 HAF, 14 HAF, 20 HAF).
[b]Percentages = % reduction in calories compared to AL.
HAF: hours after feeding; AL: *ad libitum*; DR: dietary restricted.

hour light/12-hour dark photoperiod cycle with lights on from 02:00 to 14:00 hrs daily. They were fed at 14:00 hrs, at the onset of the dark period, so that the feeding cycles and circadian rhythms for the AL and DR groups could be closely synchronized.

Animal sacrifice and experimental procedures

At the end of each experiment (6, 12, or 24 months), subsets of each of the four DI groups (AL, 10% DR, 25% DR, and 40% DR) were quietly removed from the animal room at one of four different circadian stages (06:00, 11:00, 15:00, and 23:00 hrs) and taken to an adjacent room where they were humanely sacrificed by decapitation. The various internal organs were rapidly removed and weighed on a digital balance, and head and body length (tail not included) measurements were also made and recorded. Prostate, left epididymis, and left testis weights were measured in the 6-month group only to determine the effect of DR on male reproductive potential. Whole left testis and epididymus were dissected. Additionally, the ventral prostate was dissected from the anterior to the pubic symphysis junction. The organs were then wrapped in aluminum foils and frozen in liquid nitrogen. The specific biochemical analyses of these tissues will be reported elsewhere. Although gross necropsies and histopathologies were not performed at this phase of the experiment, any unusual signs of pathology were noted and recorded at this time.

Comparisons of various anatomical parameters were made among the various DI and age groups using a two-way analysis of variance (ANOVA) where age, dietary DI, and age by DI interactions were the primary factors to be analyzed. The coefficient of variation (CV) for body weight was determined for the various DI and age groups, and the results were analyzed using ANOVA to determine differences in variability. Multiple comparisons of means were made using a Tukey-Kramer adjustment. Orthogonal contrasts were constructed to examine linear trends with age and DI. The linear trend in survival with dose was tested using Tarone's procedure which is a modification of the log-rank test for a dose effect in survival (23).

Additionally, Kaplan-Meier survival curves were plotted and pair-wise comparisons, testing the homogeneity of the survival curves among the various nutritional groups, were performed using log-rank statis-

Table 2A - *Summary of survival data for male SD rats.*

Time	AL	10% DR	25% DR	40% DR
Survival at 52 weeks on study (58 weeks of age)	96.6%	95.0%	92.5%	100%
Survival at 104 weeks on study (110 weeks of age)	63.4%	87.5%	87.5%	97.5%
Survival at 108 weeks on study (114 weeks of age)	51.7%	87.5%	85.0%	95.0%
Survival at 112 weeks on study (118 weeks of age)	40.0%	–	–	–

Table 2B - *Effect of dietary restriction in male Sprague-Dawley rats: Probability of survival at 104 weeks on study.*

Comparison (pair-wise)	Change in survival	Odds ratio	95% CI
AL to 10% DR	increase	8.03	[6.70, 9.66]
AL to 25% DR	increase	5.67	[4.75, 6.78]
AL to 40% DR	increase	17.47	[14.07, 21.89]
10% DR to 25% DR	none	0.95	[0.71, 1.24]
10% DR to 40% DR	increase	2.18	[1.71, 2.79]
25% DR to 40% DR	increase	2.32	[1.74, 3.08]

AL: *ad libitum;* DR: dietary restricted; CI: upper and lower confidence intervals.

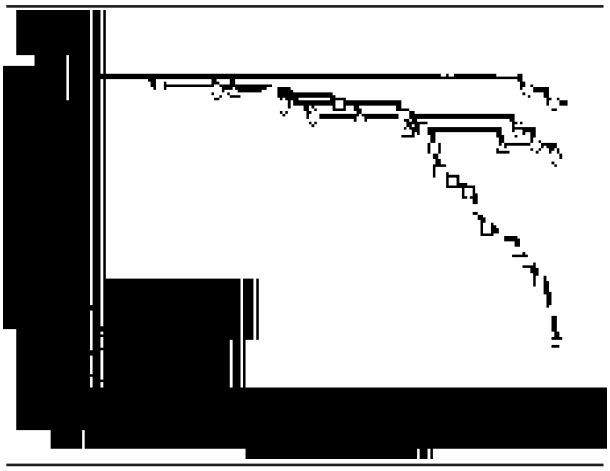

Figure 1 - *Kaplan-Meier curves representing the probability of survival in ad libitum (AL) and 10%, 25%, and 40% dietary restricted (DR) male SD rats.*

