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Short communication

Drosophila diet restriction in practice: Do flies consume fewer nutrients?

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Abstract

Although many studies of *Drosophila melanogaster* report an effect of diet upon lifespan, it is assumed rather than know that adults maintained on restricted diet acquire fewer nutrients. Diet is restricted in practice by feeding flies a medium where nutrients are diluted and some early reports suggest that flies on diluted medium compensate with increased food intake to consume equal calories on all diets. Here we measure the feeding rate of adult *Drosophila* upon a yeast-restricted diet that increases survival and reduces fecundity. We directly assessed food intake from the volume of consumed dye-marked medium and from the quantity of marked fecal pellets. Females were longest lived on diet with intermediate yeast concentration but food intake was greatest on diet with abundant yeast. Rather than compensatory feeding upon diluted diet, females increased food intake on the diet with elevated nutrient concentration. Consequently, females on diluted yeast-limited media will consume fewer calories as well as less yeast. To understand the importance of specific nutrients relative to calories as mediators of *Drosophila* aging, we must directly measure food intake and control for the feeding stimulation of nutrients.

Keywords: *Drosophila*; Feeding; Diet restriction; Longevity

Lifespan of many organisms including yeast, nematode, fruit fly, and rodents can be extended when nutrient availability is experimentally limited (Bertrand et al., 1999; Masoro, 2000; Koubova and Guarente, 2003). These manipulations are called 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, dilute essential nutrients (glucose, bacteria, yeast) in an otherwise constant volume of inert media. Since this method does not directly control of caloric intake these manipulations are simply referred to as diet restriction (DR) (Lin et al., 2002; Johnson et al., 1990; Chippindale et al., 1993; Chapman and Partridge, 1996; Magwere et al., 2004; Mair et al., 2003). One consequence of how DR is implemented is that we do not know whether diet dilution actually reduces nutrient acquisition. In this note, we determine how operational diet restriction with *Drosophila melanogaster* influences nutrient acquisition when measured by the intake of marked food and the deposition of marked fecal pellets.

Adults of *D. melanogaster* are typically maintained on media containing an agar–water matrix with sugar (or molasses), yeast, and with optional cornmeal. Diet restriction has been implemented by decreasing yeast concentration alone (Chippindale et al., 1993) or by reducing the concentration of both sugar and yeast (Chapman and Partridge, 1996; Magwere et al., 2004; Mair et al., 2003). A growing literature shows that life span is extended when adults are maintained on media at intermediary nutrient concentrations, but it remains unknown whether flies actually consume fewer nutrients under these conditions. They may not; adults maintained upon diluted media may feed more often or at a higher rate and thus acquire the same nutrition as adults fed a more concentrated diet. Clearly, whether or not compensatory feeding behavior occurs will have a profound impact on how we understand the mechanisms of diet restriction in *D. melanogaster*.

Few studies, to our knowledge, have assessed the feeding rate of adult *D. melanogaster* as a function of diet concentration. Driver et al. (1986) measured feeding rate by fecal output across a range of diets and reported that feeding varied with the inverse of nutrient concentration such that

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adults acquired the same energy on all diet types. More recently, Mair et al. (2005) estimated food intake from the duration of proboscis extension and concluded there were no differences in acquisition among flies maintained on diets that varied in concentration of sugar and of yeast. The relationship between proboscis extension and food intake under these conditions, however, is unknown.

