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Significant life extension by ten percent dietary restriction

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Although it is well documented that dietary restriction (DR) increases the life span of rodents and other animals, this increase is observed at relatively high levels of DR, in which rodents are typically fed 40% less than that consumed by rodents fed *ad libitum*. It is generally assumed that lower levels of DR will have a lesser impact on life span; however, there are very little published data on the effect of low levels of DR on life span. In this study, we show that 10% DR increased life span to almost the same extent as 40% DR. While both 10% and 40% DR resulted in similar changes in non-neoplastic lesions, 10% DR had no significant effect on the incidence of neoplasia (except for pituitary adenoma), and 40% DR resulted in a significant reduction (40%) in neoplasia. These data clearly demonstrate that the life span of F344 rats does not increase linearly with the level of DR; rather, even a low level of DR can substantially affect life span. This rodent study has important translational implications because it suggests that a modest reduction in calories might have significant health benefits for humans.

Keywords: 10% dietary restriction; calorie restriction; life span; neoplasia

Introduction

Dietary restriction (DR) has become the gold standard to which manipulations that increase life span and appear to retard aging are compared. DR has been shown to increase life span and reduce or delay the increase in age-related pathologies and the decline in most physiological functions in numerous genotypes of laboratory rodents. DR increases the life span of a wide variety of other organisms ranging from invertebrates, such as yeast, *Caenorhabditis elegans,* and *Drosophila*, as well as spiders and rotifers, to various strains of rats and mice (for reviews, see Refs. 1 and 2), and has also been reported to increase the life span of Labrador retrievers.[3] These data have led to the view that the effect of DR on longevity and aging is universal, a view that was reinforced in 2009 when Coleman *et al.*[4] reported the first data showing that DR significantly decreased the incidence of age-related deaths and delayed the onset of age-related pathologies in rhesus monkeys.

The universality of the effect of DR on longevity was called into question in 2010 when Liao *et al.*[5] reported the effect of DR on approximately 40 different recombinant inbred lines of male and female mice. Surprisingly, approximately one-third of the mice showed a decrease in life span on the DR diet; one-third showed no effect of DR on life span; and only one-third showed the expected increase in life span. These data contradicted the prevailing view that DR is a universal, beneficial intervention with respect to life span and aging. Similarly, a few previous studies, which had largely gone ignored, also reported that some mouse strains did not show an increase in life span when fed a DR diet (e.g., male wild-caught mice[6] and male DBA/2 mice[7]). In addition, Mattison *et al.*[8] reported that long-term DR in rhesus monkeys had no effect on survival outcomes; however, DR appeared to delay the onset of certain diseases.

One possible explanation for the recent contradictory data on DR is that the level of DR required

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to increase life span is genotype dependent, and because the previous studies used only one, relatively high, level of DR, which might have had a negative effect (instead, lower levels of DR might increase life span). The standard DR diet that is usually used in DR rodent studies is the Masoro diet,[9] in which rodents are fed 60% of the diet consumed by animals fed *ad libitum* (AL) (i.e., 40% DR). This is the level of restriction used by the National Institute on Aging (NIA) for their aged rodent colonies, which have been available to investigators studying aging. It is generally believed that the increase in life span is directly related to the level of DR, that is, increasing the level of restriction leads to a greater increase in life span up to a certain point (e.g., around 60% DR)[10] where further restriction is harmful.[11] However, there are only limited data to support this view. In 1986, Weindruch *et al.*[10] compared the effects of approximately 25%, 55%, and 65% DR on the life span of female C3B10RFl mice and reported that life span increased continuously from 25% to 65% DR. In contrast, when Duffy *et al.*[12] compared the life spans of Sprague–Dawley rats receiving 10%, 25%, and 40% DR, they found that the extension in life span at 10% and 25% DR was almost the same as at 40% DR. It should be noted that the survival data in the study by Duffy *et al.*[12] were not complete since the study was terminated when approximately 55% of the AL rats had died. At this time, more than 80% of all of the DR rats were still alive. Therefore, it is unclear whether the rats in the 10% and 25% DR groups in this study would have continued to show reduced mortality later in life.

