Endocrine, *vol. 11, no. 1, 41–48, August 1999* 0969–711X/99/8:41–48/$12.00

Antioxidative Mechanisms and Plasma Growth Hormone Levels

Potential Relationship in the Aging Process

Holly M. Brown-Borg,[1] Ann M. Bode,[2] and Andrzej Bartke[3]

[1]*Department of Physiology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND;*
[2]*Department of Exercise and Movement Science, University of Oregon, Eugene, OR;*
and [3]*Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL*

Factors affecting longevity are complex and poorly understood. We have recently found that Ames dwarf mice (*df/df*), which are deficient in growth hormone (GH), prolactin, and thyroid-stimulating hormone, live significantly longer than their normal siblings whereas transgenic mice that overexpress GH exhibit reduced life-spans and various indices of premature aging. The production of reactive oxygen species increases with aging and is associated with DNA damage to the tissues. However, several cellular oxygen scavenging/detoxifying systems exist that improve the antioxidative defense capacity of cells. We evaluated the activity of enzymes involved in this defense system in liver, kidney, and heart tissue from dwarf, phosphoenolpyruvate carboxykinase–bovine GH transgenic, and corresponding groups of normal mice. Liver glutathione and ascorbate levels were lower ($p < 0.0025$) in dwarf animals compared to normal and GH transgenic mice. By contrast, the level of catalase activity, which detoxifies hydrogen peroxide, in dwarf liver and kidney was significantly higher when compared to the other groups. Animals deficient in GH (dwarf) live longer and exhibit enzyme activities and levels that may combat oxidative stress more efficiently than normal mice and those overexpressing GH.

Key Words: Dwarf; delayed aging; antioxidative mechanisms; hormones.

Introduction

Once the reproductive stage of life has ended, most multicellular organisms exhibit a gradual decline in a variety of physiological processes *(1)*. The average life-span of individuals belonging to the same species depends on a host of genetic and environmental influences and on complex and poorly understood interactions among these factors *(2,3)*. Studies of aging in the human suggest that maximal life-span may represent a relatively invariable genetically determined species characteristic and that environmental factors (e.g., improved sanitation, nutrition, and health care) can increase the proportion of individuals reaching or approaching the limit of longevity but cannot alter this limit *(2,3)*. Studies in laboratory populations of rats and mice indicate that the life-spans of these animals can be extended by reducing their daily food consumption, the so-called caloric restriction effect *(4,5)*. Caloric or dietary restriction (CR) has been shown to modulate most aspects of free-radical metabolism and thereby reduces overall oxidative stress *(6)*.

We recently reported *(7)* that genetically dwarf mice (Ames dwarf, *df*/*df*) characterized by diminutive body size and delayed puberty (owing to deficiencies in anterior pituitary function) live much longer than normal animals from the same strain when maintained under standard laboratory conditions (ad libitum food and water) and, indeed, can outlive their normal counterparts by more than 50%. We suspect that GH deficiency is particularly relevant to the extended survival of Ames dwarf mice because overexpression of GH in transgenic mice is associated with reduced life-span (PEPCK.bGH, 12 mo; *[8,9]*). Importantly, GH overexpression is also associated with increased indices of free radical processes *(10)*.

Thus, the increased longevity of Ames dwarf mice may be linked to their reduced body size: the underlying endocrine defects, in particular GH deficiency; and potential differences in free-radical indices and oxidative damage. To investigate the role of plasma GH status in oxidative metabolism, we evaluated the oxidative scavenging/detoxifying capacity of tissues from mice exhibiting GH deficiency and GH overexpression.

Received March 15, 1999; Revised June 1, 1999; Accepted June 11, 1999.
Author to whom all correspondence and reprint requests should be addressed: Holly M. Brown-Borg, Department of Physiology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58203-9037. E-mail: brownbrg@medicine.nodak.edu

