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The U-Shaped Response of Initial Mortality in *Caenorhabditis elegans* to Mild Heat Shock: Does It Explain Recent Trends in Human Mortality?

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U-shaped dose-response relationships (hormesis) have been documented in numerous biological, toxicological, and pharmacological investigations. For example, in response to a mild 35°C heat shock, the longevity of *Caenorhabditis elegans* exhibits an inverted U-shaped dose-response. By applying the demographic concept of heterogeneity, we find that this U-shaped curve for longevity response is driven by a U-shaped dose-response of initial mortality. When worms are subjected to mild heat shock, the initial mortality decreases compared to the control. This initial mortality benefit increases with moderate increases in the length of heat shock, peaking at a point that coincides with the induction of damage to the worms. The dose of heat shock that coincided with this benefit in initial mortality did not affect the rate of increase in mortality.

Key Words: Hidden heterogeneity—Initial mortality—U-shaped response—Heat shock—*Caenorhabditis elegans*.

U-SHAPED dose-response relationships were reported as early as 1887 by Hugo Schulz, who found that numerous toxins enhanced fungal metabolism at low concentrations even though they were inhibitory at higher concentrations (for review, see 1,2). Nearly 60 years after Schulz's initial discovery, Southam and Ehrlich (3), unaware of the work of Schulz, made a similar observation and coined the term ''hormesis'' to describe the phenomenon (2–4). Since then, the term hormesis has been widely used to describe a dose-response relationship characterized by low-dose stimulation and high-dose inhibition or the converse. Many such U-shaped dose-response relationships have been documented in biological, toxicological, and pharmacological investigations (4–6). The mechanisms involved in forming hormetic response are not clear.

In aging research, mild heat shock is a widely recognized environmental intervention that produces hormesis (manifested as extended longevity), provided the duration of heat shock is brief. In 1995, Lithgow and colleagues (7) reported that brief exposure to elevated levels of heat resulted in a 15% increase in the mean life span of *C. elegans*, compared to nonheat-shocked controls. Subsequently, mild heat shock was observed to extend longevity in several species, including the worm *C. elegans* (8–12); the flies *Drosophila melanogaster* (13–15), *Drosophila buzzatii*, and *Drosophila koepferae* (16); the yeast *Saccharomyces cerevisiae* (17); and cultured human cells (18,19). However, when the duration of heat shock was too long, harmful effects manifested as decreased longevity. According to Yashin and colleagues (20), the mean life span of worms heated at 35°C on agar plates for 1–2 hours increased significantly as compared to unshocked controls; the life span following 3–4 hours of heat shock was not significantly different from controls, and life span following 5 hours of heat shock decreased significantly. These observations demonstrate an inverted U-shaped response to heat shock in specifying longevity of *C. elegans*.

Hormetic mechanisms after mild heat shock have been studied at the demographic level. Khazaeli and coworkers (13) investigated age-specific mortality of 28,000 *D. melanogaster* adults after mild heat stress, and found that life expectancy was extended as a demographic consequence of reduced age-specific mortality over a period of up to several weeks after the heat treatment. Their explanation did not account for hidden heterogeneity within a population (21,22). Based on the idea of Strehler and Mildvan (23), Michalski and colleagues (24) used the concept of homeostatic protection after a stress in the analyses of experimental data on populations of worms that were heat-shocked at 35°C. The authors concluded that, in this case, hormesis was driven by more effective protection of animals at ages younger than 27 days. Note that the concept used by Michalski and coworkers (24) is applicable to findings of Khazaeli and colleagues (13). The role of heat shock proteins (HSPs) in these effects remains questionable, because their level decreases quickly after heat shock (25). Yashin and colleagues (26) proposed that isogenic populations of worms are heterogeneous and consist of three subpopulations with Gompertz's mortality patterns, which include frail, normal, and robust. The authors concluded that both the hormetic effect and the harmful effect of mild heat shock were caused by the transfer of worms from one subpopulation to another. Wu and coworkers (27) confirmed the involvement of hidden heterogeneity in manifestation

of the hormesis effect after a mild heat shock. They also performed an experimental study that visualized such heterogeneity.

In another work, Strehler and Mildvan (23) studied the two parameters (initial mortality, *a,* and the rate of increase of mortality with age, *b*) of the Gompertz curve $\mu(x) = ae^{bx}$ describing mortality in human populations exposed to different environmental and living conditions. They showed that *a* and *b* are strongly negatively correlated—that is, when initial mortality (*a*) was high, the rate of increase of mortality with age (*b*) was low. Similar correlations between Gompertz parameters have been found in populations of laboratory animals exposed to different ambient conditions (28). Our preliminary studies show that mortality response to stressful conditions does not show these relationships, and deserves special investigation.

