Dietary Restriction and Cold Temperature Both Acutely Reduce Senescence in *C. elegans*

K. Yen and C.V. Mobbs*

Department of Neuroscience, Mount Sinai School of Medicine, New York, NY USA

Abstract: Cold/hypothermic induced longevity (CHIL) increases maximum lifespan, but the acute effects of CHIL on senescence, herein defined as the acceleration in mortality rate with age, have not been examined. Furthermore, dietary restriction (DR) also increases maximum lifespan, but the effect of DR on senescence remains controversial. Here, we demonstrate that in *C. elegans*, both DR and CHIL significantly reduce senescence. Furthermore, even at midlife, transfer to or from a life-extending condition acutely changes the rate of senescence. These results are consistent with the hypothesis that previous temperature or dietary conditions cause irreversible damage to the organism, but future acceleration of this damage is solely dependent on the current temperature or dietary regimen.

INTRODUCTION

Lifespan is determined by mortality rate, which can be accurately quantified by the two-variable Gompertz equation [1, 2]:

$$M(t)=Ae^{Gt}$$

(as described below, a potential third variable, M0, failed to improve the model). G, the Gompertz variable, quantifies the age-dependent acceleration of mortality rate and therefore constitutes a mathematical definition of senescence, whereas A quantifies the initial mortality rate [2].

Many manipulations and mutations increase average and maximum lifespan, but whether they do so by reducing initial mortality rate or age-dependent acceleration of mortality rate (i.e., senescence) remains controversial and may depend on genotype. For rats, it is clear that dietary restriction (DR) decreases senescence [3] (Yen *et al.* unpublished) but not initial mortality rate, but for mice and fruit flies, the relationship is more ambiguous. One study has shown that DR in fruit flies decreases initial mortality rate and not the age-dependent acceleration of mortality rate [4], but another study in flies suggests the opposite [5]. Studies in mice have also produced conflicting results about the effects of DR on initial mortality rate and age-dependent acceleration of mortality rate (Yen *et al*. unpublished) [6, 7]. A recent report [8], also suggests that DR may reduce the age-dependent acceleration in mortality rate in *C. elegans*.

Cold/hypothermic induced longevity (CHIL) is another environmental manipulation that increases lifespan in poikilotherms [9, 10] and mammals [11]. According to several versions of the highly influential rate-of-living theory [12] or free-radical theory [13]**,** the effects of DR and CHIL on lifespan might be mediated by reduced metabolic rate, but several lines of data suggest that this is not the case [14]. For example, metabolic rates between long-lived flies and wild-type flies are not significantly different [15, 16]. In addition, increasing metabolic rate by exposing mammals to cold temperatures has no negative effects on lifespan even though there is an apparent increase in oxidative stress [17,18]. Furthermore, dietary restriction does not reduce lifetime metabolic rate per unit metabolically active tissue in rats [19]. Indeed, DR actually increases mass specific metabolism in *C. elegans* [20, 21] and yeast [22, 23]. Conversely, reducing metabolism by metabolic manipulations in *C. elegans* does not extend lifespan when initiated in adult animals [24]. Although mechanisms by which CHIL increases lifespan have not been as extensively investigated, studies in fish similarly indicate that reducing temperature may also increase metabolic activity in association with increased lifespan [25, 26].

Nevertheless, whether DR and CHIL increase lifespan through similar demographic patterns remains unresolved. To clarify this question, we compared long-term and short-term effects of DR and CHIL on components of mortality rate in *C. elegans*.

MATERIALS & METHODOLOGY

Animals

N2 strain of *C. elegans* were used for all experiments and maintained on nematode growth media (NGM) plates seeded with OP50 strain of *E. coli* or axenic media supplemented with cholesterol. OP50 is a uracil-auxotrophic strain that has limited growth ability to facilitate the counting of the worms on the plate. Axenic media is a liquid media devoid of any bacteria and is made of 3% w/v soy peptone, 3% w/v yeast extract, 0.5 mg/ml hemoglobin, and 5 μg/mL cholesterol. This media was supplemented with 100 μg/ml ampicillin and 12.5 μg/ml tetracycline to prevent bacterial growth.

