Effect of the AIN-93M Purified Diet and Dietary Restriction on Survival in Sprague-Dawley Rats: Implications for Chronic Studies[1]

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ABSTRACT Survival, growth and dietary intake (DI) variables were monitored in a chronic 114-wk study in which male Sprague-Dawley rats [$n = 120$; National Center for Toxicological Research (NCTR) colony] consumed the AIN-93M purified diet ad libitum (AL), or an amount reduced by 31% of total AL intake inclusive of all macro- and micronutrients. The main objectives were to ascertain the survival characteristics of rats fed the AIN-93M diet and to determine whether dietary restriction (DR) increases longevity of rats fed this casein-based diet compared with the use of mixed-protein sources of the NIH-31 cereal-based diet in an earlier study. Body, liver, brain, the brain/body ratio, spleen, thymus and kidney weights, body length and body density were decreased ($P < 0.05$) by DR, whereas testis weight and skull length were not altered by DR. Significant age effects at 58 and 114 wk were found for body, brain, the brain/body ratio, liver and testis weights, and body density. Survival rates for the AL and 31% DR groups were 43.3 and 57.5%, respectively. Survival curves were not significantly different. The survival rate for AL rats fed the AIN-93M diet was not different from that of AL rats fed the NIH-31 diet (43.3 and 51.7%, respectively). However, the survival rate for 31% DR rats fed the AIN-93M diet was significantly lower than 25% DR rats fed the NIH-31 diet (57.5 and 87.5%, respectively) although both groups had similar body weights and energy intake at various ages. Nutritional components in the NIH-31 diet that are missing and/or reduced in the AIN-93M diet may interact with DR to increase 114-wk survival. Although the survivability, growth and anatomical results of this study suggest that the AIN-93M diet is suitable for chronic rodent studies, additional studies such as comprehensive histopathologic and physiologic investigations must be undertaken to complete the evaluation process. J. Nutr. 132: 101–107, 2002.

KEY WORDS: • *AIN-93M* • *purified* • *dietary* • *restriction* • *survival* • *rats*

Laboratory diet formulation and the selection criteria for cereal-based, purified or chemically defined diets can alter experimental outcomes such as nutrient-compound metabolic interaction, survivability, disease onset and pathology (1–4). A need for nutritionally adequate purified diets that would allow for standardized research among global laboratories led to the formulation of the AIN-76 rodent diet (1,2). However, concerns about nutritional deficiencies were noted. Pathologic studies in mice and hamsters revealed that severe amyloid protein A deposition in the kidneys, spleen, stomach and liver was associated with the feeding of high casein-protein diets, resulting in a decreased rate of survival (5,6). Lowering casein levels or replacing casein with soy protein increased survivability and delayed amyloid A–related pathologies (5–7). The AIN-76 diet was subsequently linked to kidney calcification in female rats (8).

To improve the long-term survival performance of a purified diet, an ad hoc committee was formed in 1989 to review the AIN-76 diet for the purpose of formulating a purified diet that would satisfy rodent growth and maintenance requirements (3,9). The need for a maintenance diet formulation was based on concerns of toxicologists and oncologists who suggested a lower protein, fat and carbohydrate content that would be more suitable for long-term studies. A complete description of the newly formulated AIN-93G (growth) and AIN-93M (maintenance) diets and their comparison to the AIN-76 purified diet were reported elsewhere (3) and only a summary is reported here. Major changes were made in the new AIN-93 diet compared with the previous AIN-76 formulation. As a carbohydrate source, glucose and cornstarch were substituted for sucrose, or a combination of the two was used. Soybean oil replaced corn oil as the recommended source of lipid, and *tert*-butylhydroquinone was used as an antioxidant.

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Additionally, the AIN-93G diet was formulated with 17% casein protein, whereas the AIN-93M diet was formulated with 12% casein. The major changes in the AIN-93 mineral mix compared with the AIN-76 formulation (3) included lowering the phosphorus and manganese contents, changing the amount and form of selenium and adding trace elements, such as molybdenum, boron, fluoride, lithium, nickel, silicon and vanadium. Three changes were made in the vitamin mix, which included increased amounts of vitamins K, E and B-12 (4).

