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Growth hormone alters methionine and glutathione metabolism in Ames dwarf mice☆,☆☆

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Abstract

Reduced signaling of the growth hormone (GH)/insulin-like growth factor-1(IGF-1)/insulin pathway is associated with extended life span in several species. Ames dwarf mice are GH and IGF-1 deficient and live 50–64% longer than wild type littermates (males and females, respectively). Previously, we have shown that Ames mice exhibit elevated levels of antioxidative enzymes and lower oxidative damage. To further explore the relationship between GH and antioxidant expression, we administered GH or saline to dwarf mice and evaluated components of the methionine and glutathione (GSH) metabolic pathways. Treatment of dwarf mice with GH significantly suppressed methionine adenosyltransferase (40 and 38%) and glycine-*N*-methyltransferase (44 and 43%) activities (in 3- and 12-month-old mice, respectively). Growth hormone treatment elevated kidney gamma-glutamyl-cysteine synthetase protein levels in 3- and 12-month-old dwarf mice. In contrast, the activity of the GSH degradation enzyme, gamma-glutamyl transpeptidase, was suppressed by GH administration in heart and liver. The activity of glutathione-*S*-transferase, an enzyme involved in detoxification, was also affected by GH treatment. Taken together, the current results along with data from previous studies support a role for growth hormone in the regulation of antioxidative defense and ultimately, life span in organisms with altered GH or IGF-1 signaling.

Keywords: Ames dwarf mice; Hormones; Aging; *S*-adenosylmethionine; Glycine-*N*-methyl transferase; Glutathione; γ-Glutamyl cysteine synthetase

1. Introduction

The factors that affect aging and their regulation are not well understood. One physiological system in particular, the endocrine system, exhibits specific and predictable age-related changes. Menopause and andropause are two well-characterized events in which estrogen and testosterone, respectively, decline with age, albeit more abruptly in the case of estrogen. Other hormones decline with age including growth hormone (GH) and insulin-like growth factor-1 (IGF-1) beginning around age 30 in humans. This loss of youthful levels of GH and IGF-1 has been strongly implicated in the physical changes that occur as one ages including decreased muscle mass and strength, increased fat mass and decreased skin thickness (Rosen, 2000).

Ames dwarf mice are exceptionally long lived (3–4 years) when compared to normal, wild type mice and

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Table 1
Phenotypic characteristics of mutant mice

Phenotype	Dwarf (*prop-1*, *pit-1*, GHR/BP KO)	Growth hormone overexpressing
GH/IGF1/insulin signaling	↓	↑
Body size	↓	↑
Reproduction	↓	↓
Glucose metabolism	↓	↑
Stress resistance	↑	↓
Longevity	↑	↓

multiple other strains of mice exhibiting average life spans of around 2 years. These mice lack three pituitary hormones (GH, prolactin and thyrotropin) because of a point mutation in a gene coding for a protein that drives development of certain cell types in the anterior pituitary. Several reports from this lab and others have implicated the GH-deficiency, in particular, as a major player in the extension of life span in mammals.

Current literature has established a compelling link between the GH/IGF-1/insulin signaling pathway and aging (Table 1-mutant mice). Reduced signaling through this pathway confers longevity in mammals (Brown-Borg et al., 1996; Flurkey et al., 2001; Coschigano et al., 2000; Holzenberger et al., 2003), flies (Tatar et al., 2001; Clancy et al., 2001), nematodes (Kimura et al., 1997) and yeast (Fabrizio et al., 2001). One potential mechanism of decelerated aging that appears to be consistently affected in these long-living mutants is reduced oxidative stress. Various enzymes involved in countering oxidative stress are elevated in longevity mutants. In dwarf mice, catalase is elevated in numerous tissues (Brown-Borg et al., 1999; Brown-Borg and Rakoczy, 2000; Hauck and Bartke, 2000). In turn, mitochondrial hydrogen peroxide production and protein oxidative damage are significantly lower in liver tissues from dwarf mice (Brown-Borg et al., 2001a,b). Both in vitro and in vivo studies suggest that GH status modulates antioxidative mechanisms (Brown-Borg et al., 2002; Brown-Borg and Rakoczy, 2003). Growth hormone decreases the expression of key components of the antioxidative defense system. Tissues from mice overexpressing GH have severely reduced catalase activity, protein and mRNA (Brown-Borg and Rakoczy, 2000) along with increased tissue oxidative damage (Brown-Borg et al., 2001a; Rollo et al., 1996). These factors are thought to contribute to the 50% reduction in life span in GH transgenics compared to wild type mice. In contrast, tissues from GH-deficient Ames dwarf mice exhibit enhanced antioxidative capacity and less protein and DNA oxidative damage (Brown-Borg and Rakoczy, 2000; Hauck and Bartke, 2001; Brown-Borg et al., 2001a; Sanz et al., 2002) compared to age-matched wild type mice. Moreover, both in vivo and in vitro evidence indicates that GH suppresses catalase, glutathione peroxidase (GPX) and manganese superoxide dismutase (MnSOD) protein levels and activity in tissues and hepatocytes from Ames dwarf and wild type mice, respectively (Brown-Borg et al., 2002; Brown-Borg and Rakoczy, 2003).

