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Original Contribution

EFFECTS OF GROWTH HORMONE ON HYPOTHALAMIC CATALASE AND Cu/Zn SUPEROXIDE DISMUTASE[1]

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Abstract—Age-associated changes in hypothalamic catalase activity and level, and Cu/Zn superoxide dismutase (Cu/Zn SOD) activity were examined in Ames dwarf mice with growth hormone (GH) deficiency and prolonged lifespan, in PEPCK-hGH transgenic mice with overexpression of GH and reduced lifespan, and compared to values measured in normal controls. Hypothalami from young (3–4 months), middle-aged (9–10 months), and old (19–23 months) male mice were examined using spectrophotometric assay and Western blot. In dwarf mice, Cu/Zn SOD and catalase activities declined with age, and were higher than the corresponding normal values in young and middle-aged groups. Catalase levels also declined with age, but were similar to values in normal controls. In GH transgenic mice, age-associated decline of both catalase and Cu/Zn SOD occurred earlier than in normal animals. Catalase levels and activities in transgenic animals were similar to controls, whereas Cu/Zn SOD activity was higher in transgenics than in normal mice. The present results suggest that dwarf mice, during early life, have enhanced hypothalamic free radical defenses, which may contribute to their extended lifespan. However, from the present results in GH transgenic mice, it is impossible to conclude whether early decline of hypothalamic catalase and Cu/Zn SOD in these animals represents a correlate of accelerated aging, or contributes to their reduced lifespan. © 2000 Elsevier Science Inc.

Keywords—Hypothalamus, Superoxide Dismutase, Catalase, Hormones, Aging, Free Radicals

INTRODUCTION

Despite the essential nature of oxygen for life in aerobic organisms, free radicals of oxygen, formed as a normal product of cellular metabolism, are known to cause widespread damage to macromolecules. The mitochondria represent the largest free radical producers. However, due to the high efficiency of mitochondrial cytochrome *c* oxidase, which directly reduces ground state oxygen to water, free radical production is limited to an estimated 2–3% of total oxygen consumed [1]. Nevertheless, a small fraction of oxygen is reduced by single electron transfer to generate reactive oxygen species (ROS), which when unchecked, have nearly unlimited deleterious potential to the cell, and thus, the entire organism. The potent destructive power of ROS necessitates their rapid and efficient removal in order to prevent extensive damage to macromolecules, and is accomplished through two general defense systems: small antioxidant molecules and the antioxidant enzymes (AOEs). These systems act in concert to minimize the damage done by ROS within the cell, however, the AOEs represent the primary line of defense.

The initial reaction in free radical scavenging involves superoxide dismutase (superoxide: superoxide oxidoreductase, EC 1.15.1.1), which catalyzes the dismutation of two molecules of superoxide radical anion into hydrogen peroxide and diatomic oxygen. The high catalytic efficiency of this enzyme allows for the instant removal of most of the radicals generated in the cell and limits the evolution of more highly reactive ROS. The hydrogen peroxide formed from this initial reaction, however, may generate potentially lethal radicals unless it is also efficiently removed. Two enzymes are used to remove this radical species, catalase and the peroxidases. These enzymes catalyze the conversion of hydrogen peroxide into water thus completing the reduction of oxygen to water. Catalase (hydrogen peroxide: hydrogen peroxide oxidoreductase, EC 1.11.1.6), found mainly in the

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peroxisomes, is the most effective AOE in H_2O_2 removal. Peroxidases work in tandem with catalase to additionally scavenge endogenous peroxides including H_2O_2, lipid peroxides, and other free radicals.

Given the potential for ROS to cause substantial insults to biological systems including inactivation of proteins, lipid peroxidation, and alterations in gene expression, most researchers agree that they have a central role in the aging process [2]. Numerous studies have demonstrated the increasing imbalance between pro-oxidants and antioxidant defenses with increasing age [3–6]; however, there is a great deal of controversy as to whether declines in these defenses parallel an increase in oxidative damage [7–11]. In addition, the small body of research that establishes a direct causal relationship between oxidative stress and aging adds support to the free radical theory of aging [12–14].

