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Genetic Variation in the Murine Lifespan Response to Dietary Restriction: from Life Extension to Life Shortening

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Summary

Chronic dietary restriction (DR) is considered among the most robust life-extending interventions, but several reports indicate that DR does not always extend and may even shorten lifespan in some genotypes. An unbiased genetic screen of the lifespan response to DR has been lacking. Here we measured the effect of one commonly used level of dietary restriction (DR: 40% reduction in food intake) on mean lifespan of virgin males and females in 41 recombinant inbred (RI) strains of mice. Mean strain-specific lifespan varied 2- to 3-fold under *ad libitum* (AL) feeding and 6- to 10-fold under DR, in males and females, respectively. Notably, DR shortened lifespan in more strains than those in which it lengthened life. Food intake and female fertility varied markedly among strains under AL feeding, but neither predicted DR survival: therefore, strains in which DR shortened lifespan did not have low food intake or poor reproductive potential. Finally, strain-specific lifespans under DR and AL feeding were not correlated, indicating that the genetic determinants of lifespan under these two conditions differ. These results demonstrate that the lifespan response to a single level of DR exhibits wide variation amenable to genetic analysis. They also show that DR can shorten lifespan in inbred mice. Although strains with shortened lifespan under 40% DR may not respond negatively under less stringent DR, the results raise the possibility that life extension by DR may not be universal.

Keywords

calorie restriction; lifespan; food restriction; longevity; nutrition

In 1935 McCay *et al.* (1935) reported that underfed rats “attained extreme ages beyond those of either sex that grew normally.” Since then, chronic reduction of food intake (dietary restriction or DR) has become the most common environmental intervention used to extend lifespan and probe mechanisms specifying longevity. DR extends lifespan across a variety of taxa (Weindruch & Walford, 1988; Finch, 1990; Masoro, 2003) and is considered to be among the most robust life-extending interventions (Weindruch & Walford, 1988; Masoro,

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2005). Clinical studies are underway to test the effect of DR on various mortality risk factors in humans (Holloszy & Fontana, 2007), and members of one organization, the Calorie Restriction Society, practice self-imposed DR in an effort to extend their lives (Fontana *et al.*, 2008).

However, life extension by DR may not be universal (Carey *et al.*, 2002; Cooper *et al.*, 2004). Several reports indicate that DR does not extend lifespan or has minimal effects in some rodent strains (Weindruch & Walford, 1988; Harper *et al.*, 2006; Turturro *et al.*, 1999). Others even report that DR shortens lifespan in some strains (Barrow & Roeder, 1965; Fernandes *et al.*, 1976; Harrison & Archer, 1987; Forster *et al.*, 2003), but these studies have not been conclusive given that other studies have shown lifespan extension under different conditions (Weindruch & Walford, 1988; Turturro *et al.*, 1999). A systematic, unbiased screen to determine the efficacy of moderate DR across a range of genotypes is lacking. Here, we undertook such a study -- testing the hypothesis that the lifespan response to DR is subject to naturally-occurring genetic variation encompassing null or even negative effects.

This study used 41 ILSXISS recombinant inbred (RI) mouse strains (Williams *et al.*, 2004) (formerly called LXS) originally developed to analyze genetic variation in alcohol sensitivity (Bennett *et al.*, 2006). Mice were typically maintained 5/cage (Supplementary Table S1) and started at 2–5 months of age fed *ad libitum* (AL) or DR diets (60% of strain-specific AL intake) in a specific-pathogen-free vivarium dedicated to murine aging research (Ikeno *et al.*, 2005). The DR rations, which were not implemented gradually, were calculated on the basis of AL food intake measured weekly for each strain, adjusted for wastage (Ikeno *et al.*, 2005), and the rations were given daily just before lights out. At 12 months of age, the DR rations were fixed to avoid tracking the reduction of food intake that occurs during aging. We have followed this DR protocol at 60% of AL intake for over 30 years (Ikeno *et al.*, 2005; Yu *et al.*, 1982; McCarter *et al.*, 2007). This level of restriction is one of the most common (Turturro *et al.*, 1999; de Cabo *et al.*, 2005), although DR levels from 40% to 80% of AL intake have been used to achieve life extension (Weindruch & Walford, 1988).