With regard to hormone modulation, one pathway that is vastly understudied in aging involves methionine (MET) metabolism and provision of the substrates, cysteine and MET, for the GSH system (Fig. 1). We previously reported that several components of the methionine metabolic pathway are significantly altered in the Ames dwarf mouse (Uthus and Brown-Borg, 2003). Specifically, liver concentrations of *S*-adenosylmethionine (SAM) are lower while *S*-adenosylhomocysteine (SAH) concentrations are higher in dwarf mice when compared to age-matched, normal, wild type mice. Although the global DNA methylation status of liver is not different between dwarf and wild type mice, the activity of several enzymes involved in methionine metabolism differ significantly. The activities of methionine adenosyltransferase (MAT) and glycine-*N*-methyltransferase (GNMT) are markedly elevated in the long-living dwarf compared to wild type mice. Others have shown that GH decreases the activities of GNMT (Aida et al., 1997) and MAT (Pan and Tarver, 1967) in hypophysectomized rats. Importantly, this pathway is the major provider of substrates for the synthesis of GSH.

Glutathione functions as an antioxidant and coenzyme and plays a role in detoxification. This tripeptide is elevated in dwarf mouse liver at 3-, 12- and 24-months of age when compared to age-matched wild type mouse tissue (Brown-Borg et al., 2001b). However, the oxidized form of GSH, GSH-disulfide (GSSG), is also higher indicating a larger overall GSH pool in these mice, but also suggesting a potential for increased oxidative stress. The elevations of components of both the MET and GSH pathways in dwarf mice, taken together with the upregulated antioxidant defense, suggest that the endocrine system likely plays a role in the modulation of these metabolic pathways and may contribute to the long life span of dwarf mice.

Our previous work showed that both GSH levels and GSH/GSSG ratios are significantly elevated in liver, brain and muscle tissues of GH-treated dwarf mice (Brown-Borg and Rakoczy, 2003). Concurrently, the levels of GPX activity and protein are significantly lower in liver and brain tissues following GH treatment while no changes in glutathione reductase activities are detected. We also reported that although GSH and GSSG levels in kidney tissues were not affected by GH treatment, GPX protein and activity were markedly decreased, and glutathione reductase (GR) activity was elevated following hormone treatment (Brown-Borg and Rakoczy, 2003). These findings prompted the evaluation of components of GSH synthesis and degradation and MET metabolism in the current study. Therefore, the objective of this study was to determine the role of GH on various components involved in the metabolism of MET and GSH in GH-deficient Ames dwarf.

