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Do longevity mutants always show trade-offs?

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Abstract

A number of genetic mutations that substantially increase longevity have been discovered in model organisms. Although these long-lived mutants have provided many insights into the factors that affect longevity, the results from such studies should be interpreted with caution. In particular, at least some of these mutations may be poor guides to human medical intervention because they often have deleterious side effects on important biological functions.

Keywords: Aging; *Caenorhabditis elegans*; *Drosophila melanogaster*; *Mus musculus*

1. Introduction

It is now clear that a variety of genetic mutations can substantially increase longevity in yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the house mouse *Mus musculus* (Kenyon, 2005). What is not yet clear, however, is whether these mutants have delayed aging or simply a physiological slowing that gives them more time but without an increase in lifetime biological activity (Martin, 2002). In other words, do these mutants sustain youthful function longer, or are they merely "refrigerated", with lower metabolic rates or reproductive activity? It also is unclear whether there are always major fitness costs associated with genetic mutations that extend longevity. In this review we will take a broad view in evaluating the various complications that are often associated with mutations that extend longevity. These complications include metabolic effects, fitness costs, environmental effects, and the importance of genetic background when assaying the effects of genetic mutations on longevity.

The theme common to this review is that knowledge of such complications is critical, particularly if long-lived mutants are to be considered as models for intervention in human aging. The problem of determining whether an increase in chronological longevity results in a corresponding increase in physiological function is illustrated by the effect of growth temperature on ectotherms. It has been known for almost 100 years that chronological longevity can be greatly increased in most ectotherms by simply reducing the temperature at which the organism is reared (Loeb and Northrop, 1917; McArthur and Sohal, 1982). Despite the increased chronological lifespan of ectotherms reared at lower temperatures, their lifetime metabolic output is the same or even reduced compared to animals reared at higher temperatures (Miquel et al., 1976; Van Voorhies and Ward, 1999). Thus, the long-lived mutants might, in principle, be living as though they are at a low temperature, even when cultured at higher temperatures, functioning as so-called "refrigerator mutants" (Martin, 2002).

2. Refrigeration: dormancy and inhibited reproduction

Lifespan in ectotherms is not by itself a straightforward or complete guide to their aging without additional information about reproduction and other functional characters.

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Many ectotherms readily undergo dormancy, surviving over a far wider range of metabolic depression than endothermic organisms. For example, starved *C. elegans* larvae can enter into a metabolically and reproductively dormant state called the *dauer*. As dauer larvae, *C. elegans* can live up to 10 times longer than *C. elegans* grown under conditions optimized for rapid growth (Klass and Hirsh, 1976). This increase in lifespan is even greater than that typically found for the "aging longevity mutants" that have been obtained in this species.

Many, if not all, of the long-lived *C. elegans* mutants show some compromise in fitness compared to wild-type worms. For example, the first long-lived *C. elegans* mutant that was identified, *age-1*, has reduced fitness when exposed to intermittent food levels (Walker et al., 2000). Another long-lived *C. elegans* mutant that has been extensively studied, *daf-2*, would be expected to be quickly replaced by wild-type worms if forced to complete for resources in a common environment (Jenkins et al., 2004). In addition to being out-competed in tests of reproductive fitness, it also appears that *daf-2* mutants are less capable of withstanding stresses that they are likely to encounter in their natural habitat. While *daf-2* mutants can live approximately twice as long as wild-type when reared under relatively benign laboratory conditions, these mutants actually die sooner than wild-type worms when placed in conditions that more closely approximate their natural environment (Van Voorhies et al., 2005). These results make it apparent why *daf-2* mutants are not found in wild populations – in natural conditions the mutants would be expected to have both a reduced reproductive output and be less able to survive the stresses they would likely encounter.

This result is somewhat surprising in view of the fact that the long-lived *C. elegans* mutants are commonly reported to be more "stress resistant" than wild-type worms (Kenyon, 2005). However, examination of the data reveals that the correlation between the extended longevity of a *C. elegans* mutant and its ability to withstand stress is often weak or non-existent (Lee et al., 2003). A potential limitation of such stress tests is that they may be testing the ability of the animal to withstand stresses to which it is never exposed in its natural environment. For example, since *C. elegans* is a soil dwelling nematode it is unlikely to experience UV stresses used in some stress tolerance experiments. As such it is unclear how relevant such a test would be to the ability of the animal to withstand stresses associated with aging.