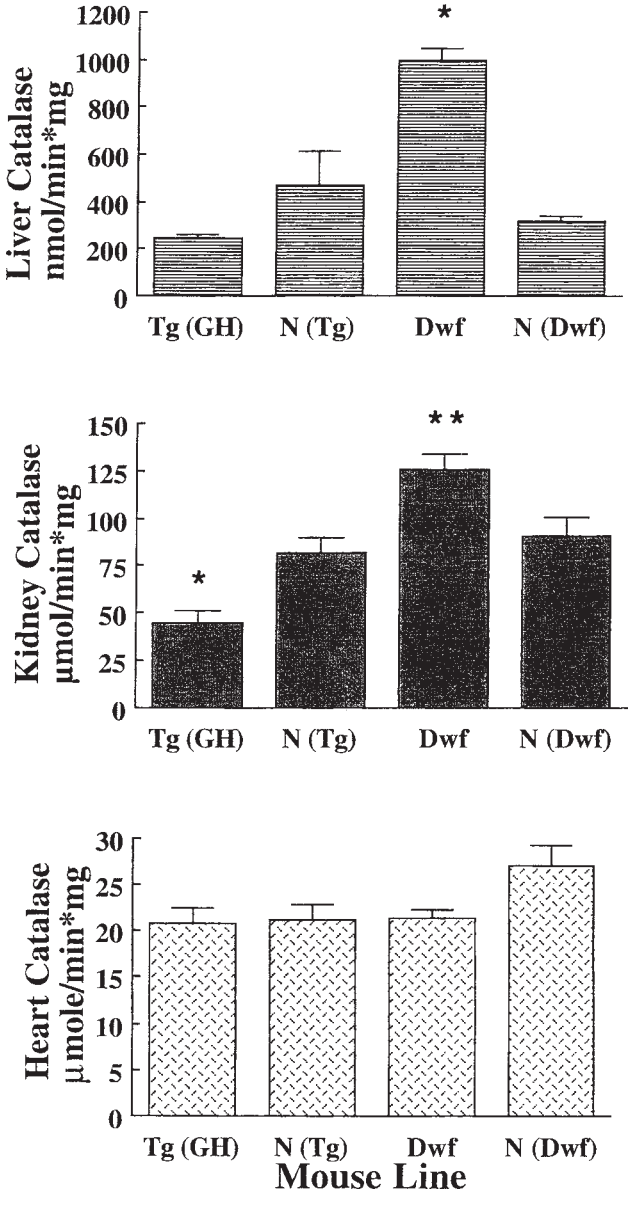

Fig. 1. Catalase activity in liver, kidney, and heart tissues from Ames dwarf (Dwf), GH transgenic (Tg), and corresponding groups of normal mice (N [Dwf] and N [Tg], respectively). Bars represent means ± SEM. *$p \leq 0.001$; **$p \geq 0.05$.

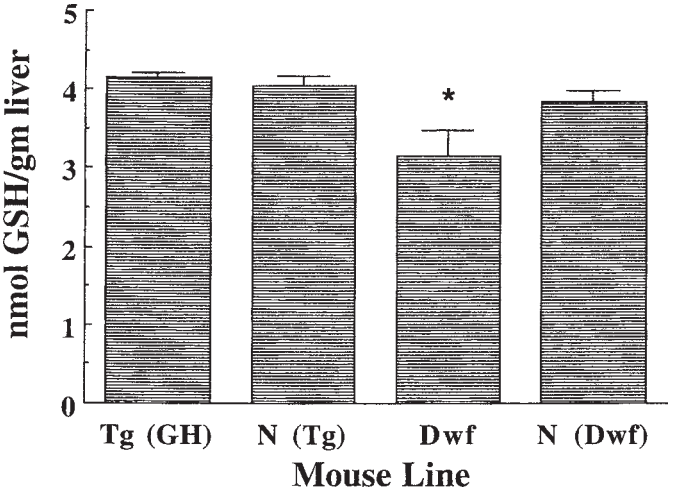

Fig. 2. Liver glutathione levels in dwarf (Dwf), GH transgenic (Tg), and corresponding groups of normal mice (N [Dwf] and N [Tg], respectively). Bars represent means ± SEM. *$p \leq 0.0025$.

Results

The level of catalase activity (Fig. 1) in dwarf liver and kidney was significantly higher ($p \leq 0.001$) when compared to the other groups. GH transgenic animals had reduced levels of catalase activity in kidney tissue ($p \leq 0.05$) compared to corresponding normal animals (Fig. 1). The level of catalase activity in heart tissue was much lower than that detected in kidney in each group of mice examined. However, no significant differences in heart catalase activity were detected in dwarf, GH transgenic, and corresponding groups of normal animals (Fig. 1).

Liver glutathione (GSH) concentrations (Fig. 2) were lower ($p \leq 0.0025$) in dwarf animals compared to normal siblings and GH transgenic mice. Enzymes involved in the generation and recycling of GSH were also evaluated. Normal mice from each line exhibited higher liver glutathione peroxidase (GPX) activity compared to both dwarf and GH transgenic mice ($p \leq 0.001$; Fig. 3). However, in both kidney and heart tissue, GPX activities were lower in the dwarf line compared to the transgenic line of mice ($p \leq 0.002$; Fig. 3). Glutathione reductase (GR) activity in the heart was significantly elevated in dwarf mice compared to normal animals, whereas the kidneys of mice from the dwarf line exhibited higher GR when compared to the transgenic line ($p < 0.05$; Fig. 4). No significant differences in GR activity between dwarfs and transgenics and their respective normal control animals were detected in the liver (Fig. 4). The level of glucose-6-phosphate dehydrogenase (G6PDH) activity tended to be higher in the hearts of longer-living dwarf mice when compared to both normal animals from the same line and animals from the transgenic line (Fig. 5). Significant differences in this enzyme were not detected in either liver or kidney tissues of any line.

The concentration of the antioxidant ascorbic acid in liver tissues was significantly decreased in GH-deficient mice compared to normal controls (Fig. 6). By comparison, mice expressing the GH transgene had elevated ($p \leq 0.0003$) liver ascorbic acid concentrations. Dehydroascorbate reductase activity (DHAAR) was elevated in heart tissue of GH transgenic animals compared to normal animals from the same strain but did not differ between dwarfs and normal animals (df strain), nor were differences observed in liver and kidney tissues (Fig. 7).

