Induced Overexpression of Mitochondrial Mn-Superoxide Dismutase Extends the Life Span of Adult *Drosophila melanogaster*

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ABSTRACT

A transgenic system ("FLP-*out*") based on yeast FLP recombinase allowed induced overexpression of MnSOD enzyme in adult *Drosophila melanogaster*. With FLP-*out* a brief heat pulse (HP) of young, adult flies triggered the rearrangement and subsequent expression of a *MnSOD* transgene throughout the adult life span. Control (no HP) and overexpressing (HP) flies had identical genetic backgrounds. The amount of MnSOD enzyme overexpression achieved varied among six independent transgenic lines, with increases up to 75%. Life span was increased in proportion to the increase in enzyme. Mean life span was increased by an average of 16%, with some lines showing 30–33% increases. Maximum life span was increased by an average of 15%, with one line showing as much as 37% increase. Simultaneous overexpression of catalase with MnSOD had no added benefit, consistent with previous observations that catalase is present in excess in the adult fly with regard to life span. Cu/ZnSOD overexpression also increases mean and maximum life span. For both *MnSOD* and *Cu/ZnSOD* lines, increased life span was not associated with decreased metabolic activity, as measured by O_2 consumption.

CURRENT theory suggests that aging results from the decreased force of natural selection acting on older individuals (Rose 1991; Partridge and Barton 1993; Charlesworth 1994; Kirkwood 1995; Kirkwood and Austad 2000). Reduction in the force of natural selection with age allows each generation to inherit mutations that have deleterious effects at late ages. The accumulation of such mutations in the germ line results in reduced fitness of old organisms, including an imbalance between damage and repair and a failure to maintain somatic tissue structure and function.

Reactive oxygen species (ROS) are produced as a toxic byproduct of normal cellular metabolism, and oxidative damage is hypothesized to be one cause of aging in metazoans (Harman 1956; Stadtman 1992; Martin *et al.* 1996; Sohal and Weindruch 1996; Wallace 1999; Finkel and Holbrook 2000). The enzymes catalase and superoxide dismutase (SOD) are major defenses against ROS in all cells. SOD exists in two forms inside the eukaryotic cell, Cu/ZnSOD primarily in the cytoplasm and MnSOD in the mitochondria. The major ROS produced by the cell is mitochondrial superoxide, which is converted by SOD to H_2O_2. Catalase converts H_2O_2 to molecular oxygen and water. These and other cellular defenses against ROS are not completely efficient. Oxidatively damaged macromolecules accumulate in every aging organism examined, and oxidative damage is implicated in the etiology of most human aging-related diseases.

Numerous studies demonstrate the correlation between aging and oxidative damage in Drosophila. In Drosophila, aging has been found to correlate with increased levels of protein carbonyls and 8-oxo-Guanine in the DNA (Orr and Sohal 1994; Sohal *et al.* 1995). Drosophila lacking either catalase or Cu/ZnSOD have reduced life span (Mackay and Bewley 1989; Phillips *et al.* 1989; Orr *et al.* 1992; Griswold *et al.* 1993). Moreover, the stress response protein hsp70 is induced in muscle tissue during Drosophila aging and this expression is increased or accelerated in *catalase* and *Cu/ZnSOD* null mutant flies (Wheeler *et al.* 1995, 1999). Finally, genetic selection of Drosophila populations for increased life span in the laboratory correlates with increased expression of oxidative stress resistance enzymes and increased oxidative stress resistance (Arking *et al.* 1991, 2000).

One way to directly test the hypothesis that oxidative damage causes aging is to engineer transgenic organisms with increased oxidative stress defenses and determine the effects on life span. In pioneering experiments Drosophila were created that contained *catalase* and *Cu/ZnSOD* transgenes under the control of their native promoters or a constitutive heterologous promoter (Seto *et al.* 1990; Reveillaud *et al.* 1991; Orr and Sohal 1992, 1993, 1994; Griswold *et al.* 1993; Sohal *et al.* 1995). While in certain cases increased stress resistance and life span were reported, current interpretation of

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the data suggests that increased life span was not demonstrated (Tower 1996; Kaiser *et al.* 1997; Tatar 1999). In contrast, when tissue-specific (GAL4/UAS; Parkes *et al.* 1998) or inducible (FLP-*out*; Sun and Tower 1999) transgenic systems were used to cause overexpression of Cu/ZnSOD, it resulted in life span increases of up to 48%. Catalase overexpression increased the resistance of flies to H_2O_2 but had neutral or slightly negative effects on life span, both alone and in combination with Cu/ZnSOD overexpression (Sun and Tower 1999). The data suggest that with regard to life span Cu/ZnSOD activity is limiting while catalase exists in excess. Consistent with this idea, analysis of multiple *catalase* mutant strains demonstrates that catalase activity must be reduced to $< \sim 10\%$ of wild-type levels before reduced life span is observed (Mackay and Bewley 1989; Orr *et al.* 1992; Griswold *et al.* 1993).

Mitochondria are thought to be the primary cellular source of ROS and may also be primary targets of oxidative damage. Because of this, the exclusively mitochondrial form of superoxide dismutase, MnSOD, may be a particularly important additional defense against oxidative damage during aging. In mice MnSOD appears to be the more critical enzyme, as *MnSOD* mutations have more severe phenotypes than *Cu/ZnSOD* mutations (Huang *et al.* 1999; Wallace 1999). In this study Drosophila were engineered to overexpress mitochondrial MnSOD, using the FLP-*out* system to assay for effects on life span.

Environmental manipulations that decrease metabolic rate in Drosophila, *e.g.*, isolation from mates (Partridge *et al.* 1987), dietary restriction (Chippindale *et al.* 1993), or decreased temperature (Miquel *et al.* 1976), also increase life span. To test whether SOD overexpression was enhancing life span through a dampening effect on metabolic rates, O_2 consumption was assayed throughout the life span of the flies overexpressing MnSOD or Cu/ZnSOD, as well as Oregon-R wild-type controls.

MATERIALS AND METHODS

Plasmid construction and transformation: The 800-bp *Eco*RI fragment containing the full-length *Drosophila melanogaster MnSOD* cDNA was isolated from plasmid pcMnSOD (Duttaroy *et al.* 1994) and substituted for the *Cu/ZnSOD* cDNA fragment in construct pActStopSOD (Sun and Tower 1999) to generate construct mSOD. Six independent transgenic lines were generated for mSOD using published methods (Rubin and Spradling 1982; Sun and Tower 1999), and each was confirmed to contain a single *P*-element insertion using Southern analysis (data not shown). Each transgenic construct insertion chromosome was made homozygous by crossing to the same set of inbred balancer stocks. The names of all transgenic lines indicate the transgenic construct (*e.g.*, mSOD), followed by the chromosome of insertion (*X*, *2*, or *3*), followed by a letter or number (or a letter/number combination) indicating the particular independent insertion on that chromosome. For example, *mSOD2C* is an insertion of the mSOD construct on the second chromosome, independent line "C," and *CAT2A2* is an insertion of the previously reported catalase FLP-*out* construct CAT on the second chromosome, independent line "A2" (Sun and Tower 1999).

General: Drosophila culture, transformation, and life span assays were performed as previously described (Sun and Tower 1999), with the modification that aging cohorts of flies consisted of 250–400 individuals for each of heat-pulsed (HP) and control (Co) groups, and were transferred to fresh food vials every 48 hr. The heat pulse regimen was as previously described for use with the FLP3 strain. All of the data presented here are novel and have not been previously reported. For strains that have previously been described, all data represent novel reassays of those strains and were essentially identical to previous results (Sun and Tower 1999).