Although complex genetic, nutritional, and husbandry variables are involved, a reduction in energy intake by diet restriction (DR)[3] can significantly promote good health, decrease disease and increase longevity in a variety of animals (10–16). Obesity, resulting from ad libitum (AL) consumption, has been shown to promote early onset pathologies, such as chronic renal disease and cardiomyopathies, which are unrelated to drug-induced carcinogenesis (10,17). Recent evidence suggests that under DR conditions, the capacity of a number of carcinogens to induce cancer is reduced (18,19). Additionally, the relative toxicity and mortalities associated with prescription drugs were significantly reduced by DR, whereas toxicity was altered dramatically by the aging process (20,21).

Although the efficacy of the AIN-93 purified diet has been tested in a few short-term studies (4), little is known about its long-term performance characteristics i.e., rodent survival, body weight maintenance performance or pathologic outcomes relative to age-related disease processes. Additionally, the DR effects of reduced AIN-93M intake have not been examined previously. Therefore, the objectives of this study were to evaluate growth performance and survival potential for AL and 31% DR male rats fed the AIN-93M diet. Additional comparisons were made to Sprague-Dawley (SD) rats that were fed the widely used NIH-31 cereal-based diet during a previous study (22).

MATERIALS AND METHODS

Animal husbandry and feeding regimens. Animal husbandry procedures used in this study were reported previously (23–26). Briefly, the original stock of SD rats [CRl:CD (SD)BR] was obtained from the Charles River Laboratory in 1972; subsequent colony rats were bred and raised in a specific pathogen-free environment at the National Center for Toxicological Research (NCTR). All rats were treated humanely in accordance with Institutional Animal Care and Use Committee guidelines. The male rats used in this study ($n = 120$) were maintained at 23°C and were conditioned to a 12-h light:dark cycle with lights on from 0600 to 1800 h daily. An AL consumption regimen employing pelleted NIH-31 cereal-based diet was used for all rats from weaning through 6 wk of age when they were assigned to the experimental protocol. Rats were given free access to purified water and were housed singly in standard polycarbonate rat cages with wire lids. Body weight (BW) measurements were taken weekly.

At 7 wk of age, rats were switched to pelleted AIN-93M diet to evaluate its usefulness in a chronic bioassay study. The composition of the AIN-93M diet is presented elsewhere (3). At this time, rats were separated into two groups, a control group ($n = 60$) that consumed food AL and a DR group ($n = 60$) that consumed 69% of the AL ration, thus reducing overall energy intake (31% diet restriction). A 31% DR level of AIN-93M was initially selected to approximate the energy intake of SD rats that were fed the NIH-31 cereal-based diet at 25 and 40% DR in a previous study (22) and studies in which Fischer 344 male rats were fed the NIH-31 diet at 40% DR (23–26). Thus, a diet utilization comparison between SD rats DR-fed either a purified or cereal-based diet was possible. The AIN-93M diet was not formulated to contain additional vitamins and minerals to accommodate the lower intake. Therefore, both AL and DR rats fed the AIN-93M diet received the same amounts of vitamins and minerals per gram of diet. The NIH-31rats fed at DR intakes were provided a formulation of the diet that contained 1.67 × additional vitamin mix, a protocol used previously with 40% DR rats (22–26). All rats were fed at 1000 h daily, which corresponded to 4 h after the onset of the light period. The sample sizes ($n = 60$) consisted of 20 rats for both AL and DR groups for 1 y and 40 rats for each of the AL and DR groups for 2 y.

Experimental procedures. At the end of the 58- or 114-wk protocols, AL and 31% DR rats were quietly and quickly removed from the animal room and taken to an adjacent room where they were humanely killed via carbon dioxide overdose. The various internal organs were rapidly removed and weighed on a digital balance, and head and body length measurements were recorded. Gross necropsies were performed and tissues from the various organs were prepared for histopathologic examination.

Statistical analysis of diet and age effects. Comparisons of various anatomical measurements were made among the two dietary intake levels and age groups by using two-way ANOVA in which age and intake level were the main effects. Because organ weights and BW were often positively correlated, ratios between these variables were calculated to remove the effect of BW from the summary statistics. The coefficient of variation (CV) for BW was determined for the various DI and age groups, and the results were analyzed by using ANOVA to determine differences in variability. Independent *t* tests were employed to discern differences in survival at wk 108 of the study among the different intake level groups. To correct for multiple tests, adjusted *P*-values were computed using Tukey's method (27). Kaplan-Meier survival curves were plotted and pair-wise comparisons testing the homogeneity of the survival curves among the various intake level groups were performed by using log-rank statistics. The results of the various statistical tests were considered to be significant when $P < 0.05$.