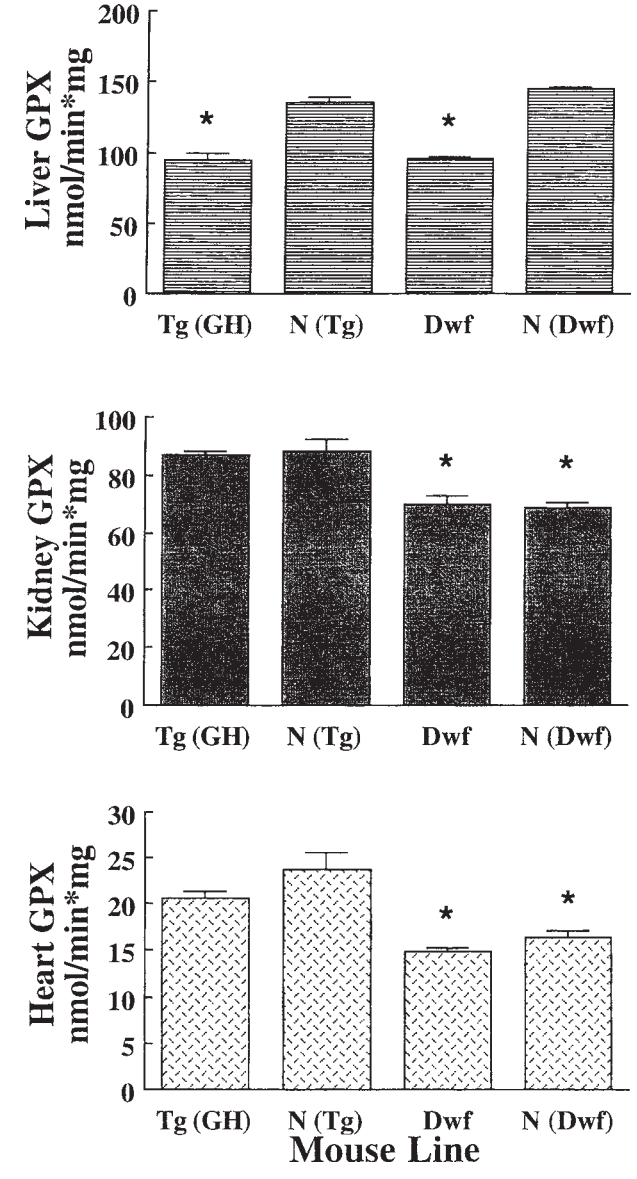

Fig. 3. GPX activity in liver, kidney, and heart tissues from Ames dwarf (Dwf), GH transgenic (Tg), and corresponding groups of normal mice (N [Dwf] and N [Tg], respectively). Bars represent means ± SEM. *p ± 0.002.

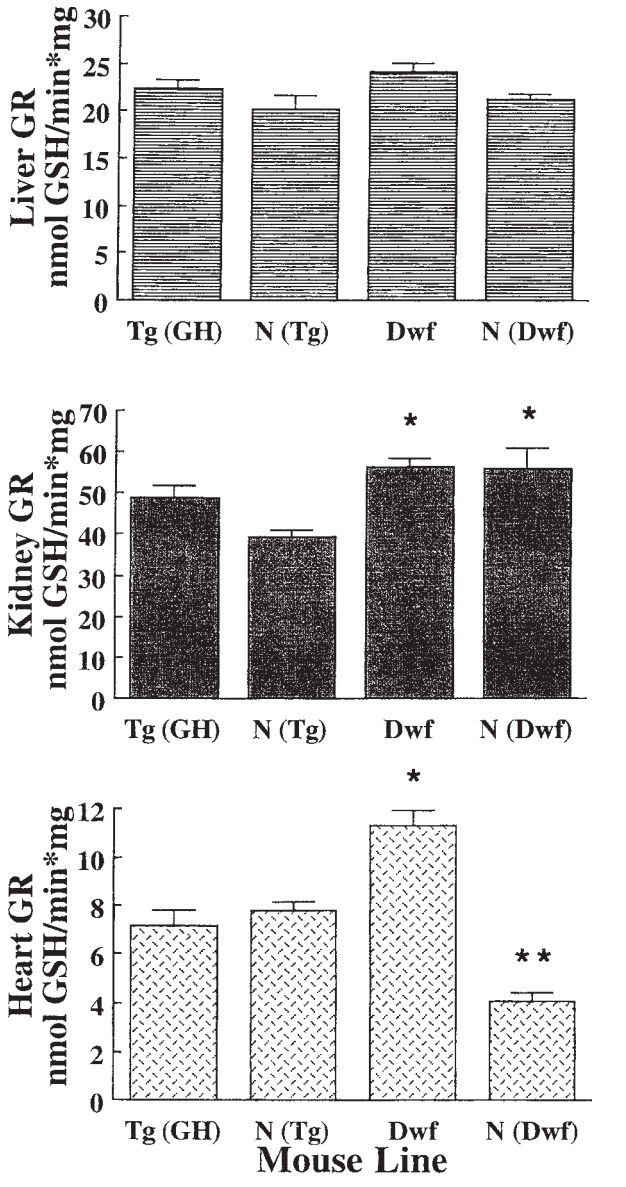

Fig. 4. Glutathione reductase (GR) activity in liver, kidney and heart tissues from Ames dwarf (Dwf), GH transgenic (Tg) and corresponding groups of normal mice (N [Dwf] and N [Tg], respectively). Bars represent means ± SEM. *$p \leq 0.05$; **$p < 0.001$ – N (Dwf) compared to dwarf mice.

Discussion

A progressive decline in physiological systems occurs in all organisms. One explanation for this decline involves oxidative stress and subsequent oxidative damage to DNA, proteins, and lipids. Most important, relatively longer life expectancy is associated with a lower accrual of oxidative damage *(11–13)*. Two major endogenous mechanisms appear to be important in reducing the overall detrimental effects of oxidants on cells and in the maintenance of homeostasis. Antioxidants and enzymes are the key defense systems that control the levels of reactive oxygen species (ROS) that cause oxidative damage to lipids, proteins, and DNA.

In this study, we evaluated the antioxidant defense capacity of the main organs involved in the detoxifying processes, liver and kidney, and heart tissues in animals with different life-spans and associated GH status. We found that the Ames dwarf mouse, which lives 50–65% longer than normal counterparts, exhibits higher levels of catalase (an enzyme that detoxifies H_2O_2) activity in both liver and kidney tissue. In conjunction with higher catalase activity, we have previously shown that hepatic levels of inorganic peroxides (a measure of H_2O_2) are reduced in the dwarf *(14)*. Catalase's activity and mRNA decrease with age whereas its activity increases following CR *(15–18)*.