Drosophila culture, FLP-*out* heat pulse regimen and life span assays: To obtain adult flies of defined age, the crosses described below were cultured at 25° in urine specimen bottles. Prior to eclosion of the majority of pupae, bottles were cleared of adults and newly eclosed flies were allowed to emerge over the next 48 hr. The majority of the males will have mated during this time. The males only were then removed, designated as 1 day old, maintained at 25° at 40/vial in culture vials with food, and passaged to new vials every 48 hr. At 5 days of age the males were pooled, separated into control and experimental groups of 250–400 flies each, and placed in culture vials with food at 40/vial. The control group was maintained at 25° and the experimental group was subjected to a heat pulse of 90 min at 37°. The experimental group was returned to 25° and then 24 hr later (day 6) the experimental group was subjected to a second heat pulse. The control and experimental groups were then maintained at 25° and passaged to fresh vials every 48 hr. Dead flies were counted at each passage, and the number of vials was progressively reduced to maintain approximately 40 flies/vial. To calculate mean life spans for the experimental and control groups, each fly's life span was tabulated, their life spans were averaged, and the SD and SEM were calculated. Maximum life span for the control and experimental cohorts was calculated as the amount of time in days required to reach 90% mortality. Life span was assayed at least twice and for several genotypes three times, using independently cultured flies in experiments separated in time by >2 weeks. Life span assays done simultaneously are indicated as Exp 1, Exp 2, Exp 3, and Exp 4 in the Tables.

SOD enzyme activity assays: Cu/ZnSOD activity was measured as total SOD activity as previously described (Sun and Tower 1999), except that EDTA concentration of assays was 0.08 mm. MnSOD activity was measured in the same way, except that assays contained 0.1 mm potassium cyanide to inhibit Cu/ZnSOD activity. SOD-specific activities are expressed as percentage inhibition of quercetin oxidation per microgram extract protein per 10-min reaction. Catalase-specific activity was measured as previously described (Sun and Tower 1999) and is expressed in units of ΔOD_{240}/min/μg extract protein. SOD and catalase activities are presented as the mean and standard deviation of triplicate extracts.

Metabolic rate assay: Data for oxygen consumption experiments are presented in Table 4. The genotypes, culture, and heat pulse regimen were as described above. On day 6, following the second heat pulse, one-half of each cohort of SOD overexpressing flies was kept at University of Southern California in Los Angeles for the life span assays of Exp 3 and Exp 4, and one-half was moved to University of California-Irvine at controlled temperature for oxygen consumption assay. The oxygen consumption assay for the SOD overexpressing flies was therefore done on sibling flies of those used for life span assays Exp 3 and Exp 4. For the oxygen consumption assay,

adult male flies from each treatment (control and HP) were divided into six replicate groups composed of 32–41 flies each and placed in maintenance vials containing food. Flies were transferred to new food vials every 2–3 days. Measurements of oxygen consumption were made using a flow-through respirometry system (Sable Systems, Henderson, NV) in a temperature-controlled room at 25° ± 1°. Flies were anesthetized with CO_2 and transferred from the maintenance vials into respirometry chambers (volume 10 ml) containing 0.5 ml food. Following recovery of the flies from anesthesia (20 min), dry, CO_2-free air was allowed to flow through the respirometry chambers at a rate of 6 ml/min, and oxygen decrement was measured using an Ametek (Pittsburgh, PA) S-3A/1 oxygen analyzer. Data were recorded and analyzed using Sable Systems' Datacan software. Metabolic rate was estimated using data from the final 10 min of the 30-min assay period to avoid the initial washout period.

The number of flies per chamber varied depending upon mortality, but initial numbers ranged from 32 to 41. Accurate estimates of metabolic rate/fly could be made within fly groups composed of as few as 5–10 flies each. The order in which flies were assayed for metabolic rate was varied systematically and experimental and control groups were measured in the same assay cycle. The food itself was found to have no measurable rate of oxygen uptake.

Oxygen consumption for all flies was measured once a week. Oxygen consumption in all *Cu/ZnSOD* lines and their controls was assayed over a 4-week period. *MnSOD* lines and their controls were assayed for 6–7 weeks, due to their increased longevity.

To estimate mean metabolic rate per fly, total oxygen consumption from each experimental chamber was divided by the total number of flies within each chamber and averaged over total number of weeks. Averages and SD were then calculated from the six replicate vials per treatment (Co and HP). These values were compared between control and experimental flies using two-sided *t*-tests and are presented in Table 3.

Statistical analyses: *t-tests:* Mean life span and enzyme activities were compared between control and heat pulse populations using unpaired two-sided *t*-tests for each individual experiment. Maximum life span (time to 90% mortality) was compared between control and heat pulse populations by combining the paired data from two experiments using paired two-sided *t*-tests. Results are presented in Tables 1 and 3.

ANOVA: The following two standard ANOVA models were used (Neeter *et al.* 1990).

1. Two-factor fixed-effects ANOVA model:

$$Y_{ijk} = \mu_{..} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where

$\mu_{..} = \Sigma_i\Sigma_j\mu_{ij}/ab$ is a constant,

α_i are constants subject to the restriction $\Sigma\alpha_i = 0$,

β_j are constants subject to the restriction $\Sigma\beta_j = 0$,

$(\alpha\beta)_{ij}$ are constants subject to the restrictions

$$\sum_i (\alpha\beta)_{ij} = 0 \text{ and } \sum_j (\alpha\beta)_{ij} = 0,$$

ε_{ijk} are independent $N(0, \sigma^2)$,

$i = 1, \ldots, a; \quad j = 1, \ldots, b; \quad k = 1, \ldots n.$

2. Three-factor fixed effects ANOVA model:

$$Y_{ijkm} = \mu_{...} + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ikj} + \varepsilon_{ijkm},$$

where

$\mu_{...} = \Sigma\Sigma\Sigma\mu_{ijk}/abc$ is a constant,

α_i, β_j, γ_k, $(\alpha\beta)_{ij}$, $(\alpha\gamma)_{ik}$, $(\beta\gamma)_{jk}$, $(\alpha\beta\gamma)_{ijk}$ are constants subject to the restrictions

$$\sum_i \alpha_i = \sum_j \beta_j = \sum_k \gamma_k = 0$$

$$\sum_i (\alpha\beta)_{ij} = \sum_j (\alpha\beta)_{ij} = \sum_i (\alpha\gamma)_{ik} = 0$$

$$\sum_k (\alpha\gamma)_{ik} = \sum_j (\beta\gamma)_{jk} = \sum_k (\beta\gamma)_{jk} = 0$$

$$\sum_i (\alpha\beta\gamma)_{ijk} = \sum_j (\alpha\beta\gamma)_{ijk} = \sum (\alpha\beta\gamma)_{ijk} = 0,$$

$i = 1, \ldots, a; \; j = 1, \ldots, b; \; k = 1, \ldots c; \; m = 1, \ldots, n.$

The term "randomized block ANOVA" refers to an ANOVA design where experimental units within each block are randomly assigned treatment. Mathematically, a randomized block design with fixed blocks is equivalent to a fixed-effects ANOVA model where the blocks are treated as one of the fixed effects.

Induced expression of MnSOD transgene increases MnSOD enzyme activity: To generate genotypes 1–6 of Tables 1 and 3, strains homozygous for the indicated mSOD insertions were crossed to the FLP3 stock containing the hs:FLP construct on the third chromosome (*w;FLP3 Sb/TM2 Ubx*). Progeny were selected that inherited the *FLP3* chromosome marked with the dominant mutation *Sb* and were therefore heterozygous for both constructs. The MnSOD enzyme activity data (Table 1) were analyzed using randomized-blocks ANOVA. The model for analysis was the two-factor fixed-effects ANOVA model. α_i was the effect for genotype i, where $i = 1, \ldots 6$ (genotypes 1–6 of Table 1). β_j was the effect for group (control *vs.* heat pulse) j, where $j = 1, 2$. There were three replications of the enzyme activity assay, $k = 1, \ldots 3$. The responses were denoted as Y_{ijk}, which indicates the activity level for genotype i, group j, and repetition k. The interaction between genotype i and group j was denoted as $(\alpha\beta)_{ij}$. ANOVA table results are presented in Table 2.

Heat pulse alone does not increase MnSOD enzyme activity: Genotypes 7–9 of Tables 1 and 3 are controls that do not contain a SOD transgene. Genotype 7 is the progeny generated by crossing flies homozygous for the FLP-*out* lacZ expression construct on the third chromosome (Sun and Tower 1999) to the *FLP3* stock. Genotype 8 is the progeny generated by crossing the *FLP3* stock to Oregon-R wild type. Genotype 9 is Oregon-R wild type. The data for the control genotypes 7–9 (Table 1) were analyzed using the two-factor fixed-effects ANOVA model as described above, where $i = 7, 8, 9$. Results are presented in the text.