Nutritional data. A comparison of the calculated digestible energy for male SD rats fed the AIN-93M and NIH-31 diets is presented in **Table 1** for different age groups. The estimated digestible energy of the AIN-93M diet was determined to be 95% on the basis of digestion coefficients of ingredient composition (28). Energy digestibility of the NIH-31 diet was estimated to be 80% on the basis of digestibility findings of the NIH-31 diet fed to Fischer 344 rats (S. Lewis, unpublished data). The metabolizable energy density (physiologic fuel value estimates) of the AIN-93M diet is 15.1 kJ/g (3); gross energy density of the NIH-31 diet is 18.1 kJ/g by bomb calorimetry and ~14.5 kJ in metabolizable energy.

TABLE 1

Comparison of estimated energy metabolized by male Sprague-Dawley rats fed the AIN-93M or NIH-31 diets

	Age, *wk*		
Intake group[1]	32	58	110
	kJ/d		
AIN-93M, AL	290[2]	278	285
AIN-93M, 31% DR	200	192	196
NIH-31, AL	276	271	272
NIH-31, 25% DR[3]	207	203	204
NIH-31, 40% DR[3]	166	162	163

[1] AL, ad libitum intake; DR, percentage dietary restriction compared with AL intake.

[2] Calculated values: AIN-93M, adjusted for 95% digestibility of energy nutrients (28), NIH-31 adjusted for 80% digestibility of energy nutrients.

[3] Adapted from (22).

[3] Abbreviations used: AL, ad libitum; BW, body weight; DI, dietary intake; CV, coefficient of variation; DR, dietary restriction; NCTR, National Center for Toxicological Research; SD, Sprague-Dawley.

RESULTS

Survival data. A summary of survival data for AL and DR male rats fed the AIN-93M purified diet is compared with previous results for the standard NIH-31 cereal-based diet (22) in **Table 2**. At 110 wk of age (wk 104 of the study), the survival rates for male rats fed the AIN-93M diet were 45.0 and 62.5% for the AL and 31% DR rats, respectively, compared with 63.4, 87.5 and 97.5% for the AL, 25% DR and 40% DR rats, respectively, fed the NIH-31 diet. Therefore, survival ranking at 110 wk of age, the established time-evaluation criterion for chronic bioassays, was (40% DR NIH-31) > (25% DR NIH-31) > (31% DR AIN-93M) > (AL NIH-31) = (AL AIN-93M). Kaplan-Meier survival curves for AL and 31% DR rats fed the AIN-93M diet are given in **Figure 1**. The mortality rates for both groups were very similar up to 98 wk of age. However, the mortality rate for AL rats was slightly greater than that for 31% DR rats (22) from 99 wk to the end of the study. The survival curves of AL rats fed either the AIN-93M or NIH-31 diets (22) are compared in **Figure 2**. The first mortality occurred at 18 wk in AL rats fed the NIH-31 diet; no mortalities were seen in rats fed the AIN-93M diet until 53 wk. The mortality rate for both groups was similar up to 101 wk, when the mortality curves started to separate (mortality of AL AIN-93M > AL NIH-31). However, the mortality curves converged again at the end of the study.

The effects of DR and diet formulation on the probability of survival at 114 wk of age are given in **Table 3** for male SD rats. The survival data for AL and DR rats fed the NIH-31 cereal diet were reported in a previous study (22). The odds of survival for rats fed the AIN-93M were improved (1.78) by a 31% reduction in energy; the odds of survival for rats fed the NIH-31 diet were greatly increased (17.4) by a similar reduction (25%) in energy intake (22). When rats consumed either the AIN-93M or NIH-31 diet ad libitum, there was no difference in survival probability at 114 wk of age (Table 3). The probability of survival decreased significantly ($P < 0.001$) when 31% DR AIN-93M data were compared with the 40 and 25% DR NIH-31 levels of intake (Table 3).

Comparisons of the effects of DR and diet formulation on overall survival curves are given in **Table 4** for male SD rats. The survival curves for AL and DR rats fed the NIH-31 diet were reported previously (22). Although the odds of survival at wk 104 of the study were marginally increased by DR in rats that were fed the AIN-93M diet, the overall survival curves for AL and DR rats were not significantly different (Table 4).

