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Multiple mild heat-shocks decrease the Gompertz component of mortality in *Caenorhabditis elegans*

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ABSTRACT

Exposure to mild heat-stress (heat-shock) can significantly increase the life expectancy of the nematode *Caenorhabditis elegans*. A single heat-shock early in life extends longevity by 20% or more and affects lifelong mortality by decreasing initial mortality only; the rate of increase in subsequent mortality (Gompertz component) is unchanged. Repeated mild heat-shocks throughout life have a larger effect on life span than does a single heat-shock early in life. Here, we ask how multiple heat-shocks affect the mortality trajectory in nematodes and find increases of life expectancy of close to 50% and of maximum longevity as well. We examined mortality using large numbers of animals and found that multiple heat-shocks not only decrease initial mortality, but also slow the Gompertz rate of increase in mortality. Thus, multiple heat-shocks have anti-aging hormetic effects and represent an effective approach for modulating aging.

1. Introduction

A mild heat-shock is an effective way to induce an increase in longevity in a widely recognized environmental intervention that we have termed heat-induced hormesis (Cypser et al., 2006). In early studies, we measured the effect of single heat-shocks on life expectancy. In 1995, Lithgow and colleagues reported that exposures of a few hours to elevated levels of heat resulted in about a 15% increase in the mean life span of the nematode *Caenorhabditis elegans*, compared to non-heat-shocked controls (Lithgow et al., 1995). Subsequently, a single mild heat-shock was observed to extend longevity in several species, including *C. elegans* (Butov et al., 2001; Michalski et al., 2001; Yashin et al., 2001, 2002; Cypser and Johnson, 2002, 2003; Rea et al., 2005; Wu et al., 2008), several species of flies: *Drosophila melanogaster* (Khazaeli et al., 1997; Le Bourg et al., 2001; Hercus et al., 2003), *D. buzzatii*, and *D. koepferae* (Scannapieco et al., 2007), as well as in the yeast *Saccharomyces cerevisiae* (Shama et al., 1998; Swieciło et al., 2000). In *C. elegans*, and *D. melanogaster,* multiple heat-shocks throughout life extend longevity even more than does a single mild heat treatment early in life (Hercus et al., 2003; Olsen et al., 2006). Multiple heat-shocks have been reported to delay the onset of several age-related changes and to have anti-aging hormetic effects on growth and various cellular and biochemical characteristics of human skin fibroblasts *in vitro* (Rattan 1998, 2005, 2008; Verbeke et al., 2000, 2001, 2002; Rattan et al., 2004; Beedholm et al., 2004).

Here, we assess age-specific mortality, which has major advantages over simple assessment of life expectancy as a measure of aging: (1) there is an age interval where mortality increases exponentially with chronological age in most species, including humans, (2) mortality captures information about the entire life span distribution, (3) mortality responds instantaneously to interventions (Mair et al., 2003; Partridge et al., 2005), (4) it is central to many definitions of aging (Finch, 1990; Vaupel et al., 1998; Carey, 2003), and (5) it is altered by many interventions that extend life, especially by genetic alterations in *C. elegans* (Johnson, 1987, 1990; Vaupel et al., 1998; Johnson et al., 2001).

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A few studies of the effect of single heat-shock have used demographic approaches, in that the populations were large enough to measure age-specific mortality, rather than merely life expectancy. Khazaeli et al. (1997) investigated age-specific mortality of 28,000 *D. melanogaster* adults after a mild heat-stress, and found that life expectancy was extended as a demographic consequence of reduced age-specific mortality over a period of several weeks after the heat treatment. In *C. elegans*, both hormesis after short exposure to heat-shock and debilitation after long exposure were observed. In order to describe these survival patterns, Michalski et al. (2001) constructed a biphasic survival model with Gamma-distributed frailty; Butov et al. (2001) proposed a stochastic model of stress which describes the accumulation of damage in the cells of the worm as the worm ages; and Yashin et al. (2001) developed an adaptation model of stress. Later, Yashin et al. (2002) proposed a discrete heterogeneity model in which isogenic populations of *C. elegans* consist of three subpopulations with distinct Gompertz mortality patterns (Gompertz, 1825). They concluded that the hormetic effect of a single mild heat-shock could be explained by the transfer of worms from one subpopulation to another. We investigated age-specific mortality following a single mild heat-shock in a large population of worms, and found that the hormetic effect on longevity is driven by a decrease of initial mortality only (Wu et al., 2008).