Lifespan Assays

Eggs were collected from gravid nematodes by standard hypochlorite treatment and grown on standard NGM plates until L4-adult stage. The worms were then transferred to media that is supplemented with 5-fluorodeoxyuridine (FUDR) to inhibit egg hatching [27] and transferred to fresh media every week or month for monoxenic or axenic cultures respectively. Worms were scored at least every 3 days to check for dead worms. Worms that were not moving or did not respond to gentle prodding from a platinum wire were scored as dead. Any worms that died of internal hatch-

*Address correspondence to this author at the Department of Neuroscience, Mount Sinai School of Medicine, New York, NY USA;
E-mail: Charles.Mobbs@mssm.edu

1876-326X/07

ing or crawled off the plate were censored on the date that they were last observed. All worms were maintained at 25°C for control temperature or 16°C for CHIL.

Survival Analysis

Survival curves were truncated when only 5% of the population remained because of the limitations of the Gompertz equation to fit the tails of survivor curves [28]. Maximum likelihood estimates were determined using the procedures described by Garg *et al*. [29] and implemented in R, a freely available statistical environment, as well as through the use of WinModest [30]. Likelihood ratio tests were used for hypothesis testing of G and A values. Initial mortality rates were compared using t=0 at the day of transfer. Kaplan-Meier estimates of survivorship curves were used and log rank test used afterwards to test for significant differences between survival curves. Changes in median lifespan were initially tested for significance by the nonparametric Kruskal-Wallis test on the remaining worms at transfer time and if there was a significant difference between groups, a Mann-Whitney test was performed to determine which groups were significantly different.

RESULTS

CHIL

Lifelong CHIL significantly increased median lifespan and significantly reduced G, the age-dependent acceleration of mortality rate (Fig. **1** and Table **1**). Transfer to 16°C from 25°C on any day tested caused a significant change in the survival curve and median lifespan ($p < .05$) when compared to worms that were maintained at 25°C for life or CHIL conditions (Fig. **1a**). Gompertz analysis indicated that transfer from 25°C to 16°C on any day tested significantly reduced G, the age-dependent acceleration of mortality rate (i.e., senescence); CHIL had no detectable effect on short-term risk, as indicated by the lack of change in A. Furthermore, the Gompertz variable of midlife transferred worms was statistically indistinguishable from those that were maintained in a lifelong CHIL environment. Linearized mortality rates are shown in Fig. **1b** and Gompertz parameters can be found in Table **1**.

The converse experiment, transfer from CHIL conditions to control temperatures, produces the expected converse results. Survival curves, median lifespan, and the mortality rate slope were all significantly changed when worms were transferred to control temperature (Fig. **2a**). When compared to worms maintained at 25°C their entire lives, the mortality rate slope was significantly higher for those transferred on days 15 or 26 but not day 10 (Fig. **2b**). There were no significant changes in short term risk by transfer from CHIL conditions except for the transfer on day 26 which caused a significant decrease in A. Values for Gompertz parameters can be found in Table **2**.

DR

Transfer to DR from ad lib conditions resulted in a significant change in survival curve and median lifespan in all cases (Fig. **3a**, Table **3**). Correspondingly, there was also a