We found that the RI strains exhibited marked genetic variation in lifespan under both AL and DR conditions (Figs. 1 A, B; Supplementary Table S1). Mean lifespan under AL feeding ranged two- to three-fold: 504 to 1152 days in males and 407 to 1208 days in females. This variation in AL lifespan is comparable to that of 31 inbred strains selected for their genetic diversity (Yuan *et al.*, 2009) (Supplementary Fig. S1). Strain variation of mean lifespan in mice under DR was even greater, ranging six- to ten-fold: 217 to 1215 days in males and 113 to 1225 days in females. Effect of strain on lifespan was significant for both sexes under both feeding conditions ($p < 1\times10^{-6}$, ANOVA). Heritability of lifespan under AL feeding was 28% (males) and 36% (females) and under DR was 55% (males) and 53% (females).

Strikingly, the majority of strains showed no extension of lifespan under the level of DR used in this study (Figs. 1C, D). Only 5% of the strains for males and 21% of the strains for females showed statistically significant life extension under DR, using single strain p values < 0.05. DR shortened lifespan in more strains (27% and 26%; males and females, respectively; $p < 0.05 – 0.001$). Although sample sizes were small, mean lifespans of males and females were significantly correlated under both AL (r = 0.50, p = 0.002) and DR (r = 0.42. p = 0.012) conditions. In addition, doubling sample size by combining the two sexes yielded a similar result: DR shortened life in more strains than showed lengthened life (Supplementary Fig. S2). Maximum lifespan (age at death of oldest mouse) was highly correlated with mean lifespan across strains under both AL and DR regimens (AL males, r = 0.81; AL females, r = 0.82; DR males, r = 0.92; DR females, r = 0.94; all $p < 1\times10^{-9}$),

indicating that the strain variation in mean lifespan was not disproportionately affected by early deaths that can arise in DR mice. That early deaths in DR mice contributed to lifespan shortening is not supported by the finding that exclusion of deaths occurring before 12 months of age had negligible effect on the frequency of lifespan shortening (Supplementary Fig. S3). These results, using a large genetic screen, buttress previous but often overlooked results showing no extension or shortening of lifespan by DR (Weindruch & Walford, 1988; Harper *et al.*, 2006; Turturro *et al.*, 1999; Barrow & Roeder, 1965; Fernandes *et al.*, 1976; Harrison & Archer, 1987; Forster *et al.*, 2003). However, whether strains showing no increase in lifespan under 40% or other fixed level of DR show no increase in lifespan under less stringent level of DR remains to be determined.

Of note, the longest lifespans achieved under DR did not exceed the longest achieved under AL feeding (Figs. 1A, B). The average of the mean lifespans of the five longest-lived strains under DR (1103±40 and 1108±32 days in males and females) did not exceed that of the five longest-lived, albeit different, strains under AL feeding (1098±20 and 1088±31 days). Future studies are needed to determine why DR cannot further extend the lifespan of long-lived strains in this RI panel. One testable hypothesis is that the lifespan extending biochemical pathways modulated by DR are already maximally modulated in strains that are long-lived under AL conditions.

The biological basis for the strikingly different responses of lifespan to the commonly used level of DR, including life shortening, is important to determine. For example, some lines in this study may have unusual nutritional needs, and thus 40% DR could cause nutritional deficiencies that might outweigh the beneficial effects of DR. However, the possibility that some strains are vulnerable to a mineral or vitamin deficiency under DR is unlikely because, with the exception of selenium and choline, the diet used (Harlan-Teklad 7912) exceeded by several fold the minimum requirements established by the National Research Council (Nutrient Requirements of Laboratory Animals, 1995) (Supplementary Table S2). Also, even with diets supplemented with vitamins, the lifespan of male DBA/2J mice was either not extended (Forster *et al.*, 2003) or minimally lengthened (Turturro *et al.*, 1999). There also was no correlation between DR lifespan and the large strain variation in absolute food intake (Table 1), suggesting that the strains most likely to encounter deficiency were not more likely to have reduced survival under DR.