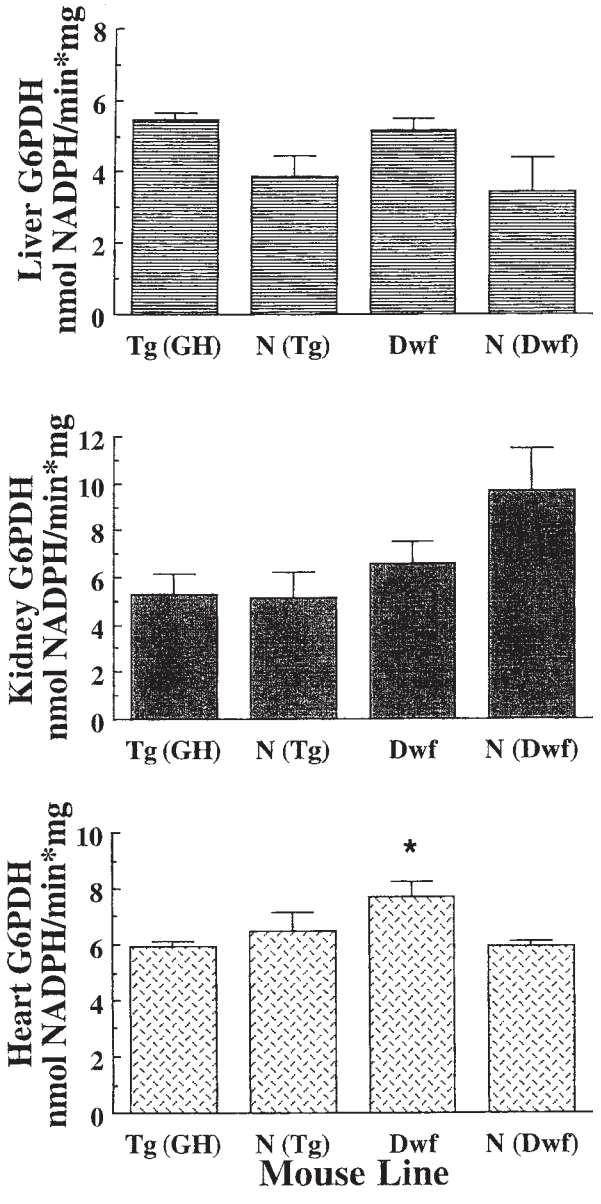

Fig. 5. Glucose-6-phosphate dehydrogenase (G6PDH) activity in liver, kidney and heart tissues from Ames dwarf (Dwf), GH transgenic (Tg) and corresponding groups of normal mice (N [Dwf] and N [Tg], respectively). Bars represent means ± SEM. $*p = 0.06$.

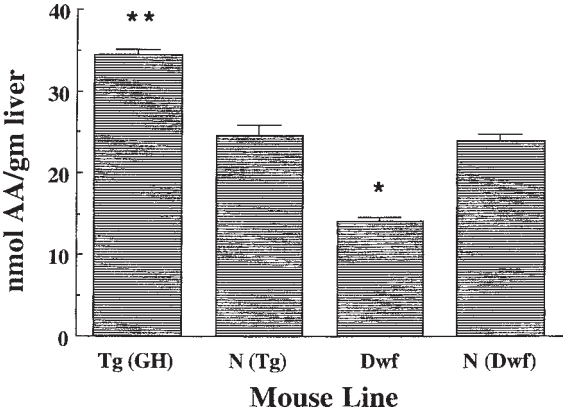

Fig. 6. Ascorbic acid (AA) concentrations in liver tissue from Ames dwarf (Dwf), GH transgenic (Tg) and corresponding groups of normal mice (N [Dwf] and N [Tg], respectively). Bars represent means ± SEM. $*p < 0.0025$; $**p < 0.0003$.

When catalase and another detoxification enzyme, superoxide dismutase (SOD), are overexpressed in *Drosophila melanogaster*, a significant extension of life-span is observed *(19)*. Our laboratory has preliminary evidence suggesting that SOD activity is also higher in dwarf liver tissues compared to normal age-matched control mice (Brown-Borg, H. M., unpublished observations). The increased activity of catalase in liver and kidney tissues of dwarf mice may represent a defense system that has been upregulated via the absence of GH thus resulting in an extended life-span. Oxidative damage may be greater in tissues that are vulnerable to oxidative stress owing to their metabolic activity (i.e., generate more ROS). This may explain the lack of differences in heart tissue catalase activity when compared to metabolically active tissues such as liver and kidney. The antioxidative defense systems in these tissues rely on repair processes to maintain efficient functioning. GH may impair repair processes, or detoxification/elimination processes leading to greater oxidative insults in these tissues and decreased catalase activity. In addition, GH may be involved in the upregulation of ROS-generating processes, again leading to increased oxidative stress in certain tissues.

In this study, we found reduced catalase activity in mice with elevated plasma GH. Additional evidence supporting the notion that GH is involved in modulating oxidative metabolism stems from work by Rollo and coworkers *(10)*, showing that the levels of superoxide radical and lipid peroxidation are elevated in rodents overexpressing GH. Therefore, GH may downregulate catalase directly or augment ROS production, leading to an exhaustion of this enzyme in animals with elevated plasma GH. The Ames dwarf lacks GH, and thus catalase levels are elevated, leading to greater protection. Lower levels of nonenzymatic antioxidants (glutathione and ascorbic acid) in liver tissues from these mice suggest that catalase may play the major role in the elimination of ROS or that utilization of these antioxidants is greater in dwarf liver tissue compared to normal animals.