TABLE 2

Survival data for male Sprague-Dawley rats fed the NIH-31 or AIN-93M diets

Time		NIH-31[1]			AIN-93M	
On study	Age	AL[2]	25% DR	40% DR	AL	31% DR
wk		*Survival, %*				
52	58	96.6	92.5	100.0	95.0	95.0
104	110	63.4	87.5	97.5	45.0	62.5
108	114	51.7	87.5	95.0	43.3	57.5
111	117	40.0	NA[3]	NA	40.0	NA

[1] NIH-31 data adapted from (22).
[2] AL, ad libitum intake; DR, percentage dietary restriction compared with AL intake.
[3] NA, not applicable; DR rats were killed at 114 wk of age and no data are available at 117 wk of age.

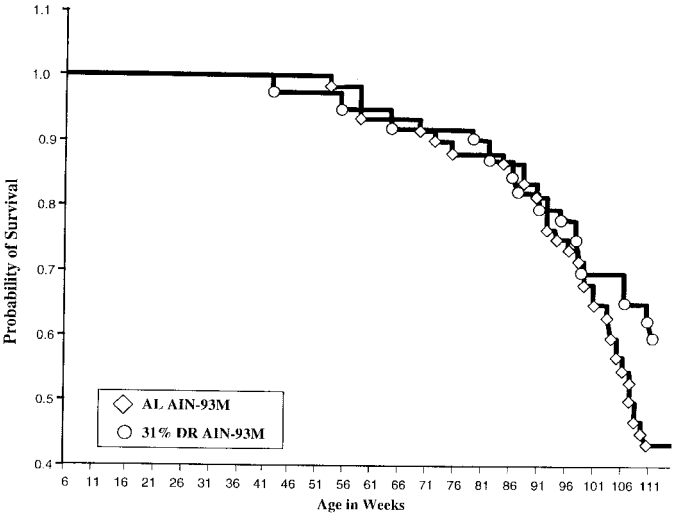

FIGURE 1 Kaplan-Meier curves representing the probability of survival for ad libitum (AL) and 31% dietary restricted (DR) male Sprague-Dawley rats fed the AIN-93M diet.

Conversely, DR altered the survival curves for rats that were fed the NIH-31 diet, thereby significantly increasing their survival potential beyond all other groups (22). The survival curves for AL and DR rats fed the AIN-93M diet and AL rats fed the NIH-31 diet did not differ significantly from one another (Table 4). There was, however, a more precipitous decrease in survival between wk 110 and 114 for the AL NIH-31 males than observed for the AL AIN-93M males, 11.7 vs. 1.7%, respectively (Table 4).

Organ weight, body weight and body length. Organ weights, BW, body length and body density at 58 and 114 wk for AL and DR male rats fed the AIN-93M diet are given in **Table 5**. The weights of organs such as brain, liver, spleen, thymus, kidney, as well as the ratios of brain, testis, spleen, thymus and kidney to BW were significantly decreased in DR rats compared with their AL counterparts. The comparative summary statistics related to the effects of age, dietary intake and intake by age interactions on organ weights are given in

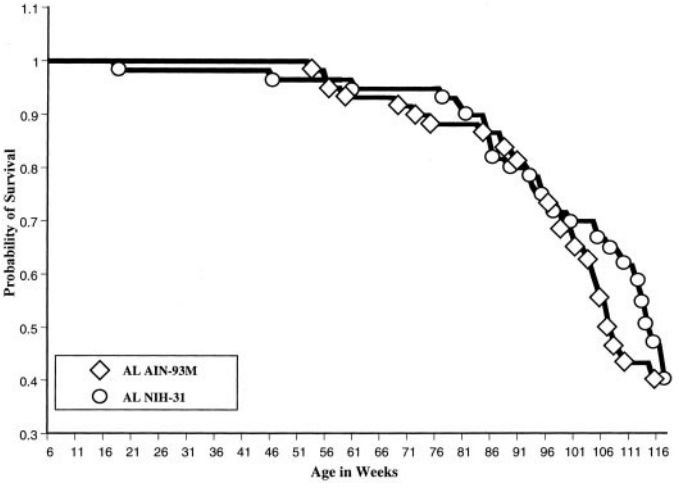

FIGURE 2 Kaplan-Meier curves representing the probability of survival for ad libitum (AL) male Sprague-Dawley rats fed the AIN-93M diet or the NIH-31diet. NIH-31 data adapted from (22).