The purpose of this study was to determine whether a modest level of DR (10% DR) could increase the life span of male F344 rats and compare its effects on life span and pathology to the effects of 40% DR. We found that 10% DR significantly increased mean life span, and surprisingly, the increase in mean life span obtained by 10% DR was similar to that observed with 40% DR. However, we observed differences in the effects of 10% and 40% DR on the incidence of fatal neoplasia; 40% DR resulted in a significant reduction in fatal neoplastic diseases, especially leukemia, which was the most common neoplastic disease in the rats.

Methods

Life-span analysis

Male F344 rats ($n = 120$), weaned at 29 days of age, were obtained from Charles River Laboratories and were housed singly throughout life under temperature-controlled, specific pathogen–free conditions in a barrier facility on a 12:12 h light–dark cycle, as previously described.[13,14] Initially, the rats were fed AL a semisynthetic diet consisting of 21% protein (soy), 10% fat (corn oil), 58% carbohydrate, 5% Ralston–Purina mineral mix, and 2% Ralston–Purina vitamin mix. At 6 weeks of age, the rats were randomly divided into three groups (40 rats/group): (1) AL group; (2) 10% DR group fed 90% of the food consumed by the AL group; and (3) 40% DR group fed 60% of the food consumed by the AL group. The amount of food ingested by the AL group was measured twice per week for 3- and 4-day periods, and the average amount of diet consumed by the AL rats was calculated. The 10% and 40% DR rats were then fed 90% and 60% of the diet consumed by the AL rats the previous week. The rats were not disturbed except to check on their health status twice each day, change cages, and to obtain body weights. All rats were weighed until approximately 32 months of age when most of the AL rats had died. The rats were permitted to live out their lives until death due to natural causes without censoring, and the date of deaths were recorded. The mean, 10%, and maximum life span were collected for each group from the survival data. This survival study and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Pathological analysis of rats that die spontaneously

All rats were inspected at least twice daily (from 0700 h to 0800 h and from 1500 h to 1600 h). The rats that spontaneously died were removed from the cage and either necropsied immediately or refrigerated for a brief period. Autolysis was observed in only one rat (out of the 40 rats); hence, it was not severe enough to prevent histopathologic evaluation. After the rats were examined for gross pathological lesions, the following organs and tissues were excised and fixed in 10% neutral-buffered formalin: brain; pituitary gland; heart; lung; trachea; thymus;

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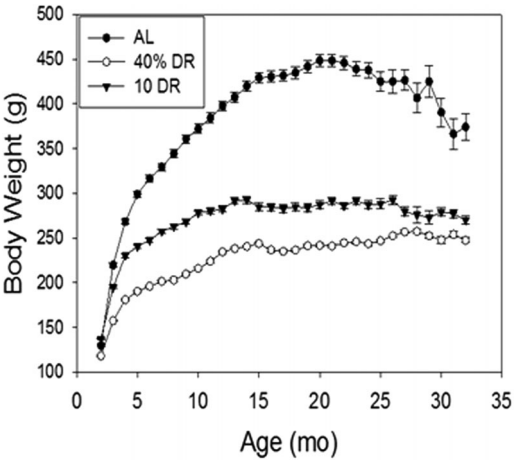

Figure 1. Body weights of rats in AL, 10% DR, or 40% DR groups. The data presented are the mean ± SEM of 40 animals/group. The rats reach maximum body weight at 15–20 months of age, and the body weight of the rats fed AL declines after 20 months of age when they begin to die. This decrease in body weight has been previously reported in male F344 rats.[17]

thoracic and abdominal aortas; esophagus; stomach; small intestine; ascending, descending and sigmoid colon; liver; pancreas; spleen; kidneys; urinary bladder; prostate; testes; epididymis; seminal vesicles; thyroid, adrenal, and parathyroid glands; psoas muscle; sternum; and lumbar vertebrae. The brain, heart, lungs, kidneys, testes, liver, spleen, pancreas, and adrenal glands were weighed before fixation. All organ tissues in which lesions were observed by gross inspection were excised and fixed, and the excised organs were examined histologically as described by Iwasaki *et al.*[9]