Here, we directly assess food intake under nutrient conditions that retard aging. Laboratories use various diets to rear *D. melanogaster* larvae and to maintain adults. We routinely rear and maintain flies with a cornmeal–sugar–yeast (CSY) diet supplemented with several grains of live yeast. Our standard CSY diet has 11% sugar (dextrose), 2% dry inactive-yeast (SAF yeast), and 5.2% cornmeal in a matrix of water and 0.79% agar (Elgin and Miller, 1980). Chippindale et al. (1993) report that restriction of yeast alone is sufficient to extend lifespan. Therefore, to manipulate life span through diet restriction we maintained adults (160 males and females/1 L demography cage; three to four replicate cages/cohort) on CSY diet where sugar (11%) and cornmeal (5.2%) were held constant but inactive-yeast was 1%, 2%, 4%, 8%, or 16% (w/v); live yeast was not added to the surface. Lifespan was greatest

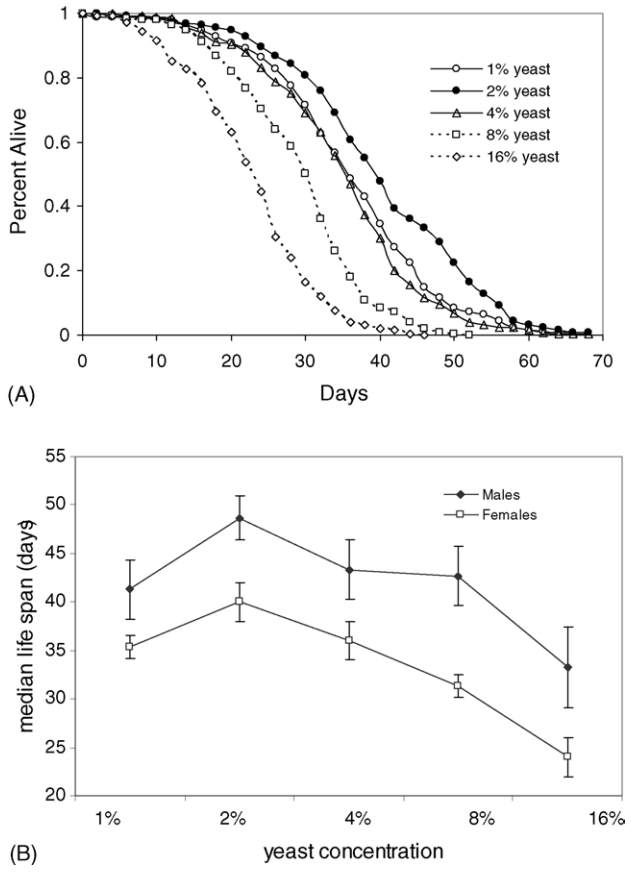

Fig. 1. Demographic patterns upon diet restriction. (A) Female survivorship when maintained on standard cornmeal–sugar–yeast (2% CSY) and on alternative CSY diet where yeast concentration was 1%, 4%, 8% or 16%. Survivorship is plotted with data of replicate cages combined. Males produce similar patterns of survivorship (data not shown). (B) Among replicate mean (with S.E.) of cage median life span as a function of yeast concentration in CSY media for males and females.

with yeast at an intermediate concentration in both males and females (Fig. 1A). Magwere et al. (2004) reported similar patterns although the diet response in females was markedly stronger than in males. In our trials, mortality was significantly reduced when adults were maintained on 2% yeast relative to 8% or 16% yeast (Log-rank test, female, $\chi^2 > 49.8$, $p < 0.0001$; male, $\chi^2 > 44.6$, $p < 0.0001$). When maintained on diets from 2% to 16% yeast, adult median life span decreased monotonically from 40 to 24 d in females and from 48 to 32 d in males. In both sexes, survivorship on 1% yeast was reduced, presumably because this diet incurs some degree of malnutrition (Good and Tatar, 2001). Diet restriction was equally effective in males and females (Fig. 1B; sex-by-yeast interaction $F_{(4,4)} = 0.93$, $p = 0.46$).