TABLE 3

Effect of dietary restriction in male Sprague-Dawley rats fed NIH-31 or AIN-93M diets; odds of survival at 114 wk of age

Pair-wise comparison among dietary groups[1]	Change in survival[2]	Odds ratio[2]	95% Confidence interval
AL[3] NIH-31[4] to AL AIN-93M[5]	NC[6]	0.88	[0.756, 1.025]
AL NIH-31[4] to 40% DR NIH-31[5]	Increase	17.47	[14.065, 21.885]
31% DR AIN-93M[4] to AL NIH-31[5]	Decrease	0.64	[0.536, 0.761]
31% DR AIN-93M[4] to 25% DR NIH-31[5]	Increase	4.14	[2.971, 4.417]
AL AIN-93M[4] to 31% DR AIN-93M[5]	Increase	1.78*	[1.491, 2.121]
25% DR NIH-31[4] to AL AIN-93M[5]	Decrease	0.14	[0.130, 0.185]
40% DR NIH-31[4] to AL AIN-93M[5]	Decrease	0.05*	[0.046, 0.071]
40% DR NIH-31[4] to 31% DR AIN-93M[5]	Decrease	0.09*	[0.070, 0.113]

[1] NIH-31 data adapted from (22).
[2] Results are expressed as the change in survival of second group compared with the first group; odds ratio, * $P < 0.05$.
[3] AL, ad libitum intake; DR, percentage dietary restriction compared with AL intake.
[4] First group.
[5] Second group.
[6] NC, no change.

Table 6. With the exception of left testis and the liver/BW ratio, there was a significant overall diet effect of DR feeding for the AIN-93M diet formulation. There was a significant age effect (decreased weight with age) for organs such as liver and left testis, ratios of brain/BW, liver/BW, spleen/BW and kidney/BW, but no age effect was found for brain, spleen, thymus and kidney, or testis/BW and kidney/BW. Also, there was a significant dietary intake level by age interaction for organs such as left testes and liver/BW, but no dietary intake level by age interaction was seen for other organs. Weekly BW data for AL and DR rats throughout the study are shown in **Figure 3** for rats fed the AIN-93M diet. There was a significant overall age effect, DI effect and DI by age interaction for the variability in BW as measured by CV ($P < 0.05$). The body weight CV was significantly decreased by DR and significantly increased by the aging process ($P < 0.05$).

Rats demonstrated a normal growth response during the first 7 wk of the study, the time when half the population was assigned to the DR regimen (Fig. 3). The AL rats continued to grow at a slow, but slightly increasing rate until 97 wk of age after which there was no notable change in BW (Fig. 3). The DR rats, however, demonstrated a growth plateau after the onset of DR (Fig. 3). There was a decrease in BW for AL rats during the last 16 wk before the completion of the study (Fig. 3). Average BW (±SEM) throughout the study was 717.8 ± 88.2 g for AL rats compared with 489.6 ± 24.4 g for DR rats, suggesting reduced growth and reduced statistical variability. Maximum BW of 871 g at 98 wk of age and 544 g at

TABLE 4

Comparison of life-time survival curves for male Sprague-Dawley rats fed NIH-31 or AIN-93M diets

Pair-wise comparison among dietary groups[1]	Change in survival[2]	Significance (*P*-value)[2]
AL[3] NIH-31[4] to AL AIN-93M[5]	NC[6]	0.601
AL NIH-31[4] to 40% DR NIH-31[5]	Increase	0.001*
31% DR AIN-93M[4] to AL NIH-31[5]	NC	0.659
31% DR AIN-93M[4] to 25% DR NIH-31[5]	Decrease	0.012*
AL AIN-93M[4] to 31% DR AIN-93M[5]	NC	0.241
25% DR NIH-31[4] to AL AIN-93M[5]	Decrease	0.001*
40% DR NIH-31[4] to AL AIN-93M[5]	Decrease	0.001*
40% DR NIH-31[4] to 31% DR AIN-93M[5]	Decrease	0.001*

[1] NIH-31 data adapted from (22).
[2] Results are expressed as the change in survival of second variable compared with the first variable; log-rank significance, * $P < 0.05$.
[3] AL, ad libitum intake; DR, percentage dietary restriction compared with AL intake.
[4] First variable.
[5] Second variable.
[6] NC, no change.