Results

Life-span experiments

Male F344 rats were maintained on three dietary regimens over their life spans, starting at 6 weeks of age: AL, 10% restriction (10% DR), and 40% restriction (40% DR). Figure 1 shows the body weights of the three groups of rats over most of their life spans. As would be expected, the rats on the DR diets showed a major reduction in body weight. The maximum body weight (i.e., the weight at 15–20 months of age) of rats on 40% DR was approximately 45% of that observed for the rats fed AL. Interestingly, the maximum body weight reached by the rats on 10% DR was approximately 35% of the rats fed AL, much greater than would have been predicted from a 10% decrease in food consumption.

The survival curves for three groups of male F344 rats are shown in Figure 2A, and the survival data are presented in Table 1. Analysis of the entire survival curves shows that 10% and 40% DR resulted in a statistically significant ($P < 0.001$) increase in life span over that observed for rats fed AL. Ten percent and 40% DR increased the mean survival to 15% and 19%, respectively, over that observed for the rats fed AL (Table 1). Iwasaki *et al.*[9] previously reported that 40% restriction of the same diet increased the life span of F344 rats approximately 14%. No statistical difference was observed in the mean life spans of rats on 10% and 40% DR. When the entire life-span curves were analyzed by the Kaplan–Meier log-rank test, we observed a P value of 0.057. Because the log-rank test is conservative, the difference between the survival curves of the 10% and 40% DR groups might be different. It is also apparent from the survival curves that 40% DR had a greater effect on survival during the last quarter of the life span compared to the rats on 10% DR. In other words, some of the rats on 40% DR showed improved health compared to rats on 10% DR. As shown in Table 2, both 10% and 40% DR significantly increased the 10% survival (when 90% of the rats had died) compared to the AL group ($P < 0.001$). However, the increase in 10% survival was much greater for the rats on 40% DR (e.g., the 10% survival was increased 26% for the 40% DR mice compared to AL rats while the increase in 10% survival for the rats fed 10% DR was only 12%). The difference in 10% survival for the rats fed 10% and 40% DR was statistically significant ($P = 0.016$).

We also compared the survival of the rats fed 10% and 40% DR to AL fed rats using the Gompertz plot, which analyzes age-specific mortality rates. We used the maximum likelihood estimation, to rule out the possibility that the changes in the Gompertz curves are the result of a poor fit to the Gompertz model. Using the likelihood estimation, we did not reject the Gompertz model in favor of the complex logistic model for any of the three groups. As shown in Figure 2B, the estimated Gompertz parameters showed differences between the effect of 10% and 40% DR on mortality trajectories. Forty percent DR appeared to alter primarily the Gompertz slope (i.e., the rate of increasing mortality over the adult life span). In contrast, 10% DR primarily altered the initial mortality rate (i.e., it lowered the elevation of the curve).