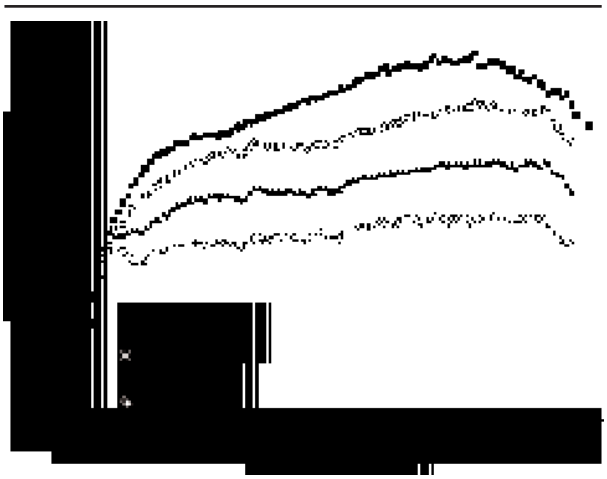

Figure 2 - *Body weight in ad libitum (AL) and 10%, 25%, and 40% dietary restricted (DR) male SD rats.*

tics. The results of the various statistical tests were considered to be significant when *p*-values were <0.05.

RESULTS

Survival data

A summary of survival data for male SD rats at various levels of DR is provided in Table 2A, and weekly changes in mortality throughout the study are shown in Figure 1. Survival at 104 weeks on study (110 weeks of age) was found to be 63.4%, 87.5%, 87.5%, and 97.5% for the AL, 10% DR, 25% DR, and 40% DR groups, respectively. Survival at 114 weeks of age was 51.7%, 85%, 87.5%, and 95% for the AL, 10% DR, 25% DR, and 40% DR groups, respectively. Survival at 118 weeks of age was 40.0% for the AL group. Additionally, the average life span of AL rats was 115 months. The effect of the level of DR on the probability of survival at 104 weeks on study is given in Table 2B for SD rats. The probability of survival increased as the level of food consumption decreased for all pair-wise comparisons with the exception of the 10% DR to 25% DR comparison which was not significantly different. Comparisons of survival curves for AL and various groups of DR rats are given in Table 2C. The pair-wise comparisons of survival curves for AL and 10% DR, AL and 25% DR, and AL and 40% DR were significantly different. All other pair-wise comparisons were not significantly different. Additionally, when all four DI groups were analyzed together, a significant diet effect was found ($p<0.0001$), and there was a significant linear (positive) trend between survival and level of DR ($p<0.0001$).

Growth and food intake data

Body weight. Weekly changes in mean body weight throughout the study are shown in Figure 2 for SD rats at the various levels of DI. Additionally, body weight data are compared in Table 3A for SD rats at 6, 12, and 24 months on study. There was a significant decrease in body weight at a given age group (6, 12, or 24 months) as the level of DR increased. In addition to a

Table 2C - *Comparison of survival curves for male Sprague-Dawley rats.*

Comparison (pair-wise)	Change in survival	Significance (*p*-value)
AL to 10% DR	increase	< 0.01
AL to 25% DR	increase	< 0.001
AL to 40% DR	increase	< 0.0001
10% DR to 25% DR	none	NS
10% DR to 40% DR	none	NS
25% DR to 40% DR	none	NS

AL: *ad libitum*; DR: dietary restricted; NS: not significant.

significant overall DI effect and DI effects among all of the individual age groups for body weight, there was also a linear trend with DI within all age groups. Since body weight increased with age, there was a significant overall age effect, a significant age effect for all individual DI groups, and a linear trend with diet intake for all of the DI groups. Additionally, there was a DI by age interaction for body weight.

Body weight CV data for the various DI and age groups are compared in Table 3B.

There was a significant overall age effect, DI effect, and DI by age interaction for the variability in body weight as measured by CV. Although body weight CV was smallest in the 40% DR and 25% DR groups, a significant reduction also occurred between AL and the lowest level, 10% DR.