In this article, we continue studying the role of hidden heterogeneity in longevity hormesis effects after a mild heat shock. In particular, we investigate which mortality characteristics are modified by heat shock conditions. For these purposes, we use different heat shock protocol on a large number of worms. Then we investigate the dose-response relationships in isogenic populations of *C. elegans*. We describe population data from stress experiments using a model of heterogeneity that makes no assumptions about mortality.

Methods

Strain and Age-Synchronization of Populations

TJ375 (*gpIs1 [hsp-16.2::GFP]*), which is a strain of the *hsp-16.2* promoter driving expression of green fluorescent protein (GFP) integrated in the genome of the N2 standard background strain, was used to predict longevity of *C. elegans* (29) and to examine the theory of population hidden heterogeneity (27). We used the same strain. Animals were maintained as frozen stocks as described (30) until needed. To establish age-synchronous populations, worms were grown at 20°C on nematode growth medium (NGM) agar plates seeded with live *Escherichia coli* (OP50). Gravid adult worms were put onto fresh NGM plates for 5 hours 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.

Heat Shock

Young adults were transferred into a 1-liter flask containing S Basal solution, *E. coli* strain OP50 (1×10^9 cells/mL) and cholesterol (10 μg/mL) (liquid medium) that had been preheated to 35°C. After .5 hour, 1 hour, or 2 hours at 35°C with rotation (100 rpm) in a New Brunswick Scientific Co. [Edison, NJ], Series 25D incubator), worms were transferred to 50 mL tubes, pelleted under gravity (~ 5 min), and then transferred to fresh medium that had been precooled to 20°C. Recovering cultures were maintained at 20°C with rotation in liquid medium for 10 hours.

Assessment of Longevity

After recovery from heat shock, worms were transferred to fresh liquid medium for longevity assessment, as described previously (30). Eighty worms per set of conditions were cultured in plastic petri plates containing 4 mL of liquid medium (described above) but with the addition of 5-fluoro-2′-deoxyuridine (FUdR; Sigma, St. Louis, MO) at a final concentration of 25 μM to prevent development of progeny. Previous experiments (31,32) and our own unpublished data indicate that FUdR does not significantly affect life span. Worms were transferred to fresh medium every second day, and the numbers of worms alive, dead, or censored (lost or accidentally killed) were recorded using the criteria of death described in Johnson and Wood (30).

Gompertz Model and Model of Discrete Heterogeneity

Worms are heterogeneous in the ability to respond to stress (27,29). We used the discrete heterogeneity model proposed in Yashin and colleagues (26)—that is, a heterogeneous population of worms is assumed to consist of one or more subpopulations. If there is only one subpopulation, the hidden heterogeneity does not significantly affect longevity; otherwise, it does. Within a given subpopulation, the Gompertz model is used to measure the force of mortality, as follows.

Formula (1) describes the force of mortality of subpopulation i in the Gompertz model. Parameters a_i and b_i are initial mortality and the rate of increase in mortality, respectively. For a population consisting of N subpopulations, the average survival function is given by formula (2).

$$\mu_i(x) = a_i e^{b_i x} \tag{1}$$

$$\overline{S}(x) = \sum_{i=1}^{N} p_i e^{-\frac{a_i}{b_i}(e^{b_i x}-1)} \tag{2}$$

where x is an individual's age, i designates the subpopulation, and p_i is the initial proportion of the ith subpopulation within the overall population.

To obtain estimates for model parameters a_i, b_i, and p_i, we used a maximum-likelihood estimation procedure (26). The log-likelihood function can be expressed in the form

$$LogLik = \sum_x (d_x \ln q_x + (n_x - d_x)\ln(1 - q_x)) \tag{3}$$

where d_x is the number of deaths on day x, n_x is the number of worms at risk at the beginning of day x, and q_x is the probability of death on day x. The probability of death is described by the equation $q_x = 1 - \frac{\overline{S}(x+1)}{\overline{S}(x)}$. The effect of the length of the heat shock was tested using the likelihood ratio test.

Results

Inverted U-Shape of Mean Life Span

Worms were treated under four sets of conditions: no heat, 0.5-hour heat, 1-hour heat, or 2-hour heat, and three independent experiments were conducted (Table 1). In all, 527 worms were not heated, and 532, 524, and 612 worms were heat shocked for .5 hour, 1 hour, and 2 hours,

Table 1. Mean Life Span and Descriptive Statistics

Experiment No.	Heat Length (h)	Sample Size	Mean Life Span (d)	Percentage of Control	*SE* (d)	*p* Values for Comparison With Control*
I	0	179	18.0	100.0	0.4	–
	0.5	180	20.4	113.3	0.5	.0030
	1	183	21.3	118.3	0.5	1.2E-05
	2	193	9.7	53.9	0.6	1.0E-15
II	0	173	18.5	100.0	0.5	–
	0.5	176	21.2	114.6	0.5	.0001
	1	171	22.4	121.1	0.6	5.9E-07
	2	221	8.4	45.4	0.5	1.0E-15
III	0	175	18.9	100.0	0.4	–
	0.5	176	20.9	110.6	0.5	.0017
	1	170	21.6	114.3	0.5	2.4E-05
	2	198	8.5	45.0	0.5	1.0E-15
Total	0	527	18.6	100.0	0.3	–
	0.5	532	20.8	111.8	0.3	2.1E-07
	1	524	21.8	117.2	0.3	4.6E-14
	2	612	8.9	47.8	0.3	1.0E-15