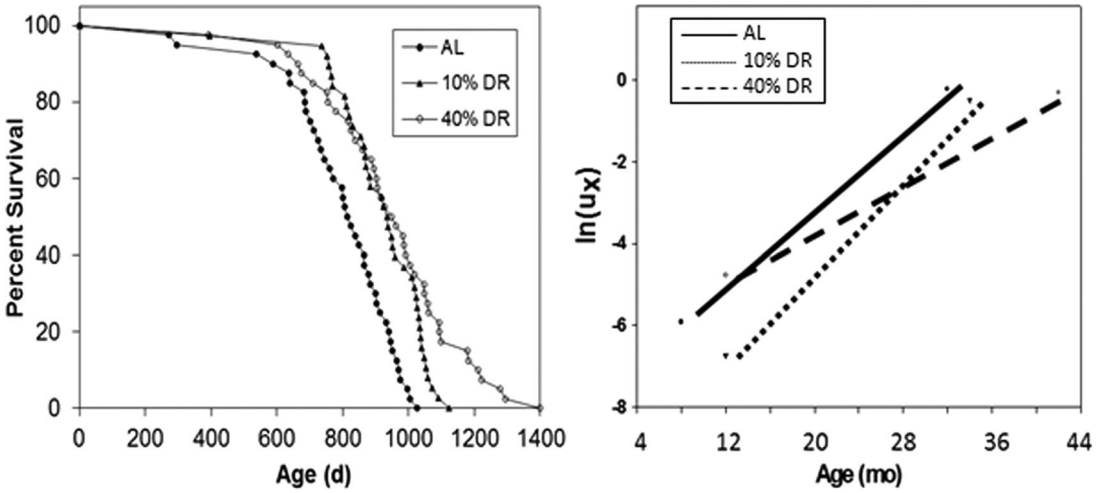

Figure 2. The survival of rats in AL, 10% DR, or 40% DR groups. Shown on left are Kaplan–Meier plots of survival data for the AL (●), 10% DR (▲), and 40% DR (○) groups. Using the Kaplan–Meier log-rank test, we found that the 10% and 40% DR curves were significantly different from the survival curve for AL rats at the $P < 0.001$ level. Statistical comparison of the survival curves for the 10% and 40% DR groups resulted in a P value of 0.057. Shown on right is the Gompertz plot for survival data of the AL (—), 10% (····), and 40% DR (----) groups. The Gompertz mortality analysis of life span was conducted using the distributions of ages at death by the equation $u_x = ae^{bx}$, where u_x is the instantaneous age-specific mortality. The Gompertz parameters a and b are the initial rate of mortality and the rate of increase in the hazard for mortality (i.e., the mortality rate), respectively.

End-of-life pathology

We also compared end-of-life pathology for the rats fed AL versus those on either 10% or 40% DR. The pathology data in Table 2 are expressed in two ways: (1) the probable cause of death, which lists all causes of death, including multiple fatal lesions; and (2) the incidence of each of the major specific fatal lesions separately. When the cause of death from all neoplastic diseases was compared, we did not observe a significant difference between the AL and 10% DR groups; however, the 40% DR group showed a significantly lower incidence (40% less) of fatal neoplastic lesions compared to the AL group ($P < 0.05$). The specific incidence of mononuclear cell leukemia, which was the most common pathological lesion observed, was significantly reduced in the 40% DR group. Interestingly, the 10% DR group showed a higher incidence of leukemia compared to AL and 40% DR groups ($P < 0.05$). This unexpected higher incidence of leukemia in the 10% DR group compared to the AL group could be explained by the extended life span of the rats on 10% DR; approximately 40–45% of the fatal leukemia in the rats on 10% DR occurred after the age at which all of the AL rats died. However, the 40% DR rats, who showed an even greater extension of life span, showed no increase in leukemia (i.e., the 40% DR rats appeared to live longer with less leukemia than the 10% DR rats). The incidence of fatal pituitary tumors also occurred more in the AL group than in the 10% and 40% DR groups ($P < 0.05$).

The incidence of all non-neoplasm lesions was lower in the AL than in the DR groups. Nephropathy has been shown to be a major cause of death in male F344 rats.[15] However, Iwasaki *et al.*[9] showed that the replacement of the protein source from casein to soy (as was used in this study) greatly reduced the incidence of fatal chronic nephropathy in F344 rats. Only 18% of rats in the AL group had fatal chronic nephropathy, and none of the rats in the 10% and 40% DR groups died of chronic nephropathy ($P < 0.05$). There was a nonsignificant trend for rats fed either 10% or 40% DR to die more often of unknown causes of death; for example, many of these rats showed little evidence of severe tissue pathology at death, compared to rats fed AL.