Considering the derivation of the ILSXISS strains, we tested whether the lifespan variation in response to DR might be related to the segregation of alleles for extreme differences in ethanol sensitivity, which could potentially reflect differences in vitality or stress resistance. However, there was no correlation between sensitivity to this stressor and lifespan in DR mice (Table 1). Another potential measure of vigor, female fertility, also showed no correlation with DR lifespan (Table 1). These results argue against the notion that strains in which DR shortened lifespan lacked overall vitality.

Many other testable possibilities exist to explain life-shortening of some strains under DR. These include vulnerability a) to stresses requiring energy expenditure, such as cold stress; b) to inbreeding depression (recessive alleles) not reflected by the variation in AL lifespan or fertility; and c) to a 40% reduction in food intake that would not be present at a 30% or 20% reduction. Nevertheless, the variable response of these strains to DR provides a valuable tool for identifying quantitative trait loci (genes) that modulate DR's mechanism of action. In addition, mechanistic traits hypothesized to underlie the lifespan modulating effect of DR should correlate positively with the variation in the lifespan response to DR.

In summary, these findings, coupled with earlier reports, show that even though DR extends lifespan across a variety of taxa, a prolongevity effect may not be a foregone conclusion for

many genotypes. The marked genetic variation among RI strains provides a tool for identifying genes and biochemical pathways that mediate lifespan modulation by DR. Finally, the results raise a cautionary note concerning the application of DR to humans and a critical need for predictors of efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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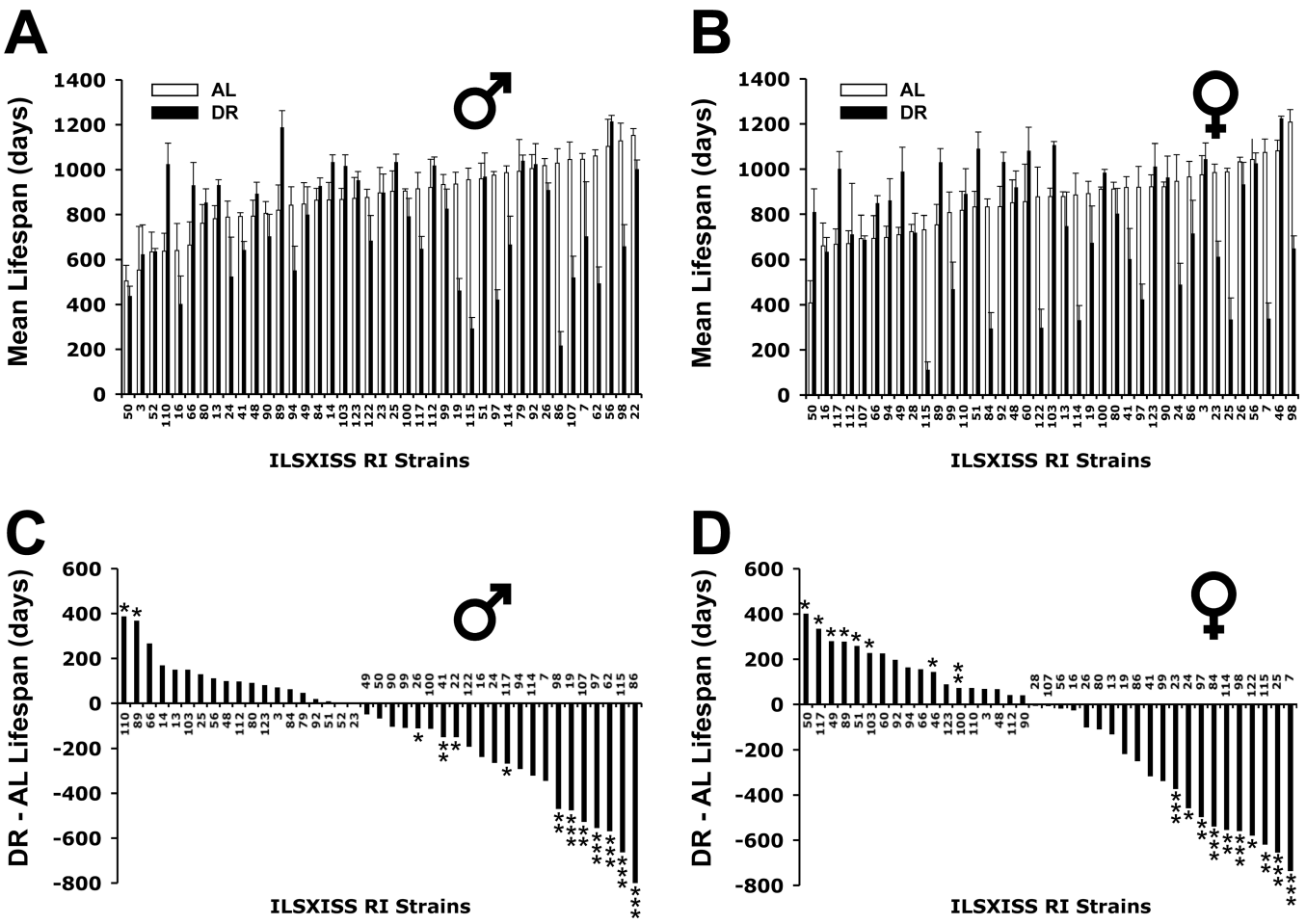