Induced expression of *MnSOD* transgene increases mean and maximum life span: Mean life span data for the MnSOD overexpressing genotypes 1–6 (Table 3) were analyzed using the two-factor fixed-effects ANOVA model as described above, except that the responses Y_{ijk} were life span, and there were two replications $k = 1, 2$. Maximum life span data for the MnSOD overexpressing genotypes 1–6 (Table 3) were analyzed using the same model.

Heat pulse alone does not increase life span: Mean life span data for the control genotypes 7–9 (Table 3; $i = 7, 8, 9$) were analyzed using the two-factor fixed-effects ANOVA model as described above. Responses Y_{ijk} were life span, and there were two replications $k = 1, 2$. Maximum life span was analyzed in the same way.

Life span is increased in proportion to the increase in MnSOD enzyme activity: The percentage increase in life span (mean and maximum) was plotted *vs.* the percentage increase

in MnSOD enzyme activity (Figure 2), revealing a linear relationship for both. The data were analyzed using a standard linear regression model, namely, $Y_i = \alpha + \beta x_i + \varepsilon_i$, where ε_i was normally distributed with mean 0 and variance σ^2. The subscript i indicated genotype, where $i = 1, \ldots 6$. The life span for genotype i was denoted as Y_i, while the enzyme activity for genotype i was denoted as x_i. The intercept of the regression line was denoted as α, while the slope was denoted as β.

Catalase overexpression is not associated with increased life span: Genotype 10 is the progeny generated by crossing flies homozygous for the previously described *catalase* FLP-*out* expression construct on the second chromosome (*CAT2A2*; Sun and Tower 1999) to the FLP3 stock. Genotype 11 was generated by crossing a strain homozygous for both the *mSODX1* insertion and the *CAT2A2* insertion to *FLP3*. Genotype 12 was generated by crossing a strain homozygous for both the *CAT2A2* insertion and the *mSOD3C* insertion to *FLP3*. The data for genotypes 1, 5, 11, and 12 of Tables 1 and 3 were analyzed using three-way factorial ANOVA with mSOD genotype (genotype), presence or absence of the CAT construct (cattype), and group (Co *vs.* heat pulse) as the three cross-classified main effects.

SOD overexpression and oxygen consumption: Genotypes 1–5 of Table 4 are the same as genotypes 13, 14, 2, 4, and 9, respectively, of Tables 1 and 3. Recall that there are 12 replicate vials for each genotype assayed for oxygen consumption at multiple time points (dates), and 6 vials were control and 6 vials were heat pulsed. First, the two-factor fixed-effects ANOVA model described above was used to confirm that heat pulse caused increased life span in this experiment, and results are presented in the text.

Second, oxygen consumption was analyzed across all five genotypes (Table 4) using a three-way factorial ANOVA with main effects of genotype, date of assay, and group (Co *vs.* heat pulse), and results are presented in the text.

Third, the relationship between life span and oxygen consumption was analyzed across all five genotypes (Table 4) using ANCOVA of the line means, and results are presented in the text. The analysis was done in three steps: (i) correlation analysis of the means for oxygen consumption and life span; (ii) a model of "oxygen consumption = genotype + group"; and (iii) life span was included as a covariate in a model of "oxygen consumption = life span + genotype + group."

The more appropriate analysis for the conditional system was to analyze the relationship between the change in life span and the change in oxygen consumption caused by the heat pulse. An ANCOVA model was used that combined a continuous independent variable [percentage change in oxygen consumption (oxyper)] with a categorical variable (genotype) as

$$Y_i = \mu_{..} + \beta_i + \gamma X_i + \varepsilon_i,$$

where Y_i is the percentage change in life span based on means (heat pulse − Co), $\mu_{..}$ is an overall mean term, β_i is the genotype effect, γ is the coefficient for X_i (is it significant?), and X_i is the percentage change in oxygen consumption. In this case genotype had two values: experimental (genotypes 1–4 of Table 4) and control (genotype 5 of Table 4). Results are presented in the text.

RESULTS

FLP-*out*: In the FLP-*out* approach, the yeast FLP recombinase is expressed under the control of the *hsp70* heat-inducible promoter in one transgenic construct, called FLP. A brief heat stress causes tissue-general expression of FLP recombinase. A second transgenic construct (the "target" construct) contains the gene of interest downstream of the constitutive, tissue-general Drosophila *actin5C* promoter. Transcripts initiating at the *actin5C* promoter are prevented from reaching the gene of interest by a transcriptional "stop" sequence. This transcriptional stop sequence is itself flanked by FRTs, which are the target sites for FLP recombinase. After FLP expression is induced by the heat pulse, the FLP recombinase protein causes the precise excision of the transcriptional stop sequence in the target construct. This results in direct fusion of the *actin5C* promoter to the gene of interest and thus constitutive expression of the gene of interest from that point in time onward in all tissues of the fly. The particular site of chromosomal insertion affects the expression level of transgenes, and it was anticipated that *MnSOD* transgene expression levels would vary between lines. For this reason, six independent transgenic lines were generated, each containing the mSOD target construct integrated at one unique location in the genome.

Induced expression of *MnSOD* transgene increases MnSOD enzyme activity: MnSOD enzyme activity was assayed in extracts of flies containing both *FLP3* and the mSOD target construct. Induced overexpression of MnSOD enzyme varied from 29 to 75%, depending on the particular transgenic line (Table 1, genotypes 1–6). The MnSOD enzyme activity assay was somewhat variable, and for three of the six MnSOD overexpressing lines (genotypes 2, 3, and 6) the increase in enzyme activity had a P value slightly >0.05 by two-sided t-test. However, when considered as a group the MnSOD overexpressing lines had a highly significant increase in enzyme (average 45%) that was not observed in the control lines, as confirmed by two-factor fixed-effects ANOVA (Table 2).

Genotype 1 differs from the others in that the insert is on the *X* chromosome. Repeat of the ANOVA analysis of enzyme activity excluding genotype 1 yields essentially the same results: group $F = 57.00$, $P < 0.0001$; genotype $F = 10.52$, $P < 0.0001$; group × genotype $F = 0.81$, $P = 0.5343$.

Heat pulse alone does not increase MnSOD enzyme activity: The control lines (Table 1, genotypes 7–9) do not contain a MnSOD transgene, and for these lines there was an average 14% increase in MnSOD enzyme activity that was not significant. Two-factor fixed-effects ANOVA results were group $F = 4.34$, $P = 0.0592$; genotype $F = 147.41$, $P < 0.0001$; group × genotype $F = 0.43$, $P = 0.5587$.

Induced expression of *MnSOD* transgene increases mean and maximum life span: Mean life span was assayed in control and experimental (HP) populations of ~300 flies each, for each *MnSOD* transgenic line (Table 3, genotypes 1–6). Mean life span was increased by an average of 16%, with some lines showing 30–33% increases. Maximum life span was assayed by determining