Discussion

The data from this study clearly demonstrate that a 10% restriction of food significantly increases the life span of male F344 rats and, surprisingly, that the increase in life span is comparable to what was

Table 1. Longevity data for rats in AL, 10% DR, and 40% DR groups

	AL	10% DR	40% DR
Survival data:			
Mean ± SEM	796 ± 27 days	918[a] ± 22 days	947[a] ± 33 days
10% Survival	974 days	1090[a] days	1223[a,b] days
Maximum	1026 days	1180 days	1400 days
Gompertz data:			
Parameter A	0.0004	0.00131	0.00006
	(0.00009, 0.00199)	(0.00043, 0.00398)	(7.86E-06, 0.00048)
Parameter B	0.24486	0.15128	0.29037
	(0.189, 0.318)	(0.118, 0.194)	(0.224, 0.376)

Note: For survival data, the mean, 10%, and maximum (last animal to die) survival were obtained from the data used to generate the curves presented in Figure 2A. A standard Student's *t*-test was used to statistically analyze the mean survival, and the statistical analysis of the 10% survival (when 90% of the rats died) was conducted as described by Wang *et al.*[19] using the Boschloo test.
[a]Significantly different from the AL group ($P < 0.001$); [b]significantly different from the 10% DR group ($P = 0.016$).
For the Gompertz data, the Gompertz parameters A and B are the initial rate of mortality and the rate of increase in the hazard for mortality (i.e., the mortality rate), respectively. The values shown are the estimates of each parameter with the lower and upper confidence intervals.

observed for rats that were restricted 40%. These data were surprising because of the general view that increasing the level of DR up to 40% would result in a continuous increase in life span, as reported by Weindruch *et al.*[10] for female C3B10RF1 mice in which a significant increase (over 20%) in the mean survival occurred between approximately 25% and 55% DR. The differences in the results of our study and those of Weindruch *et al.*[10] could be due to differences in the way rat and mouse sex and genotype respond to DR (e.g., differences in pathophysiology). In general, rat longevity is enhanced more by DR than is mouse longevity.[2] However, Rafael deCabo's laboratory has found in mice that 20% DR results in an increase in life span comparable to 40% DR (personnel communication). These data in combination with the data from Duffy *et al.*,[12] which reported that feeding rats 10% and 25% DR was as effective as 40% DR in reducing the early mortality of male Sprague–Dawley rats, demonstrate that the life span of certain strains of rats and mice does not increase linearly up to 40% DR. Most of the extension of life span appears to be achieved by levels of DR much lower than 40% DR.

Although the survival of the 10% and 40% DR groups was similar, the effect of 10% and 40% DR on life span was not identical. Most notable was the difference in maximum survival and the Gompertz analysis. While the mean survival was essentially the same for 10% and 40% DR, the increase in the maximum survival of the 40% DR rats was twice that observed for 10% DR rats. The Gompertz analysis shows that 10% and 40% DR have quite different effects on the slope of the Gompertz curves. For 10% DR, the curve is shifted to the right but parallel to the Gompertz curve for the rats fed AL. There are multiple possible interpretations of these data, including that the rats on 10% DR show increased health throughout life, even when young, but not a difference in the rate of declining health with age. It is also possible that the onset of aging is delayed in the 10% DR rats. In contrast, 40% DR altered the slope of the Gompertz curve, which demographers typically refer to as slowing the aging rate. However, one could argue that because of heterogeneity arising from epigenetic changes in the inbred population of the F344 rats that we were studying, a subset of rats responded poorly to 40% DR and died early, while those that tolerated 40% DR responded very well. Because the Gompertz equation was developed for studying human populations, which are highly heterogeneous, we believe that the most likely explanation for the shift in the slope of the Gompertz curve is due to a slowing of the aging rate.