The accumulating evidence of a central role for free radicals in aging and the attenuation of age-associated decline by increased efficacy of AOEs has led many to question whether the body has systemic control over these enzymes, and perhaps, the aging process. One possible modulator of AOE activity is the brain, which consumes ~20% of total oxygen intake, but has the lowest level of protection from ROS, relatively high concentrations of easily oxidizable unsaturated fatty acids, and limited regeneration potential [15]. Recently, Mo et al. [16] has shown that in the mouse brain, declines in AOEs do correlate with increased oxidative damage. One specific brain region, which may mediate age-associated decline of physiological function and thus represent a "control center" for aging, is the hypothalamus. As the primary link between the neural and endocrine systems, the hypothalamus controls many of the homeostatic and developmental mechanisms throughout the body. Evidence suggests that age-related changes in the hypothalamus may be responsible for the concomitant alterations of biological rhythms. The activity of neurons in the suprachiasmatic nucleus has been reported to have the capacity to terminate reproductive capability, diminish endocrine hormone release, and perhaps initiate the shift to a senescent state [17]. Because of its control over most all systems of the body, several groups have suggested that the hypothalamus is critical in regulating aging processes [18,19].

The evidence of hormonal regulation of AOEs is growing rapidly. This is partially based on the predominant phenomenon of decreased endocrine function with a parallel decrease in AOEs as a function of age. Thyroid hormones were among the first to be linked to the aging process. It has been demonstrated that in rats rendered moderately hypothyroid, aging is retarded and mean lifespan is extended [20]. In contrast, lifelong hyperthyroidism accelerates aging in young and middle-aged rats and significantly reduces lifespan [21]. Similarly, hyperthyroidism enhances lipid peroxidation while hypothyroidism diminishes lipid peroxidation [22]. A correlation between overexpression of GH in transgenic mice and reduced lifespan has also been demonstrated [23]. In addition, a link between oxidative stress-induced aging and endocrine function has been proposed from evidence that GH and PRL levels positively correlate with mammary Cu/Zn SOD activity, and TSH levels with hepatic Cu/Zn SOD activity [24]. This suggests that as an animal ages, decreased endocrine function may be responsible for the concomitant decrease on AOE activity, thus increasing the rate of accumulation of oxidative damage. Although GH administration to mice has been shown to elevate catalase and glutathione peroxidase (GPX) activities, chronically high levels of GH, such as those exhibited by GH transgenic mice, are associated with increased age-dependent pathologies, generalized decreases in AOE activities, and decreased lifespan [25–27]. A common feature of both GH and TSH are their metabolism inducing actions, which increase oxidative processes. PRL has also been shown to affect AOE status. Administration of PRL completely reversed the sharp decline in Cu/Zn and Mn SOD mRNA in the rat corpora lutea following hypophysectomy [28]. During the aging process, previous researchers have generally seen a decline in AOE activity in most brain regions; however, in aging rats the activity of SOD in the hypothalamus is reported to be unchanged [29]. Given that PRL has the potential to increase the expression of SOD, the age-associated increase in PRL in certain rat strains may be responsible for the maintenance of hypothalamic SOD during aging. To attempt to further clarify the endocrine role in free radical processes, Ames dwarf mice, which are PRL, TSH, and GH deficient and live significantly longer than their genetically normal siblings have been utilized [30–32]. The findings demonstrated that a deficiency in these hormones correlates with a significant elevation of catalase activity in liver and kidney tissues, and reduced levels of lipid hydroperoxide [26,33].

Studies summarized here establish a solid basis for implicating endocrine decline in accelerated oxidative processes during aging. The objective of this research was to determine the extent to which differences in GH status associated with differences in longevity influence catalase and Cu/Zn SOD, two key AOEs in the hypothalamus. This was approached by using GH, PRL, and TSH deficient Ames dwarf mice and PEPCK-hGH transgenic mice that overexpress human GH, which has both PRL and GH activities [34–36]. GH transgenic mice have a significantly reduced lifespan, higher levels of renal and hepatic superoxide radical and lipid peroxidation, and elevated glial fibrilary acidic protein, an aging

Table 1. Lifespan of Ames Dwarf Mice, PEPCK-hGH Transgenic Mice, and the Corresponding Normal Animals From These Same Lines [32,37]