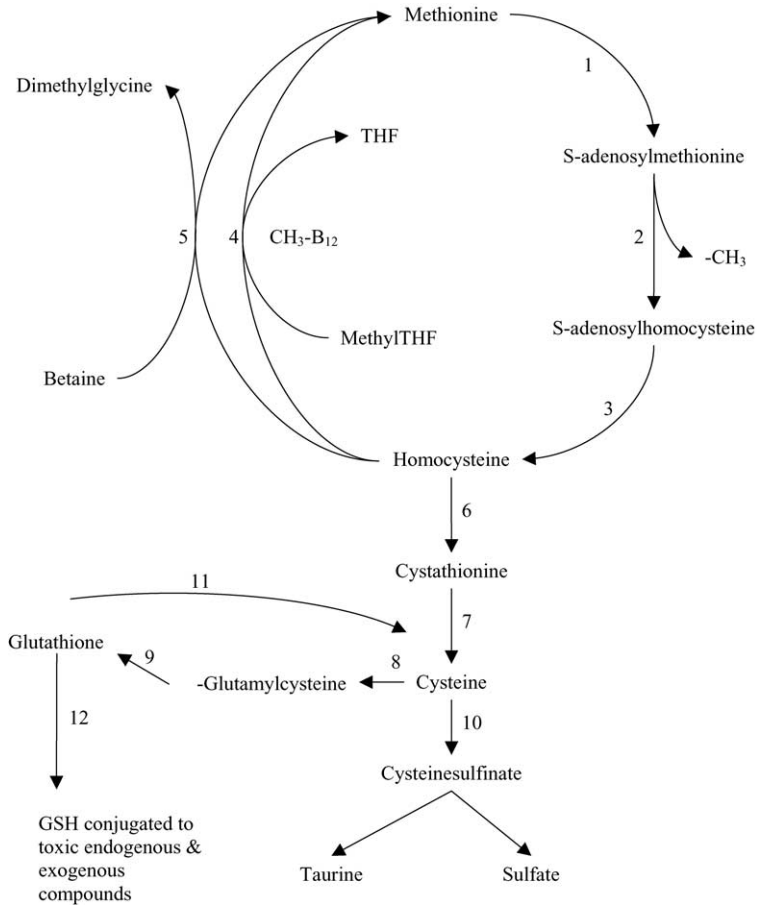

Fig. 1. Methionine and glutathione metabolism. Enzymes: (1) MAT (*S*-adenosylmethionine synthase); (2) SAM-dependent transmethylase; (3) *S*-adenosylhomocysteine hydrolase; (4) methionine synthase; (5) betaine homocysteine methyltransferase; (6) cystathionine synthase; (7) γ-cystathionase; (8) γ-glutamylcysteine synthetase; (9) glutathione synthetase; (10) cysteine dioxygenase, (11) γ-glutamyl transpeptidase and dipeptidases; (12) glutathione-*S*-transferase (modified from Uthus and Brown-Borg, 2003).

2. Materials and methods

Ames dwarf mice were bred and maintained at the University of North Dakota (UND) vivarium facilities under controlled conditions of photoperiod (12 h light:12 h dark) and temperature (22 ± 1 °C) with ad libitum access to food (PMI Intl., St. Louis, MO; Lab diet ≥ 23% crude protein ≥ 4.5% fat) and water (standard laboratory conditions). The Ames dwarf (df/df) mice used in this study were derived from a closed colony with heterogeneous background (over 20 years). Dwarf mice were generated by mating either homozygous (df/df) or heterozygous (df/+) dwarf males with carrier females (df/+). All procedures involving animals were reviewed and approved by the UND Institutional Animal Care and Use Committee. For reference, the average lifespan of the wild type mice in our colony is 23–24 months (Brown-Borg et al., 1996). All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise noted.

Porcine GH was employed as adequate amounts of recombinant mouse GH are lacking. Porcine GH administration was limited to 7 days because mice develop an immune response to foreign antigen (non-mouse GH) with long-term use (>8 days). Porcine GH was obtained from Dr. A.F. Parlow (NIDDK National Hormone and Pituitary Program) and administered to 3- and 12-month-old Ames dwarf mice. Porcine GH [25 μg/injection in alkaline saline (pH 9) mixed with 50% polyvinylpyrrolidone (PVP) in saline (1:1, saline-PVP)] was injected s.c. (50 μl/injection) into one group of dwarf mice (n = 6–7/age; repetitions = 2) or saline-PVP into another group of dwarf mice (n = 6–7/age; r = 2) two times daily (8:00 a.m. and 5:00 p.m.) for 7 days (total of 13 injections). On the morning of the seventh day, 1 h following the last injection of saline-PVP or GH, the mice were killed and tissues removed and frozen. This dosing regimen for GH increases plasma IGF-1 levels in dwarf mice from non-detectable levels to near normal mouse values (Chandrashekar and Bartke, 1993) and increases body (1–4 g) and liver weights of dwarf mice (Brown-Borg and Rakoczy, 2003). Body weight was recorded prior to the first injection and following the last injection. Liver weights were recorded on the final day of the experiment. This study

was part of a larger study in which the body and liver weights and GSH, GSSG, glutathione peroxidase (GPX), and glutathione reductase (GR) data were previously reported (Brown-Borg and Rakoczy, 2003). Therefore, these data will be presented and cited accordingly.

3. Glutathione metabolic pathway

For most of the enzyme assays, frozen tissue (liver, kidney, heart, brain) samples were homogenized on ice with a Teflon pestle in buffer (CPE buffer; Brown-Borg and Rakoczy, 2000). A supernatant fraction (following centrifugation) was used for analysis of enzyme activities. The activities of γ-glutamyl transpeptidase (GGT; EC 2.3.2.2; Meister et al., 1981) and GSH-*S*-transferase (GST; EC 2.5.1.18; Mannervick et al., 1987; Habig and Pabst, 1979), which are involved in the synthesis and degradation of GSH, were determined.

Mouse γ-glutamylcysteine synthetase (GCS) antibody (Neomarkers; Fremont, CA) was used to detect GCS protein levels using standard immunoblotting procedures with chemiluminescence (BioRad; Hercules, CA) and densitometry (BioRad Imaging Densitometer and Molecular Analyst software) as previously described (Brown-Borg and Rakoczy, 2000). Protein concentrations were determined by the method of Bradford (Bradford, 1976).

4. Methionine metabolism

Liver was the only tissue used for evaluation of MET metabolism because of the very limited amounts of tissue from dwarf mice. Methionine adenosyltransferase (*S*-adenosylmethionine synthase, MAT) activity was determined by the method of Cantoni (1955) with modifications that were previously described (Uthus and Brown-Borg, 2003). Liver was homogenized in 5 volumes of cold 0.4 M perchloric acid and prepared for SAM and SAH analysis according to Davis and coworkers (2000) and measured with a Dionex 4000i HPLC (Dionex Corp., Sunnyvale, CA; Wagner et al., 1984) as previously reported (Uthus and Brown-Borg, 2003). Liver glycine-*N*-methyltransferase (GNMT) activity was determined as previously described (Uthus and Brown-Borg, 2003) employing the methods of Cook and Wagner (1984).