Notes: **t* test.
SE = standard error.

respectively. When worms were heat shocked for .5 hour, the mean life span increased from 18.6 days to 20.8 days, and the increase of 11.8% was highly significant ($p = 2.1 \times 10^{-7}$). After the heat shock of 1 hour, the mean life span of worms was extended further, to 21.8 days, an increase of 17.2% as compared to controls. However, the life span of worms receiving a heat shock of 2 hours was shortened to 8.9 days, a significant decrease (52.2%), as compared to the controls. Thus, worms received a hormetic benefit from heat shocks of .5 hour or 1 hour, but were damaged by a heat shock of 2 hours. This inverted U-shape of mean life span was observed in each replication of all three experiments (Table 1).

Age-Specific Mortality

The mean life span represents mortality averaged across all ages. In contrast, age-specific mortality allows us to look at the segmental effects on mortality at each age. Figure 1 shows the age-specific probability of death within populations with no heat shock or heat shock for .5 hour, 1 hour, or 2 hours, in each experiment. In examining the combined data (Figure 1D), the population heated for .5 hour displays a mortality curve parallel to the control curve, and has lower mortality than the control at all ages. When worms were heated for 1 hour, the age-specific probability of death was higher before day 6, but much lower after day 6, as compared to the unheated control group. After a 2-hour heat shock, mortality before day 6 was high, then fell until day 10, and then increased gradually with age, such that, at all ages, the probability of death was higher than that of the controls. These patterns of age-specific probability of death were also observed in each of the three individual experiments (Figure 1A–C). These curves of mortality reveal that (a) all worms received a hormetic benefit from the .5-hour heat shock; (b) some worms were harmed and died soon after the 1-hour heat shock, but others received a hormetic benefit; and (c) all worms were harmed by the 2-hour heat shock, and some were severely damaged.

Heterogeneity Analysis

We used a discrete heterogeneity model to separately fit the longevity data of the control, 0.5-hour-, 1-hour-, and 2-hour-heated populations. To determine the minimal number of subpopulations within each of these heated populations, we used a likelihood ratio test. The *p* values of the likelihood ratio tests and the corresponding estimates of parameters of each heterogeneity model are listed in Tables 2 and 3, respectively. For both the control and the .5-hour-heated population, models with two subpopulations did not fit the data significantly better than did the models with only one subpopulation (Table 2). This was true using either combined or individual experiments. In contrast, for the 1-hour-heated population, the two-subpopulation model fits significantly better than the model with only one subpopulation, and the model with three subpopulations failed to converge. For the 2-hour-heated population, the model with three subpopulations fits significantly better than the model with two subpopulations, and the model with four subpopulations failed to converge. To summarize, the likelihood ratio test showed only one population in the control, only one population in the 0.5-hour-heated population, two subpopulations in the 1-hour-heated population, and three subpopulations in the 2-hour-heated population.

We calculated the life expectancy of each subpopulation (Figure 2) by using life table methods (34). Based on the combined data (Figure 2D), all worms received a hormetic benefit from the .5-hour heat shock, with life expectancy increasing from 18.9 days to 21.2 days. After the 1-hour heat shock, 93.8% (Table 3) of the worms increased their life span further to 23.3 days (Figure 2D), but 6.2% of them were damaged, resulting in a life expectancy of only 5.1 days. After the 2-hour heat shock, all worms were damaged. One subpopulation (38.3%, Table 3) displayed a decrease of life span from 18.9 days to 16.8 days (Figure 2D), a second (29.8%) to 6.0 days, and a third subpopulation (31.9%) displayed a decrease to only 3.5 days. All three individual experiments (Figure 2A–C) show the same pattern as the