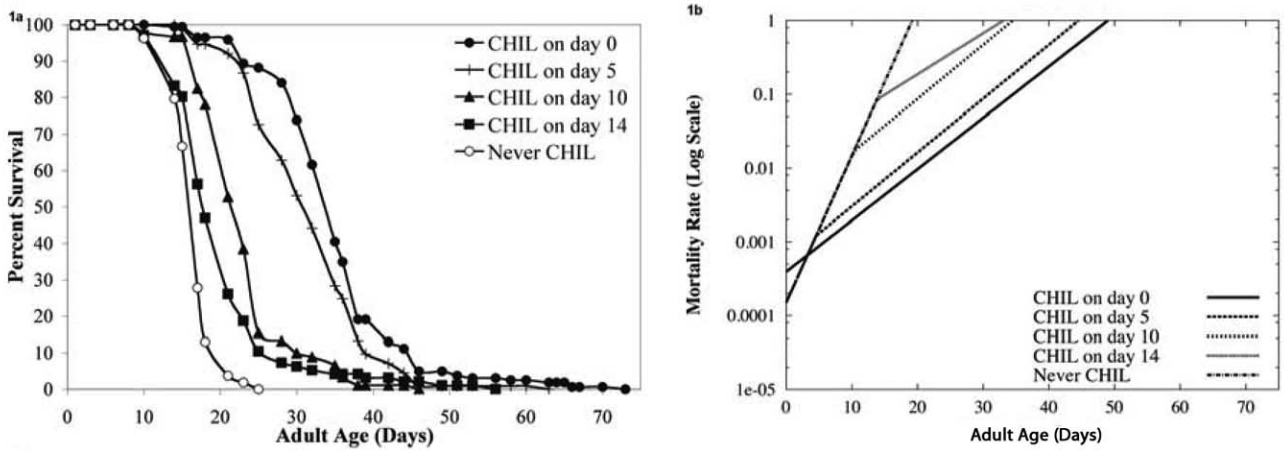

Fig. (1). Transfer to CHIL conditions **a**. Survival curves of midlife transfers to CHIL conditions. All survival curves are significantly different from each other ($p < .05$). **b**. Plot of estimated mortality rate.

Table 1. Estimates of Gompertz Parameters for Transfers from 25°C to 16°C. Significantly Different from: * 25°C, + 16°C

Transfer On Day	Median Lifespan	Initial Mortality Rate (A)	Slope (G)	N at Day of Transfer
Never	17+	1.50E-04+	0.459+	60
5	32*+	5.66E-04	0.168*	117
10	23*+	2.99E-03+	0.168*	90
14	18*+	1.43E-02+	0.128*	84
0	35*	3.95E-04*	0.165*	180

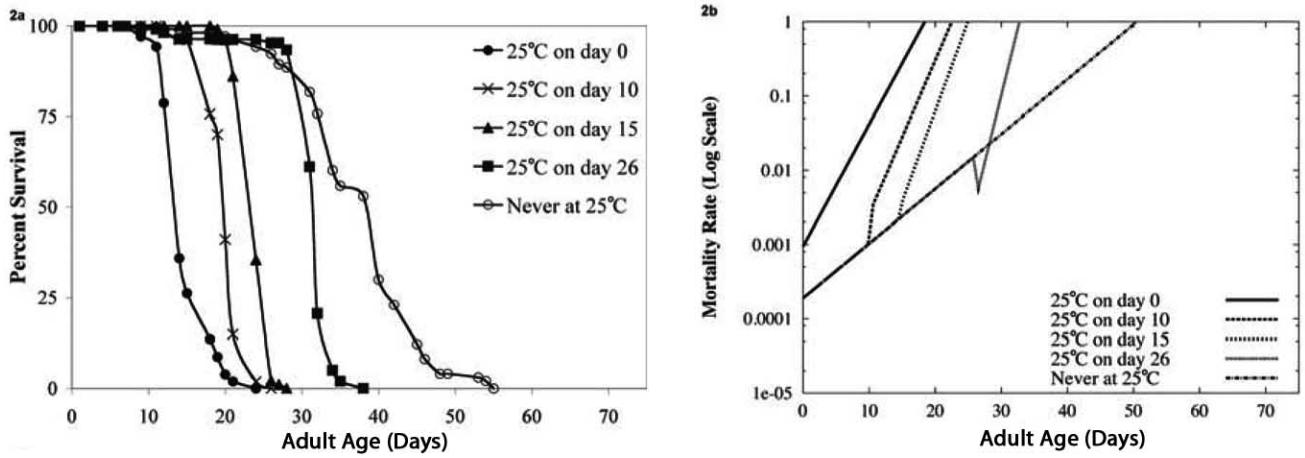

Fig. (2). Transfer from CHIL conditions **a**. Survival curves of midlife transfers from CHIL conditions. All survival curves are significantly different from each other ($p < .05$). **b**. Plot of estimated mortality rate.