In *D. melanogaster*, lifespans increase and metabolic rates decline in response to lower environmental temperatures (Loeb and Northrop, 1917; Berrigan and Partridge, 1997). Such a "refrigeration" effect may be caused by reduced overall metabolic rate, or it may involve reduced reproduction. The *ovariless* and *grandchildless* mutants of *Drosophila* greatly increase longevity in conjunction with reduced fecundity, as do other methods of reducing fecundity (Maynard Smith, 1958). The long-lived *Drosophila chico* mutants produce sterile females (Richard et al., 2005). The *chico* mutant is also more sensitive to food levels than wild-type flies, and the longevity of the *chico* mutant is less than that of wild-type flies when fed a diluted diet (Leroi et al., 2005). It is also worth noting that while longevity in *D. melanogaster* can be increased by upregulating endogenous levels of heat–shock proteins, there are fitness costs, such as a reduction in reproductive rate, directly associated with such manipulations (Leroi et al., 2005). Similarly, dietary restriction leads to increased longevity in association with substantially decreased reproduction (Chippindale et al., 1993). While *Drosophila* bred for increased longevity have increased total reproduction, along with increased stress resistance and normal metabolic rates, they also have reduced early reproduction under at least some laboratory conditions (Rose et al., 2004). As a result, when these flies are returned to normal laboratory culture, they undergo a rapid decline in average longevity (Rose et al., 2004).

Extensive progress has been made in characterizing the insulin-like signaling pathway in organisms ranging from yeast to mice (Carter et al., 2002). In general, mutations or treatments that decrease the levels or the effectiveness of insulin growth factors (IGF) often result in an increase in longevity. In addition to their involvement in aging, insulin/IGF pathways are also important factors in the growth, reproduction, bone mineralization, and the energy balance of an organism. The fact that the insulin/IGF pathways are involved in so many basic physiologic processes means that interventions involving insulin/IGF modulation must be viewed with caution. Systemic or neuronal disruption of insulin-like signaling typically impairs fertility; a substantial body of literature demonstrates beneficial actions of these hormones in young and old animals (Carter et al., 2002). IGF levels naturally decrease with age in many animals; increasing IGF levels in older humans and rodents has been associated with decreased muscle wasting and improved mobility (Hursting et al., 2003). As such reducing IGF levels in the elderly could potentially result in an increased longevity but at the cost of an increased risk of bone fracture due to reduced bone and muscle mass (Hursting et al., 2003).

Inverse relationships between longevity, growth, and reproduction are commonly encountered in mammals. Mice overexpressing the *Klotho* gene and *Dwarf* mutants live longer than laboratory "wild-type" mice, but often have a reduction in fertility or are even sterile (Bartke et al., 2003; Kurosu et al., 2005). Klotho is a circulating hormone that binds to cell-surface receptors to repress intracellular signaling of insulin and insulin-like growth factor. Thus, increased levels of Klotho would be expected to reduce the effect of insulin-like growth factor. Klotho also appears to affect insulin sensitivity. Male *Klotho* mice are less sensitive to insulin, a condition associated with human type II diabetes (Kurosu et al., 2005). IGF levels can be altered by genetically disabling the genes that produce IGF. Female mice genetically "knocked-out" for the IGF-1 receptor grow to normal adult size and are longer lived than their wild-type counter parts (Holzenberger et al., 2003). However, the side effects of disrupting the

IGF-1 receptor gene include an apparent predisposition to diabetes (Holzenberger et al., 2003).

Even though it does appear possible to increase longevity in mice by disrupting insulin/IGF-like pathways, extended longevity frequently leads to reduced reproductive success, among other functional impairments. This cost will not necessarily be apparent in laboratory experiments, particularly those in which the sexes are kept apart, but may be revealed by assaying long-lived animals in more natural environments.

In view of the deleterious side effects often associated with increased longevity, we pose the following question: will we eventually find that all large-effect longevity mutants in fact have "refrigeration" side effects, such as greatly reduced reproduction? The potential difficulties of identifying such side effects are illustrated in the recent unexpected finding that yeast with increased Sir2 expression have an increased replicative lifespan, but reduced chronological longevity under stressful conditions (Fabrizio et al., 2005). Another example of the unexpected side effects that may occur in treatments that extend longevity is the observation that, while mutations in the phosphatidylinositiol-3-OH kinase (PI(3)K) pathway are often associated with an increased longevity in model organisms, mutations in PI(3)K are also associated with an increased risk of colorectal cancer in humans (Parsons et al., 2005). Understanding the subtle side effects associated with life extension will be a critical component of attempts to postpone aging in humans (Austad, 2004; Leroi et al., 2005; Jafari and Rose, 2006).