TABLE 5

Organ weight, body weight, body length and body density of AIN-93M–fed male Sprague-Dawley rats at 58 and 114 wk of age[1]

Organ	Age, *wk*	AL[2]	31% DR
		g	
Brain, *g*	58	$2.40^{y} \pm 0.03$	$2.23^{z} \pm 0.03$
	114	2.37 ± 0.03	2.30 ± 0.04
Liver, *g*	58	$20.75^{a,y} \pm 0.70$	$12.78^{a,z} \pm 0.29$
	114	$15.9^{b,y} \pm 0.66$	$9.94^{b,z} \pm 0.23$
L testis, *g*	58	$1.72^{a} \pm 0.03$	1.57 ± 0.05
	114	$1.4^{b} \pm 0.05$	1.51 ± 0.07
Spleen, *g*	58	$1.19^{y} \pm 0.05$	$0.87^{z} \pm 0.07$
	114	$1.28^{y} \pm 0.08$	$0.94^{z} \pm 0.09$
Thymus, *g*	58	$0.69^{y} \pm 0.07$	$0.32^{z} \pm 0.04$
	114	$0.62^{y} \pm 0.09$	$0.30^{z} \pm 0.02$
Kidneys, *g*	58	$4.13^{y} \pm 0.14$	$2.81^{z} \pm 0.03$
	114	$4.16^{y} \pm 0.19$	$3.17^{z} \pm 0.08$
Body weight, *g*	58	$863.75^{a,y} \pm 20.56$	$498.84^{z} \pm 4.77$
	114	$747.10^{b,y} \pm 34.14$	$476.95^{z} \pm 6.52$
Head length, *cm*	58	6.22 ± 0.09	6.15 ± 0.09
	114	6.31 ± 0.13	6.12 ± 0.08
Body length, *cm*	58	$19.28^{y} \pm 0.28$	$18.39^{z} \pm 0.20$
	114	18.95 ± 0.14	18.42 ± 0.19
Total length, *cm*	58	$25.50^{y} \pm 0.27$	$24.54^{z} \pm 0.17$
	114	$25.27^{y} \pm 0.19$	$24.53^{z} \pm 0.18$
Body density,[3] *g/cm*	58	$33.96^{a,y} \pm 0.92$	$20.32^{z} \pm 0.18$
	114	$29.57^{b,y} \pm 1.23$	$19.42^{z} \pm 0.27$

[1] Values are means ± SEM, $n = 60$. [a,b] Means in a column across age groups with different superscripts differ ($P < 0.05$); [y,z] means in a row across diet groups with different superscripts differ ($P < 0.05$).
[2] AL = ad libitum intake; DR = percentage dietary restriction compared to AL intake.
[3] g body/total cm.

TABLE 6

Organ weight, body weight, body length and total density statistics for AIN-93M–fed male Sprague-Dawley rats at 58 and 114 wk of age

	Effect type		
Organ	Age	Intake level	Intake level by age
		P-value	
Brain	0.495	0.001*	0.101
Liver	0.001*	0.001*	0.064
L Testis	0.001*	0.664	0.027*
Spleen	0.306	0.001*	0.870
Thymus	0.436	0.001*	0.715
Kidney	0.159	0.001*	0.223
Brain/Body[1]	0.001*	0.001*	0.625
Liver/Body[1]	0.001*	0.627	0.013*
Body weight	0.003*	0.001*	0.036*
Head length	0.761	0.203	0.521
Body length	0.460	0.001*	0.383
Total length	0.565	0.001*	0.590
Body density[2]	0.003*	0.001*	0.046*

[1] g organ/g body.
[2] g body/total cm.
* $P < 0.05$.

96 wk were obtained in AL and DR rats fed the AIN-93M diet, respectively. Weekly variations in food consumption are given in **Figure 4** for the AL and DR groups. There was a significant decrease in food consumption between 11 and 52 wk of age ($P < 0.05$) followed by a gradual increase in food consumption between 53 and 76 wk ($P < 0.05$). Additionally, there was a small decrease in food consumption in both groups at the end of the study.

DISCUSSION

As reported previously (22), the results clearly show that the SD rats (NCTR colony) used in this study were extremely long-lived compared with other strains such as [CRl:CD-