Table 2. Estimates of Gompertz Parameters for Transfers from 16°C to 25°C. Significantly Different from: * 25°C, + 16°C

Transfer On Day	Median Lifespan	Initial Mortality Rate (A)	Slope (G)	N at Day of Transfer
0	14+	9.43E-04+	0.378+	120
10	20*+	2.57E-03*	0.476+	107
15	23*+	3.78E-03	0.561*+	106
26	32*+	3.67E-03+	0.832*+	99
Never	40*	1.89E-04*	0.170*	121

significant decrease in the slope of mortality rate (Table **3**) and there was no evidence of any short-term decrease in mortality rate for 2 of the groups. The slopes for worms that were transferred to DR on day 10 or 14 were not statistically different from those that were on DR their entire lives (Fig. **3b**), but unusually, those worms that were transferred on day 5 to DR had a slope slightly higher, but significantly different from the other cohorts in axenic media. Even with a higher slope than other DR groups, the worms transferred to DR on day 5 were still able to achieve a greater median lifespan than any other DR group due to a significant decrease in initial mortality rate.

Transfer to ad lib conditions from DR also caused a significant change in median lifespan, survival curves, and mortality rate slope (Fig. **4a**). The slopes for day 15 and 20 were not significantly different than the slope of worms that were maintained at ad lib their entire lives, but the slope for those transferred on day 10 was significantly steeper (Fig. **4b** and Table **4**). There was no evidence of any change in A for any of the groups.

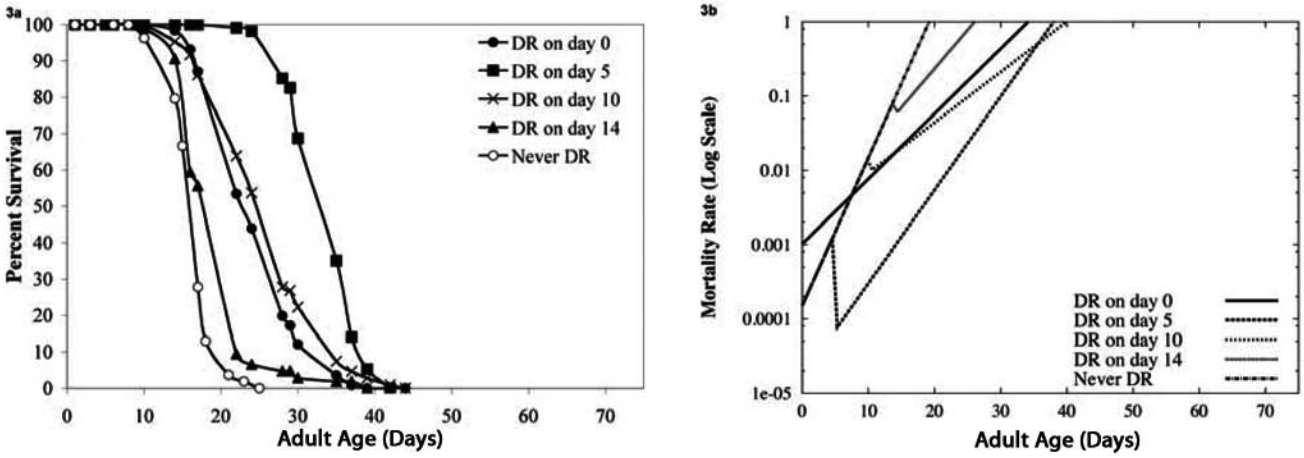

Fig. (3). Transfer to dietary restriction (DR) **a**. Survival curves of midlife transfers to dietary restriction. All survival curves are significantly different from each other ($p < .05$). **b**. Plot of estimated mortality rate.