Diet consumption and caloric intake. Weekly patterns of diet consumption at different ages throughout the study are shown in Figure 3 for male SD rats from the various DI groups. Additionally, food consumption and caloric intake data are given in Table 3A for SD rats at 6, 12, and 24 months on study. The amount of energy (kilocalories) consumed per gram of body weight is shown in Figure 4 for male rats from the different age and DR groups. Although the total calories consumed were progressively reduced by increasing the level of DR, there was no significant difference in the caloric consumption per gram of body weight among any of the DI groups between 26 and 114 weeks of age. However, at the onset of DR, the energy intake per gram of body weight was significantly lower in the DR groups than in the AL group at the earliest ages (7-14 weeks).

Brain weight. Mean values and standard errors for various organ weights, which are parameters that accurately characterize growth potential, are given in Table 4 for the various DI groups of SD rats at 6, 12, and 24 months on study. Unlike body weight, brain weight was unaffected by various levels of DR. Therefore, there was no significant overall DI effect, no DI effect for any individual age groups, and no overall linear trend with DI. However, there was a significant overall age effect, a significant age effect for the 10% DR group, and a significant overall linear trend with age. There appeared to be no DI by age interaction for brain weight.

Liver weight. The effects of DI and age on liver weight (Table 4) were similar to those seen in body weight. Since liver weight decreases with increasing levels of DR, there was a significant overall DI effect, a significant DI effect for all individual age groups, and a significant overall linear trend with DI. Also, there was a significant overall age effect, a significant age effect for all individual DI groups, and a significant overall linear trend with age. However, no significant DI by age interaction was found for liver weight.

Brain/Body weight ratio. The brain/body weight ratio (Table 4) was found to increase with increasing levels of DR. There was a significant overall DI effect, a significant DI effect for all individual age groups, and a significant overall linear trend with DI. Also there was a significant overall age effect, a significant age effect for the 10%, 25%, and 40% DR groups, and a significant overall linear trend with age. As was seen in body weight, there was a significant DI by age interaction for the brain/body weight ratio.

Liver/Body weight ratio. The liver/body weight ratio tended to decrease slightly with increased levels of DR. There was a marginally significant overall DI effect and a significant DI effect for the 6-month group

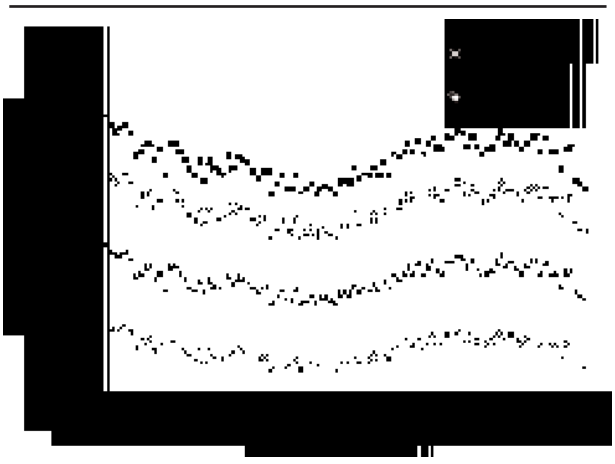

Figure 3 - *Food consumption in ad libitum (AL) and 10%, 25%, and 40% dietary restricted (DR) male SD rats.*

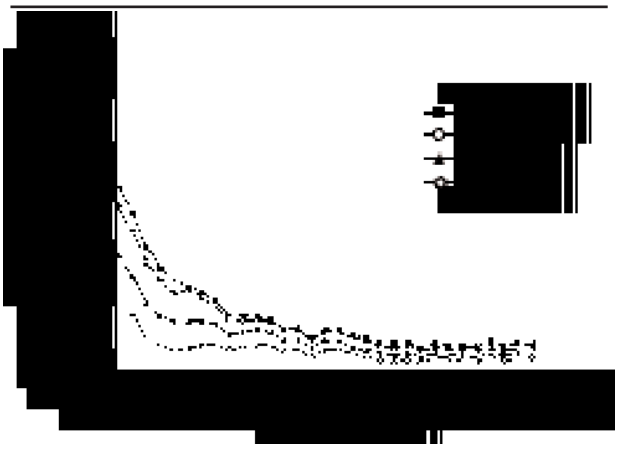

Figure 4 - *Energy consumption (kilocalories) per gram of body weight in ad libitum (AL) and 10%, 25%, and 40% dietary restricted (DR) male SD rats.*

Table 3A - *Body weight and food consumption at various time intervals on study.*