5. Statistical analysis

In each experiment, differences between means were assessed utilizing Prism (Graphpad, San Diego, CA). For activity assays, two-way analyses of variance (ANOVA) and when appropriate, Bonferroni post hoc testing was used to determine significant differences among means. For comparison of protein levels within age based on densitometric analysis, Students *t*-tests were employed. Data are reported as mean ± S.E.M.

6. Results

The administration of growth hormone to GH-deficient mice altered several parameters in the methionine and glutathione metabolic pathways. Although our efforts have focused on liver, other tissues were also analyzed to evaluate tissue specificity of some components in the glutathione pathway for direct comparison with earlier observations. As previously reported (Brown-Borg and Rakoczy, 2003), growth hormone administration significantly increased body (2–3 g) and liver weights of dwarf mice (Table 2). Treatment with GH did not significantly affect concentrations of SAM in dwarf liver tissue when compared to saline-treated dwarfs at either age tested (Fig. 2A). In contrast, liver SAH concentrations were significantly decreased 24 and 30% in GH-treated animals at 3- and 12-months of age, respectively, when compared to mice receiving saline (ANOVA-treatment-$P = .0002$; Fig. 2B). An age-related decline in the levels of SAH was also observed in the saline-treated mice ($P = .04$). Although the liver SAH levels were significantly decreased by GH treatment, no significant difference was detected in the SAM/SAH ratio (Fig. 2C). No significant treatment × age interactions were detected.

The activities of enzymes involved in the methionine pathway were clearly affected by GH treatment. Liver MAT activity was decreased 40 and 38% in 3- and 12-month-old dwarf mice, respectively when compared to saline controls (Fig. 3; $P = .0002$ and $P = .0016$). In addition, MAT activity declined 20 and 22% with age in both GH- and saline-treated mice (Fig. 3; GH-$P = .09$; Saline-$P = .008$). The activity of GNMT was also significantly suppressed by GH administration in both 3- and 12-month-old (44 and 43% decrease) dwarf mice relative to saline-injected control mice ($P < .0002$ and $P = .012$, respectively; Fig. 4). Between

Table 2
Body weight change (difference before and after GH treatment) and liver weights in 3- and 12-month-old dwarf mice treated with GH or saline for 7 days

Treatment and age	Saline 3-months	GH 3-months	Saline 12-months	GH 12-months	*P*-value		
					Treatment	Age	Interaction
Body weight change (g)	0.783 ± 0.185	3.167 ± 0.109	0.153 ± 0.154	2.305 ± 0.240	<.0001	.0005	.5230
Liver weight (g)	0.525 ± 0.026	0.703 ± 0.019	0.550 ± 0.024	0.820 ± 0.139	.0058	.3403	.5345

Growth hormone administration increased body weight and liver weights in mice of both ages. Values represent mean ± S.E.M. *P*-values represent results of a two-way ANOVA. Data adapted from Brown-Borg and Rakoczy (2003).