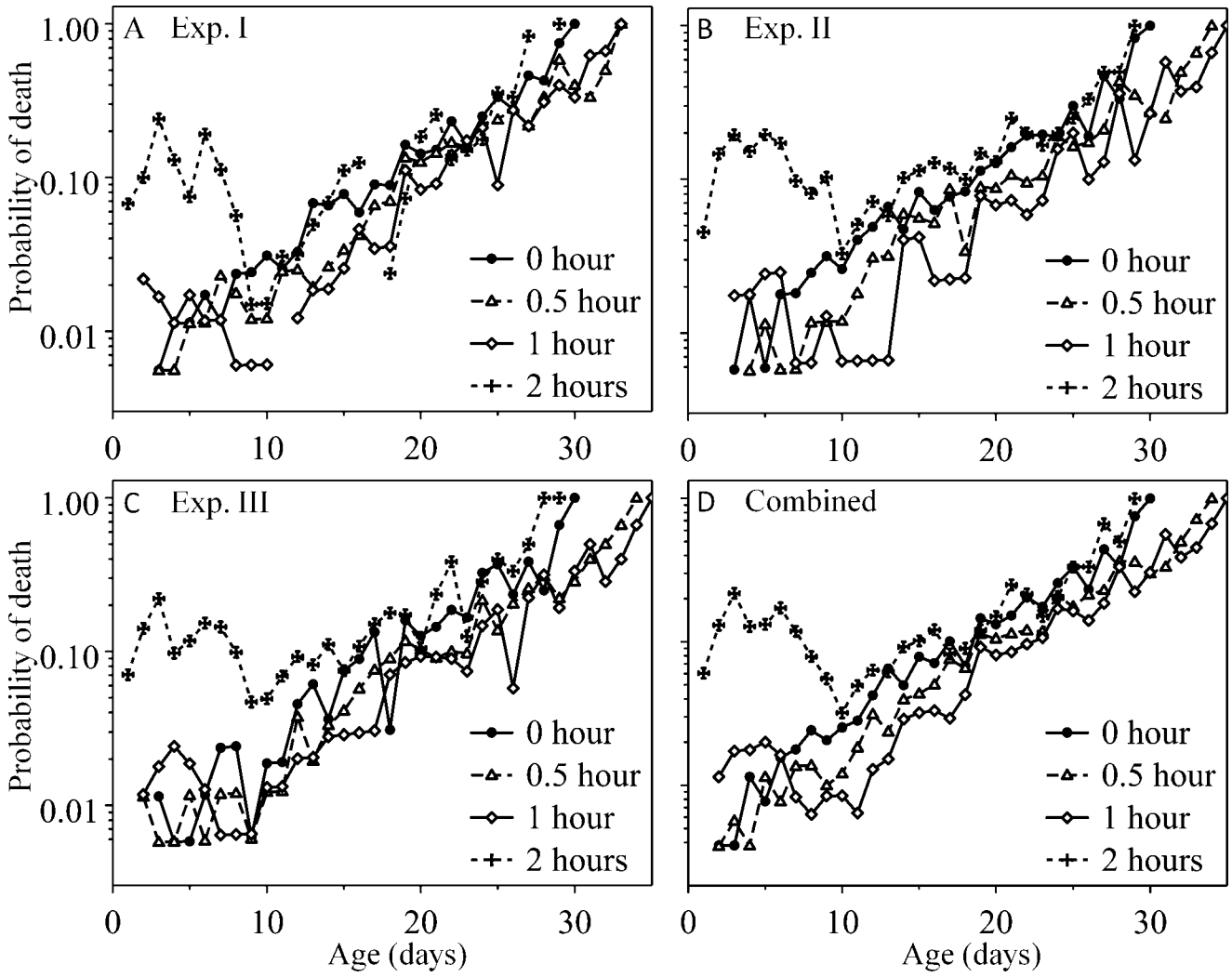

Figure 1. Age-specific probability of death of worms exposed to a mild heat shock at 35°C for 0 hour, 0.5 hour, 1 hour, or 2 hours. **A,** Experiment I. **B,** Experiment II. **C,** Experiment III. **D,** Combined data. The sample size of each experiment is given in Table 1. *y*-axis is in log scale.

combined data. This is consistent with the previous analysis of age-specific mortality.

U-Shaped Relationship Between Heat Dose and Initial Mortality

By examining the combined data of all three experiments, we found that the rates of increase in mortality for the control ($b = 0.175$), for the .5-hour-heated population ($b = 0.168$), for the first subpopulation of the 1-hour-heated population ($b = 0.186$), and for the first subpopulation of the 2-hour-heated population ($b = 0.146$) are very close (Table 3). We also found that the initial mortalities for the second subpopulation of the 1-hour-heated population ($a = 0.012$), and for the second and third subpopulations of the 2-hour population ($a = 0.011$ and $a = 0.016$, respectively) are very close (Table 3). The likelihood ratio test showed that the rates of increase in mortality for the above four populations (subpopulations) were not significantly different and that the initial mortalities for the above three subpopulations were not significantly different ($p = .067$).