	AL Mean ± SD		10% DR Mean ± SD		25% DR Mean ± SD		40% DR Mean ± SD	
Body weight (g)[a,b,c]								
6 months	625.7^{w}	55.6	542.4^{dx}	43.2	472.7^{dy}	21.1	400.8^{z}	20.7
12 months	610.0^{w}	68.7	595.4^{ew}	43.1	519.0^{ex}	22.5	422.4^{y}	18.6
24 months	656.7^{w}	76.9	601.7^{ex}	53.3	524.9^{ey}	38.6	416.6^{z}	27.4
Food intake (g/day)[b]								
6 months	24.5^{dw}	2.2	22.1^{dx}	–	18.4^{dy}	–	14.7^{dz}	–
12 months	22.9^{ew}	2.2	20.6^{ex}	–	17.2^{ey}	–	13.7^{ez}	–
24 months	25.1^{dw}	2.8	23.2^{dx}	–	19.4^{dy}	–	15.5^{dz}	–
Food intake (g)/body weight (g)								
6 months	0.040^{dw}	0.007	0.039^{dwx}	–	0.037^{dxy}	–	0.036^{dy}	–
12 months	0.034^{ew}	0.003	0.034^{ew}	–	0.033^{ewx}	–	0.031^{ex}	–
24 months	0.037^{dew}	0.004	0.036^{ex}	–	0.034^{ex}	–	0.033^{ex}	–
Caloric intake[b] (kcal/day)								
6 months	88.9^{dw}	8.0	80.2^{dx}	–	66.8^{dy}	–	53.4^{dz}	–
12 months	83.1^{ew}	8.0	74.8^{ex}	–	62.4^{ey}	–	49.7^{ez}	–
24 months	91.1^{dw}	10.2	84.2^{dx}	–	70.4^{dy}	–	56.3^{dz}	–
Caloric intake (kcal)/body weight (g)								
6 months	0.146^{dw}	0.012	0.142^{dwx}	–	0.135^{dxy}	–	0.130^{dy}	–
12 months	0.122^{ew}	0.012	0.125^{ew}	–	0.120^{ewx}	–	0.114^{ex}	–
24 months	0.133^{dew}	0.015	0.129^{ex}	–	0.125^{ex}	–	0.121^{ex}	–

[a] Significant age effect ($p<0.05$), [b] significant diet effect ($p<0.05$), [c] significant age by diet effect ($p<0.05$).
[def] Means in the same column across age groups with different superscripts are significantly different ($p<0.05$).
[wxyz] Means in the same row across diet groups with different superscripts are significantly different ($p<0.05$).
AL: *ad libitum*; DR: dietary restricted.

only. Also, there was a significant overall linear trend for liver/body weight ratio. The liver/body weight ratio decreased with age especially in the 25% and AL groups. Therefore, there was a significant overall age effect, a significant age effect for the 25% DR and AL groups, and a significant overall linear trend with age. Additionally, there was a significant DI by age interaction for liver/body weight ratio.

Reproductive variables. Prostate weight (Table 4) was decreased by DR, and was found to be statistically different ($p<0.05$) among the DI groups, with the exception of the 10% DR and 25% DR comparison. Similarly, left epididymis weight (Table 4) was decreased by DR, and was found to be significantly different among the DI groups, with the exception of the AL to 10% DR and the 10% DR to 25% DR com-

Table 3B - *Variability in body weight: comparison of various time intervals on study.*

Body weight variability (CV)	AL Mean ± SD		10% DR Mean ± SD		25% DR Mean ± SD		40% DR Mean ± SD	
Age groups								
6 – 58 weeks	9.1^{aw}	0.6	8.0^{x}	0.5	5.1^{y}	0.4	5.7^{z}	0.8
59 – 114 weeks	11.6^{bw}	0.9	8.0^{x}	0.6	5.0^{y}	0.8	5.1^{y}	0.5
6 – 114 weeks	10.2^{cw}	1.0	8.0^{x}	0.6	5.0^{y}	0.7	5.4^{y}	0.6

CV: coefficient of variation: (body weight standard deviation / mean body weight) x 100.
[abc] Means in the same column across age groups with different superscripts are significantly different ($p<0.05$).
[wxyz] Means in the same row across diet groups with different superscripts are significantly different ($p<0.05$).
AL: *ad libitum*; DR: dietary restricted.