Mouse line	Mean lifespan (months)	10% Survival (months)	*p* value	Reference
Normal (non-inbred closed colony)	23.8 ± 1.8	30	—	[32]
Ames dwarf	35.4 ± 1.8	43	0.0001[a]	
Normal (C57Bl/6 X C3H)	22.2 ± 2.4	31	—	[37] and unpublished
PEPCK-hGH Tg	8.3 ± 0.6	12	0.0001[a]	observations[b]

[a] Compared to normal animals from the same line.
[b] These data are derived, in part, from records from our production colony and, therefore, may underestimate the mean lifespan of both normal and transgenic animals.

indicator in the brain, in areas including the hypothalamus [37–39]. The aim was to further elucidate the role of endocrine function in the aging process as it relates to antioxidant defense status and to test the hypothesis that the hypothalamus may have a central role in the onset of aging.

MATERIALS AND METHODS

Animals

Male PEPCK-hGH transgenic (Tg), Ames dwarf (df/df), and matched normal (N) mice were bred and maintained in the Southern Illinois University animal facility. They were maintained on a 12:12 light-dark cycle at 20–24°C and fed and watered ad libitum. Male mice of each genotype were selected by age corresponding to one of three groups: young (3–4 months old), middle-aged (9–10 months old), and old (19–23 months old). Each age group contained four groups of mice ($n = 6–13$/group): transgenic and normal mice from the PEPCK-hGH line, and dwarf and normal mice from the Ames dwarf line. Transgenic and normal mice from the PEPCK-hGH line were produced by mating C57Bl/6 transgenic males from each generation to C3H F_1 hybrid females. Dwarf and normal mice from the Ames dwarf line were produced from a noninbred closed colony unrelated to the transgenic background. Dwarf mice from this line have a significantly longer mean and maximum lifespan than their normal littermates [32]. In contrast, PEPCK-hGH transgenic mice have a significantly shorter lifespan compared to their normal littermates [37] and (unpublished observations) (Table 1). Due to the drastically reduced lifespan of the transgenic mice, only normal mice, from both lines, and dwarfs were available for analysis in the oldest age group.

Samples

Mice were killed by decapitation, the brain was rapidly removed from the skull and the hypothalamus was dissected out and stored at −60°C. Hypothalami were sonicated in ice cold buffer (1:5 w/v, 50 mM Tris-HCl plus 1% Triton X-100, pH 7.3) in two, 15 s bursts with cooling at 0°C between each burst. Samples were centrifuged at 13,000 × *g* for 25 min at 4°C, and the supernatant was collected for determination of catalase and Cu/Zn SOD status. Total hypothalamic protein was determined by colorimetric method using BCA protein assay reagent (Pierce, Rockford, IL, USA). In addition, trunk blood was collected in tubes containing EDTA for measurement of plasma levels of IGF-1. After collection, blood was centrifuged at 6000 × *g* for 15 min at 4°C, the plasma was collected and stored at −60°C.

Catalase and Cu/Zn SOD activity

Total Cu/Zn SOD activity was determined by adaptation of the method described by Sun et al. [40,41]. One arbitrary unit (au) of SOD activity was defined as the amount of hypothalamic protein causing 50% inhibition of the rate of nitroblue tetrazolium reduction. Catalytic activity of the samples was determined by comparison to a standard curve of commercial Cu/Zn SOD (Sigma, St. Louis, MO, USA).

Catalase activity was determined by UV method (Spectronic Genesys 5 spectrophotometer: Milton Roy, Rochester, NY, USA), as previously described [42]. One unit of the enzyme was defined as μmol H_2O_2 disproportionated/min/mg protein.

Western blot and/or slot blot

Catalase levels were quantified by Western blot and slot blot using a polyclonal antibody raised against human erythrocyte catalase in rabbit (Calbiochem, La Jolla, CA, USA) [43,44]. Slot blots were performed only after a test Western blot detected low to absent non-specific binding, which might be misinterpreted as catalase. Test blot results were compared against the slot blot for further assurance against nonspecific binding or sample deterioration. For Western blots, 50 μg total protein from