Thus with the four rates of increase in mortality identical and the three initial mortalities identical, the parameters of each subpopulation were re-estimated by using the maximum likelihood method. As shown in Figure 3, worms given a .5-hour mild heat shock displayed the same rate of increase in mortality as the control (0.173), but also displayed lower initial mortality (0.0028) than the control (0.0045). Worms given a 1-hour heat shock displayed a bifurcation of reactions: 94.3% of these worms displayed initial mortality (0.0020) even lower than that of the 0.5-hour group, with their rate of increase in mortality matching that of the control (0.173). In contrast, the minor subpopulation in the 1-hour group (5.7%) displayed higher initial mortality (0.0147) and a higher rate of increase in mortality (0.762). After a 2-hour heat shock, we observed a further separation of the worms into three distinct subpopulations: 36.1% of worms had higher initial mortality

Table 2. Likelihood Ratio Test

Experiment No.	Hypothesis	Length of Heat Shock (h) 0	0.5	1	2
I	Hypothesis A*	0.4235	0.3705	0.0016	
	Hypothesis B†				1.8E-07
II	Hypothesis A	0.2214	0.0873	0.0003	
	Hypothesis B				5.3E-05
III	Hypothesis A	0.4825	0.4716	0.0081	
	Hypothesis B				2.3E-07
Total	Hypothesis A	0.0531	0.0719	9.6E-09	
	Hypothesis B				1.0E-17

Notes: *Null hypothesis (H0): the model with two subpopulations fits the data equally well as the model with one subpopulation.

†Null hypothesis (H0): the model with three subpopulations fits the data equally well as the model with two subpopulations.

(0.0055), with no change in the rate of increase in mortality (0.173) compared to controls; 32.4% of worms had even higher initial mortality (0.0147) and a higher rate of increase in mortality (0.587) compared to controls; and 31.5% of worms had even higher initial mortality (0.0147) and even higher rate of increase in mortality (1.328) compared to controls. In summary, we observed a U-shaped curve in survival response among worms after mild heat shock. When worms were mildly heated, initial mortality decreased whereas the rate of increase in mortality is unchanged. As long as the heat shock was not severe, the initial mortality decreased further. As the duration of heat shock increased into severity, initial mortality started to increase again. Heat shocks severe enough to produce maximal initial mortality also increase the subsequent rate of increase in mortality.

Similarly, in each individual experiment, the likelihood ratio test showed that the rates of increase in mortality for the control, for the .5-hour-heated population, for the first subpopulation of the 1-hour-heated population, and for the first subpopulation of the 2-hour-heated population (Table 3) were not significantly different ($p = .43$, .035, .44 in experiments I, II, and III, respectively). With these four identical rates of increase in mortality, the parameters of each subpopulation in each experiment were re-estimated by using the maximum likelihood method, and the initial mortalities for the above four populations (subpopulations) are shown in Figure 4. A clear U-shaped curve of initial mortality varying with the length of heat-shock was observed in each experiment as well.

Maximum Hormetic Effect

At the minimum of the U-shaped curve (Figure 3, Figure 4), worms displayed the lowest initial mortality and the lowest rate of increase in mortality. These parameters combine to produce the maximum life span and thus define the maximum hormetic effect. The methods of heat shock used here produced a maximum life expectancy of 22.9 days, 24.2 days, 23.0 days, and 23.3 days in the individual experiments and the combined data, respectively, as represented by the life expectancy of the first subpopulation of the 1-hour-heated population. This maximum hormetic effect is equivalent to a 24.5%, 29.4%, 19.2%, and 23.3% increase in longevity, as compared to the respective controls.

Discussion

The data presented here and previous data (20) showed that *C. elegans* exhibits an inverted U-shaped response in longevity after mild 35°C heat shock. By separately examining the two parameters (initial mortality and the rate of increase in mortality) of the widely-used Gompertz model (34,35), we showed that the inverted U-shaped response in longevity can be explained by the U-shaped response in initial mortality. After a mild heat shock, the initial mortality

Table 3. Maximum Likelihood Estimates of Parameters of Heterogeneity Model

Experiment No.	Heat Length	Parameters of Each Subpopulation: Subpopulation 1 α*	b†	%‡	Subpopulation 2 α	b	%	Subpopulation 3 α	b	%
I	0	0.0041	0.178	100						
	0.5	0.0027	0.182	100						
	1	0.0011	0.208	93.5	0.034	0.56	6.5			
	2	0.0033	0.187	37.5	0.003	0.85	23.3	0.015	1.27	39.2
II	0	0.0049	0.166	100						
	0.5	0.0030	0.167	100						
	1	0.0012	0.189	93.1	0.003	1.00	6.9			
	2	0.0186	0.114	41.8	0.012	0.74	35.0	0.003	2.24	23.2
III	0	0.0036	0.182	100						
	0.5	0.0035	0.161	100						
	1	0.0021	0.172	94.3	0.009	0.92	5.7			
	2	0.0118	0.144	39.8	0.010	0.64	28.0	0.018	1.36	32.2
Total	0	0.0042	0.175	100						
	0.5	0.0031	0.168	100						
	1	0.0016	0.186	93.8	0.012	0.79	6.2			
	2	0.0093	0.146	38.3	0.011	0.64	29.8	0.016	1.31	31.9

Notes: *Gompertz parameter, initial mortality.