Driver et al. (1986) estimated feeding rate from the rate of fecal deposition. Because this method measures excretion associated with osmotic regulation as well as with feeding, to focus on food intake we marked the food medium with a stable dye and measured intake as the deposition of dye-marked fecal pellets and as amount of consumed dye. As well, here, we ask whether the rate of food intake varies across diets with demonstrated affects upon adult survival; for this purpose it is sufficient to test diets with 2% and 16% yeast. Newly eclosed wildtype (Canton-S) adults were maintained on CSY diet with 2% and 16% yeast; each vial contained 30 adults and food vials were changed each 2 days. Sexes were tested and prepared separately. On day 5 and 20, we provided fresh food vials with Blue No. 1 added to the media at a concentration of 0.5%. Food dye FD&C Blue No. 1 is not affected by gut pH and digestive enzymes, and remains in the digestive tract until it is passed in the fecal pellet (Tanimura et al., 1982; Shimada et al., 1987). After 24 h upon the marked food adults were anesthetized and scored individually for the presence of dye within their guts. The total sample from each treatment was divided into subsamples of 20 and homogenized in PBS. After centrifugation, absorbance of the supernatant was measured at 625 nm to estimate the quantity of ingested dye (Edgecomb et al., 1994). In addition, a subset of females given marked diet on day 5 were left undisturbed for 48, after which we counted all blue-marked fecal deposits within each of two 1 cm^2 quadrants upon the glass surface midway between the food and vial cap.

By every measure, young females on the 16% yeast diet fed at a higher rate than females upon the 2% yeast diet. After 24 h, more then 70% of the females on the yeast-rich diet had marked digestive tracts compared to less than 30% among females on the restricted diet (Fig. 2A top, $F_{(1,8)} = 40.5$, $p < 0.001$). Among young females the total quantity of consumed marked-food was four-fold greater in females upon the 16% yeast diet (Fig. 2B top, $F_{(1,8)} = 30.65$, $p < 0.001$). Although total feeding is reduced among 20 day-old females, those on yeast-rich food continued to consume the most diet (Fig. 2A bottom, $F_{(1,8)} = 16.2$, $p < 0.01$; Fig. 2B bottom, $F_{(1,8)} = 10.12$, $p = 0.012$). These measures of food ingestion correspond to estimates from dye-marked excreta. Females maintained on high yeast diet produced about three times more deposits than females on restricted medium (Fig. 2C $F_{(1,18)} = 32.14$, $p < 0.001$). As judged from the ingestion of marked-dye