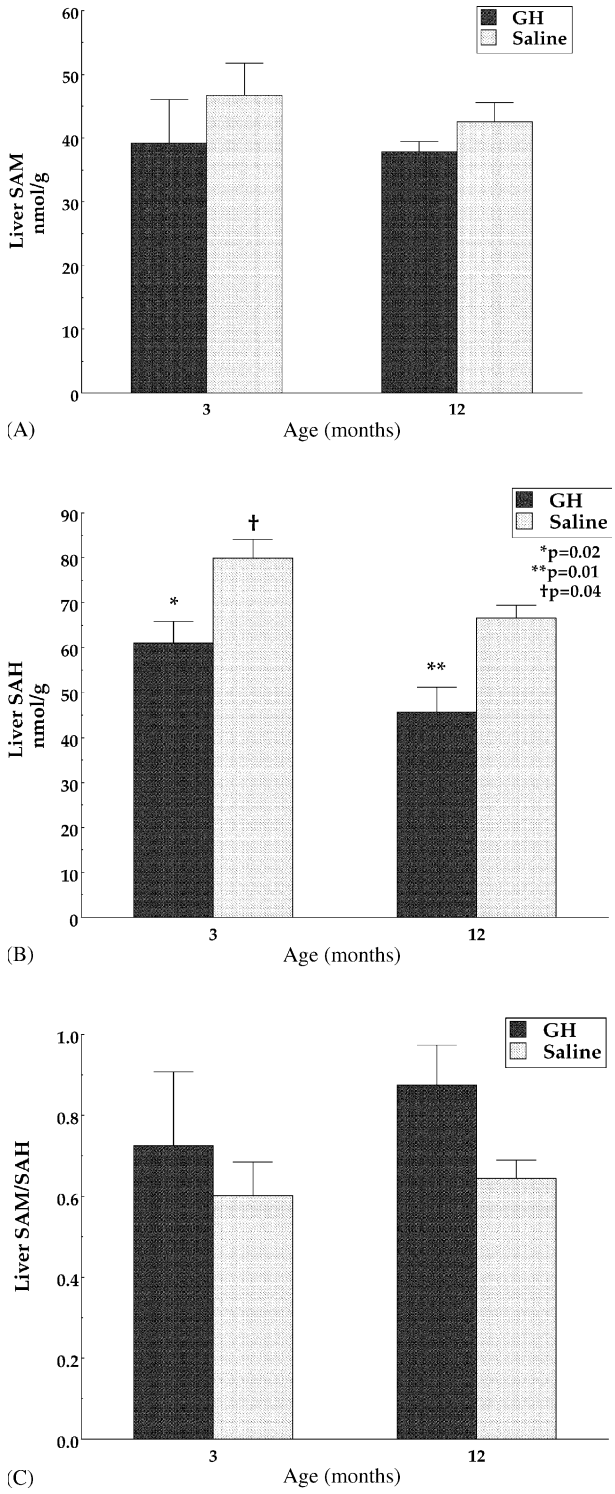

Fig. 2. S-adenosylmethionine (SAM) (A) and S-adenosylhomocysteine (SAH) (B) levels in 3- and 12-month-old dwarf mice treated with GH or saline for 7 days. (C) SAM/SAH ratio. Values represent mean ± S.E.M. Levels are expressed as nmol/mg protein. Treatment: $^{*}P = .02$; $^{**}P = .01$. Age: $^{\dagger}P = .04$.

3- and 12-months of age, liver GNMT activity levels decreased 28% in both treatment groups (Fig. 4; $P < .03$). No treatment × age interactions were detected for MAT or GNMT activities.

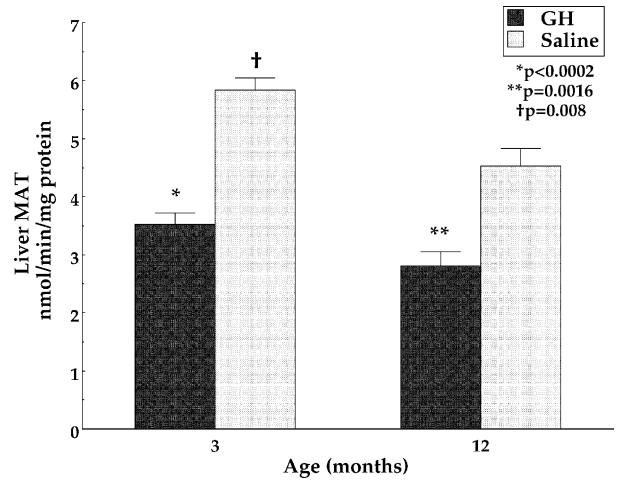

Fig. 3. Methionine adenosyltransferase activity (MAT; nmol/min/mg) levels in dwarf mice receiving GH or saline for 7 days. Values represent mean ± S.E.M. Treatment: $^{*}P < .0002$; $^{**}P = .0016$. Age: $^{\dagger}P = .008$.

Focusing on liver, protein levels of GCS, the rate limiting step in GSH biosynthesis, were not found to be different in GH-treated 3- and 12-month-old dwarf mice, relative to mice receiving saline treatment (Table 3). Gamma-glutamyltranspeptidase, which initiates the breakdown of GSH, was decreased 23% in 3-month-old GH-treated dwarf mice compared to dwarf mice receiving daily saline treatment (Table 3). At 12 months, GH administration did not significantly alter GGT activity in liver. In addition, no age-related change in GGT activity levels were observed between the 3- and 12-month-old dwarf mice in either treatment (Table 3). Liver GST activity was suppressed 24% in 12-month-old GH-treated mice while no difference was detected at 3 months of age when compared to saline controls. However, GST activity in the liver increased 36% between 3- and 12-month-old GH-treated dwarf mice and 50% between 3- and 12-month-old saline-treated mice

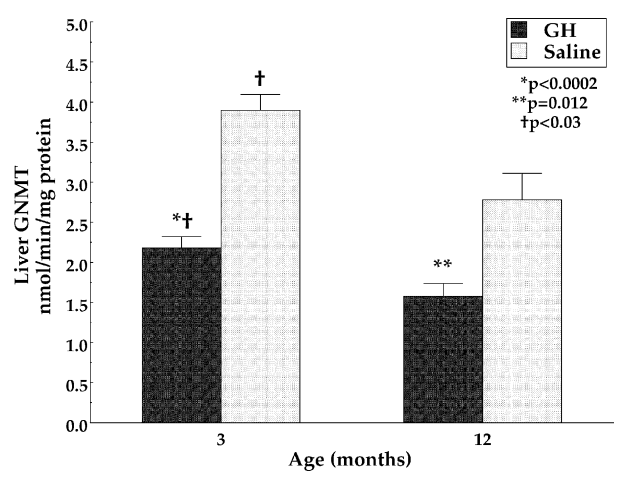

Fig. 4. Glycine-*N*-methyltransferase activity (GNMT; nmol/min/mg) levels in 3- and 12-month-old dwarf mice treated with GH or saline for 7 days. Values represent mean ± S.E.M. Treatment: $^{*}P = .0002$; $^{**}P = .012$. Age: $^{\dagger}P < .03$.