†Gompertz parameter, the rate of increase in mortality with age.

‡Initial proportion of the subpopulation to the whole population after heat shock (%).

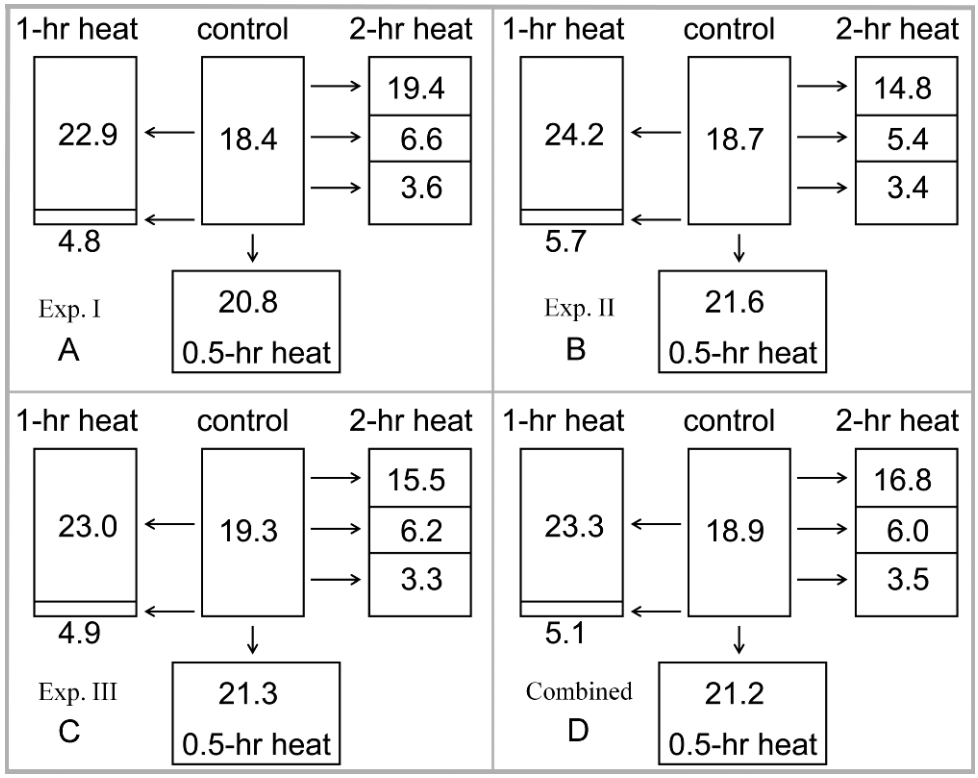

Figure 2. Subpopulations of worms and their life expectancies. There are four populations: the control, 0.5-hour-, 1-hour-, and 2-hour-heated populations. The number of subpopulations in each population is based on the likelihood ratio test (for details, see Table 2), and mortality in each subpopulation is assumed to follow a Gompertz distribution. Estimates of parameters of each subpopulation are given in Table 3. Life expectancy of each subpopulation is calculated based on the life table technique (34). Each rectangle represents a subpopulation, and the number in (out) of each rectangle is the life expectancy. **A,** Experiment I. **B,** Experiment II. **C,** Experiment III. **D,** Combined data.

of worms decreased whereas the rate of increase in mortality remained the same as the control, resulting in an increase in overall longevity (a hormetic effect), as compared to the control. But as the duration of the heat shock increased, the initial mortality of worms started to increase. When the initial mortality surpassed that of the control, worms began to exhibit damage.

Our results demonstrate that the increase in longevity after a mild heat shock results entirely from decreased initial mortality. This finding is consistent with the observed induction kinetics of HSPs (25). HSPs are induced as part of the response of an organism to heat shock and other forms of stress; many aspects of the heat shock response have been found to be highly conserved among eukaryotic cells (36,37). The nematode *C. elegans* exhibits a typical response to heat shock, and at least eight major HSPs are induced (38). The most prominent HSPs in *C. elegans* include polypeptides of masses 16,000 and 18,000 Da (39). Link and colleagues (25) constructed a novel *hsp-l6.2::GFP* reporter transgene to monitor HSP16.2 expression in response to heat shock and found that HSP16.2 levels increased and reached a maximum 24 hours after heat shock, and then decreased over the course of the next several days. As chaperones and/or contributors to proteolysis, HSPs form the front line of a cell's defenses against protein damage, functioning to refold, sequester, or aggregate damaged proteins or to target proteins for degradation when damaged past repair (40–44). The decrease in initial mortality could be caused by upregulated HSPs dealing successfully with a transient stress. We suggest that the levels of HSP16.2 (and other HSPs) increased quickly and fall too soon to affect the long-term rate of accumulation of damage, and thus had no significant effect on the rate of increase in mortality.