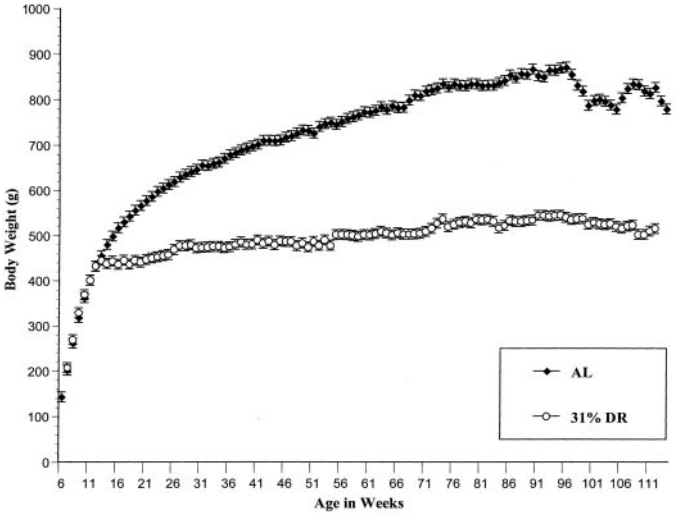

FIGURE 3 Body weights of ad libitum (AL) and 31% dietary restricted (DR) male Sprague-Dawley rats fed the AIN-93M diet. Values are means ± SEM, $n = 60$. Groups differed, $P < 0.05$.

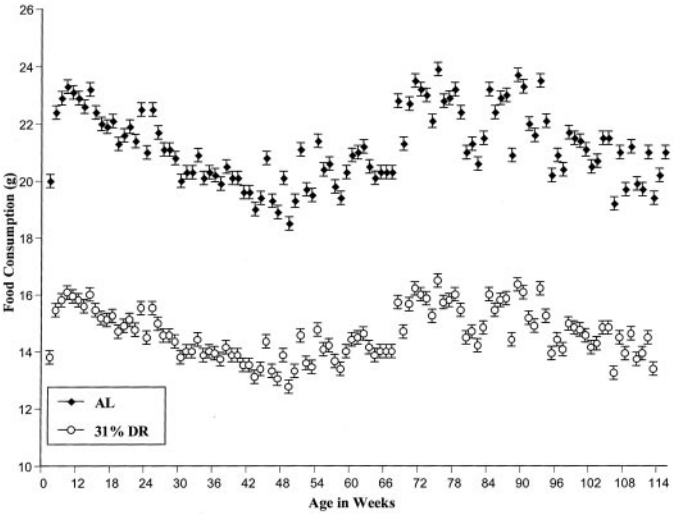

FIGURE 4 Food consumption by ad libitum (AL) and 31% dietary restricted (DR) male Sprague-Dawley rats fed the AIN93-M diet. Values are means ± SEM, $n = 60$. Groups differed, $P < 0.05$.

(SD)BR] rats from Charles River Laboratory (18). The survival rates reported here for SD rats are among the highest in recent literature, regardless of diet fed. Recent studies have shown that survival of AL male rats in 2-y chronic bioassays varied from as low as 7% in one study (17) to 39% in another (29).

On the basis of the results of this study, it is evident that many of the issues with previous purified diets (AIN-76A) that led to poor survival (30) have potentially been resolved with the AIN-93M diet formulation, at least with SD rats. We conclude that the reductions in the percentage of casein and lipid, and the partial substitution of cornstarch for sucrose, resulting in an overall decrease in soluble carbohydrates, are at least partially responsible for this beneficial effect.

An important finding of this study, i.e., that the average CV for BW was higher in AL rats fed the AIN-93M diet than their DR counterparts, was similar to the results of a previous study using the NIH-31 cereal diet (22). Decreased individual variation has been reported to have a significant positive effect in the bioassay because it increases the power to resolve potential risk and decreases the sample size required to obtain statistically significant results (31–33).

The fact that DR had a disproportionately larger effect on the survival rate of rats fed the NIH-31 diet (40% DR) than those fed the AIN-93M purified diet (31% DR) was unexpected. Previous rodent survival studies that used cereal-based diets reported that the increased longevity of DR rats was the direct result of a concurrent decrease in BW (17,22,31–34). The 114-wk survival rate for these rats fed the AIN-93M diet at 31% DR was 57.5% compared with a 87.5% survival rate for rats fed the NIH-31 diet at 25% DR, even though their BW were similar, 476.9 and 524.9 g (22), respectively. Conversely, AL rats fed the NIH-31 diet had the same lifetime survival curve as 31% DR rats fed the AIN-93M diet, whereas their average BW at 110 wk were significantly ($P < 0.05$) different, 695.1 and 502.4 g, respectively (22). As noted in **Table 1**, daily energy intakes between rats restricted to 31% AIN-93M and 25% NIH-31 differed less than an average of 5% at 32, 58 and 110 wk of age. Daily energy intakes of rats restricted to 40% NIH-31 differed by 16.5% compared with the group fed 31% AIN-93M at 32, 58, and 110 wk of age. Thus, energy intake at 31% DR of the AIN-93M diet more closely approximates 25% DR of the NIH-31 diet than that of 40% DR of