Table 4 - *Organ weights and body length at various time intervals on study.*

	AL Mean ± SD		10% DR Mean ± SD		25% DR Mean ± SD		40% DR Mean ± SD	
Brain weight (g)[a]								
6 months	2.08	0.10	2.09^{de}	0.11	2.09	0.13	2.09	0.13
12 months	2.10	0.19	2.05^{d}	0.17	2.08	0.13	2.07	0.21
24 months	2.11	0.10	2.17^{e}	0.13	2.12	0.13	2.10	0.13
Liver weight (g)[a,b]								
6 months	18.38^{w}	1.02	15.27^{x}	1.02	13.81^{x}	1.02	10.74^{y}	1.02
12 months	17.98^{w}	1.04	16.48^{w}	1.02	14.44^{x}	1.02	11.26^{y}	1.02
24 months	17.00^{w}	1.03	16.28^{w}	1.02	13.19^{x}	1.02	11.04^{y}	1.02
Brain weight (g)/body weight (g)[a,b,c]								
6 months	0.0033^{w}	0.0003	0.0042^{dx}	0.0004	0.0044^{dy}	0.0002	0.0052^{z}	0.0003
12 months	0.0034^{w}	0.0003	0.0034^{ew}	0.0005	0.0040^{ex}	0.0003	0.0049^{y}	0.0005
24 months	0.0032^{w}	0.0005	0.0036^{dew}	0.0003	0.0040^{ex}	0.0005	0.0050^{y}	0.0005
L Testis weight (g)								
6 months	1.78^{w}	0.29	1.78^{w}	0.21	1.75^{wx}	0.19	1.70^{x}	0.16
L Epididymis weight (g)[b]								
6 months	0.75^{w}	0.15	0.70^{wy}	0.08	0.66^{xy}	0.09	0.63^{x}	0.06
Prostate weight (g)[b]								
6 months	0.87^{w}	0.22	0.68^{x}	0.17	0.63^{x}	0.14	0.53^{y}	0.11
Head length (cm)[a]								
6 months	6.41	0.38	6.26	0.43	6.23	0.32	6.34	0.34
12 months	6.58	0.67	6.57	0.62	6.43	0.45	6.33	0.53
24 months	6.34	0.42	6.30	0.28	6.35	0.27	6.16	0.35
Body length (cm)[a,b,c]								
6 months	19.01^{dw}	1.12	18.68^{dw}	1.02	18.43^{dwx}	1.02	17.74^{dx}	1.08
12 months	22.06^{ew}	1.22	22.10^{ew}	1.01	21.63^{ew}	0.89	20.21^{ex}	0.68
24 months	19.45^{dw}	0.80	19.22^{dw}	1.70	19.13^{dwx}	0.98	18.37^{dx}	0.83
Total length (cm)[a,b,c]								
6 months	25.42^{dw}	1.11	24.94^{dwx}	1.03	24.67^{dwx}	1.03	24.08^{dx}	1.07
12 months	28.63^{ew}	1.26	28.66^{ew}	1.08	28.08^{ew}	0.88	26.57^{ex}	0.87
24 months	25.80^{dw}	0.94	25.52^{dw}	1.75	25.48^{dw}	0.92	24.53^{dx}	0.81

[a] Significant age effect ($p<0.05$), [b] significant diet effect ($p<0.05$), [c] significant age by diet effect ($p<0.05$).
[def] Means in the same column across age groups with different superscripts are significantly different ($p<0.05$).
[wxyz] Means in the same row across diet groups with different superscripts are significantly different ($p<0.05$).
AL: *ad libitum*; DR: dietary restricted.

parisons, which were not significantly different. Therefore, the effect of DR on growth profiles for prostate and left epididymis was similar to those for liver and body weight. Conversely, left testis weight (Table 4) was not significantly different among the various DI groups, with the exception of the AL to 40% DR comparison, which was significantly different.

Head and body (total) length. Unlike body weight (Fig. 2) which increased rapidly between birth and one year on study, and then plateaued or increased slightly between one and two years on study, head and body length (Table 4) increased rapidly from birth to one year on study during the skeletal growth phase, and then decreased significantly between one and two years when aging became the dominant factor. Therefore, there was a significant overall DI effect, a significant DI effect for all individual age groups, and a significant overall linear trend with DI. Also, there was a significant overall age effect for head and body length and a significant DI effect for all individual age groups. However, unlike body weight, there was no overall linear trend with age. There was a marginal-

ly significant DI by age interaction for head and body length.