GPX, which is also involved in the elimination of peroxides *(20)*, is lower in dwarf liver compared to that of normal animals and in kidney and heart of animals from the dwarf line. The activity of GPX is coupled to GR, both of which act to maintain reduced GSH levels *(21)* and thus are of interest in models of aging. GR catalyzes the NADPH-dependent reduction of GSSG, thus providing essential GSH in vivo *(22)*. This enzyme is higher in the hearts of dwarf mice compared to normal animals. Since enzymatic

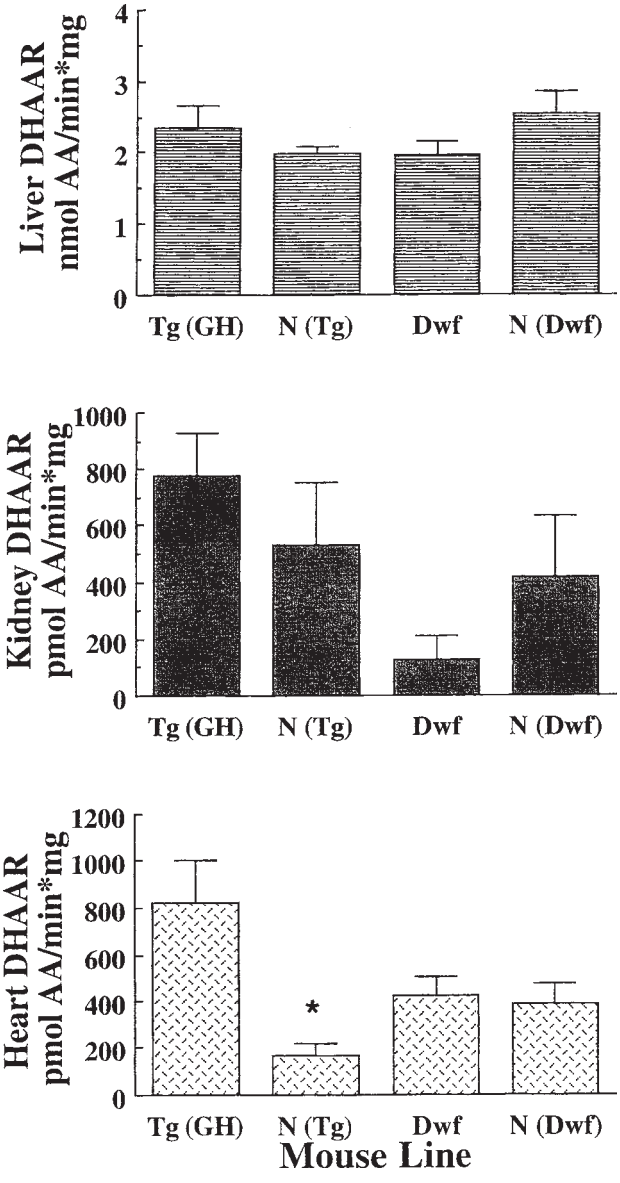

Fig. 7. Dehydroascorbate reductase (DHAAR) activity in liver, kidney and heart tissues from Ames dwarf (Dwf), GH transgenic (Tg) and corresponding groups of normal mice (N [Dwf] and N [Tg], respectively). Bars represent means ± SEM. *$p < 0.05$. AA, ascorbic acid.

reactions involving G6PDH provide reducing equivalents for reductive processes including the recycling of the antioxidants, GSH and ascorbic acid *(23)*, we also measured the activity of this enzyme. A slight increase in G6PDH was detected in dwarf heart but not the other tissues tested. Enzyme activity differs across tissues depending on the tissue function, metabolic activity, and amount of substrate available in a specific tissue. Activity could be decreased by negative feedback owing to surplus substrate or from modifications resulting from oxidative damage *(24)*. Muscle tissue exhibits marked changes in antioxidant enzyme activity in young vs old rodents *(25)*; thus, heart tissue collected from these mice may not show differences at this specific age but changes may be detected over time. Obviously, the balance between catalase and perhaps SOD (or another enzyme or antioxidant) may be the key determinant in the ability to combat oxidative stress and ultimately extend life-span. These possibilities are currently under study since these enzymes have been implicated in the aging process *(17,26)*.

The role of nonenzymatic antioxidative mechanisms in this model of delayed aging may also be influenced by GH status. GSH is oxidized to GSSG following the conversion of hydrogen peroxide to water via GPX. GSH and ascorbic acid concentrations are reduced in livers from the long-lived GH-deficient dwarf. The relevance of ascorbic acid in aging is controversial for two reasons. First, this vitamin is generated in vivo in rodents but not in humans, because ascorbate is provided by dietary supplementation. Second, ascorbic acid can also act as a potent prooxidant in conditions in which mineral status (iron storage disease; *[27]*) is altered and may actually stimulate pathogenesis of these types of diseases. Nevertheless, these antioxidants undergo redox reactions and thus are constantly recycling; therefore, reduced levels of these products indicate that the dwarf produces lower amounts of antioxidants, that these products are being rapidly recycled, or that utilization is greater. The level of inorganic peroxides is lower in dwarf liver compared to normal liver *(14)*, suggesting that synthesis may be responsible for the lower amounts of GSH and ascorbic acid found. Lower production of ROS and less oxidative damage leading to life extension is possible because metabolism may be reduced in these mice (Bartke, A., unpublished observations). In addition, core body temperature is 1.5°C lower in dwarf mice when compared to normal siblings *(28)*. Also, GSH levels have been found to be reduced in aging rodents *(29–31)* and humans *(32)*.

In contrast to the GH-deficient dwarfs, the animals that express a GH transgene and exhibit significantly reduced life-spans have lower catalase activity in liver and kidney and higher liver ascorbic acid levels. Other evidence is available suggesting that animals overexpressing GH and living reduced life-spans, compared to normal controls, exhibit signs of premature aging including reduced replicative potential of cells in vitro *(33)*, as well as indications of premature central nervous system aging including reduced catecholamine turnover *(8)*, increased astrogliosis *(34)*, and impaired memory and learning *(35)*. In addition, reduced life-span has been reported in patients with acromegaly and pituitary gigantism (both exhibit supraphysiological levels of GH; *[36,37]*).