each sample was subjected to electrophoresis, in duplicate, on a 10% polyacrylamide gel. The separated gel proteins were then transferred to nitrocellulose membrane by electroblotting. For slot blots, 50 μg total protein was applied, in duplicate, directly to the nitrocellulose mounted in the slot blot apparatus (MilliBlot-S: Millipore, Bedford, MA, USA) under strong vacuum until nearly dry. The membrane was incubated in appropriately diluted antibody (1:2000) overnight at 4°C. After incubation, the nitrocellulose was washed 3× in Wash buffer (TBS plus 0.05% Tween-20) and incubated in secondary antibody (1:10,000) (goat antirabbit immunoglobin) conjugated with horseradish peroxidase for 2 h. The wash was repeated following secondary antibody incubation and the blots were developed on Kodak (Rochester, NY, USA) radiographic film by using an Amersham ECL kit to illuminate catalase bands (Amersham, Arlington Heights, IL, USA). Catalase was identified by comparison with molecular weight standard (Bio-Rad, Hercules, CA, USA) run under the same conditions and with human erythrocyte catalase standards (Sigma) in 0.5, 1.0, and 2.5 ng quantities. Quantification of catalase bands was performed by laser densitometry (Personal Densitometer SI: Molecular Dynamics, Sunnyvale, CA, USA). Amount of enzyme was calculated by linear regression to the standard curve.

IGF-1 measurement

The function of the GH−IGF-1 axis was assessed by quantifying plasma levels of IGF-1, the mediator of GH actions. These levels were measured by a commercial rat IGF-1 ELISA kit (Diagnostic Systems Laboratories Inc., Webster, TX, USA).

Statistical analysis

Data from each experiment were compiled and analyzed by ANOVA. For statistical analysis of IGF-1 levels, the data were log transformed before ANOVA analysis, due to lack of homogeneity of variance among groups. Fisher's protected least significant difference post hoc test was used to determine specific differences between means. $p < .05$ was considered significant, and data are presented as mean ± SEM.

RESULTS

IGF-1 concentration

Plasma levels of IGF-1 in both age groups of PEPCK-hGH transgenic mice were significantly higher than in all other groups (Fig. 1). In contrast, Ames dwarf mice had significantly lower levels of IGF-1 than other groups. No significant age-associated differences were detected.

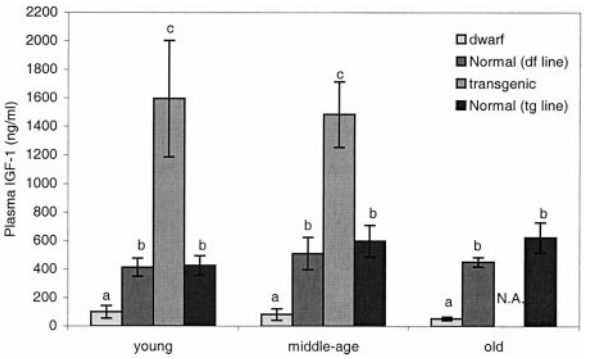

Fig. 1. Plasma IGF-1 in aging male PEPCK-hGH transgenic, Ames dwarf, and matched normal mice [from both Ames (df) and Transgenic (Tg) lines]. IGF-1 levels were measured by commercial ELISA kit (DSL Inc., Webster, TX, USA). Data reported are mean ± SEM. a, b, and c = Mean activities that do not share a common superscript are significantly different ($p < .05$). N.A. = Due to the reduced lifespan of transgenic mice, no animals were available for examination in the old age group.

Cu/Zn superoxide dismutase

In general, Cu/Zn SOD activity declined with age, however these declines occurred at different chronological ages and were not always significant despite numerically large percent differences between age groups. In Ames dwarf mice, a significant age effect was seen (Fig. 2) with a significant reduction in Cu/Zn SOD activity in old as compared to young dwarf mice ($p < .0121$), and a 67% lifespan decline. In old normal mice, there was an apparent 38% decline in Cu/Zn SOD activity from values measured in young normal mice, but this difference was not statistically significant ($p < .1060$). In comparison to