It is worth emphasizing that trade-offs between adult survival and reproduction are not necessarily apparent under all environmental conditions. The magnitude of effects, as well as secondary effects, may depend significantly on the environment. Khazaeli et al. (2005) were unable to reproduce longevity increases previously reported for the *Drosophila* mutations *methuselah* or *Indy*. Mockett and Sohal (in press) found that the longevity of the *Drosophila methuselah* mutant is the same as that of wild-type flies under many environmental conditions. Benign environments in which longevity mutants thrive may not reveal losses of function that are apparent under less benign or less artificial conditions (Walker et al., 2000; Jenkins et al., 2004; Van Voorhies et al., 2005). For example, the long-lived *Drosophila Indy* mutant appears to be physiologically identical to wild-type flies under normal rearing conditions, but is more sensitive to reduced food levels than wild-type (Marden et al., 2003). Further, there are theoretical reasons to think that populations can experience significant trade-offs that are nonetheless individually undetectable (Houle, 1991). Examples of substantial differences in the degree to which a mutation extends longevity are not uncommon. Several *C. elegans* clk mutants originally identified as being long-lived relative to wild-type worms (Lakowski and Hekimi, 1996) had the same or reduced longevity as wild-type in a separate study under what appear to be very similar conditions (Larsen and Clarke, 2002). Similarly, reported values for the effect of *C. elegans daf-2* (e1370) mutation on longevity vary nearly threefold under similar assay conditions (Kenyon et al., 1993; Van Voorhies and Ward, 1999; Yasuda et al., 1999). While a *clk-1/daf-2* double mutant was reported to live almost six times longer than wild-type, at the time the largest increase in longevity reported for a mutant (Lakowski and Hekimi, 1996), the extension in longevity for the same mutant was a much more modest 65% at a slightly lower temperature (Van Voorhies and Ward, 1999).

It is notable that while the large-scale *Drosophila* study of Khazaeli et al. (2005) found relatively short lifespans among some so-called "longevity" mutants, substantially increased longevity was detected in the selected lines of Rose and Luckinbill (Luckinbill and Clare, 1985; Rose et al., 2004), which were studied at the same time. Artificially selected stocks might have more robust longevity phenotypes. This raises the possibility that "longevity" mutants may increase lifespan by mitigating the deleterious effects of alleles accidentally fixed in highly inbred laboratory animals. If this is correct, such mutants may lose their beneficial effects when they are placed in a different genetic background. Such a result was found when superoxide dismutase levels were overexpressed in *Drosophila* lines of different genetic backgrounds. While SOD overexpression did generally increase lifespan, the effect was both genotype- and sex-specific; the greatest increase in longevity was found in strains with the shortest lifespan, with some lines showing no increase in longevity (Orr et al., 2003; Spencer et al., 2003).

3. Can a mutant be better at everything?

There are also theoretical reasons for expecting a rarity of large-effect mutations that robustly improve longevity without significant costs. Alleles that enhance survival rates substantially over a wide range of ages and environments, without any deleterious effects on other components of fitness, are likely to be strongly favored by natural selection. We expect such alleles to be present at high frequencies in natural populations. There should be far fewer *de novo* mutations with such generally beneficial effects in outbred populations, simply because most such mutations will have already occurred in the evolutionary past of these populations and then gone to fixation.

On the other hand, alleles that enhance longevity while greatly reducing important components of fitness will NOT have been strongly favored in the prior history of a model organism. Mutagenesis may produce such alleles, and when it does they may substantially increase longevity. Nonetheless, such alleles will be eliminated by selection in natural populations. Thus, the importance of such alleles in the context of interventions to the human aging process may be limited, both from the perspectives of medicine and evolutionary biology. This view is also relevant to the interpretation of selection experiments for extended life (e.g., Rose et al., 2004). However,

life-extending alleles that increase in frequency among populations selected for postponed aging may ultimately be more valuable than the alleles produced by mutant screens. The former are more difficult to characterize, but have been tested by selection over many generations in genetically heterogeneous populations; the latter need only survive in isolated culture.

In conclusion, we urge great caution in the interpretation of data from *de novo* mutants that substantially increase longevity. While the identification of mutations that extend longevity has generated a great deal of interest in both the popular and scientific literature, such mutants may be less promising as potential keys to the control of human longevity than they appear.

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