TABLE 1

Enzyme activity

Genotype[a]	Exp	Enzyme activity[c] Enzyme	Co	HP	%	P^d
1. *mSODX1/Y;FLP3/+*	1	MnSOD	0.560 ± 0.055	0.982 ± 0.049	+75.3	0.0006
2. *mSOD2C/+;FLP3/+*	1	MnSOD	0.857 ± 0.028	1.36 ± 0.249	+58.2	0.0720
3. *mSOD3A/+;FLP3/+*	1	MnSOD	0.650 ± 0.134	0.968 ± 0.176	+48.9	0.0561
4. *mSOD2A/+;FLP3/+*	1	MnSOD	1.008 ± 0.038	1.401 ± 0.051	+39.0	0.0007
5. *mSOD3C/FLP3*	1	MnSOD	1.025 ± 0.135	1.391 ± 0.157	+35.7	0.0390
6. *mSOD2E/+;FLP3/+*	1	MnSOD	0.820 ± 0.029	1.057 ± 0.115	+28.9	0.0626
7. *LacZ/FLP3*	1	MnSOD	1.275 ± 0.158	1.427 ± 0.121	+11.92	0.2607
	2	Cu/ZnSOD	16.60 ± 3.46	17.00 ± 2.26	+2.46	0.8735
8. *FLP3/+*[b]	1	MnSOD	0.667 ± 0.068	0.722 ± 0.037	+8.18	0.3066
	2	Cu/ZnSOD	18.91 ± 0.19	20.42 ± 1.69	+7.98	0.2615
9. Oregon-R	1	MnSOD	0.392 ± 0.065	0.468 ± 0.071	+19.14	0.2486
	2	Cu/ZnSOD	3.71 ± 0.85	3.55 ± 1.16	−4.12	0.8473
10. *CAT2A2/+;FLP3/+*	2	Catalase	0.00258 ± 0.00006	0.00354 ± 0.00009	+37.12	0.0002
11. *mSODX1/Y;CAT2A2/+;FLP3/+*	2	MnSOD	0.438 ± 0.541	0.541 ± 0.019	+23.34	0.0995
	2	Catalase	0.00261 ± 0.00006	0.00343 ± 0.00035	+31.67	0.0508
12. *CAT2A2/+;mSOD3C/FLP3*	2	MnSOD	0.543 ± 0.127	0.812 ± 0.020	+49.44	0.0635
	2	Catalase	0.00299 ± 0.00012	0.00412 ± 0.00026	+37.95	0.0001
13. *cSOD3A1/FLP3*	2	Cu/ZnSOD	7.06 ± 0.44	9.29 ± 1.31	+31.69	0.0849
14. *cSOD2A/+;cSOD3A1/FLP3*	2	Cu/ZnSOD	11.52 ± 2.36	16.93 ± 0.41	+47.01	0.0539

[a] All transgenic constructs are heterozygous.
[b] Progeny of cross of *FLP3* and Oregon-R.
[c] Mean ± SD of triplicate assays, units defined in MATERIALS AND METHODS.
[d] Unpaired two-sided *t*-test.

the time until 90% mortality. Maximum life span was increased by an average of 15%, with one line showing as much as 37% increase. Analysis of all the *MnSOD* transgenic lines using ANOVA confirmed that both mean and maximum life span were significantly increased.

Two-factor fixed-effects ANOVA results for mean life span were group $F = 353.80$, $P < 0.0001$; genotype $F = 531.96$, $P < 0.0001$; group × genotype $F = 18.93$, $P < 0.0001$. Because the group × genotype interaction was significant, it may be informative to repeat the ANOVA separately for group = control and group = HP. When this was done the genotype effect was unchanged in each case ($P < 0.0001$).

Repeat of the ANOVA analysis of mean life span excluding genotype 1 yielded essentially the same results: group $F = 261.63$, $P < 0.0001$; genotype $F = 363.60$, $P < 0.0001$; group × genotype $F = 21.78$, $P < 0.0001$. Because the group × genotype interaction was significant, it may be informative to repeat the ANOVA separately for group = control and group = HP. When this was done the genotype effect was unchanged in each case ($P < 0.0001$).

TABLE 2

Enzyme induction using two-factor fixed-effects ANOVA

Source	d.f.	MS	*F*	*P*
Group	1	1.249	84	<0.0001
Genotype	5	0.226	15	<0.0001
Group × genotype	5	0.012	0.81	0.55
Error	24	0.015		

MS, mean square.

Maximum life span data for the MnSOD overexpressing genotypes 1–6 (Table 3) were analyzed using the same two-factor fixed-effects ANOVA model. Results were group $F = 20.97$, $P = 0.0006$; genotype $F = 34.67$, $P < 0.0001$; group × genotype $F = 1.77$, $P = 0.1943$.

Repeat of the analysis of maximum life span excluding genotype 1 yielded essentially the same results: group $F = 16.49$, $P = 0.0023$; genotype $F = 46.50$, $P < 0.0001$; group × genotype $F = 1.54$, $P = 0.2629$.

As expected, the transgenic lines varied in their starting life spans, due to unavoidable small differences in genetic background (KAISER *et al.* 1997; SUN and TOWER 1999; TATAR 1999; TOWER 1996, 2000). The largest increase in mean life span (avg. 33%) was observed for line *mSODX1* (Table 3, genotype 1), which has the lowest starting life span of 26 days. The possibility cannot be ruled out that, for this particular strain, MnSOD overexpression is rescuing some unique genetic defect. However, the starting life spans of genotypes 2–4 range from 40 to 56 days, which is typical for Drosophila controls, and these strains exhibit reproducible 12–29% increases in life span. In addition, ANOVA analysis of the *MnSOD*

TABLE 3

Life span

Genotype[a]	Exp	Mean life span (days)[c] Co	HP	%	*P*[d]	Avg. %	90% mortality (days) Co	HP	%	Avg. %	*P*[e]
1. *mSODX1/Y;FLP3/+*	1	28.02 ± 0.64	36.83 ± 0.97	+31.45	<0.0001	+32.97	44	60	+36.4	+37.6	0.0424
	2	23.53 ± 0.55	31.65 ± 0.86	+34.48	<0.0001		36	50	+38.9		
2. *mSOD2C/+;FLP3/+*	1	41.40 ± 0.98	51.93 ± 1.00	+25.44	<0.0001	+28.98	64	72	+12.5	+14.3	0.0704
	2	40.66 ± 0.87	53.88 ± 1.02	+32.52	<0.0001		62	72	+16.1		
3. *mSOD3A/+;FLP3/+*	1	44.22 ± 0.76	56.28 ± 0.78	+27.27	<0.0001	+14.19	58	70	+20.7	+16.8	0.1257
	2	48.85 ± 0.65	54.68 ± 0.94	+11.93	<0.0001		62	70	+12.9		
4. *mSOD2A/+;FLP3/+*	1	54.70 ± 0.86	60.05 ± 0.93	+9.79	<0.0001	+11.27	76	78	+2.6	+2.6	<0.0001
	2	55.15 ± 0.97	62.18 ± 0.92	+12.75	<0.0001		76	78	+2.6		
5. *mSOD3C/FLP3*	1	30.95 ± 0.75	34.62 ± 1.02	+11.86	0.0035	+7.44	52	62	+19.2	+11.7	0.3743
	2	36.35 ± 0.56	37.44 ± 0.73	+3.01	0.2292		48	50	+4.2		
6. *mSOD2E/+;FLP3/+*	1	35.59 ± 0.69	34.83 ± 0.74	−2.14	0.4497	+1.47	51	50	−2.0	+3.0	0.6560
	2	35.40 ± 0.67	37.19 ± 0.78	+5.06	0.0814		50	54	+8.0		
7. *LacZ/FLP3*	1	46.45 ± 1.04	45.94 ± 1.16	−1.09	0.7442	+1.55	64	67	+4.7	+5.7	0.0903
	2	44.28 ± 0.94	46.14 ± 1.23	+4.20	0.2290		60	64	+6.7		
8. *FLP3/+*[b]	1	46.40 ± 0.91	44.53 ± 0.85	−4.03	0.1314	−0.78	62	62	0.0	+1.6	0.5000
	2	50.74 ± 0.89	51.99 ± 0.83	+2.46	0.3053		64	66	+3.1		
9. Oregon-R	1	41.64 ± 1.02	41.32 ± 0.97	−0.77	0.8235	−5.15	60	56	−6.7	−8.2	0.1257
	2	45.54 ± 0.86	41.20 ± 0.90	−9.53	0.0006		62	56	+9.7		
10. *CAT2A2/+;FLP3/+*	1	50.61 ± 0.62	47.13 ± 0.79	−6.88	0.0005	−7.54	62	62	0.0	−4.5	0.5000
	2	53.04 ± 0.81	48.69 ± 0.89	−8.20	0.0003		66	60	−9.1		
11. *mSODX1/Y;CAT2A2/+;FLP3/+*	1	32.18 ± 0.70	34.57 ± 0.68	+7.43	0.0149	+7.45	44	48	+9.1	+6.8	0.2048
	2	32.35 ± 0.65	34.77 ± 0.72	+7.48	0.0127		44	46	+4.5		
12. *CAT2A2/+;mSOD3C/FLP3*	1	40.83 ± 0.70	42.16 ± 1.02	+3.26	0.2862	−1.85	52	62	+19.2	+10.6	0.4365
	2	40.79 ± 0.83	37.95 ± 1.12	−6.96	0.0381		53	54	+1.9		
13. *cSOD3A1/FLP3*	2	36.20 ± 0.55	40.55 ± 0.74	+12.02	<0.0001	+12.88	48	54	+12.5	+19.4	<0.0001
	3	29.10 ± 0.46	33.10 ± 0.65	+13.75	<0.0001		38	48	+26.3		
14. *cSOD2A/+;cSOD3A1/FLP3*	2	35.09 ± 0.77	40.32 ± 0.91	+14.90	<0.0001	+11.04	46	56	+21.7	+15.0	0.2578
	3	34.86 ± 0.59	37.36 ± 0.68	+7.17	0.0057		49	53	+8.2		

