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Short communication

Restriction of amino acids extends lifespan in *Drosophila melanogaster*

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Abstract

Dietary restriction extends adult *Drosophila melanogaster* life span when the concentration of dietary yeast is diluted in a media with abundant carbohydrates. Here we vary the concentration of casein as a source of amino acids in adult diet to uncover a quality of nutrient yeast responsible for longevity control. Longevity is maximized upon diet with intermediary levels of casein. Differences in survival are not caused by elevated age-independent mortality; the longevity maximum at intermediate casein does not arise because casein is non-specifically harmful at higher concentrations. Furthermore, fecundity increases when the level of dietary casein is elevated. The demographic phenotypes of adult *Drosophila* maintained on intermediate levels of casein resemble their response to limited dietary yeast. Dietary restriction through dilution of yeast may extend longevity because this limits the intake of amino acids.

Keywords: Amino acids; Dietary restriction; Nutrition; *Drosophila*; Longevity

Reduction of food intake without starvation extends life span in many organisms including yeast, nematode, fruit fly and rodents (Bertrand et al., 1999; and reviews in Masoro, 2000; Koubova and Guarente, 2003; Partridge et al., 2005; Tatar, in press). These manipulations are referred to as caloric restriction (CR) when applied to rodents because longevity may be extended through control of calorie intake alone (Masoro, 2000). Studies with non-vertebrates, in contrast, typically dilute all components of the diet and thus vary all nutrients in an otherwise constant volume of inert media (e.g., Lin et al., 2002; Johnson et al., 1990; Chippindale et al., 1993; Chapman and Partridge, 1996; Mair et al., 2003). These manipulations are referred to as dietary restriction (DR) because they do not directly regulate the intake of calories, and it remains generally unknown whether calories or a specific component of the adult diet is responsible for longevity extension.

Limiting calories, in fact, may not be the feature of nutrition responsible for extended life span in *Drosophila melanogaster*. Longevity can be extended in males and females maintained on a carbohydrate media supplemented with a relatively low concentration of live yeast (Chippindale et al., 1993). Likewise, lifespan is increased by reducing the concentration of inactive dry yeast in constant base medium of sugar and cornmeal (Min and Tatar, 2006). Variation in yeast may be sufficient to modulate aging but because caloric intake is increased on yeast-rich medium, yeast concentration and total caloric intake can be positively correlated. Mair et al. (2005) have recently manipulated both dietary yeast and sugar to disentangle these nutrient effects. Reduction of either yeast or sugar alone improved survival in females but flies were markedly more sensitive to variation in yeast, and across diets longevity did not correlate with the food energetic value. Since feeding rate was thought to be constant across these diets at least in terms of proboscis extension (but see Carvalho et al., 2005; Min and Tatar, 2006), the increase in longevity with restricted diet was argued to be independent of net caloric intake. The data of Chippindale et al. (1993) and of Mair et al. (2005) suggest that limiting a specific nutrient component of the yeast mediates longevity in *Drosophila*.

Dietary yeast is a complex source of nutrition, containing essential and non-essential amino acids and fatty acids, nucleic acids, minerals, vitamins and carbohydrates. Although any of these components could influence longevity, here we focus on amino acids, which are the most abundant nutrient metabolite of dry yeast (~45%) (Schulze, 1995). In rodents, restriction of the methionine alone is sufficient to extend life span and to

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retard the progression of age-dependent degenerative pathology (Zimmerman et al., 2003; Miller et al., 2005). Amino acids are also implicated in recent molecular studies in *Drosophila* and *C. elegans* where longevity is extended by repressing the cellular amino acid responsive Target of Rapamycin (TOR) pathway (Kapahi et al., 2004; Vellai et al., 2003; Kaeberlein et al., 2005).

Amino acids as a potential mediator of dietary restriction in *D. melanogaster* have been investigated in two early studies through manipulation of casein as a dietary nutrient. Hollingsworth and Burcombe (1970) compared survival on an adult diet of sugar alone relative to sugar plus casein. The addition of amino acids improved longevity. Van Herrewege (1974) compared survivorship across several casein concentrations in a base medium with sugar, vitamins, nucleic acids and essential lipids. Mean lifespan was maximized at intermediate concentrations of casein but these data did not address whether mortality and reproduction changed in ways consistent with slow aging as induced by dietary restriction. In particular, high levels of a refined nutrient such as casein may be toxic and contribute to mortality that is independent of aging. Here we follow-up on these early reports and describe not only the overall survivorship across varied casein diets but also the trajectories of mortality and the patterns of age-specific fecundity.