Our findings confirm earlier results that mild heat shock for 1 hour extends longevity of *C. elegans* (9,20,26). However, worms heat-shocked for 2 hours exhibit different effects. Whereas hormesis was still observed in the earlier experiments (20,26), in the current experiments longevity was decreased and mortality increased. The reason is that the heat shock protocols used are different: In earlier experiments worms were heated on NGM plates at 35°C for 2 hours and then recovered for up to 24 hours at 20°C—that is, with the temperature increasing and decreasing gradually; whereas in the current experiments worms were transferred to a 2-liter flask containing S-Basal, 1×10^9 OP50/mL, and 10 μg cholesterol/mL (liquid medium), which had been preheated to 35°C, for 2 hours of heat shock, and were then immediately transferred back to liquid medium at 20°C; thus temperature was increased and decreased quickly.

The discrete heterogeneity model was proposed and applied successfully to the analysis of heat shock in *C. elegans* by Yashin and colleagues (26). By using the model, we found that worms given a 2-hour-heat shock separated

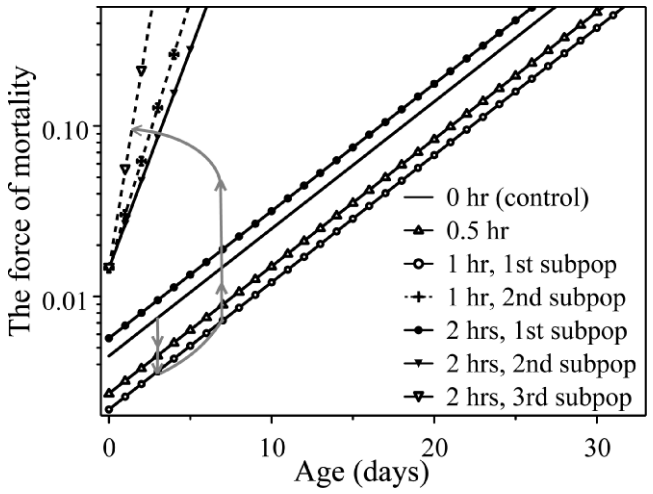

Figure 3. The force of mortality (log scale) of each subpopulation and the U-shaped curve of initial mortality of the combined data. There were one, one, two, and three subpopulations in those worms heated for 0 hour (control), 0.5 hour, 1 hour, and 2 hours, respectively. The control (*solid line without symbol*), the 0.5-hour-heated population (*solid line, empty upward triangle*), the first subpopulation (94.3%, *solid line, empty circle*) of 1-hour-heated worms, and the first subpopulation (36.1%, *solid line, solid circle*) of 2-hour-heated worms had the same rate of increase in mortality (slope). The second subpopulation (5.7%, *dashed line, cross*), and the second (32.4%, *solid line, solid downward triangle*) and third (31.5%, *dashed line, empty downward triangle*) subpopulations of 2-hour-heated worms had the same initial mortality. After heat shock, initial mortality decreased and then increased, whereas the rate of increase in mortality kept the same as the control. When initial mortality reached a high level, the rate of increase in mortality started to increase.

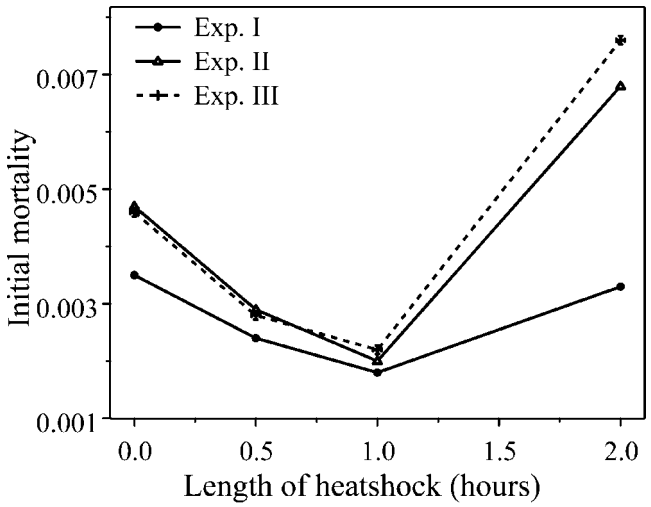

Figure 4. The U-shape of the initial mortality of the control, the 0.5-hour-heated population, the first subpopulation of the 1-hour-heated population, and the first subpopulation of the 2-hour-heated population in each experiment. The rates of increase in mortality of the four populations (subpopulations) are the same.