Table 3. Estimates of Gompertz Parameters for Transfers from ad lib to Dietary Restricted (DR) Conditions. Significantly Different from: * Ad lib, - DR

Transfer On Day	Median Lifespan	Initial Mortality Rate (A)	Slope (G)	N at Day of Transfer
Never	17-	1.50E-04	0.459-	60
5	35*-	1.66E-05*-	0.290*-	110
10	28*-	1.92E-03	0.157*	108
14	22*-	1.92E-03	0.240*	96
0	24*	1.02E-03	0.202*	180

DISCUSSION AND CONCLUSIONS

CHIL has been shown to consistently decrease senescence in poikilotherms [4, 31] and the present study supports that view. Switching between temperatures solely changes the age-associated acceleration of mortality rate, with no detectable effect on the short-term risk. While the underlying mechanism of CHIL is unknown, its relevance to mammalian species has already been demonstrated by Conti *et al*. [11].

DR has been shown to extend lifespan by greater than 100% in many cases, but only extended lifespan by approximately 50% in the present study [32]. The difference is likely due to the difference in protocols used by most studies. Although most studies have initiated DR when the worms were still in their larval stage, the present initiated DR in adults to avoid developmental confounds as the generational time has shown to be longer in worms maintained in axenic media [20].

Gompertz analysis of the effects of DR on worms has shown that as in rats and some strains of mice, DR decreases the age-associated acceleration of mortality rate [3, 7] (Yen *et al*. unpublished). This is in contrast to one study in flies, in which DR only changed the short-term risk/initial mortality rate with no change in age-dependent acceleration of mortality rate [4], but consistent with a different study in flies [5]. While the present manuscript was being prepared, results consistent with the present study were published [8]. As with

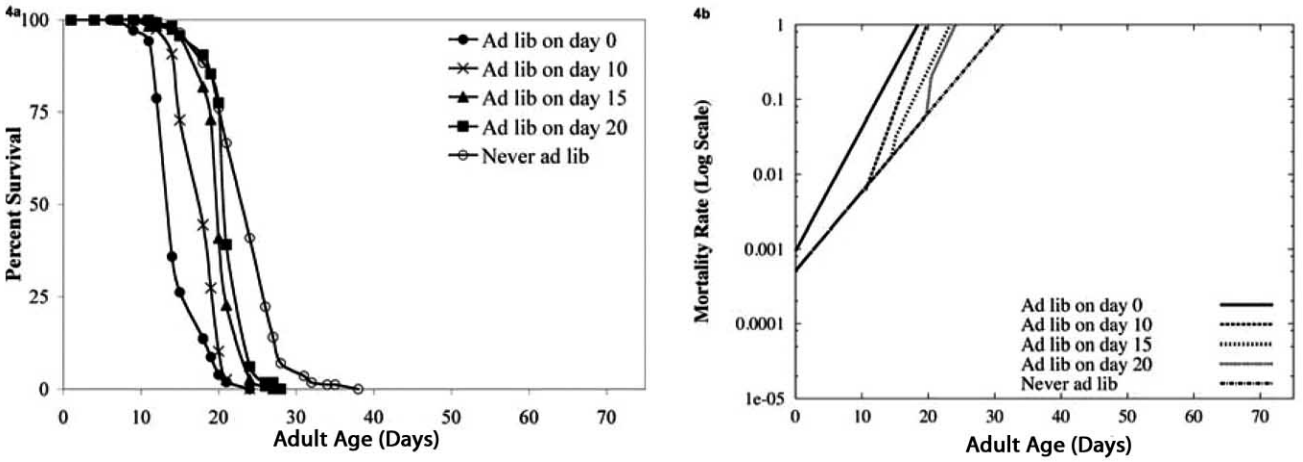

Fig. (4). Transfer from dietary restriction (DR) **a**. Survival curves of midlife transfers from dietary restriction. All survival curves are significantly different from each other ($p < .05$). **b**. Plot of estimated mortality rate.