[a] All transgenic constructs are heterozygous, *mSOD* = *MnSOD* construct, *cSOD* = *Cu/ZnSOD* construct.
[b] Progeny of cross of *FLP3* and Oregon-R wild type.
[c] Mean ± SEM.
[d] Unpaired two-sided *t*-test.
[e] Paired two-sided *t*-test.

transgenic lines, omitting line mSOD(X)1, yielded qualitatively identical results. Therefore the data demonstrate that MnSOD overexpression extends life span regardless of the starting life span of the strain. Similar results were obtained in the previous study of Cu/ZnSOD overexpression; *i.e.*, life span was extended in both long- and short-lived genetic backgrounds with the largest increases observed in the short-lived background (Sun and Tower 1999).

Heat pulse alone does not increase life span: The control strains (Table 3, genotypes 7–9) that do not contain a *MnSOD* transgene did not exhibit a significant increase in mean or maximum life span due to the heat pulse, as confirmed by two-factor fixed-effects ANOVA. Results for mean life span were group $F = 1.02$, $P = 0.3125$; genotype $F = 37.06$, $P < 0.0001$; group × genotype $F = 2.91$, $P = 0.0544$. Results for maximum life span were group $F = 0.02$, $P = 0.8911$; genotype $F = 8.59$, $P = 0.0173$; group × genotype $F = 4.67$, $P = 0.0598$. Therefore the heat pulse alone does not increase life span, and increased life span must result from MnSOD overexpression. Representative survival curves are presented for three MnSOD overexpressing lines (Figure 1, A–C) and three control lines (Figure 1, D–F).

Life span is increased in proportion to the increase in MnSOD enzyme activity: Inspection of Tables 1 and 3 suggests that the largest increases in life span are observed in the lines yielding the largest increases in enzyme activity. Each transgenic line varies in its starting enzyme activity and life span. Plotting the percentage change in life span *vs.* the percentage change in enzyme activity corrects for this variation. The plot reveals a strikingly linear correlation between enzyme activity and both mean and maximum life span for all six *MnSOD* transgenic lines (Figure 2). Regression analysis demonstrates that this relationship is significant for both enzyme activity and mean life span and enzyme activity and maximum life span. Results for mean life span were $R^2 = 0.9375$, the intercept term was significant $P = 0.0184$, and the slope term was significant $P = 0.0015$. Results for maximum life span were $R^2 = 0.8283$, the intercept term was not significant $P = 0.0790$, and the slope term was significant $P = 0.0118$.

Catalase overexpression is not associated with increased life span: FLP-*out* using the *FLP3* insertion allowed induced overexpression of catalase up to 37%, as measured by enzyme assay of fly extracts (Table 1). Increased catalase activity has previously been shown to increase resistance to killing by H_2O_2 feeding, but to have neutral or slightly negative effects on life span on its own or when coexpressed with Cu/ZnSOD (Sun and Tower 1999). In the experiments presented here, catalase was again found to have neutral or negative effects on life span on its own (Table 3, genotype 10) and to have neutral or negative effects on life span when coexpressed with MnSOD (Table 3, compare genotypes 1 and 5 with genotypes 10 and 11). The neutral or slightly negative effects of catalase when combined with MnSOD may be due in part to the fact that MnSOD overexpression was significantly reduced when the *catalase* construct was also present. A similar effect was previously observed for simultaneous overexpression of catalase and Cu/ZnSOD (Sun and Tower 1999). Taken together, the data support the conclusion that catalase activity is already in excess in the adult fly, at least with regard to life span.

The results were confirmed by three-factor fixed-effects ANOVA. Results for MnSOD enzyme activity were cattype $F = 114.00$, $P < 0.0001$; genotype $F = 67.47$, $P < 0.0001$; cattype × genotype $F = 10.65$, $P = 0.0049$; group $F = 58.06$, $P < 0.0001$; cattype × group $F = 7.52$, $P = 0.0145$; genotype × group $F = 0.52$, $P = 0.4801$; cattype × genotype × group $F = 2.15$, $P = 0.1624$.

Results for mean life span were cattype $F = 4.89$, $P = 0.0271$; genotype $F = 452.39$, $P < 0.0001$; cattype × genotype $F = 22.11$, $P < 0.0001$; group $F = 87.18$, $P < 0.0001$; cattype × group $F = 34.71$, $P < 0.0001$; genotype × group $F = 11.74$, $P = 0.0006$; cattype × genotype × group $F = 0.33$, $P = 0.5665$.

Results for maximum life span were cattype $F = 0.00$, $P = 0.9610$; genotype $F = 9.47$, $P = 0.0152$; cattype × genotype $F = 0.74$, $P = 0.4161$; group $F = 8.86$, $P = 0.0177$; cattype × group $F = 1.59$, $P = 0.2428$; genotype × group $F = 0.43$, $P = 0.5304$; cattype × genotype × group $F = 1.35$, $P = 0.2794$.

SOD overexpression and oxygen consumption: The effect of SOD overexpression on metabolic rate of the flies was examined by assaying O_2 consumption at time points throughout the adult life span. In addition to the *MnSOD* lines, two *Cu/ZnSOD* lines were also examined, and reassay of enzyme activity and life span for the *Cu/ZnSOD* lines yielded results essentially identical to those previously reported (Sun and Tower 1999).

ANOVA (materials and methods) was first used to confirm that life span was significantly increased in both the Cu/ZnSOD and MnSOD overexpressing lines (Table 4, genotypes 1–4) but was not increased in the Oregon-R controls (genotype 5). When the two *Cu/ZnSOD*-containing genotypes (Table 4, genotypes 1 and 2) were analyzed together for mean life span the increase was significant: genotype $F = 66.79$, $P < 0.0001$; group $F = 31.19$, $P < 0.0001$; genotype × group $F = 1.47$, $P = 0.2257$. When the two *MnSOD*-containing genotypes (Table 4, genotypes 3 and 4) were analyzed together for mean life span the increase was significant: genotype $F = 140.28$, $P < 0.0001$; group $F = 71.39$, $P < 0.0001$; genotype × group $F = 2.11$, $P = 0.1465$. When all four SOD-containing genotypes (Table 4, genotypes 1–4) were analyzed together the increase was significant: genotype $F = 536.17$, $P < 0.0001$; group $F = 102.63$, $P < 0.0001$; genotype × group $F = 7.16$, $P < 0.0001$.

Second, oxygen consumption was analyzed across all five genotypes (Table 4) using a three-way factorial ANOVA with main effects of genotype, date of assay, and

