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Effects of caloric restriction are species-specific

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Abstract

This article addresses two questions: (1) ‘can caloric restriction (CR) extend the life spans of all species of experimental animals’, and (2) ‘is CR likely to slow the human aging process and/or extend the human life span?’ The answer to the first question is clearly ‘no’, because CR decreases the life span of the housefly, *Musca domestica*, and fails to extend the life span of at least one mouse strain. The answer to the second question is unknown, because human CR has not yet been shown either to increase or curtail the human life span. However, recent efforts to develop insect models of CR have been unsuccessful and/or relatively uninformative, so any insights regarding the relationship between CR and human aging are more likely to arise from studies of established, mammalian models of CR.

Keywords

Caloric restriction; Aging; Life span; Insect; *Drosophila*

Introduction

The effects of caloric restriction (CR), i.e. restriction of food intake while avoiding malnutrition, have been investigated in mammals for more than 70 years and in lower animals for more than 40 years (reviewed by Masoro 2003). A general conclusion arising from these studies is that CR can diminish the incidence of disease, delay the aging process and extend the life spans of organisms from a wide variety of phylogenetic groups, extending even outside the animal kingdom. The question has arisen whether CR can increase longevity in all animal species, but CR fails to extend the life span of DBA/2 mice (Forster et al. 2003). Contrasting results from three dipteran insect species—the housefly (*Musca domestica*), medfly (*Ceratitis capitata*), and fruit fly (*Drosophila melanogaster*)—demonstrate that extension of life span by CR is not a universal phenomenon. Among these species, the effect of CR on mean life span is either negative (*Musca*), neutral (*Ceratitis*), or reportedly positive, but in fact unknown (*Drosophila*).

Insect caloric restriction—failure to establish a model

Musca domestica

CR was studied in male houseflies after comparing various food sources, in order to select those associated with the longest control life spans (Cooper et al. 2004). The longest life span was attained by flies consuming crystalline sucrose *ad libitum* (AL). CR, based on direct measurement of the weight of sucrose consumed by young adult flies, and adjusted daily to reflect decreasing numbers of surviving flies, had a progressive, deleterious effect on the mean life span. When the weight of sucrose was adjusted to reflect an age-related decrease in food intake (unpublished), CR also progressively diminished the maximum life span. Diets containing other nutrients, i.e. proteins and lipids, decreased the life span in comparison with 100% sucrose, and moderate or severe CR ($\leq 80\%$ of normal food consumption) on these diets caused an additional decrease in survival times. Mild CR (90% of normal food consumption) was not consistently detrimental in comparison with the same diets fed AL, but none of the populations attained survival times equal to those of flies maintained on AL sucrose.

Ceratitis capitata

The life expectancies of both male and female medflies were examined as a function of the total quantity of food consumed (Carey et al. 2002). Under starvation conditions, associated with diminished lifetime reproductive output, the life span was curtailed. As food intake was increased above the starvation threshold (70% of a 40 mg/day control), there was neither any further increase nor decrease in reproductive output (up to 100% of the control) or in life span (up to 100% of the control for males and 200% for females). As in houseflies and rodents, the caloric intake was controlled by providing a known, fixed amount of food, which was fully consumed each day. The absence of any association between life expectancy and food intake, in either of two trials representing a total of 24 food concentrations, demonstrates that CR does not extend the life span of the medfly.

Drosophila melanogaster

CR has been reported to extend the life span of the fruit fly (Pletcher et al. 2002; Mair et al. 2003), but there are at least four outstanding problems with the validation of the *Drosophila* model of CR:

1. Identification of an optimal food source—Mair et al. (2005) reported that restriction of the amount of sucrose in the medium had very little effect on life span, whereas a large extension was observed by decreasing the amount of yeast. Thus, as in house-flies, sources of nutrients other than sucrose might diminish the life span, for reasons unrelated to the total caloric intake. In *Drosophila*, food consumption appears to be significantly greater on a high fat versus high carbohydrate diet, suggesting that carbohydrates are utilized more efficiently than fats (Driver and Lamb 1980).

2. Compensatory feeding in response to food dilution—In *Drosophila*, “CR” conditions are achieved by dilution of a food source present in excess amounts, whereas in *Musca*, *Ceratitis* and mammalian species, CR is achieved by daily feeding of a fixed volume or mass of food, with a known caloric content, which is completely consumed before the next feeding time. Houseflies fed sucrose in solution exhibit dramatic increases in the volume of food consumed in response to dilution (Cooper, unpublished results), but *Drosophila* CR protocols used prior to 2005 did not even attempt to quantify the caloric intake. Consequently, they could not establish that the effects of CR had been tested in *Drosophila*, much less that it extended the life span. Min and Tatar (2005) sought to address this issue by administering media containing food dye and subsequently quantifying abdominal dye content and counting marked fecal pellets in 1 cm² sectors of the vial. They were unable to demonstrate differences

in abdominal dye content in male flies on 16% versus 2% yeast, but the amount was increased in females on 16% yeast. However, there was no indication that fecal pellets “midway between the food and vial cap” represented a fixed proportion of the total fecal quantity deposited. Older flies and females carrying more eggs are less able to ascend when vials are in an upright position (unpublished observations).