Table 4. Estimates of Gompertz Parameters for Transfers from Dietary Restricted (DR) to ad Lib Conditions. Significantly Different from: * Ad lib, - DR

Transfer On Day	Median Lifespan	Initial Mortality Rate (A)	Slope (G)	N at Day of Transfer
0	14-	9.43E-04	0.378-	120
10	18*-	4.51E-03*	0.553*-	118
15	20*-	3.08E-02	0.420-	111
20	21*-	1.65E-01	0.441-	89
Never	24*	5.15E-04	0.243*	121

low temperature, DR only influenced age-associated acceleration of mortality rate, not acute mortality rate, with one exception. Surprisingly, worms that were transferred to DR several days after adulthood lived longer than those that were transferred immediately to DR upon reaching adulthood. These results have been replicated numerous times on different genetic backgrounds with similar results (unpublished). These results are consistent with a recently reported study in which a delay later in adulthood increased lifespan more than initiation of DR early in adulthood (it should be noted that the method of DR in this case was complete removal of food and the control condition initiated DR at the L4 stage) [33].

In addition to the Gompertz analysis, use of the midlife transfer paradigm independently demonstrates that DR reduces senescence and not acute mortality rate. By examining the median lifespan of groups switched to or from a life-extending condition before any deaths occur, one can determine if the life-extending condition affects senescence. If there were only an effect on initial mortality rate, then median lifespan should be the same as if the cohort had been at the final condition their entire life. Conditions that reduce age-associated mortality rate should result in an increased median lifespan compared to a cohort maintained at the final condition their entire life. The present results demonstrate that switching to both DR and reduced temperature at midlife increase median lifespan, and corroborating the conclusion of the Gompertz analysis and supporting the view that DR and CHIL both decrease senescence.

In general, switching to or from DR causes the age-dependent acceleration of mortality rate to rapidly reflect the current treatment, but does not reduce the absolute mortality rate, to levels observed at earlier ages. This suggests that DR only helps reduce further accumulation of damage and does not reverse the effects of a previous dietary regimen as reported in flies [4]. CHIL is similar to DR in that transfer to cold temperature decreases the acceleration of mortality rate, but not the absolute mortality rate, to the same degree as animals that have been maintained at CHIL conditions their entire life. On the other hand, worms switched from CHIL to control temperature displayed a significantly steeper mortality rate slope than both CHIL worms and those maintained at control temperatures. This may imply that the thermal adaptations to CHIL, although beneficial at cold temperatures, may have detrimental effects when the worms are returned to normal temperatures.

ACKNOWLEDGEMENTS

These studies were supported by the Ellison Medical Foundation. Some nematode strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR).

REFERENCES

[1] Finch CE. Longevity, senescence, and the genome. 1st Ed. Chicago: The University of Chicago Press; 1990.

[2] Johnson TE. Increased life-span of age-1 mutants in Caenorhabditis elegans and lower Gompertz rate of aging. Science 1990; 4971: 908-12.

[3] Pletcher SD, Khazaeli AA, Curtsinger JW. Why do life spans differ? Partitioning mean longevity differences in terms of age-specific mortality parameters. J Gerontol A Biol Sci Med Sci 2000; 8: B381-389.

[4] Mair W, Goymer P, Pletcher SD, Partridge L. Demography of dietary restriction and death in drosophila. Science 2003; 5640: 1731-1733.

[5] Bross TG, Rogina B, Helfand SL. Behavioral, physical, and demographic changes in Drosophila populations through dietary restriction. Aging Cell 2005; 6: 309-317.

[6] Cheney KE, Liu RK, Smith GS, Leung RE, Mickey MR, Walford RL. Survival and disease patterns in C57BL/6J mice subjected to undernutrition. Exp Gerontol 1980; 4: 237-258.

[7] Harper JM, Leathers CW, Austad SN. Does caloric restriction extend life in wild mice? Aging Cell 2006; 6: 441-449.

[8] Lenaerts I, Eygen SV, Vanfleteren JR. Adult-limited dietary restriction slows gompertzian aging in *Caenorhabditis elegans*. Ann N Y Acad Sci 2007; 1: 442-448.

[9] Klass MR. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. Mech Ageing Dev 1977; 6: 413-29.

[10] Hosono R, Mitsui Y, Sato Y, Aizawa S, Miwa J. Life span of the wild and mutant nematode *Caenorhabditis elegans*. Effects of sex, sterilization, and temperature. Exp Gerontol 1982; 2: 163-72.