into three subpopulations: the first subpopulation (38.3%, Table 3) displayed a life expectancy of 16.8 days, the second (29.8%) 6.0 days, and the third subpopulation (31.9%) 3.5 days. The first, second, and third subpopulations are assumed to have, respectively, a high, medium, and low ability to respond to stress. Rea and colleagues (29) used a COPAS Biosort 250 Worm Sorter (Union Biometrica Inc./Harvard Biosciences, Holliston, MA) to sort populations of worms displaying high, medium, or low levels of HSP16.2 and found that the differences in stress resistance and life span among these populations were highly significant. The data of Rea and colleagues (29) lend molecular support to our model of distinct subpopulations. Similarly, our analysis of the discrete heterogeneity model showed that worms given a 1-hour-heat shock separated into two subpopulations: 93.8% of worms displayed a life expectancy of 23.3 days, whereas 5.7% of worms had a life expectancy of 5.1 days. Rea and colleagues (29) sorted 1% of worms with a high ability to respond to stress and 1% of worms with a low ability to respond to stress, and found that the difference in mean life span between these groups was very significant. Here again, Rea and colleagues (29) support our model in which worms subjected to a 1-hour heat shock separate into two subpopulations.

Age-specific mortality of *C. elegans* increases exponentially with chronological age, and can be accurately fitted to the Gompertz model (45,46). The Gompertz model has two parameters: initial mortality and the rate of increase in mortality. Life span could be extended by a reduction in the rate of increase in mortality with age, by a lowering of initial mortality, or by both (45,46). For example, *age-1* mutants display a decrease in the rate of increase in mortality by 30%–40% without change in initial mortality as compared to wild-type strains (46,47) and thus extended longevity; recombinant inbred line TJ143 of *C. elegans* increased longevity by lowering both initial mortality and the rate of increase in mortality (45). Here we report that, as an environmental intervention, mild heat shock extends longevity solely by decreasing initial mortality without changing the rate of increase in mortality.

When worms received a hormetic benefit from mild heat shock, initial mortality of worms decreased whereas the rate of increase in mortality remained unchanged, as shown in the .5-hour-heated population and the first subpopulation of the 1-hour-heated population. When worms were slightly damaged, their initial mortality increased but their rate of increase in mortality did not change as compared to the control, as exemplified by the first subpopulation of the 2-hour-heated population. When worms were severely damaged, their initial mortality reached a high level and their rates of increase in mortality increased, as shown in the second subpopulation of the 1-hour-heated population and the second and third subpopulations of the 2-hour-heated populations. Therefore, the first parameter of the Gompertz model—initial mortality—is revealed as being the more sensitive to environmental intervention via hormetic heat shock. This is consistent with other data (Wu D, Rea SL, Cypser JR, Johnson TE, 2008, unpublished data) regarding the timing of changes in mortality in response to a variety of conditions. In those experiments, worms were shifted from a variety of high-mortality conditions to a corresponding low-mortality state and vice versa. We found that a short period (several days) of exposure to the new condition changed initial mortality only, with no significant effect on the subsequent rate of increase in mortality.

Numerous studies of survival data confirm the observation that the mortality curve is elastic, that is, it responds to environmental challenges by changing its pattern with age. In analyzing human mortality data, Strehler and Mildvan (23) found that changes in the human mortality curve are not arbitrary, but follow important regularity: The parameters of the Gompertz curve (describing the trajectory of human mortality) experience strong negative correlation (called the SM correlation) during the interval between 40 and 80 years of age. Many researchers have confirmed the presence of this correlation in humans (48,51) and laboratory animals (28). Yashin and colleagues (52,53) showed that, for humans, the SM correlation took place only at the first half of the last century. During this period, improved survival followed a rectangularization pattern. However, such correlation ceased during the second half of the century, when a parallel shift to the right of the entire survival curve was observed. This shift was characterized by substantial changes in the intercept parameter of the Gompertz curve (i.e., the initial mortality), but almost no changes in the slope of the logarithm of the mortality curve were observed.

These observations indicate that changes in mortality, occurring in accordance with the pattern of the SM correlation in humans and animals, represent a population response to specific environmental challenges. The spectrum of such challenges for humans is likely to have changed during the second part of the last century. Although many new factors may have contributed to the mortality decline during the second half of the last century (e.g., antibiotics, reduced prevalence of hypertension, development of emergency care, etc.), the exact relationship between these factors and observed trends is not yet established. The results of our studies also show that the spectrum of external conditions experienced by populations of worms in the stress groups differs from those corresponding to the SM correlation. Just as it was observed in humans during the second half of the last century, only the intercept parameter of the Gompertz curve changed in response to the magnitude of stress experienced by nematode worms earlier in life. This fact raises the possibility of the presence in human populations of the cohort effect, which describes variation in health status and mortality arising from different causal factors to which each birth cohort in a population is exposed at the beginning of their life as environment and society change. Because it is unlikely that the decline in human mortality observed during the last decades is a population response to mild stress, the similarity of two patterns of survival changes indicates that such changes may result from many different factors. This finding shows that the SM correlation is not a universal response of mortality to changes in external conditions. More studies are needed to evaluate effects of stressful conditions experienced during the life course on the trajectory of the mortality curve with age.

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