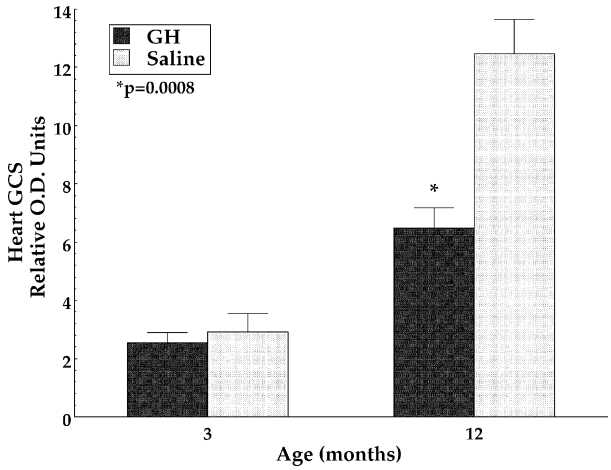

Fig. 5. γ-Glutamylcysteine synthetase protein (GCS; relative optical density units) levels in 3- and 12-month-old dwarf mice receiving GH or saline for 7 days. Values represent mean ± S.E.M. $^{*}P = .0008$.

(Table 3; $P = .02$ and $P = .004$, respectively) suggesting that GH treatment suppressed the age-related rise in GST activity.

In the current study, GCS was elevated 64 and 46% in kidney tissues of 3- and 12-month-old mice, respectively, following 7 days of GH treatment (Table 4; 3 month–$P = .0003$; 12 month–$P = .02$). Growth hormone treatment of GH-deficient dwarf mice increased GGT activity 51 and 27% in kidney tissues at 3- and 12-months of age, respectively (Table 4). An age-related increase in GGT activity was also observed in both treatment groups (Table 4). The activity of GST in the kidney was reduced by GH administration in Ames dwarf mice (33 and 16% reduction in 3- and 12-month-old GH-treated dwarf mice, when compared to age-matched saline injected mice, respectively (Table 4). However, in the saline-treated mice, kidney GST activity decreased 25% between 3- and 12-months resulting in a significant interaction between treatment and age ($P = .036$, Table 4).

In heart tissue, protein levels of GCS in GH-treated mice were nearly half (52%) that of mice receiving saline ($P = .0008$) at 12-months of age (Fig. 5). Similar to liver, GGT activity levels in the heart were reduced 31% in GH-treated 3-month-old mice relative to saline-treated mice (95.38 ± 6.91 versus 138.55 ± 13.04 nmol/mg protein, respectively). However, a 20% increase in GGT activity was detected between GH and saline-treated 12-month-old mice (GH–142.02 ± 13.12 versus saline–118.48 ± 7.85 nmol/mg protein) resulting in a significant treatment × age interaction ($P = .0077$). GST activity was not evaluated in heart tissues due to limited sample availability.

The effects of GH treatment on whole brain tissue were much less pronounced compared to peripheral tissues. The administration of GH to dwarf mice did not affect brain GCS protein levels at either age examined (data not shown). The activity of GGT in brain tissue was not significantly affected by 7 days of GH treatment in 3- or 12-month-old dwarf mice (3-month GH–114.0 ± 2.9 versus saline–133.6 ± 9.0; 12-month GH–122.0 ± 7.0 versus saline–121.3 ± 3.9 nmol/mg protein). The level of GST activity in dwarf brain was similarly unaffected by GH treatment at 3- or 12-months of age compared to brain levels observed in dwarf mice receiving only saline (3-month GH–428.5 ± 23.2 versus saline–516.4 ± 32.0; 12-month GH–438.5 ± 37.5 versus saline–471.0 ± 49.5 nmol/min/mg protein). No age-related differences in the activities of GGT or GST were observed.