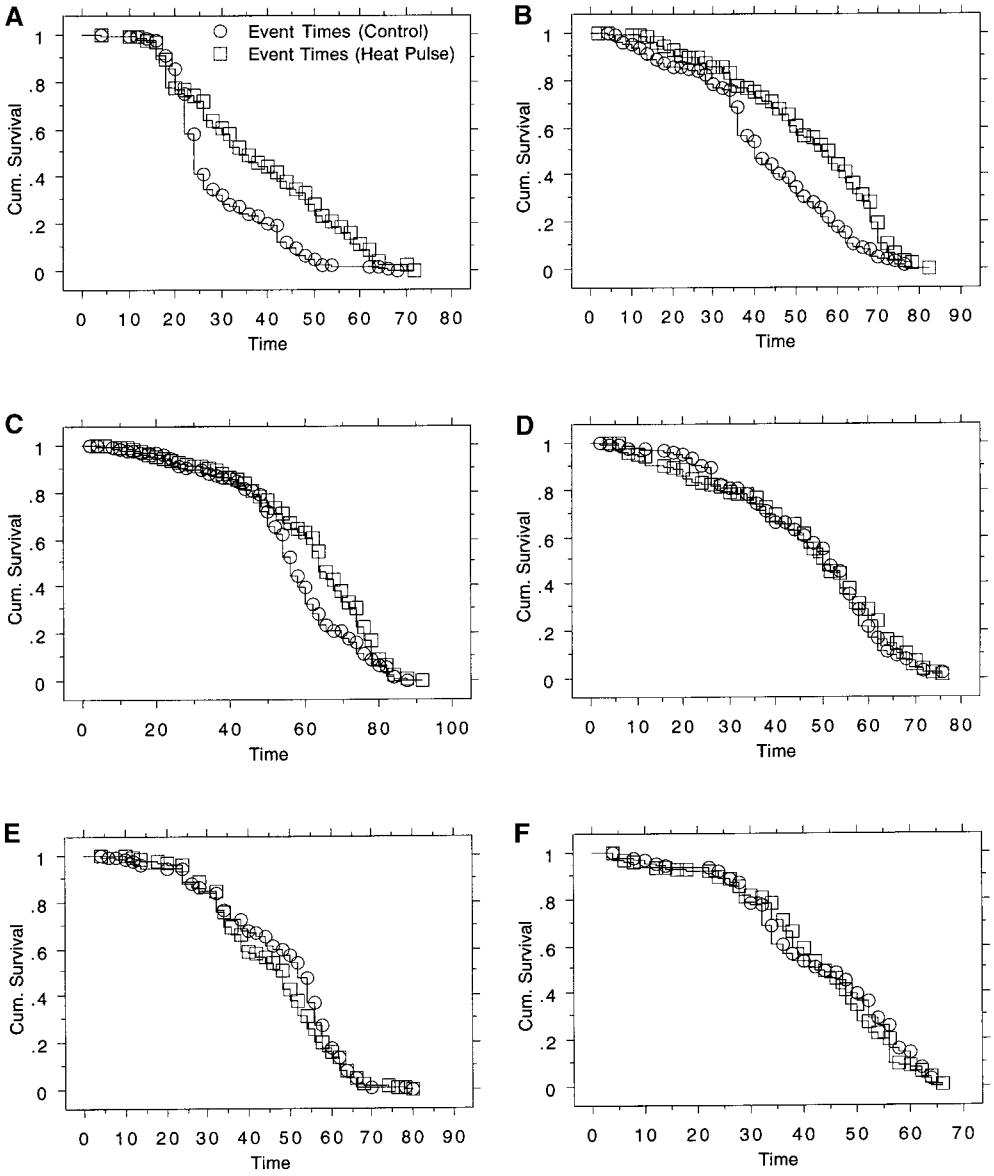

FIGURE 1.—Cumulative survival as a function of time for selected control and MnSOD overexpressing lines. The data are presented for life span assay, experiment 1, genotypes 1, 2, 4, 7, 8, and 9 of Table 3. Circles, control flies; squares, heat-pulsed flies. (A) *mSODX1/Y;FLP3/+* genotype 1 of Table 3. (B) *mSOD2C/+;FLP3/+* genotype 2 of Table 3. (C) *mSOD2A/+;FLP3/+* genotype 4 of Table 3. (D) *LacZ/FLP3* genotype 7 of Table 3. (E) *FLP3/+* genotype 8 of Table 3. (F) Oregon-R genotype 9 of Table 3.

group (Co *vs.* heat pulse). Results for oxygen consumption were genotype $F = 7.16$, $P < 0.0001$; group $F = 12.54$, $P = 0.0005$; genotype × group $F = 0.94$, $P = 0.4433$; date $F = 3.61$, $P < 0.0001$; genotype × date $F = 0.73$, $P = 0.7242$; group × date $F = 0.53$, $P = 0.9131$; genotype × group × date $F = 0.37$, $P = 0.9727$.

Third, the relationship between life span and oxygen consumption was analyzed across all five genotypes (Table 4) using ANCOVA of the line means. The analysis was done in three steps.

i. Correlation analysis of the means for oxygen consumption and life span revealed a negative correlation: −0.75497.
ii. A model of "oxygen consumption = genotype + group" gave results genotype $F = 26.84$, $P = 0.0038$; group $F = 21.04$, $P = 0.0101$.
iii. Life span was included as a covariate in a model of "oxygen consumption = life span + genotype + group" with results life span $F = 0.05$, $P = 0.8322$; genotype $F = 8.47$, $P = 0.0552$; group $F = 8.82$, $P = 0.0591$.

The results revealed that heat pulse had a significant negative effect on oxygen consumption across all five genotypes and that there was a negative correlation between life span and oxygen consumption. However, the analyses did not control for the different starting life spans and oxygen consumption values of the various genotypes. These base line life spans and oxygen consumption values are expected to correlate strongly and may mask the effects caused by transgene expression. The more appropriate analysis for the conditional system was therefore to analyze the relationship between the change in life span and the change in oxygen consumption caused by the heat pulse. An ANCOVA model was used that combined a continuous independent variable (oxyper) with a categorical variable (genotype)

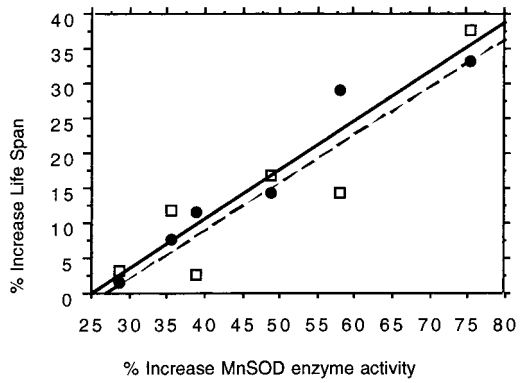

FIGURE 2.—Correlation between MnSOD overexpression and life span extension. For all six independent transgenic lines, the percentage increase in MnSOD enzyme activity was plotted *vs.* the percentage increase in mean life span (solid circles, solid line; $r^2 = 0.9375$) and maximum life span (open squares, dashed line; $r^2 = 0.8283$, 90% mortality); data are from Tables 1 and 3.

as described in MATERIALS AND METHODS. In this case genotype had two values: experimental (genotypes 1–4 of Table 4) and control (genotype 5 of Table 4). Results were genotype $F = 11.36$, $P = 0.0434$; oxyper $F = 0.70$, $P = 0.4651$. Consistent with the analyses presented above, there was a genotype effect, meaning the experimental genotypes lived longer than control. In contrast, the coefficient for oxygen was not significant, meaning oxygen consumption was not a significant factor in increasing life span in experimentals relative to control. Therefore, increased life span was not associated with decreased O_2 consumption as is found when life span is altered by culture temperature (MIQUEL *et al.* 1976). The data support the conclusion that overexpression of MnSOD or Cu/ZnSOD increases life span by a mechanism that does not involve reductions in oxidative metabolism.

DISCUSSION

To further test the theory that oxidative stress is a major cause of aging, Drosophila were engineered to overexpress the antioxidant enzyme MnSOD. The FLP-*out* transgenic system yields transgene expression specifically in the adult in all tissues. The amount of MnSOD enzyme overexpression achieved varied between six independent transgenic lines, with increases up to 75%. Life span was found to be increased in proportion to the increase in enzyme. Mean life span was increased up to 33%, and maximum life span, as measured by time to 90% mortality, was increased up to 37%. Therefore, adult Drosophila life span is limited by MnSOD activity, analogous to the results previously obtained for Cu/ZnSOD (PARKES *et al.* 1998; SUN and TOWER 1999). Both Cu/ZnSOD and MnSOD were found to increase mean and maximum life span with

TABLE 4

Oxygen consumption

		O_2 consumption[a]				Mean life span[c]			
Genotype	Exp	Co	HP	%	P^b	Exp	Co	HP	%	P^b
1. *cSOD3A1/FLP3*	3	93.3 ± 11.0	82.4 ± 11.8	−11.68	0.1305	3	29.10 ± 0.46	33.10 ± 0.65	+13.75	<0.0001
2. *cSOD2A/+;cSOD3A1/FLP3*	3	91.4 ± 15.1	87.8 ± 17.0	−3.94	0.7002	3	34.86 ± 0.59	37.36 ± 0.68	+7.17	0.0057
3. *mSOD2C/+;FLP3/+*	4	74.4 ± 10.2	73.8 ± 4.6	−4.65	0.4477	4	43.73 ± 0.88	53.00 ± 1.06	+21.20	<0.0001
4. *mSOD2A/+;FLP3/+*	4	76.4 ± 6.0	65.8 ± 3.7	−13.87	0.0043	4	56.47 ± 0.92	62.92 ± 0.90	+11.42	<0.0001
5. Oregon-R	3	76.0 ± 7.5	73.2 ± 8.8	−3.68	0.5662	1	41.64 ± 1.02	41.32 ± 0.97	−0.77	0.8235
	4	72.8 ± 12.2	61.8 ± 5.7	−15.11	0.0731	2	45.54 ± 0.86	41.20 ± 0.90	−9.53	0.0006

[a] Mean ± SD, units = nl O_2/min fly.
[b] Unpaired, two-sided *t*-test.
[c] Mean ± SE. For genotypes 1, 2, and 5 mean life spans are repeated from Table 2 for comparisons.

no detectable negative effect on metabolic activity. Co-overexpression of catalase with MnSOD had no added benefit for life span, consistent with the previous conclusion that catalase is in excess in the adult fly with regard to life span (Sun and Tower 1999).