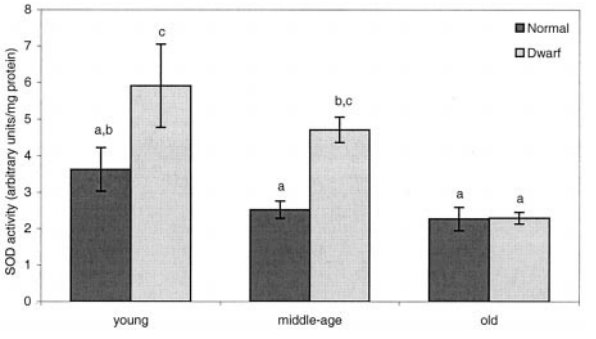

Fig. 2. Hypothalamic Cu/Zn SOD activity in aging male Ames dwarf and normal mice. Activity in hypothalamic homogenates was measured by spectrophotometry (λ = 560 nm) using xanthine/xanthine oxidase as a superoxide generator, as previously described [40,41]. Activities were determined by regression to a standard curve of known SOD activities. Data reported are mean ± SEM. a, b, and c = Mean activities that do not share a common superscript are significantly different ($p < .05$).

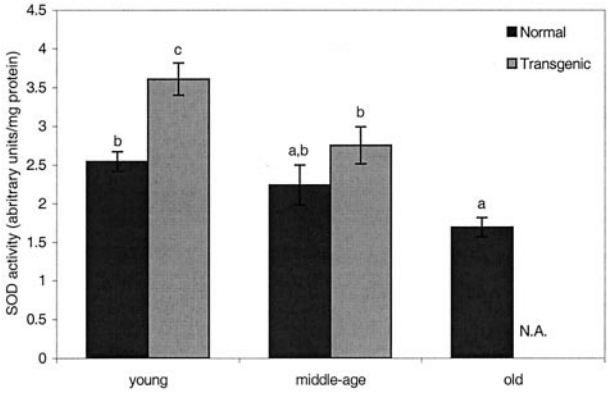

Fig. 3. Hypothalamic Cu/Zn SOD activity in aging male PEPCK-hGH transgenic and normal mice. Data reported are mean ± SEM. a, b, and c = Mean activities that do not share a common superscript are significantly different ($p < .05$). N.A. = Due to the reduced lifespan of transgenic mice, no animals were available for examination in the old age group.

normal animals, dwarf mice had significantly elevated Cu/Zn SOD activity in both young ($p < .0084$) and middle-aged ($p < .0220$) groups. However, in old mice, Cu/Zn SOD activity was essentially identical between genotypes.

Transgenic mice, as expected, did not live long enough to be included in the old age group. Cu/Zn SOD activity in transgenics declined significantly from young to middle-age ($p < .0178$), while significant declines in normal mice were not seen until old age ($p < .0047$) (Fig. 3).

Comparisons between age groups are complicated by the reduced lifespan of the PEPCK-hGH transgenic animals. If the percent of relative mean lifespan rather than chronological age is considered, 4 month old transgenics and 9 month old normals have lived roughly 33% of their typical lifespan, and thus may be considered comparable in terms of "biological age". Similarly, nine month old transgenics and nineteen month old normals may also be matched for comparison since each have lived roughly 75% of their relative lifespan. Cu/Zn SOD activity was elevated in transgenics versus controls using either age criteria, but these increases were most pronounced when the groups were matched on the basis of percent lifespan. Cu/Zn SOD activity was elevated in short-living transgenics, relative to normal levels, in a manner similar to that seen in the long-living Ames dwarfs. These elevations were significant in both 33% lifespan ($p < .0001$) and 75% lifespan ($p < .0029$) comparisons.

Catalase

Age-associated declines were generally similar to those found in Cu/Zn SOD activity. In the dwarf line, young dwarf mice had numerically 34% higher ($p <$ .0900) catalase than normals (Fig. 4A). Although these differences were not significant, dwarf mice had delayed loss of catalase activity such that significant elevations ($p < .0185$) were present at middle age. This, however, did not continue into old age. After reaching middle age, normal mice showed no further loss of catalase activity (34% overall). In contrast, dwarf mice experienced a 33% decline in catalase activity from middle to old age and a 41% lifespan decline ($p < .0097$). Interestingly, though the age-associated declines in catalase activity were similar between dwarf and normal mice, the lifespan decline in normal mice was not significant ($p <$ .0898).