Larvae of the Canton-S strain were grown on standard medium (5.2 g cornmeal, 11 g sugar, 11 g yeast, 0.79 g agar per 100 ml water) supplemented with several grains of live yeast (Elgin and Miller, 1980). Newly elose adults were collected over 48 h and were randomly assigned to three 1 l demography cages to a final density of 150 individuals (mixed sex). Food vials were affixed to each cage and changed every 2 days, at which time dead flies were removed, sexed and recorded.

Adults were short-lived (median life span: males 18 days, females 22 days) on a sucrose-only diet (11 g sugar and 1.1 g agar per 100 ml water) as expected (Good and Tatar, 2001), and the addition of 10% yeast strongly increased survival (Fig. 1A). Likewise, the addition of casein to the sucrose diet increased lifespan. In males, casein at levels of 0.5%, 1% and 2% produced survival similar to the diet with 10% yeast and significantly greater than the survival on 4% casein (Log rank test, 1% versus 4%, $\chi^2 = 233.82$, $p < 0.0001$). Male survival is reduced 38% upon 4% diet compared to one on 1% casein diet. In females survival was greatest when maintained on 0.5% or 1% casein, intermediate on 2% casein and lowest on 4% casein (Log rank test, 1% versus 2%, $\chi^2 = 42.195$, $p < 0.0001$; 1% versus 4%, $\chi^2 = 261.37$, $p < 0.0001$). Thus, as previously observed, *D. melanogaster* longevity is extended when dietary amino acids are limited.