The quantification of caloric intake in *Drosophila* was addressed relatively more rigorously by Carvalho et al. (2005). Measurements using radioactive tracers established that flies fed 1× medium (1% sucrose, 1% yeast extract, 8% cornmeal) consumed substantially larger volumes of food than flies fed 5–15× (5–15% sucrose, 5–15% yeast, 8% cornmeal). The flies on 1× medium still consumed smaller amounts of sucrose and yeast and lived somewhat longer than flies on the more concentrated media; however, assuming equally efficient uptake and digestion, the increased consumption of cornmeal would compensate fully for the decreased amounts of sucrose and yeast. The remaining differences in life span could then result from differences in the proportions of various nutrients in the cornmeal versus sucrose and yeast.

Dilution of the medium could additionally affect the rate of proliferation of bacteria excreted onto the food surface by the flies, or the muscular work required for the flies to avoid sticking to the food. In the first major reports of life extension by CR in *Drosophila*, Pletcher et al. (2002) showed that the control life span of 25.4 days was extended by >80% (to 46.2 days); however, Clancy et al. (2002) observed < 15% extension (versus 1× normal food concentration) in flies exhibiting a more typical control life span of ~50 days. Thus, the difference between control and CR groups arose largely because of the short life span of flies on the nutrient-rich medium, as opposed to an exceptionally long life span on the diluted medium. Two-fold variability in control life spans creates an enormous potential for spurious effects on “the rate of aging” by treatments which compensate for suboptimal conditions of maintenance, especially when represented as “percent extension of life span”. Although tetracycline feeding did not alter the life span or the effect of CR (Mair et al. 2005), which would appear to exclude bacterial proliferation as a cause of premature death on the nutrient-rich ‘AL’ medium, stronger evidence would have been provided if the bacterial load of the flies and medium had been quantified to establish the effectiveness of the antibiotic.

3. Definition of the control food concentration—A fundamental question is whether the AL or the CR flies should be regarded as normally fed. In *Drosophila*, the question—“which group is the control?”—is not easily addressed, as exemplified by the study of Min and Tatar (2005). These authors described a diet containing cornmeal, sugar and 2% yeast as both “our standard CSY diet” and as “our operational methods to implement diet restriction”, within the same study. Although the median life spans of female flies fed 1–4% yeast were no longer than 40 days, the authors also concluded that diet restriction (DR) increased adult survival, based on the even shorter life spans and increased food consumption on media containing 8–16% yeast. However, if DR is by definition whatever nutritional regimen yields the longest life span, then ‘extension of life span by DR’ becomes tautological: it is no longer the outcome of an experiment, but a stipulative definition with no obvious relationship to mammalian CR. Given the earlier definition of 2% yeast as “standard”, a more appropriate conclusion would have been that overfeeding leads to premature death.

4. Trade-off effects—The problem of trade-off effects associated with and potentially underlying extensions of life span in poikilothermal animals has been reviewed extensively elsewhere (Sohal et al. 2002). The absence of adaptive responses in the rate of oxygen consumption or fertility of male houseflies in CR studies could explain the absence of any beneficial effect on life span (Cooper et al. 2004). Conversely, Piper et al. (2005) state that the appropriate range of food dilution for CR studies in *Drosophila* is identified as one in which “life span is extended and daily and lifetime fecundity coordinately reduced”. This approach

raises a strong possibility that the life span is affected by a redistribution of resources from somatic maintenance to reproductive output. Similarly, the costs of reproduction are likely to account for the low median life span of female flies in mixed sex populations on nutrient-rich food (Bross et al. 2005), whereas male flies in the same study exhibited nearly 100% compensation for food dilution between 0.5× and 1× concentrations “of an arbitrary normal condition” and males fed 1×, 1.5× and 3× concentrations exhibited no difference in median life span. Given the simultaneous presence of so many trade-off effects and confounding variables, the prospect that *Drosophila* CR will elucidate mechanisms of aging in higher animals seems very remote.

Caloric restriction in other species—hazards of extrapolation to humans

The adverse effects of caloric restriction in houseflies, absence of any effect in the medfly, and various difficulties with the establishment of a CR regimen for *Drosophila* are sufficient to demonstrate that CR is not universally beneficial, and that a valid insect model for CR studies has yet to be developed. Dilution of the medium without actual quantification of food consumption is clearly not a reliable method for the restriction of caloric intake in flies, but compensatory feeding behavior might also be exhibited in other lower animals purportedly subject to CR. Consequently, exposure to a nutrient-depleted environment should not be classified as caloric restriction in any species unless the actual caloric intake can be documented. Additionally, and in order to maintain conceptual distinctions between caloric restriction, hibernation, estivation and stress resistance, extensions of life span in lower animals and nonanimal species should not be attributed to CR unless the existence of a hypometabolic state can be ruled out.

In comparison with the work in insects and other poikilothermal model organisms, more extensive and informative studies have been performed in animals that are phylogenetically closer to *Homo sapiens*. Studies in rodents show that the life span is extended by CR; however, the effect in *Mus musculus* is strain-specific (Forster et al. 2003). The species- and strain-specific nature of the effects of CR, which differ markedly even among closely related insects and rodents, illustrate the pitfalls of extrapolation to much more remotely related phylogenetic groups. It is not apparent in advance which species will or will not respond to CR. Consequently, it is premature at present to speculate about possible effects in humans.

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