7. Discussion

GH administration markedly depressed liver MAT activity yet SAM levels were not affected. In conjunction with significant reductions in GNMT activity in the presence of GH, liver SAH levels were also decreased. These changes are congruent with previous data from our lab showing that MET metabolism is significantly elevated in GH-deficient mice. For example, MAT, which synthesizes SAM from methionine, is elevated 205% in dwarf mice compared to wild type mice (Uthus and Brown-Borg, 2003). Glycine-*N*-methyltransferase activity is elevated 91% in GH-deficient mice compared to their GH-sufficient counterparts. Although there are numerous methyltransferases that remove a methyl group from SAM resulting in SAH, the synthesis of this particular enzyme appears to be under the inhibitory control of GH (Oscarsson et al., 2001). Thus, in GH-deficient animals, GNMT activity is elevated. Further along the metabolic pathway of MET are the enzymes cystathionine-β-synthase and cystathionase (also significantly elevated in dwarf mice), which are responsible for the synthesis of cystathionine and cysteine from homocysteine and cystathionine, respectively. The methionine and cysteine residues provided via this mechanism feed thiols into the GSH pathway to promote the synthesis of GSH. Our results suggest that one possible mechanism for the altered glutathione metabolism observed in dwarf mice may be the

Table 3
Components of glutathione metabolism in liver tissue of 3- and 12-month-old Ames dwarf mice treated with GH or saline for 7 days

Treatment and age	Saline 3-months	GH 3-months	Saline 12-months	GH 12-months	*P*-value		
					Treatment	Age	Interaction
GCS protein (relative OD units)	5.60 ± 0.58	4.37 ± 0.89	7.35 ± 0.56	7.46 ± 0.66	ND	ND	ND
GGT activity (nmol/mg protein)	75.61 ± 7.14	58.16 ± 4.37	69.92 ± 3.38	62.74 ± 3.23	.0203	.9110	.3040
GST activity (nmol/min/mg)	743.5 ± 49.1	727.6 ± 85.3	1487.0 ± 178.1	1136.0 ± 98.4	.1198	<.0001	.1533

Values represent mean ± S.E.M. *P*-values represent results of a two-way ANOVA for GGT and GST activities. OD = optical density; ND = ANOVA not done.

Table 4
Components of glutathione metabolism in kidney tissue of 3- and 12-month-old Ames dwarf mice treated with GH or saline for 7 days

Treatment and age	Saline 3-months	GH 3-months	Saline 12-months	GH 12-months	*P*-value		
					Treatment	Age	Interaction
GCS protein (relative OD units)	14.45 ± 1.61	23.7 ± 0.60	10.42 ± 1.32	15.21 ± 1.20	ND	ND	ND
GGT activity (nmol/min/mg protein)	0.137 ± 0.031	0.206 ± 0.037	0.233 ± 0.039	0.297 ± 0.035	.0157	.0749	.9358
GST activity (nmol/min/mg)	912.4 ± 41.1	607.3 ± 54.9	731.2 ± 42.3	616.2 ± 29.0	.0555	<.0001	.0360

Values represent mean ± S.E.M. *P*-values represent results of a two-way ANOVA for GGT and GST activities. OD = optical density; ND = ANOVA not done.

result of the distinctive methionine metabolism exhibited in these mice. The effects of GH on GSH metabolism in the Ames mice may also be a consequence of the altered MET pathway.

Glutathione plays vital roles in the defense against free radicals and toxins and in the storing and transferring of cysteine. In agreement with other reports, our data show that GCS expression and regulation is tissue specific. Liver tissue levels of GCS were not altered by GH treatment of dwarf mice (Table 3). However, GH increased the levels of this rate-limiting protein in kidney tissues of mice at both ages (Table 4) while in heart GCS expression was suppressed by GH administration. Both GCS (the rate limiting step in GSH biosynthesis) and GSH synthetase are responsible for GSH synthesis and are regulated by amino acid availability, hormones (Deneke and Fanburg, 1989; Lu et al., 1990) and feedback inhibition of GSH on GCS (Griffith, 1999). Our study is the first to report on the actions of GH on GCS protein levels. Other hormones such as insulin and the corticosteroids have been previously shown to stimulate hepatic GSH synthesis by inducing GCS (Lu et al., 1991, 1992) and are required for normal activity and transcription of GCS (Griffith, 1999). In contrast, thyroid hormones decrease GSH synthesis in liver (Videla and Fernandez, 1995). In the face of low thyroid hormones, Ames dwarf liver GCS protein is significantly elevated at 3-, 12-, and 24-months of age compared to age-matched wild type mice (Brown-Borg, unpublished data). The transsulfuration pathway present in liver and kidney tissues does not exist in all cell types which may explain, in part, the tissue differences in GCS and GSH observed in Ames and wild type mice. Levels of GSH synthesis and hence, GCS activity, have also been shown to increase in cells challenged by oxidative stress suggesting potential stress in dwarf kidney tissues versus liver and heart in the presence of GH.