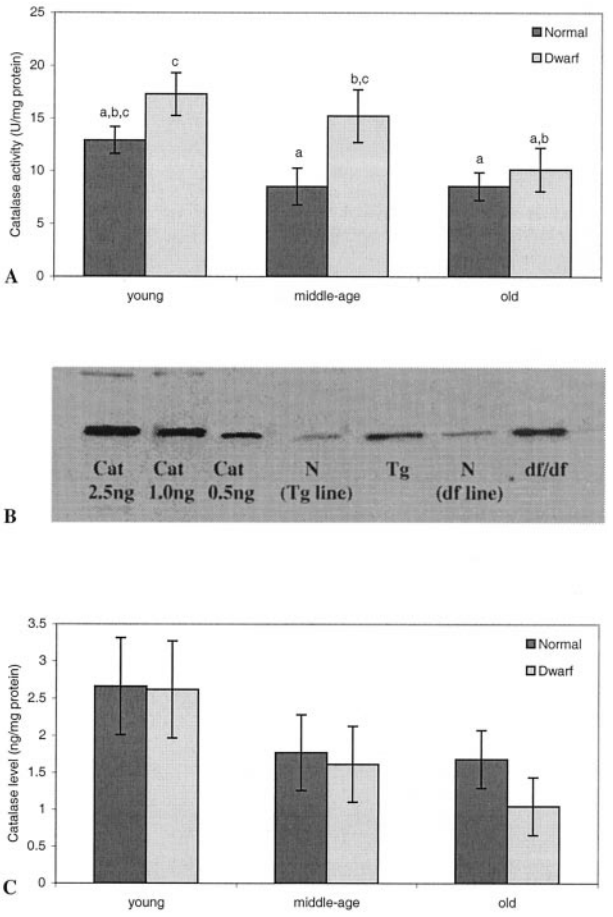

Fig. 4. Hypothalamic catalase activity and level in aging Ames dwarf and control male mice. Data reported are mean ± SEM. (A) Catalase activity was measured by spectrophotometry ($\lambda = 240$ nm) by following the decrease in absorbance of H_2O_2 over time, as previously described [42]. a, b, and c = Mean activities that do not share a common superscript are significantly different ($p < .05$). (B) Western blot of transgenic (Tg), dwarf (df/df) and matched normal (N) mice. Of note are the increasing optical densities of the catalase (Cat, in ng) standard curve, the absence of nonspecific binding in the sample lanes, and the considerable antibody cross-reactivity. (C) Catalase level was measured by Western/Slot blot, as previously described [43,44].

Catalase level, in order to minimize assay variations, was quantified by slot blot after a representative Western

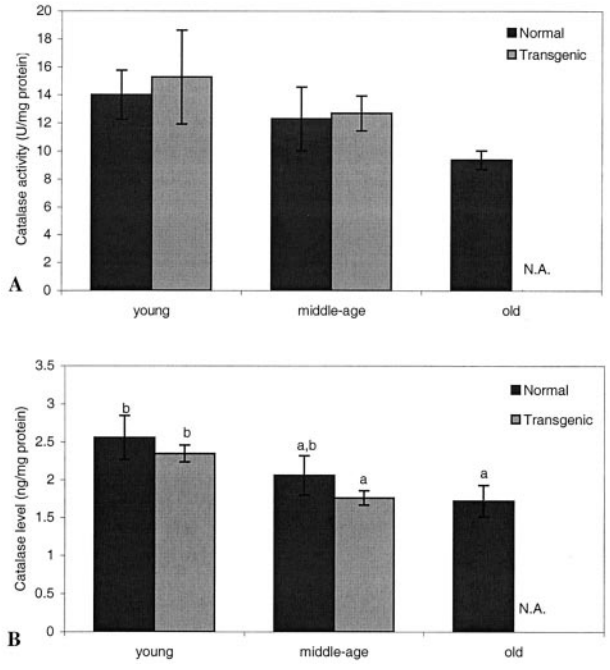

Fig. 5. Catalase activity (A) and level (B) in aging male PEPCK-hGH transgenic and normal mice. Data are reported as mean ± SEM. a and b = Mean activities that do not share a common superscript are significantly different ($p < .065$). N.A. = Due to the reduced lifespan of transgenic mice, no animals were available for examination in the old age group.

blot. Figure 4B represents a combined Western blot of hypothalamic catalase, and illustrates typical results. In dwarf mice, catalase level followed an age-associated decline, similar to catalase activity. However, catalase levels in dwarf mice were not different from normal mice at any age (Fig. 4C). Despite the lack of overall significance, an age effect was suggested by the numerical 60 and 37% age-associated loss of enzyme in dwarf and normal mice, respectively. Correlation between activity and amount of enzyme decreased greatly with age in this line. In young mice, a relationship between catalase activity and level appeared to exist, with a correlation coefficient value of $r = .6098$. However, by middle age this value declined to $r = .2688$ indicating that perhaps activity did not depend strongly on the amount of enzyme. This was further indicated by a decrease in correlation to $r = .1647$ in old mice.