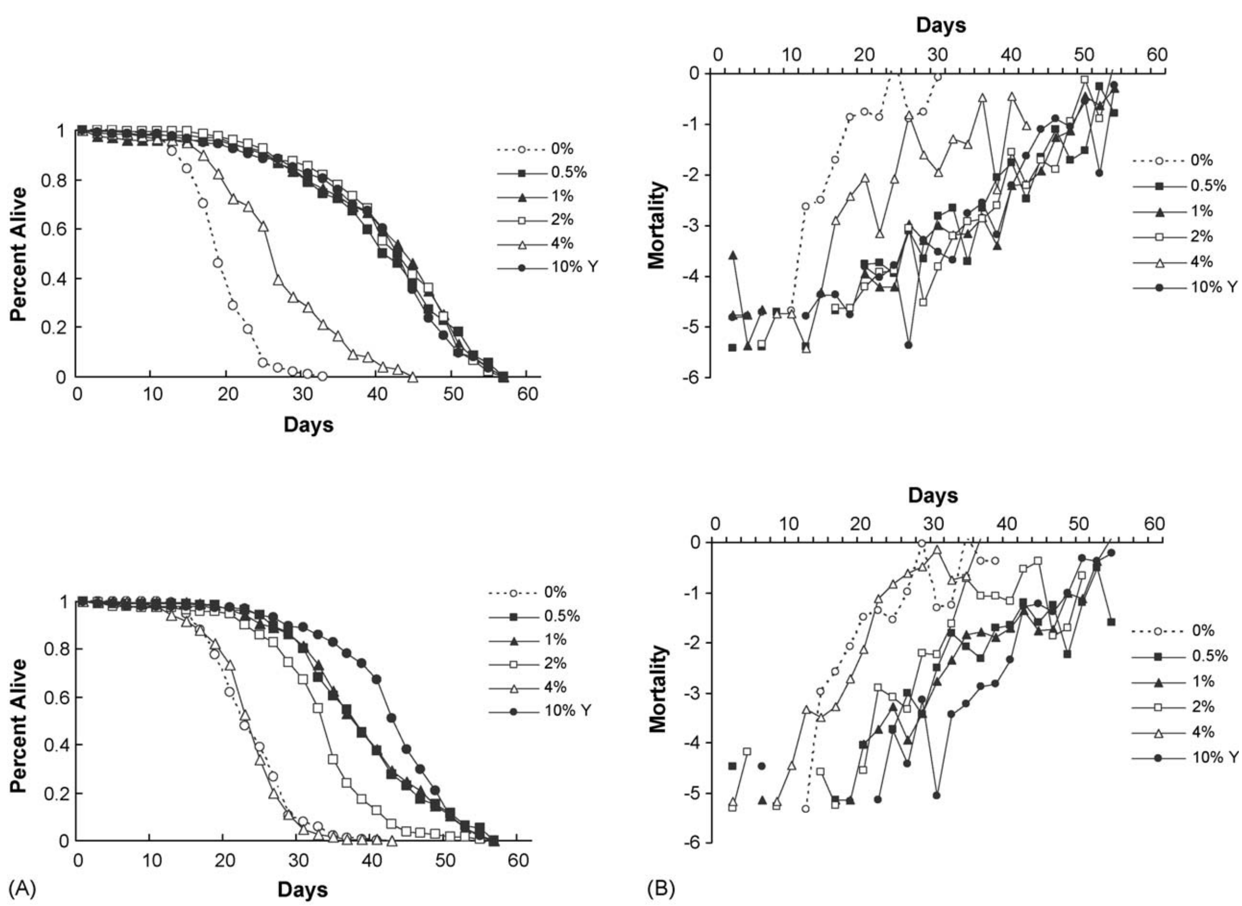

Fig. 1. Demographic patterns with amino acids addition. Male (top) and female (bottom) survivorship (A) and mortality curve (B) when maintained on no yeast diet (0%), on alternative diet with casein concentration at 0.5%, 1%, 2% and 4% and on 10% yeast diet (10% Y). Survivorship is plotted with data of replicate cages combined.

Table 1
Mortality statistics of the five different cohort flies fed different concentration of casein, with best-fit mortality models

	Best-fit model	a ($\times 10^{-3}$) (LCI, UCI)	b ($\times 10^{-2}$) (LCI, UCI)	s (LCI, UCI)	c ($\times 10^{-3}$) (LCI, UCI)	Med LS
Males						
0%	L	0.11 (0.03, 0.47)	44.45 (35.36, 55.87)	1.19 (0.72, 1.98)		18
0.5%	G	0.71 (0.42, 1.2)	11.29 (10.08, 12.64)			40
1%	M	0.12 (0.04, 0.38)	15.09 (12.80, 17.78)		3.10 (1.52, 6.32)	44
2%	G	0.28 (0.15, 0.55)	13.54 (12.07, 15.17)			42
4%	L	0.78 (0.3, 2.02)	21.33 (16.50, 27.56)	0.98 (0.54, 1.77)		26
Females						
0%	L	0.09 (0.01, 0.53)	37.40 (27.68, 50.55)	1.45 (0.77, 2.71)		22
0.5%	L	0.19 (0.05, 0.77)	17.84 (13.31, 23.92)			38
1%	L	0.28 (0.07, 1.09)	16.08 (11.70, 22.09)	0.60 (0.19, 1.82)		38
2%	LM	0.01 (0.01, 0.11)	30.85 (23.67, 40.19)	1.28 (0.77, 2.10)	2.05 (0.8, 4.77)	34
4%	L	0.21 (0.06, 0.40)	30.88 (25.14, 37.92)	0.71 (0.4, 1.24)		24

Best-fit models and parameter estimates were obtained using WinModest Software (Pletcher, 1999). Data were fitted to among Gompertz (G, $\mu x = a\,e^{bt}$), Logistic (L, $\mu x = a\,e^{bx} + f(s)$), Makeham (M, $\mu x = c + a\,e^{bx}$) or Logistic-Makeham (LM, $\mu x = c + a\,e^{bx} + f(s)$), where μx represents mortality rate at age t, a the baseline mortality, b the change in mortality rate with age, c the age-independent mortality risk and s is mortality deceleration. Median life span (Med LS, days) was also reported.