Interestingly, it has recently been reported that flies transgenic for an extra copy of the native *MnSOD* gene did not exhibit increased life span (Mockett *et al.* 1999). This difference in results is not due simply to a difference in the amount of enzyme overexpression achieved, as the levels of enzyme overexpression observed in whole fly extracts were similar to the levels of overexpression observed here. We suggest two possible models for the difference in results obtained with the native promoter approach *vs.* the FLP-*out* approach. In the first model, MnSOD overexpression during development is hypothesized to be deleterious and have negative effects on subsequent adult life span. The native promoter approach is expected to result in overexpression throughout the life cycle both during development and in the adult. Negative effects of MnSOD overexpression during development might then cancel out any positive effects on life span that might have otherwise resulted from overexpression in the adult. In contrast, with FLP-*out* MnSOD overexpression is specific to the adult stage and therefore significant increases in life span are observed. However, this model requires that the putative positive and negative effects of the native promoter constructs be almost exactly equal to yield unchanged life span for both MnSOD and Cu/ZnSOD (as described below)—a result that seems unlikely. The second model, which we therefore favor, involves possible differences in the tissue specificity of expression. With the native promoter approach the tissue and temporal specificity of overexpression is expected to be the same as, or similar to, the native expression pattern. In contrast, with FLP-*out* MnSOD overexpression is ultimately driven by the powerful, tissue-general *actin5C* promoter. We hypothesize that the *actin5C* promoter results in a novel pattern of *MnSOD* transgene expression that results in increased life span. The *actin5C* promoter may drive MnSOD expression in some critical cells or tissues where the native promoter is inactive or less active. This situation appears to be analogous to that with Cu/ZnSOD. Overexpression of Cu/ZnSOD alone with the native promoter approach does not yield increased life span, as previously reported (Orr and Sohal 1993) and in extensive subsequent analyses of the same and additional transgenic lines (B. Orr, personal communication). In contrast, overexpression of Cu/ZnSOD with the heterologous FLP-*out* system or the heterologous Gal4/UAS system does yield increased life span (Parkes *et al.* 1998; Sun and Tower 1999). In the latter case life span increase is associated with preferential expression of Cu/ZnSOD in the motorneurons. Therefore, for both Cu/ZnSOD and MnSOD overexpression using the native promoter does not yield increased life span while the heterologous expression systems do yield increased life span. It will be of great interest in the future to determine what are the unique aspect(s) of the heterologous expression systems that allow for increased life span.

The present data for MnSOD and Cu/ZnSOD combined with previous studies of Cu/ZnSOD (Parkes *et al.* 1998; Sun and Tower 1999) demonstrate that SOD activity is limiting for life span in the adult fly. Because the primary catalytic activity of SOD is to convert superoxide to H_2O_2, the current data suggest that increased life span results from more efficient conversion of superoxide and reduced superoxide levels. The first mechanism by which superoxide levels might limit life span is by directly or indirectly causing oxidative damage to cellular macromolecules and organelles. However, alternative mechanisms involving a negative signaling or regulatory effect of superoxide on life span or a positive signaling or regulatory effect of H_2O_2 on life span cannot be ruled out at this time. It will be of interest in the future to determine if overexpression of SOD and increased life span correlate with decreased and/or delayed accumulation of specific oxidative damage products as predicted by the first proposed mechanism. Identification of specific cellular molecules that are protected from oxidative damage by both MnSOD and Cu/ZnSOD overexpression may reveal critical life span-limiting targets for oxidative damage during aging. Of particular interest may be mitochondrial aconitase and the inner mitochondrial membrane protein adenine nucleotide translocase (ANT). Both proteins appear to be preferentially susceptible to oxidative damage during aging in houseflies (Yan *et al.* 1997; Yan and Sohal 1998).

The results presented here are consistent with previous and concurrent studies of genetically selected Drosophila strains. Selection of genetically heterogeneous populations of Drosophila for late life female fecundity over many generations results in populations with correlated phenotypes of increased life span and increased stress resistance (Luckinbill *et al.* 1984; Rose 1984; Partridge and Fowler 1992). Detailed analysis of one such selected strain and its matched control strain reveals that increased life span in this particular strain correlates with increased oxidative stress resistance and increased expression of antioxidant enzyme genes, including *Cu/ZnSOD* and *MnSOD* (Arking *et al.* 1991, 2000). The results presented here are also consistent with genetic selection experiments with regard to metabolic activity. Metabolic activity was assayed in the selected and control population as well as an inbred long-lived strain and inbred control strain derived from the selected and control populations. The results indicated that while aerobic efficiency declined as a function of age in all flies, metabolic activity of the long-lived and control strains was equal throughout the majority of the life span (Arking *et al.* 1988; Ross 2000; Mockett *et*

al. 2001). Therefore both genetic selection experiments and FLP-*out* overexpression of SOD appear to increase life span by a mechanism that does not involve decreased metabolic activity.

It will be of interest to determine if SOD overexpression can extend life span in mammals. Consitutive overexpression of Cu/ZnSOD has been achieved in mice; however, no increases in life span have been observed (Huang *et al.* 2000). Analogous to the situation with Drosophila, it may be found that inducible or tissue-specific expression systems (Li *et al.* 1999) will be required to observe increased life span. Finally, in *Caenorhabditis elegans* and Drosophila, and possibly also in yeast and mammals, a conserved insulin-like signal transduction pathway negatively regulates life span (Kenyon 2001). In *C. elegans* the increased life span is associated with increased expression of the gene encoding mitochondrial MnSOD (Honda and Honda 1999). The data presented here demonstrate that in Drosophila MnSOD overexpression is sufficient to significantly increase life span. These results suggest the possibility that increased MnSOD expression may be responsible for all or part of the life span increases observed upon mutation of the conserved insulin-like life span regulatory pathway in *C. elegans*, Drosophila, and perhaps other species.

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LITERATURE CITED

Arking, R., S. Buck, R. A. Wells and R. Pretzlaff, 1988 Metabolic rates in gentically based long lived strains of *Drosophila*. Exp. Gerontol. **23:** 59–76.

Arking, R., S. Buck, A. Berrios, S. Dwyer and G. T. Baker, 1991 Elevated paraquat resistance can be used as a bioassay for longevity in a genetically based long-lived strain of *Drosophila*. Dev. Genet. **12:** 362–370.

Arking, R., V. Burde, K. Graves, R. Hari, E. Feldman *et al.*, 2000 Forward and reverse selection for longevity in *Drosophila* is characterized by alteration of antioxidant gene expression and oxidative damage patterns. Exp. Gerontol. **35:** 167–185.

Charlesworth, B., 1994 *Evolution in Age Structured Populations.* Cambridge University Press, Cambridge, UK.

Chippindale, A. K., A. M. Leroi, S. B. Kim and M. R. Rose, 1993 Phenotypic plasticity and selection on Drosophila life-history evolution. I. Nutrition and the cost of reproduction. J. Evol. Biol. **6:** 171–193.