Data obtained in PEPCK-hGH transgenic mice do not support the hypothesis that catalase is affected by hGH expression (Fig. 5A). In contrast to the Cu/Zn SOD data, ANOVA did not detect differences in catalase activity (overall: $p < .3255$). However, transgenic mice tended to have higher activity than controls when compared by percent lifespan. One such trend was that catalase activity in transgenics was numerically higher than that of percent lifespan matched controls in both 33 and 75% of the relative lifespan.

Catalase level in transgenic mice, similarly to catalase activity, was not different from matched normals, demonstrating that hGH expression had little effect on catalase level (Fig. 5B). However, a significant age effect was seen in both genotypes. Old normal mice had significantly lower ($p < .0086$) catalase levels than young normal mice. Matching by percent lifespan, transgenic mice had levels that were nearly identical to the controls. Moreover, the early loss of catalase in transgenic mice was similar to that of Cu/Zn SOD activity. Catalase level in transgenic mice apparently declined 25% from young to middle age ($p < .0650$). The correlation between activity and amount of enzyme, despite the lack of significant differences from normal mice, was higher in transgenics. Correlation values for transgenics were $r = .6502$ and $r = .5966$ in young and middle-aged animals, while the corresponding values in normals were $r = .4854$ and $r = .2621$ respectively. Age-associated decline in level-activity correlation was also seen in this line. The values were highest among young mice $r = .5557$, then declined sharply to $r = .2679$ in middle-aged mice, and again in old mice to $r = .0500$.

DISCUSSION

Evidence of progressive age-associated declines in hypothalamic catalase and Cu/Zn SOD was clearly seen in this study. These declines were evident in both lines examined, however PEPCK-hGH transgenic mice lost the activity of these free radical scavenging enzymes at an earlier age than either normal, of both lines, or Ames dwarf mice. One explanation for these declines, and for the more rapid changes seen in hGH transgenic mice, is increased accumulation of free radical damage to macromolecules; particularly those involved in free radical defense systems. It is reasonable to assume that the major age-associated declines in antioxidant defenses occurring in this critical neuroendocrine structure may have consequences on endocrine function and longevity.

Of further interest, in terms of endocrine modulation of aging, was the apparent relationship between pituitary hormone expression and catalase and Cu/Zn SOD status. The models used in this study were selected because of their differing levels of expression of several pituitary hormones and because they had either delayed or accelerated aging. Measurement of plasma of IGF-1, the principal mediator of GH action, provided an easy estimation of the GH status, because IGF-1 is released under the direct influence of GH. While these data did not provide novel information, they formed a basis for attributing the changes in catalase and Cu/Zn SOD activity and/or level, to the function of the GH−IGF-1 axis. IGF-1 levels in transgenic mice were expectedly higher than all other groups, given the high level of expression of the hGH

transgene. In contrast, Ames dwarf mice, which are deficient in GH, in addition to TSH and PRL, and have increased lifespan, had expectedly lower levels of IGF-1 than all other groups. Results of the present study, show that hypothalamic Cu/Zn SOD activity was elevated in short-living transgenics and in long-living dwarfs. Thus, our results would suggest that while hormones, specifically GH, may regulate AOEs, Cu/Zn SOD activity in the hypothalamus may not be the primary determinant of lifespan.

AOE-specific genes are activated by a variety of endocrine hormones, which has led to the suggestion that humoral factors have a role in the systemic control of aging [45–48]. Given the PRL-like action of hGH in PEPCK-hGH transgenic mice, the present results may support conclusions that PRL induces SOD [28]. In transgenic mice expressing bovine growth hormone (bGH), which does not possess PRL activity, lipid peroxidation (LP) levels in the kidney are higher than in normal mice, whereas in hGH transgenic mice LP levels are similar to normals (J. Carlson and A. Bartke, unpublished observations). GH-receptor-mediated signaling also appears to stimulate AOE activity [49]. Mice administered hGH showed significant increases in glutathione and catalase activity [25]; however, we did not detect elevated catalase activity in hGH transgenic mice. Additional investigation of this possible mechanism is warranted.