We believe that GH deficiency may be pertinent to the extended life-span of Ames dwarf mice because overexpression of GH in transgenic mice is associated with reduced life-span, and other animal models (Snell dwarf) of GH insufficiency also appear to live longer. Extension of life-span in Ames dwarf and Snell dwarf mice (Dr. K.

Flurkey, personal communication, Bar Harbor, ME), which are homozygous for unrelated mutations on different chromosomes *(38,39)*, strongly suggests that this effect is not due to unknown pleiotropic effects of the mutated DW and DF genes, but to phenotypic characteristics common to the two mutants. In addition, preliminary evidence suggests that transgenic animals expressing a GH antagonist outlive their normal counterparts (J. J. Kopchick and W. Y. Chen, personal communication).

Additional observations suggest that GH may play a role in longevity of different species. Small breeds of dogs and horses live longer than larger breeds *(40)*, and shorter people may live longer than taller people from the same population *(41)*. Some of these differences have been linked to lower insulin-like growth factor-1 ([IGF-1], the main mediator of GH action on growth) in smaller breeds of animals (48-50). Reduced body size of Ames dwarfs reflects deficiencies of GH and thyroid hormones, which have well-documented effects on growth (plasma IGF-1 levels are below the detectability limit of radioimmunoassay *(45)*. A report by Ooka and coworkers *(46)* suggests that hypothyroidism induced by neonatal injection of thyroxine extends life-span by 4 mo in male rats and 2 mo in females, a modest increase in comparison to the Ames dwarf. To our knowledge, no information is available associating prolactin deficiency with life extension. There is, however, a growing body of evidence suggesting that GH plays a role in the aging process. This report, along with others *(10,47)*, links GH and oxidative metabolism with aging and suggests that GH may be involved in longevity assurance.

The role of GH in aging processes is not without controversy (for a recent review *see* ref. *48*). Our data on rodents as well as data by other investigators suggest that GH status is associated with longevity and possibly antioxidative mechanisms. However, in humans, the physiological symptoms of GH deficiency (muscle atrophy, increased adiposity, reduced cardiac function *[49,50]*) that occur in elderly individuals can be reversed by GH administration (increased lean body mass, reduced adiposity, improved general well-being *[49]*). Although these results are interesting, the studies in aging patients have been conducted on a short-term basis, and therefore it will be necessary to examine long-term, well-controlled studies to determine whether GH replacement is beneficial. Signs of premature aging are not detected in rodent models of GH deficiency as they are in GH deficient humans (Werner's syndrome *[51]*) nor is there an increase in mortality as noted in hypopituitary patients. Bates and coworkers *(52)* suggest that the observed increase in mortality in hypopituitary patients is primarily owing to pituitary tumors and the effects of treatment. As mentioned previously, GH-deficient rodents enjoy a healthy, extended life-span in comparison to normal control animals and those overexpressing this hormone. In addition, the effects of GH on aging may be related to biphasic dose-response relationships, body size, cell division, and metabolism *(48)*. Therefore, some evidence exists supporting both accelerated and decelerated aging in association with GH. It is obvious that much more research is necessary to reconcile the differences.

To date, calorie-restricted rodents have been used to explore mechanisms of delayed aging. The dwarf mouse and the calorie-restricted rodent model share many characteristics including reduced body weights, lower plasma glucose and insulin, increased plasma corticosterone concentrations, lower body temperatures, and possibly suppressed ROS production *(28,53–56)*. Our data suggest that tissues from dwarf mice have an increased ability to scavenge free radicals (increased catalase and depressed H_2O_2) and thus may be less vulnerable to oxidative damage. Therefore, the dwarf mutant may represent a unique example of a "gerontogene" in mammalian species and hence provide an important genetic model in which to study oxidative mechanisms, hormones, and delayed aging.

Materials and Methods

Ames dwarf, GH transgenic, and corresponding groups of age-matched normal littermate mice were maintained at the Southern Illinois University vivarium facilities under controlled conditions of photoperiod (12 h light:12 h dark) and temperature (22 ± 1°C) with ad libitum access to food and water. All procedures involving animals were reviewed and approved by the Southern Illinois School of Medicine Institutional Animal Care and Use Committee. The GH transgenic animals used in these studies were derived from a single male founder produced by microinjection of the phosphoenol pyruvate carboxykinase promoter region (300 bp)/bGH hybrid gene into the male pronucleus of single-cell embryos. The production and initial characterization of transgenic animals were described previously *(57,58)*.

Liver, kidney, and heart tissues were collected, rapidly frozen, and maintained at –80°C until analysis. For enzyme assays, frozen tissues were weighed, minced, and homogenized on ice with a teflon pestle in homogenizing buffer (20 m*M* MOPS, 300 m*M* sucrose, and 0.1 m*M* EDTA, pH 7.2). The homogenate was centrifuged for 30 min at 16,250*g* and the supernatant fraction used for analysis of various enzyme activities. Protein concentration was determined using the Bradford assay *(59)*.

For measurement of ascorbic acid or glutathione, frozen tissues were weighed, minced, and homogenized in either 0.5 mL ice cold 0.3% metaphosphoric acid ([MPA], with 0.1 m*M* EDTA and 1 m*M* thiourea to stabilize ascorbic acid) or 4 vol (w/v) of 1% picric acid, respectively. Acid homogenates were centrifuged at 16,250g rpm for 30 min and supernatant fractions were analyzed for ascorbate or glutathione and glutathione disulfide.