Head length. As was reported for brain weight, DR did not alter head length (Table 4). However, head length tended to increase between 6 months and one year and then decrease between one and two years, as was seen in body length. Therefore, there was no overall DI effect and no DI effect for any of the individual age groups for head length. Conversely, there was a significant overall linear trend with DI. Also, there was a significant overall age effect for head length and a significant age effect for the 25% DR group only. There was no overall linear trend with age for head length, nor a DI by age interaction.

Body weight/Body length. Body weight was divided by body length to provide an estimate of overall density and to normalize body weight for size differences among animals. The density term decreased slightly between 6 months and one year on study, and then increased significantly between one and two years with increasing age. There was a significant decrease in density with increasing levels of DR. Therefore, there was a highly significant overall DI effect for density, a significant DI effect for all individual age groups, and a significant overall linear trend with DI. Also, there was a significant overall age effect, a significant age effect for the 10% DR, 25% DR, and AL groups, and a significant overall linear trend with age. Additionally, there was a significant DI by age interaction for linear density.

DISCUSSION: IMPLICATIONS FOR CHRONIC BIOASSAYS

Previous studies have shown that high and moderate levels of DR reduce the rate of aging, disease, and drug toxicity (1-14). However, the results from the present study indicate that even low levels of DR (10%) have a significant effect on rodent survival in the chronic bioassay. Of particular importance is the finding that the largest increase in survival rate (24.1%) occurred between the AL group and the 10% DR group, with only a modest 10% increase in survival between 25% and 40% DR, and no change in survival between 10% and 25% DR. These results clearly show that incremental amounts of caloric stress do not modulate 114-week survival in a linear fashion.

The measurement of anatomical variables such as body weight, organ weights, and head and body length give us an important insight as to how DR modulates basic growth parameters. A significant factor was that several other parameters such as brain/body weight ratio, liver/body weight ratio, liver/brain weight ratio, and head and body length, had DI, age, and DI by age interaction effects that were similar to those for body weight. The fact that brain weight was not altered by DR, and that whole body, liver, epididymis, and prostate were progressively reduced by increasing levels of DR, was consistent with previous studies that used high (24) and low (19) levels of DR. The reduced caloric intake per gram of body weight during the first weeks of DR suggests that metabolic efficiency for body weight gain was initially increased to offset the reduction of calories caused by DR. The DR-dependent reduction in prostate and epididymis weight in 6-month-old rats may indicate that reproductive potential was altered by DR, even at the 10% level (19, 25). The reason why testis weight was not reduced by DR is, as yet, unknown. Unlike prostate and left epididymis, DR had little effect on growth in the left testis, suggesting that growth characteristics for brain and testis are similar.

Another interesting finding is that, although there is an overall DI effect (decrease) on body length (not tail) and head and body length, 10% DR and 25% DR have little effect on skeletal length, whereas 40% DR caused the largest decrease, demonstrating the impact of energy limitation on long-term skeletal development. The highly significant effect of DI on the density variable may suggest that normalizing body weight for differences in length may provide a useful and accurate variable to predict the survival rate of rodents in chronic bioassay studies, and to compare data among studies that use animals of different size and/or weight, much as the body mass index (weight in kg/height in meters^2) is used to evaluate obesity status in humans (26).

The results of this study indicate that the NCTR colony of SD rats is extremely long-lived with a survival rate of 63.4% after 104 months on study (110 weeks of age), compared to 7 and 39% survival in previous studies (12, 13). This clearly shows that proper animal husbandry techniques can prevent or decrease the gradual but persistent increase in body weight that has plagued many rat colonies for the past several decades. The results of this study suggest that selective breeding procedures that are unrelated to random genetic drift are responsible for increased body weight in other colonies of SD rats. Body weight has remained constant in the NCTR colony of SD rats, from the time that they were purchased from Charles River Laboratory in 1972. Based on the results of the study, it is evident that long-lived rodents, such as the NCTR strain, are well suited for chronic studies.

There has been much disagreement as to whether or not DR should be used in chronic bioassays to increase the survival rate of rodents. As a result of this contention, the toxicological community has been polarized into two different factions, consisting of those that advocate the use of DR to increase survival (12-14, 17, 19, 22), and those that support other methods such as using

smaller full-fed rodents that are long-lived, and modifying rodent diets to increase longevity (27). The fact that the 25% DR rats in a previous study (28) were approximately the same weight as the AL rats in this study, and that both had similar survival rates (63.4 to 68%), suggests that both methods may be effective.