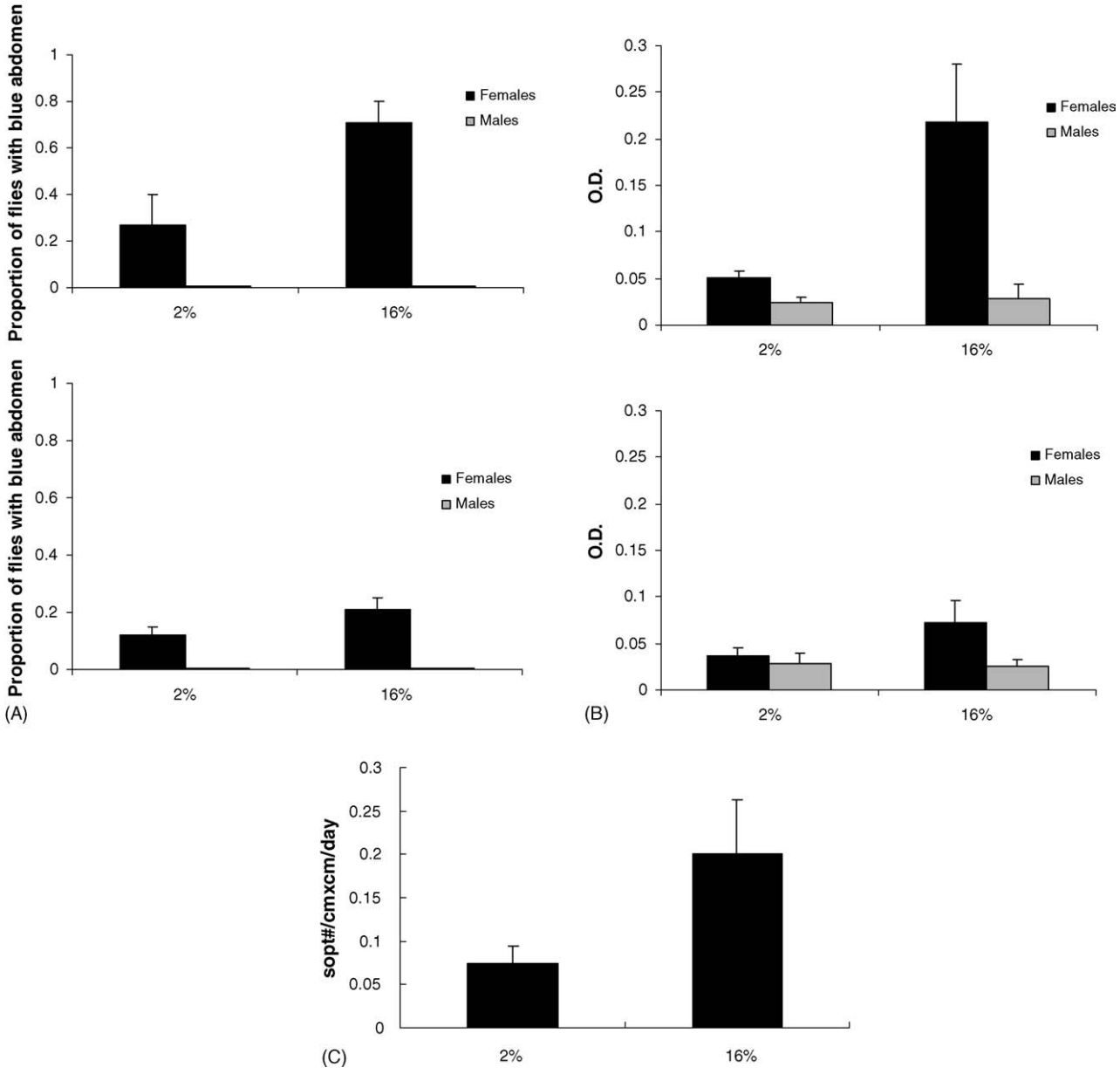

Fig. 2. Feeding rate upon diet restriction. (A) The proportion of flies with a dye-marked abdomens in flies aged 5 d (top) and 20 d (bottom) when maintained on 2% CSY or on 16% CSY diet. (B) o.d. value at 625 nm in flies aged 5 d (top) and 20 d (bottom) when maintained on 2% CSY or on 16% CSY diet. (C) The density per day of dye-marked fecal spots (standard error) from females aged 5 d when maintained on 2% CSY or 16% CSY diet. In each panel, data show the mean (with standard error) among five replicates per group.

across 24 h, males feed very little and there were no significant differences with respect to age or diet (5 d: $F_{(1,8)} = 0.43$, $p = 0.62$; 20 d: $F_{(1,8)} = 0.22$, $p = 0.64$). The time we allowed for males to feed upon the marked food may have been too short relative to their feeding behavior to provide a sensitive assay of their food intake.

Rather than increasing food intake when maintained on a yeast-restricted medium, we find that females eat more when fed a yeast-rich diet. Females on a yeast-rich diet have elevated egg production (Chippindale et al., 1993) and the associated metabolic demand may stimulate high food intake. Notably, we directly measured food intake on diets that operationally extend survival by diet restriction; diets of the sort used by Driver et al. (1986) were shown to induce compensatory feeding but whether they extended lifespan is unknown. We conclude that our operational methods to implement diet restriction in *D. melanogaster* increase adult survival without compensatory feeding. In fact, the rate of food intake is lower on a diet with diluted yeast. Females on such a dilute diet will not only consume less yeast, they will consume less sugar and acquire fewer total calories.

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