Measurement of Ascorbic Acid

For each sample, the supernatant fraction was added to 10% MPA (containing 0.1 m*M* EDTA and 1 m*M* thiourea as described previously) in triplicate. Concentration of ascorbic acid was measured by high performance liquid chromatography with electrochemical detection (ECD) *(60)*.

Measurement of Glutathione and Glutathione Disulfide

Supernatant fractions in picric acid were assayed for total glutathione (GSH) and glutathione disulfide (GSSG) by the standard recycling method *(61)*, and glutathione content was determined using a standard curve generated from known concentrations of glutathione. One-half of each sample was used for GSSG determination and the other half for GSH. Samples for GSSG determination were incubated at room temperature with 4-vinyl pyridine for 1 h, which allows for measurement of only GSSG without interference by GSH. The GSSG (as GSHx2) was then subtracted from the total GSH to determine the actual level of GSH.

Measurement of Enzyme Activities

The supernatant fractions homogenized in buffer were used to determine a variety of enzyme activities, including catalase (*[62]*; EC 1.11.1.6), G6PDH (*[23]*; EC 1.1.1.49), GR (*[22]* EC 1.6.4.2), and glutathione peroxidase (*[20]* EC 1.11.1.9). All activities were measured spectrophotometrically at varying wavelengths.

Differences between means were assessed utilizing Prism (GraphPad Software, San Diego, CA). A one-way analysis of variance (ANOVA) or a multiple ANOVA was used to determine significant differences among means. When needed, a Student Newman Keuls post hoc test was used to test for specific differences. The accepted level of significance for F_{obs} was $p < 0.05$.

Acknowledgments

We extend our appreciation to Hua Tu, Edie Green, and Shar Rakoczy for their technical assistance.

References

1. Sohal, R. S. and Weindruch, R. (1996). *Science* **273,** 59–63.
2. Harman, D. (1981). *Proc. Natl. Acad. Sci. USA* **78,** 7124–7128.
3. Finch, C. E. (1990). *Longevity, senescence, and the genome.* The University of Chicago Press: Chicago.
4. Masoro, E. J., Yu, B. P., and Bertrand, H. A. (1982). *Proc. Natl. Acad. Sci. USA* **79,** 4239–4241.
5. Weindruch, R. and Walford, R. L. (1988). *The retardation of aging and disease by dietary restriction.* CC Thomas: Springfield, IL.
6. Yu, B. P. (1994). *Physiol. Rev.* **74,** 139–163.
7. Brown-Borg, H. M., Borg, K. E., Meliska, C. J., and Bartke, A. (1996). *Nature* **384,** 33.
8. Steger, R. W., Bartke, A., and Cecim, M. (1993). *J. Reprod. Fertil. Suppl.* **46,** 61–75.
9. Wanke, R., Wolf, E., Hermanns, W., Folger, S., Buchmuller, T., and Brem, G. (1992). *Horm. Res.* **37,** 74–87.
10. Rollo, C. D., Carlson, J., and Sawada, M. (1996). *Can. J. Zool.* **74,** 606–620.
11. Stadtman, E. R. (1992). *Science* **257,** 1220–1224.
12. Ames, B. N., Shigenaga, M. K., and Hagen, T. M. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 7915–7922.
13. Sohal, R. S. and Orr, W. C. (1995). In: *Molecular aspects of aging.* Esser, K., and Martin, G. M., (eds.). Wiley: New York.
14. Brown-Borg, H. M., Borg, K. E., Bode, A. M., Carlson, J., and Bartke, A. (1998). 80th Annual Meeting of the Endocrine Society, New Orleans, LA.
15. Amicarelli, F., Di Ilio, C., Masciocco, L., Bonfigli, A., Zarivi, O., D'Andrea, M. R., Di Giulio, C., and Miranda, M. (1997). *Mech. Ageing Dev.* **97,** 215–226.
16. Ciriolo, M. R., Marasco, M. R., Iannone, M., Nistico, G., and Rotillo, G. (1997). *Neurosci. Lett.* **118,** 21–24.
17. Mura, C. V., Gong, X., Taylor, A., Villalobos-Molina, R., and Scrofano, M. M. (1996). *Mech. Ageing Dev.* **91,** 115–129.
18. Mote, P. L., Grizzle, J. M., Walford, R. L., and Spindler, S. R. (1991). *J. Gerontol.* **46,** B95–100.
19. Orr, W. C. and Sohal, R. S. (1994). *Science* **263,** 1128–1130.
20. Flohe, L. and Gunzler, W. (1984). In: *Methods in enzymology.* Packer, L. (ed.). Academic: Orlando, FL.
21. Bompart, G. J., Prevot, D. S., and Bascands, J. L. (1990). *Clin. Biochem.* **23,** 501–504.
22. Carlberg, I. and Mannervik, B. (1985). In: *Methods in enzymology.* Meister, A. (ed.). Academic: Orlando, FL.
23. Deutsch, J. (1983). In: *Methods of enzymatic analysis.* Bergmeyer, H. U., Bergmeyer, J., and Grabl, M. (eds.). Verlag Chemie: Deerfield Beach, FL.
24. Tabatabaie, T. and Floyd, R. A. (1994). *Arch. Biochem. Biophys.* **314,** 112–119.
25. Ji, L. L., Dillon, D., and Wu, E. (1990). *Am. J. Physiol.* **258,** R919–R923.
26. Xia, E., Rao, G., Van Remmen, H., Heydari, A. R., and Richardson, A. (1995). *J. Nutr.* **125,** 195–201.
27. Herbert, V., Shaw, S., and Jayatilleke, E. (1996). *J. Nutr.* **126,** 1213S–1220S.
28. Croson, W. B., Hunter, R., Sivansan, R., and Bartke, A. (1998). *FASEB J.* **12,** A120.
29. Abraham, E. C., Taylor, J. F., and Lang, C. A. (1978). *Biochem. J.* **174,** 819–825.
30. Chen, T. S., Richie, J. P. Jr., and Lang, C. A. (1990). *Drug Metab. Dispos.* **18,** 882–887.
31. Lang, C. A., Wu, W., Chen, T. S., and Mills, B. J. (1989). In: *Dietary restriction and aging.* Snyder, D. (ed.). Alan R. Liss: New York.
32. Richie, J. P. Jr. (1992). *Exp. Gerontol.* **27,** 615–626.
33. Pendergrass, W. R., Li, Y., Jiang, D., and Wolf, N. S. (1993). *J. Cell. Physiol.* **156,** 96–103.
34. Miller, D. B., Bartke, A., and O'Callaghan, J. P. (1995). *Exp. Gerontol.* **30,** 383–400.
35. Meliska, C. J., Burke, P. A., Bartke, A., and Jensen, R. A. (1997). *Neurobiol. Learn. Memory* **68,** 1–12.
36. Jadresic, A., Banks, L. M., Chid, D. F., Diamant, L., Doyle, F. H., Fraser, T. R., and Joplin, G. F. (1982). *Q. J. Med.* **202,** 189–191.
37. Thorner, M. O., Vance, M. L., Laws, Jr., E. R., Horvarth, E., and Kovacs, K. (1998). In: *Williams textbook of endocrinology*, 9th ed. Wilson, J. D., Foster, D. W., Kronenberg, H. M., and Larsen, P. R. (eds.). W. B. Saunders: Philadelphia.
38. Li, S., Crenshaw, E. B. 3rd, Rawson, E. J., Simmons, D. M., Swanson, L. W., and Rosenfeld, M. G. (1990). *Nature* **347,** 528–533.
39. Anderson, B., Pearse II, R. V., Jenne, K., Sorenson, M., Lin, S. C., Bartke, A., and Rosenfeld, M. G. (1995). *Dev. Biol.* **172,** 495–503.
40. Promislow, D. E. (1993). *J. Gerontol.* **48,** B115–B123.
41. Samaras, T. T. and Storms, L. H. (1992). *Bull. World Health Org.* **70,** 259–267.
42. Comfort, A. (1961). *Sci. Am.* **205,** 114–117.