In contrast to life span, we found major differences in end-of-life pathology between the rats on 10% and 40% DR. Both DR groups show similar changes in non-neoplastic lesions, including a decrease in the incidence of nephropathy. However, 40% DR had a greater effect on the incidence of neoplasia than 10% DR. Except for the incidence of pituitary adenoma, which was decreased by both

Table 2. End-of-life pathology for rats in AL, 10% DR, or 40% DR groups

	AL	10% DR	40% DR
Probable cause of death (including multiple causes of death)			
Neoplasm	24	23	18
Leukemia	8	16[a]	8
Leukemia + pituitary adenoma	2	0	0
Pituitary adenoma	3	3	2
Subcutaneous tumor	4	0	4
Others	7	4	4
Non-neoplasm	12	16	21
Nephropathy	4[b]	0	0
Thrombus, heart	3	5	5
Impaction (alimentary track)	0	2	6
Others	2	3	4
Neoplasma and non-neoplasm	4	1	0
Undetermined	3	6	6
Incidence of specific fatal diseases			
Neoplasm (total)	30	24	18[c]
Leukemia	11	16	8[d]
Pituitary adenoma	8[b]	3	2
Nephropathy	7[b]	0	0

Note: The end-of-life pathology was obtained from the three groups of rats used in the life-span experiments described in Figure 2 (i.e., each group contained 40 animals (except the 40% DR group, which contained 39 animals because autolysis prevented the pathological assessment of one animal)). The numbers in the table represent the number of rats in each group that showed the particular pathology listed. The total frequency of a lesion or grade of lesion was analyzed with a chi-square test. When the expected frequencies were too small for the chi-square test, the data were analyzed with Fisher's exact test for 2×2 tables.[18] [a]$P < 0.05$ compared to the AL and 40% DR groups; [b]$P < 0.05$ compared to the 10% DR and 40% DR groups; [c]$P < 0.05$ compared to the AL group; [d]$P < 0.05$ compared to the AL and 10% DR groups.

10% and 40% DR, 10% DR had no significant effect on the incidence of other neoplasia, including leukemia, the major pathological lesion found in these rats.

The possibility that lower levels of DR are as effective in increasing life span as high levels of DR could help explain the contradictory results reported on the effect of DR in rhesus monkeys in studies that were conducted at the University of Wisconsin[4] and the NIA.[8] One of the major differences in these two studies was body weight and the amount of food consumed by the AL monkeys. Body weight and food consumption were significantly greater in the AL monkeys in the study at Wisconsin compared to the AL monkeys at the NIA, suggesting that the AL monkeys at the NIA were slightly restricted compared to the AL monkeys at Wisconsin. Therefore, the lack of an increase in longevity reported by Mattison *et al.*[8] could be because of the AL rhesus monkeys in this study having achieved a level of restriction necessary for an increase in life span, and a further restriction did not further increase life span. Although Mattison *et al.*[8] did not observe an increase in life span of the DR rhesus monkeys fed a DR diet, they did observe a significant decrease in the incidence of cancer in the DR rhesus monkeys, which is also consistent with what we observed in rats with 40% DR compared to 10% DR.

The possibility that life span does not increase linearly with increasing levels of restriction up to 40% DR also offers a possible explanation for why some strains of mice have been reported to either not respond to DR, or in some cases, show a decrease in survival.[5–7] All of these studies used only one level of DR; therefore, it is possible that the level of DR that each genotype required for optimal life extension differs. For example, high levels of DR might have a negative effect on the strains of mice that did not show an increase (or a decrease) in life span, and lower levels of DR would have resulted in increased life span. For example, Rafael deCabo's laboratory has found that 20% DR results in a greater increase in life span than 40% DR in some strains of mice (personnel communication).

The data from our study have important translational implications. On the basis of results from the rodent studies, it is generally assumed that for DR to have an effect in humans comparable to that observed in rodents, a 30–40% reduction in food consumption is necessary. Adherence to such a dramatic reduction in calories is very difficult, or impossible, for most individuals. For example, in a study on the feasibility of DR in non-obese humans using dietary and behavioral interventions to reduce food consumption by 20%, Racette *et al.*[16] assessed adherence to the regime by assessing change in body mass and total energy expenditure compared with baseline using doubly-labeled water. The actual percent DR achieved over 1 year was 11.5%, which was less than prescribed. Our data would suggest that many, if not most, of the benefits of DR might be achieved by this modest level of caloric restriction.

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Conflicts of interest

The authors declare no conflicts of interest.

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