the NIH-31 diet when fed to SD male rats. These results suggest that factors other than BW such as diet composition, nutrient digestibility and efficiency of energy conversion must partially account for the difference in longevity between rats fed the cereal-based and purified diets. Additional evidence supporting this conclusion is that the survival curves for AL and DR rats fed the AIN-93M diet were not different, whereas those for AL and DR rats fed the NIH-31 diet were significantly ($P < 0.05$) different. The results of this study, taken in their entirety, suggest that under conditions of reduced food consumption, there is likely an unknown nutritional component or components in the NIH-31 diet, not present or in reduced concentrations in the AIN-93M purified diet, that interact with DR to increase longevity and to decrease onset of age-related diseases. One important distinction between the two diets was the absence of a vitamin allocation beyond the vitamins required to meet rat nutritional requirements in the diet fed to the 31% DR AIN-93M group. It must be noted that historically, all DR rodents at the NCTR were provided a formulation of the NIH-31 diet that contains 1.67 × the vitamin formulation of the NIH-31 diet fed to AL rats. The complex nature of the cereal-based, or natural-ingredient diet, with varied combinations of macro- and micronutrient elements as well as other metabolically active agents such as phytoestrogens or flavonoids may play a role in important metabolic events. The fact that organ weights, growth curves and body length are not altered by decreased concentration and type of protein (cereal protein vs. casein) suggests that the AIN-93M diet is supportive of normal growth characteristics.

Relevance to chronic studies. The results of this study clearly indicate that the AIN-93M diet has the potential to be used not only for nutritional studies but also for toxicologic and gerontologic research as well. This purified diet, which offers excellent survival characteristics, will be a useful tool for research that requires discrete nutrient, xenobiotic or a targeted dose effect response criterion. The decreased effect of DR-dependent BW on survival of rats fed the AIN-93M diet may actually be beneficial to some bioassays. Small changes in BW, resulting from adverse environmental conditions and drug-related anomalies that affect food consumption, might not significantly alter the survival of rats fed the AIN-93M diet. This could reduce the variability of BW-dependent results among comparison groups.

The nutritional studies reported here for male rats fed the AIN-93M diet must be replicated in female rats to determine comparable survival characteristics. Diet-related changes in reproductive, pathologic, growth and behavioral endpoints will be the primary focus of these studies. Efforts should be made to study the effects of the AIN-93M diet in Fischer 344 rats and B6C3F1 mice, models routinely used in National Toxicology Project chronic bioassays. Another important avenue to pursue is to modify selectively the nutrient component concentrations of the AIN-93M diet to determine whether the specific beneficial DR effects on survival, toxicity and disease that are associated with cereal diets (31,32) can also be triggered by using purified diets. If these components of cereal diets that are missing from the AIN-93M diet can be identified, it may be possible to promote extended longevity and improved health status without using DR. Additional studies must be conducted to determine basic mechanisms by which various types of nutrients interact with DR to modulate disease, aging and drug toxicity.

The comparison of BW data for rats fed the NIH-31 (22) and the AIN-93M diets suggests that an increased digestibility, subsequent energy absorption and feed conversion efficiency of the purified diet contributed to the significant increase in BW in rats fed the AIN-93M diet. Although caprophagy was observed among DR rats fed the NIH-31 diet, the fate of microbial protein in DR rats fed the AIN-93M diet has not been determined. To reduce BW gain and to perhaps improve the survival of rats fed the AIN-93M diet, an increase in the fiber component and concomitant decrease in starch would effectively lower the metabolizable energy of the diet. Although the survival rate (45%) for AL rats fed the AIN-93M diet was lower than the survival rate (63%) for AL rats fed the NIH-31 diet, and was slightly below the FDA guideline (50% survival at 24-mo), the data presented here indicate that with the appropriate changes or other minor modifications, the AIN-93M diet has the potential to meet the requirements for chronic studies that entail the precise control of nutritional components such as food additives and substitutes, as well as drugs and potentially toxic compounds. The completion of these suggested studies will advance the characterization of the AIN-93M purified diet as a standard for biomedical research protocols that would effectively minimize diet variability, a major source of variability when comparing the results of animal studies. However, the effects of the AIN-93M diet on pathologic and physiologic variables must be evaluated before this diet can be deemed suitable for chronic studies.

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