Duttaroy, A., R. Meidinger, K. Kirby, S. Carmichael, A. Hilliker *et al.*, 1994 A manganese superoxide dismutase-encoding cDNA from *Drosophila melanogaster*. Gene **143:** 223–225.

Finkel, T., and N. J. Holbrook, 2000 Oxidants, oxidative stress and the biology of aging. Nature **408:** 239–247.

Griswold, C. M., A. L. Matthews, K. E. Bewley and J. W. Mahaffey, 1993 Molecular characterization of rescue of acatalesemic mutants of *Drosophila melanogaster*. Genetics **134:** 731–788.

Harman, D. J., 1956 Aging: a theory based on free radical and radiation chemistry. J. Gerontol. **11:** 298–300.

Honda, Y., and S. Honda, 1999 The *daf-2* gene network for longevity regulates oxidative stress resistance and *Mn-superoxide dismutase* gene expression in *Caenorhabditis elegans*. FASEB J. **13:** 1385–1393.

Huang, T. T., E. J. Carlson, I. Raineri, A. M. Gillespie, H. Kozy *et al.*, 1999 The use of transgenic and mutant mice to study oxygen free radical metabolism. Ann. NY Acad. Sci. **893:** 95–112.

Huang, T. T., E. J. Carlson, A. M. Gillespie, Y. Shi and C. J. Epstein, 2000 Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. J. Gerontol. Biol. Sci. **55:** B5–B9.

Kaiser, M., M. Gasser, R. Ackermann and S. C. Stearns, 1997 P element inserts in transgenic flies: a cautionary tale. Heredity **78:** 1–11.

Kenyon, C., 2001 A conserved regulatory system for aging. Cell **105:** 165–168.

Kirkwood, T. B. L., 1995 The evolution of aging. Rev. Clin. Gerontol. **5:** 3–9.

Kirkwood, T. B. L., and S. N. Austad, 2000 Why do we age? Nature **409:** 233–238.

Li, Y., E. J. Carlson, K. Murakami, J. C. Copin, R. Luche *et al.*, 1999 Targeted expression of human CuZn superoxide dismutase gene in mouse central nervous system. J. Neurosci. Methods **89:** 49055.

Luckinbill, L. S., R. Arking, M. Clare, W. Cirocco and S. Buck, 1984 Selection for delayed senescence in *Drosophila melanogaster*. Evolution **38:** 996–1003.

Mackay, W. J., and G. C. Bewley, 1989 The genetics of catalase in *Drosophila melanogaster*. Isolation and characterization of acatalasemic mutants. Genetics **122:** 643–652.

Martin, G. M., S. N. Austad and T. E. Johnson, 1996 Genetic analysis of ageing: role of oxidative damage and environmental stresses. Nat. Genet. **13:** 25–34.

Miquel, J., P. R. Lundgren, K. G. Bensch and H. Atlan, 1976 Effects of temperature on the life span, vitality and fine structure of *Drosophila melanogaster*. Mech. Ageing Dev. **5:** 347–370.

Mockett, R. J., W. C. Orr, J. J. Rahmandar, J. J. Benes, S. N. Radyuk *et al.*, 1999 Overexpression of Mn-containing superoxide dismutase in transgenic *Drosophila melanogaster*. Arch. Biochem. Biophys. **371:** 260–269.

Mockett, R. J., W. C. Orr, J. J. Rahmandar, B. H. Sohal and R. S. Sohal, 2001 Antioxidant status and stress resistance in long- and short-lived lines of *Drosophila melanogaster*. Exp. Gerontol. **36:** 441–463.

Neeter, J., W. Wasserman and M. H. Kutner, 1990 *Applied Linear Statistical Models.* Irwin, Burr Ridge, IL.

Orr, W., and R. S. Sohal, 1994 Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science **263:** 1128–1130.

Orr, W. C., and R. S. Sohal, 1993 Effects of Cu-Zn superoxide dismutase overexpression on life span and resistance to oxidative stress in transgenic *Drosophila melanogaster*. Arch. Biochem. Biophys. **301:** 34–40.

Orr, W. C., and R. S. Sohal, 1992 The effects of catalase gene overexpression on life span and resistance to oxidative stress in transgenic *Drosophila melanogaster*. Arch. Biochem. Biophys. **297:** 35–41.

Orr, W. C., L. A. Arnold and R. S. Sohal, 1992 Relationship between catalase activity, life span and some parameters associated with antioxidant defenses in drosophila melanogaster. Mech. Ageing Dev. **63:** 287–296.

Parkes, T. L., A. J. Elia, D. Dickson, A. J. Hilliker, J. P. Phillips *et al.*, 1998 Extension of *Drosophila* lifespan by overexpression of human *SOD1* in motorneurons. Nat. Genet. **19:** 171–174.

Partridge, L., and N. H. Barton, 1993 Optimality, mutation and the evolution of aging. Nature **362:** 305–311.

Partridge, L., and K. Fowler, 1992 Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution **46:** 76–91.

Partridge, L. A., A. Green and K. Fowler, 1987 Effects of egg production and of exposure to males on female survival in *Drosophila melanogaster*. J. Insect Physiol. **33:** 745–749.

Phillips, J. P., S. D. Campbell, D. Michaud, M. Charbonneau and A. J. Hilliker, 1989 Null mutation of copper/zinc superoxide dismutase in *Drosophila* confers hypersensitivity to paraquat and reduced longevity. Proc. Natl. Acad. Sci. USA **86:** 2761–2765.

Reveillaud, I., A. Niedzwiecki, K. G. Bensch and J. E. Fleming, 1991 Expression of bovine superoxide dismutase in *Drosophila melanogaster* augments resistance to oxidative stress. Mol. Cell. Biol. **11:** 632–640.

Rose, M. R., 1984 Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution **38:** 1004–1010.

Rose, M. R., 1991 *Evolutionary Biology of Aging*. Oxford University Press, New York.

Ross, R. E., 2000 Age-specific decrease in aerobic efficiency associated with increase in oxygen free radical production in Drosophila melanogaster. J. Insect Physiol. **46:** 1477–1480.

Rubin, G. M., and A. C. Spradling, 1982 Genetic transformation of *Drosophila* with transposable element vectors. Science **218:** 348–353.

Seto, N. O. L., S. Hayashi and G. M. Tener, 1990 Overexpression of Cu-Zn superoxide dismutase in *Drosophila* does not affect life span. Proc. Natl. Acad. Sci. USA **87:** 4270–4274.

Sohal, R., A. Agarwal, S. Agarwal and W. Orr, 1995 Simultaneous overexpression of copper- and zinc-containing superoxide dismutase and catalase retards age-related oxidative damage and increases metabolic potential in *Drosophila melanogaster*. J. Biol. Chem. **270:** 15671–15674.

Sohal, R. S., and R. Weindruch, 1996 Oxidative stress, caloric restriction, and aging. Science **273:** 59–63.

Stadtman, E. R., 1992 Protein oxidation and aging. Science **257:** 1220–1224.

Sun, J., and J. Tower, 1999 FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. Mol. Cell. Biol. **19:** 216–228.

Tatar, M., 1999 Transgenes in the analysis of life span and fitness. Am. Nat. **154**(Suppl.): S67–S81.

Tower, J., 1996 Aging mechanisms in fruit flies. Bioessays **18:** 799–807.

Tower, J., 2000 Transgenic methods for increasing *Drosophila* life span. Mech. Ageing Dev. **118:** 1–14.

Wallace, D. C., 1999 Mitochondrial diseases in man and mouse. Science **283:** 1482–1488.

Wheeler, J. C., E. T. Bieschke and J. Tower, 1995 Muscle-specific expression of *Drosophila* hsp70 in response to aging and oxidative stress. Proc. Natl. Acad. Sci. USA **92:** 10408–10412.

Wheeler, J. C., V. King and J. Tower, 1999 Sequence requirements for upregulated expression of *Drosophila hsp70* transgenes during aging. Neurobiol. Aging **20:** 545–553.

Yan, L.-J., and R. S. Sohal, 1998 Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. Proc. Natl. Acad. Sci. USA **95:** 12896–12901.

Yan, L.-J., R. L. Levine and R. S. Sohal, 1997 Oxidative damage during aging targets mitochondrial aconitase. Proc. Natl. Acad. Sci. USA **94:** 11168–11172.

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