The question as to why GH transgenic mice age faster despite increases in free radical defense systems remains unanswered. One possible explanation for their accelerated aging lies in the typical causes of death in these mice. Autopsy data indicate that the primary cause of death is due to kidney pathologies including glomerular sclerosis, early development of kidney lesions, and eventual renal failure [50,51]. It has also been reported that liver and kidney catalase levels in bGH transgenic mice are reduced [52]. Thus, elevated free radical processes may in part explain the causes of advanced aging in GH transgenic mice. Results reported here however, do not support the role of oxidative processes and defenses in the hypothalamus as the determinant of aging. Rather, they suggest that aging is more associated with critical free radical damage to one or more "weak links", of which the kidney may be one. Chronic elevations of GH in the kidney causing pathological alterations, combined with reduced AOE defenses and higher ROS production, lead to early death, despite elevations of these defenses in tissues such as the hypothalamus, which might otherwise extend lifespan.

Ames dwarf mice, in contrast to the GH transgenics, have lower levels of inorganic peroxides and elevated catalase activity in the liver and kidney [26,33]. Increases in hypothalamic Cu/Zn SOD and catalase activities detected in dwarf mice in the present study are consistent with similar results of elevated AOEs in other organs of Ames dwarf mice, and with the suspected relationship of delayed aging in these mice to reduced free radical damage. Elevated free radical scavenging early in life would slow the accumulation of oxidant damage and extend life. While the differences in catalase and Cu/Zn SOD activities do not necessarily represent a causal mechanism for delayed aging in dwarf mice, the presumed decrease in free radical damage from increased AOE defenses represents one plausible explanation for their increased lifespan. Undoubtedly, other mechanisms are also involved. These may include decreased glucose and insulin levels in these animals as well as the consequences of thyroid and growth hormone deficiencies [53]. Both of these hormones have profound effects on metabolic rate. Ames dwarf mice have significantly reduced core body temperature [54], and presumably decreased metabolic rate as observed in the endocrinologically similar Snell dwarf mice [55]. Given that the production of ROS is directly related to metabolism, increased longevity of dwarf mice may perhaps be more closely associated with producing fewer free radicals, rather than having greater protection against them. All of these possible mechanisms however assume that some factor secondary to endocrine alterations modulates the aging process, which indirectly supports a central role for free radicals. Early enhanced protection against free radicals, reported here as increases in catalase and Cu/Zn SOD activities may extend lifespan by limiting accumulation of free radical-mediated tissue damage. The greater than 50% lifespan decline in Cu/Zn SOD and catalase activities in dwarf mice, versus more moderate declines detected in normal mice may be of less importance, given that dwarfs have elevated hypothalamic free radical defenses during early and middle age. Together with similar evidence of increased AOE activity in various tissues, these data support the possible role of attenuated free radical–induced damage as a putative mechanism for delayed aging in Ames dwarf mice.

In conclusion, the data have not provided support for oxidative processes in the hypothalamus having a primary role in aging of mice with reduced lifespan. However, the present findings do show consistent age-associated declines in free radical defenses systems in the hypothalamus, which would perhaps allow substantial oxidative damage to occur in this critical neuroendocrine structure. A critical question yet to be answered, and a point of further investigation, is whether the declines reported here result in concomitant elevations in oxidative damage in the brain. ROS damage to the hypothalamus from decreased catalase and Cu/Zn SOD may be manifested as systemic functional declines through depressed release of pituitary stimulating factors. Thus, the

hypothalamus may represent one of many "aging centers". The regulation of free radicals, and the aging process, may be more closely related to changes in the rate of ROS production [56]. Data presented here demonstrating elevations in Cu/Zn SOD activity in GH transgenic mice, suggest that even grossly elevated GH can have a beneficial effect on enhancing free radical defense systems. Further examination of the mechanisms of increased longevity in dwarf mice, which display a variety of characteristics that could potentially contribute to long life, including elevations of hypothalamic Cu/Zn SOD and catalase activities as reported here, deserves additional attention.

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