Fig. 1.

Strain variation in mean lifespan of ILSXISS recombinant inbred (RI) mice under *ad libitum* (AL) and dietary restriction (DR) diets. Lifespans were typically obtained from 10 AL and 10 DR mice from each strain (5 males & 5 females per treatment group); sample sizes in a few strains were either greater or less than 10 (details in Supplementary Table S1). The mean lifespans in the upper two panels are shown for each strain [AL (□) and DR (■)], ranked in ascending order according to the AL means (A: males, 41 strains; B: females, 39 strains). The lower two panels illustrate the deviation (positive and negative) of the mean DR lifespan from the mean AL for the same strains, ranked from the strain with the greatest increase in lifespan under DR to the strain with the greatest decrease (C: males; D: females). Error bars represent SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by t-test (no experiment-wise Bonferroni correction).

Table 1

Absence of correlation between lifespan under dietary restriction (DR) and lifespan, food consumption, fertility and ethanol sensitivity under *ad libitum* (AL) feeding.[f]

		AL lifespan[a]	AL food intake[b]	AL fertility[c]	AL fertility[d]	AL LORR[e]
DR lifespan[a]	Males	$r = 0.09$ $p = 0.56$	$r = -0.02$ $p = 0.91$	$r = 0.21$ $p = 0.19$	$r = -0.21$ $p = 0.19$	$r = 0.13$ $p = 0.39$
	Females	$r = -0.03$ $p = 0.86$	$r = 0.30$ $p = 0.06$	$r = 0.18$ $p = 0.27$	$r = -0.12$ $p = 0.48$	

[a] Strain-specific mean lifespan (days).

[b] The food intake (g/mouse/day) was measured on a weekly basis. The values are the average of food intake from 3–5 months to 12 months of age.

[c] Fertility (litter size) was measured in the generation of mice preceding the lifespan cohort and defined as the average litter size of the first three litters.

[d] Fertility (litter/female) defined as the average number of litters per strain.

[e] The sedative-hypnotic response to a high-dose, intraperitoneal injection of ethanol, defined as time to regain loss of righting reflex (LORR). The LORR data are from Bennett *et al.*, 2006.

[f] The p values of the Pearson correlation coefficients (r) are all from 2-tailed tests.