43. Eigenmann, J. E., Patterson, D. F., and Froesch, E. R. (1984). *Acta Endocrinol.* **106,** 448–451.
44. Eigenmann, J. E., Patterson, D. F., Zapf, J., and Froesch, E. R. (1984). *Acta Endocrinol.* **105,** 294–299.
45. Chandrashekar, V. and Bartke, A. (1993). *Biol. Reprod.* **48,** 544–551.
46. Ooka, H., Fujita, S., and Yoshimoto, E. (1983). *Mech. Ageing Dev.* **22,** 113–120.
47. Rollo, C. D. (1994). In: Phenotypes: their epipgenetics, ecology and evolution. Chapman and Hall: London.
48. Bartke, A. (1998). *Endocrine* **8,** 103–108.
49. Rudman, D., Feller, A. G., Nagraj, H. S., Gergans, G. A., Lalitha, P. Y., Goldberg, A. F., Schenkler, R. A., Cohn, L., Rudman, I. W., and Mattson, D. E. (1990). *New Engl. J. Med.* **323,** 1–6.
50. Sonksen, P. H., Cuneo, R. C., Salomon, F., McGauley, G., Wiles, C. M., Wilmshurst, P., Byrne, C., Hesp, R., Lowy, C., and Weissberger, A. (1991). *Acta Paediatr. Scand.* **379(Suppl.)**, 139–146.
51. Rubin, C. D. and Reed, B. (1996). *Exp. Gerontol.* **31**, 557–561.
52. Bates, A. S., Van't Hoff, W., Jones, P. J., and Clayton, R. N. (1996). *J. Clin. Endo. Metab.* **81,** 1169–1172.
53. Weindruch, R. and Sohal, R. S. (1997). *New Engl. J. Med.* **337,** 986–994.
54. Lane, M. A., Baer, D. J., Rumpler, W. V., Weindruch, R., Ingram, D. K., Tilmont, E. M., Cutler, R. G., and Roth, G. S. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 4159–4164.
55. Borg, K. E., Brown-Borg, H. M., and Bartke, A. (1995). *Proc. Soc. Exp. Biol. Med.* **210,** 126–133.
56. Kim, J. D., McCarter, R. J. M., and Yu, B. P. (1996). *Ageing Clin. Exp. Res.* **8,** 123–129.
57. McGrane, M. M., DeVente, J., Yun, J., Bloom, J., Park, E., Wynshaw-Boris, A., Wagner, T., Rottman, F. M., and Hanson, R. W. (1988). *J. Biol. Chem.* **263,** 11,443–11,451.
58. Steger, R. W., Bartke, A., Parkening, T. A., Collins, T., Cerven, R., Jun, J. S., and Wagner, T. E. (1994). *Transgenics* **1,** 245–253.
59. Bradford, M. M. (1976). *Anal. Biochem.* **72,** 248–254.
60. Schell, D. A. and Bode, A. M. (1993). *Biomed. Chrom.* **7,** 267–272.
61. Griffith, O. W. (1986). In: *Methods of enzymatic analysis*. Bergmeyer, H. U., Bergmeyer, J., and Grabl, M. (eds.). Verlag Chemie: Deerfield Beach, FL.
62. Aebi, H. (1984). In: *Methods in enzymology*. Packer, L. (ed.). Academic: Orlando, FL.