[11] Conti B, Sanchez-Alavez M, Winsky-Sommerer R, *et al.* Transgenic mice with a reduced core body temperature have an increased life span. Science 2006; 5800: 825-828.

[12] Pearl R. The Rate of Living. 1st Ed. New York: Alfred Knopf; 1928.

[13] Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 3: 298.

[14] Yen K, Mastitis JW, Mobbs CV. Lifespan is not determined by metabolic rate: evidence from fishes and *C. elegans*. Exp Gerontol 2004; 6: 947-9.

[15] Arking R, Buck S, Wells RA, Pretzlaff R. Metabolic rates in genetically based long lived strains of Drosophila. Exp Gerontol 1988; 1: 59-76.

[16] Hulbert AJ, Clancy DJ, Mair W, Braeckman BP, Gems D, Partridge L. Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in Drosophila melanogaster. Exp Gerontol 2004; 8: 1137-1143.

[17] Holloszy JO, Smith EK. Longevity of cold-exposed rats: a reevaluation of the "rate-of-living theory". J Appl Physiol 1986; 61: 1656-1660.

[18] Kaushik K, Kaur J. Chronic cold exposure affects the antioxidant defense system in various rat tissues. Clin Chim Acta 2003; 333: 69-77.

[19] McCarter RJ, Palmer J. Energy metabolism and aging: a lifelong study of Fischer 344 rats. Am J Physiol Endocrinol Metab 1992; 3: E448-452.

[20] Houthoofd K, Braeckman BP, Lenaerts I, *et al.* Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends life span in Caenorhabditis elegans. Exp Gerontol 2002; 12: 1371-8.

[21] Bishop NA, Guarente L. Two neurons mediate diet-restriction-induced longevity in *C. elegans*. Nature 2007; 7144: 545-549.

[22] Lin S-J, Kaeberlein M, Andalis AA, *et al.* Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. Nature 2002; 6895: 344-348.

[23] Lin S-J, Ford E, Haigis M, Liszt G, Guarente L. Calorie restriction extends yeast life span by lowering the level of NADH. Genes Dev 2004; 1: 12-16.

[24] Dillin A, Hsu A-L, Arantes-Oliveira N, *et al.* Rates of behavior and aging specified by mitochondrial function during development. Science 2002; 5602: 2398-2401.

[25] Liu RK, Walford RL. Observations on the lifespans of several species of annual fishes and of the world's smallest fishes. Exp Gerontol 1970; 3: 241-6.

[26] Liu RK, Walford RL. Mid-life temperature-transfer effects on lifespan of annual fish. J Gerontol 1975; 2: 129-31.

[27] Mitchell DH, Stiles JW, Santelli J, Sanadi DR. Synchronous growth and aging of Caenorhabditis elegans in the presence of fluorodeoxyuridine. J Gerontol 1979; 1: 28-36.

[28] Olshansky SJ, Carnes BA. Ever since Gompertz. Demography 1997; 1: 1-15.

[29] Garg ML, Rao BR, Redmond CK. Maximum-Likelihood Estimation of the Parameters of the Gompertz Survival Function. Appl Stat 1970; 2: 152-159.

[30] Promislow, Tatar, Pletcher and Carey. Below-threshold mortality: implications for studies in evolution, ecology and demography. J Evol Biol 1999; 2: 314-328.

[31] Valenzano DR, Terzibasi E, Cattaneo A, Domenici L, Cellerino A. Temperature affects longevity and age-related locomotor and cognitive decay in the short-lived fish Nothobranchius furzeri. Aging Cell 2006; 3: 275-278.

[32] Houthoofd K, Braeckman B, Johnson T, Vanfleteren J. Life extension via dietary restriction is independent of the Ins/IGF-1 signalling pathway in Caenorhabditis elegans. Exp Gerontol 2003; 9: 947-54.

[33] Kaeberlein TL, Smith ED, Tsuchiya M, *et al.* Lifespan extension in Caenorhabditis elegans by complete removal of food. Aging Cell 2006; 6: 487-494.

Received: August 01, 2007 Revised: November 26, 2007 Accepted: December 05, 2007