Reduced survival upon diet with elevated casein is not caused by elevated age-independent mortality. We assessed the relative goodness-of-fit between mortality with and without the Makeham parameter (c), which estimates the magnitude of age-independent mortality. The Makeham parameter did not significantly differ from zero in any cohort of casein-fed males or females; most cohort were best described by the Gompertz ($\mu x = A \exp(Bx)$) or Gompertz-Logistic ($\mu x = A \exp(Bx) + f(s)$) mortality model (Table 1). These patterns are clear in Fig. 1B where there is no trend of early age-independent mortality; the observed early death rates correspond to observations expected at the 'left-hand boundary' of a mortality plot (see Promislow et al., 1999), and mortality rates decelerate among the oldest-old (Vaupel et al., 1998). Reduced amino acids increases lifespan because this nutrient state consistently reduces the age-dependent trajectory of mortality, it retards demographic aging.

Fecundity was measured by locating single female with two males for 10 days. Females laid very few eggs when maintained on sugar-only medium (Fig. 2A). Sugar medium supplemented with 10% yeast elicited normal oviposition; females laid ~30 eggs/day/female by day 5 and stabilized at 18–25 eggs/day/female thereafter (mean among female fecundity for 10 days: 160 ± 11.7). Females maintained on casein produced a small number of eggs each day (Fig. 2A), with a peak of 5–10 eggs at early ages. Importantly, the total number of eggs from casein-fed females generally increased with the concentration of the amino acid nutrient (Fig. 2B, one-way ANOVA on all data points, $F_{(3,54)} = 12.77$, $p < 0.0001$; one-way ANOVA on data points after day 4, $F_{(3,54)} = 32.44$, $p < 0.001$). Although our casein diets were not optimal for egg production, the increase in reproduction with high casein represents a physiological trade-off between fecundity and lifespan. Consistent with our interpretation of the mortality patterns, flies on reduced casein were not relatively long-lived because flies on high casein suffered a pharmacological artifact which may cause decrease in fecundity.

Several protocols increase lifespan *D. melanogaster* by restricting dietary yeast (Chippindale et al., 1993; Mair et al., 2005; Min and Tatar, 2006). We now confirm that restricting the source of dietary amino acids in media with abundant carbohydrate is sufficient to increase longevity. Importantly,

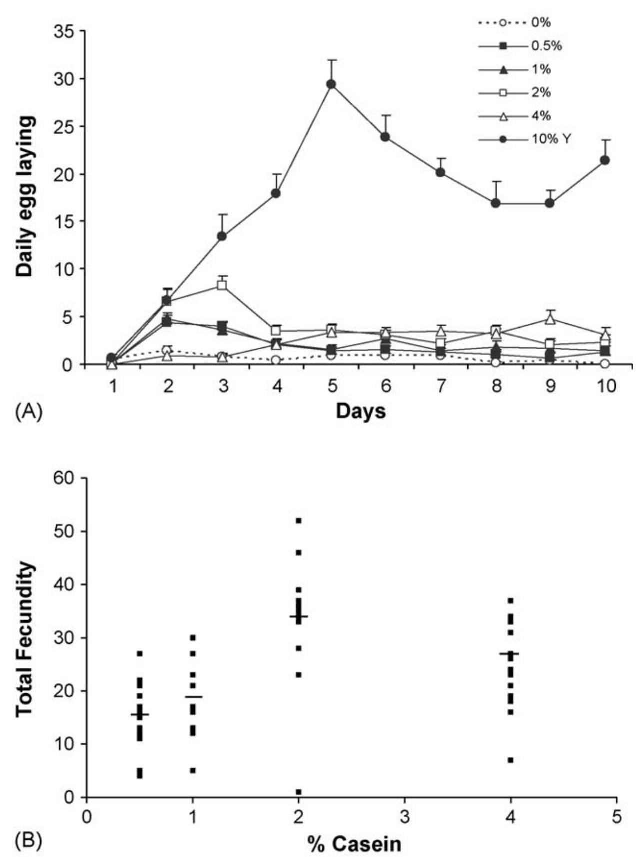

Fig. 2. Fecundity of females fed diet with various amino acid concentrations. (A) Number of eggs laid by females with amino acid addition. Error bars show standard errors. (B) Total fecundity for 10 days with amino acid addition. Bar means mean value.

this effect is consistent with physiological and demographic patterns induced by limiting the level of dietary yeast. Amino acids are at least one specific nutrient through which dietary restriction modulates aging.

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