Although multiple heat-shocks were reported to extend longevity (Hercus et al., 2003; Olsen et al., 2006), no demographic studies of the effects of multiple heat-shocks on mortality have been reported. We previously demonstrated that a single heat-shock early in life decreased *only* initial mortality without changing the subsequent rate of increase in mortality (Wu et al., 2008). Because of the close association between aging and the rate of increase in mortality (Finch, 1990; Mair et al., 2003), our earlier observations suggest that a single hormetic treatment early in life does not alter the rate of aging. We therefore questioned whether multiple heat-shocks administered during adulthood influenced the rate of increase in mortality in *C. elegans*.

2. Materials and methods

2.1. Strain and age-synchronization of populations

We used TJ375 (*gpIs1 [hsp-16.2::GFP]*), which carries the *hsp-16.2* promoter, driving expression of GFP (Green Fluorescent Protein), integrated into the genome of the N2CGCb background strain, which has been employed to predict longevity (Rea et al., 2005) and to examine mortality of worms following single mild heat-shock early in life (Wu et al., 2008). Animals were maintained as frozen stocks as described (Johnson and Wood, 1982) until needed. To establish age-synchronous populations, worms were grown at 20 °C on NGM agar plates seeded with live *Escherichia coli* (OP50). Gravid adult worms were put onto fresh NGM plates for 5 h and then removed by washing with S Basal solution. The eggs remaining on the plates were permitted to develop into young adults at 20 °C.

2.2. Heat-shock

Young adults were divided into six populations: control (no heat-shock), heat-shocked only on day 1 of adulthood within 24 h after the onset of fertility, heat-shocked on days 1 and 4, on days 1, 4, and 7, on days 1, 4, 7, and 10, and those heat-shocked on days 1, 4, 7, 10, and 13. Adults were maintained in fresh liquid medium at 20 °C for longevity assessments, which are described below. On the day of heat-shock, populations were transferred to 10 cm NGM/OP50 plates and heated at 35 °C for 1 h on day 1, 55 min on day 4, 50 min on day 7, 40 min on day 10, and/or 30 min on day 13. Preliminary results suggested that these times would yield hormetic effects on longevity at these ages.

2.3. Assessment of longevity

After heat-shock, worms were transferred to fresh liquid medium for longevity assessment, as described previously (Johnson and Wood, 1982). About eighty worms were cultured in plastic Petri plates containing 4 ml of liquid medium (1×10^9 cells/ml OP50) but with the addition of 5-fluoro-2′-deoxyuridine (FUdR, Sigma) at a final concentration of 25 μM/ml to prevent development of progeny. Previous experiments (Mitchell et al., 1979; Apfeld, 1999) and our own unpublished data indicate that FUdR does not significantly affect life span under these conditions. Worms were transferred to fresh medium every day and the numbers of worms alive, dead, or censored (lost or accidentally killed) was recorded using the criteria of death described in Johnson and Wood (1982).

2.4. Statistical analyses

We assigned the life span of worms on the first day of adulthood as zero. The calculation of mean life span and statistical tests were described in Wu et al. (2006). Age-specific probability of death was estimated and plotted to reflect mortality trajectory with age. It was estimated by the formula:

$$q_x = \frac{d_x}{n_x}$$

where q_x is the probability of death at age (day) x; d_x is the number of worms that died in the age interval $(x, x+1)$ and n_x is the number of worms at risk at the beginning of age x. Age-specific force of mortality was fitted with the following Gompertz model to reflect the initial mortality and the rate of increase in mortality with age. Gompertz parameters were estimated by using the maximum likelihood method (Wu et al., 2008). The likelihood ratio test was employed for the comparison of the rates of increase in mortality between two populations.

3. Results

3.1. Mean life span

Worms were subjected to six sets of conditions: control (not heated), and experimental groups heat-shocked 1, 2, 3, 4, or 5 times. The first heat-shock was always administered on day 1 of adulthood within 24 h after the onset of fertility and subsequent shocks were administered in consecutive fashion on days 4, 7, 10, and 13. Two independent experiments were conducted.

In the first experiment, the mean life spans of animals heat-shocked, 1, 2, 3, 4, and 5 times are 19.0, 21.9, 23.7, 24.9, 25.8, and 26.4 days, respectively (Fig. 1 and Table 1). When worms were exposed to mild heat-stress on day 1 of adulthood, the mean life span increased from 19.0 to 21.9 days, and the increase of 15.3% compared to controls was highly significant ($p < 0.0001$). When two mild heat-shocks were applied, the mean life span of worms was extended to 23.7 days, an increase of 24.7% as compared to control. When worms received 3, 4, or 5 heat-shocks, they displayed increasing extensions of mean life span: 31.1%, 35.8%, and 38.9% as compared to control, respectively. The second experiment produced similar results. A single heat-shock on day 1 of adulthood significantly increased longevity (a 16.7% increase compared to controls, $p < 0.0001$). Multiple heat-shocks of 2, 3, 4, or 5 times extended mean life span 24.2%, 29.3%, 34.3%, or 38.4% as compared to