There are distinct advantages and disadvantages to various methods of increasing survival rate. The advantage of using DR is that, as seen in the present study, survival can be maintained in a controlled and accurate fashion by adjusting the level of food intake. Additionally, the present study clearly shows that individual variation in body weight (CV) can be significantly reduced by low levels of DR, thereby increasing the statistical power of the test and reducing the sample size requirements. Another advantage of DR is that it eliminates or reduces the incidence of non-tumor related pathologies, such as chronic renal disease and cardiomyopathies, that are confounding factors in risk assessment (12, 22). Some toxicologists argue that AL obese test animals are not appropriate controls for studies that are designed to predict drug toxicity in humans because obese rodents are often sick or diseased (12).

A recent study by the Center for Disease Control and Prevention reported that a majority of Americans (55%) are overweight, and that a minority (17.9%) of this population are severely overweight (29). Therefore, since clinical trials in humans rarely use morbidly overweight subjects, a valid justification can be made for using DR rodents in chronic bioassays, rather than AL rodents, to adjust the body weight to more accurately emulate normal human conditions.

A possible disadvantage of using DR to increase survival in chronic bioassays is that improper feeding practices may decrease the sensitivity of rats to carcinogenesis, since many studies indicate that a high level of DR prevents both spontaneous tumors and those related to exposure to known carcinogens (8, 9). Conversely, some toxicologists believe that moderate DR does not adversely affect the sensitivity of the chronic bioassay (12).

The survival rate of the long-lived NCTR strain of SD rats meets all of the FDA guidelines (50% survival) for chronic bioassays. The advantage of using these low-body weight rats is that an acceptable survival rate can be achieved without using DR, thereby significantly reducing the cost and complexity of the bioassay. Additionally, the elimination of the need for DR ensures that the results of chronic bioassays are not compromised by unknown nutritional factors.

The significant disadvantage of using overweight rodent strains is that individual variability among AL animals is much higher than that seen in DR animals, which reduces the statistical power to resolve small changes in pathological profiles (17, 19). A major problem with using AL animals in chronic studies is that spontaneous behavior is not well controlled and extremely variable in these animals (20, 21). Therefore, they are difficult or impossible to synchronize with dietary strategies other than DR.

The main objective of most chronic bioassays is to use rodent data to predict the relative toxicity of specific compounds in humans. If DR is to be used in chronic bioassays, experimental protocols must be developed that increase the rate of survival, but do not adversely affect parameters such as reproduction and growth. The results of this study strongly suggest that 10% DR is the optimal level of restriction to use in small long-lived rat stocks such as the NCTR colony, since no difference in two-year survival was seen between 10% DR and 25% DR. The fact that the greatest change in head and body length occurred between 25% DR and 40% DR in this study, and that liver and body weight were greatly reduced in the 40% DR group, suggests that a low level of DR should be used in small rats. However, the degree of DR may need to be adjusted for different sized rats, since previous studies in large overweight rats required a 15-35% reduction in energy to raise the survival rate of SD rats to an acceptable 74-68% level (12, 28). A comparison of survival rates between the present and a previous study suggests that the optimal method of DR is to adjust the amount of calories to maintain a constant preset level of body weight (19). The extreme degree of variability in the survival rate of obese rats (body weight > 800 g) in previous studies indicates that, if the body weight of SD rats can be controlled in a range between 451 g and 681 g at one year on study, excellent survival rates can be maintained (12, 28).

CONCLUSIONS

It is most apparent that if DR procedures are to be adopted, they must be made as simple and uniform as possible to gain universal acceptance. This means that there has to be a compromise between extremely complex methodologies and practical solutions that are cost effective. Additionally, the results of this study clearly demonstrate that procedures and methodologies that have been developed in basic gerontological and nutritional research studies, such as the implementation of DR regimens and long-lived rodent models to increase longevity, can be used as powerful tools to provide effective and practical solutions to difficult problems related to chronic bioassay studies. Additional basic gerontological and nutritional research studies must be funded by government regulatory agencies such as the FDA, USDA, and EPA, as well as the food and pharmaceutical industry to provide a compre-

hensive scientific database that can be used to identify fundamental mechanisms by which aging and DR modulate food, drug, and chemical efficacy and toxicity.

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