Lower GCS activity may act to limit the rate of GSH synthesis and favor catabolism of cysteine to taurine or sulfate. The Ames dwarf has increased levels of liver enzymes that lead from the MET pathway through to cysteine (Uthus and Brown-Borg, 2003) and increased levels of proteins that decrease cysteine level via utilization in the γ-glutamyl cycle. We have previously shown that liver GSH levels are higher in Ames mice compared to wild type mice (Brown-Borg et al., 2001b). Moreover, GH treatment of Ames dwarf mice further elevated GSH levels in liver, brain and muscle compared to saline-treated dwarf mice (Brown-Borg and Rakoczy, 2003). Synthesis of GSH is dampened as GCS is mostly inactive when GSH is present in normal concentrations (Richman and Meister, 1975). This evidence may partially explain the lack of differences observed in GCS protein levels in the livers and brains of GH versus saline-treated dwarf mice (as the enzyme may be inactive in tissues with adequate or elevated levels of GSH).

Liver and kidney tissues secrete large amounts of GSH that are cleaved into constituent amino acids by the sequential actions of GGT and dipeptidases. The activity of GGT in the kidney is a critical determinant of the rate of disappearance of GSH and GSSG from the circulation as 80–90% of GSH catabolism occurs in the kidney (Griffith and Meister, 1979; Meister, 1983). GGT expression in mouse is regulated in a tissue-specific manner but outside of the kidney, the relative contribution of other organs is unclear. In our study, GH generally decreased liver, heart and brain degradation and liver, kidney and brain utilization of GSH (via reductions in GGT and GST activities, respectively). These alterations may lead to the elevated GSH observed in some tissues (liver and brain). Differences in responses to GH may reflect tissue specific expression of both GH receptors and GSH metabolic enzymes. GGT mobilizes cysteine from the GSH pool and makes it available to multiple tissues (Ishikawa, 1992; Monks and Lau, 1994; Lieberman et al., 1996). As expected, kidney GGT activity levels were higher when compared to activity levels in liver, heart and brain tissues. Kidney GGT is measured in a kinetic assay because of the high activity levels versus liver, brain and heart tissues that are measured in an endpoint assay. Very little other information is available regarding potential hormonal modulation of GGT expression and activity with the exception that mice deficient in GGT (via gene disruption) have profound growth deficits (non-detectable plasma IGF-1 concomitant with elevated plasma GH levels) and die by 10–18 weeks of age (Lieberman et al., 1996). The activity of GGT likely affects amino acid availability, specifically cysteine, thus during growth stages the lack of available cysteine may alter growth rates leading to lower demand of IGF-1 or that cysteine is necessary for GH actions on liver IGF-1 (GH receptor dysfunction, downstream signal disruption) synthesis or secretion.

Glutathione is a major player in detoxification of both endogenous and exogenous toxic compounds. The glu-

tathione-*S*-transferase family of proteins performs the function of conjugating GSH to a broad range of compounds resulting in less reactive, more water-soluble and readily excreted compounds (Ketterer and Meyer, 1989; Salinas and Wong, 1999; Whalen and Boyer, 1998). The expression of GST varies during development and aging, and is organ, sex and species specific (Staffas et al., 1998). Our study showed that GH administration to GH-deficient Ames dwarf mice significantly decreased GST activity in kidney and had a lesser effect on liver and brain tissues (decreasing potential utilization of GSH in detoxification reactions). Growth hormone has been shown to regulate levels of cytosolic GSTs in certain target organs of rats (Staffas et al., 1992) and it is known that GH secretion patterns underlie the sexually dimorphic expression of GSTA5 (isoenzyme 5 of GST alpha class; Staffas et al., 1998). Furthermore, in rats, hypophysectomy leads to increased GSTA5 expression in both sexes and GH administration down regulates this response, as does testosterone. Male mice constitutively express exceptionally high levels of liver GST II that is negatively regulated by plasma testosterone levels (Muskhelishvili et al., 1996). There is controversy regarding GST expression levels with aging and the influence of caloric restriction (Carrillo et al., 1989; Liu and Choi, 2000; Cho et al., 2000).

Regulation of GST expression and activity has profound effects on sensitivity to chemical insults. Dwarf mice have been shown to resist toxic chemical challenges. Ames mice, treated with paraquat, an oxidative stressor, significantly outlive corresponding wild type mice (Bartke, 2000). Snell dwarf mice (phenotypically identical to Ames mice) challenged with a chemical carcinogen develop fewer tumors than wild type mice and exhibit reduced growth of transplanted tumors (Bielschowsky and Bielschowsky, 1961; Chen et al., 1972). Fibroblasts from GH-deficient Snell dwarf mice are resistant to multiple forms of cellular stress including paraquat, hydrogen peroxide, cadmium, UV light and heat (Murakami et al., 2003). Moreover, GH-deficient dwarf rats are resistant to chemical carcinogen challenge (Ramsey et al., 2002). Recently, Ikeno and coworkers (2003) reported delayed occurrence and lower incidence of fatal neoplastic diseases in Ames dwarf mice. These reports strongly suggest that GH/IGF-1 deficiencies provide protection against exogenous and endogenous toxins. We believe that this resistance may be partially explained by differences in GSH metabolism. In addition to the results reported in this study, we have data indicating that the activity of GST is elevated 50–100% in dwarf mice at 3-, 12- and 24-months of age when compared to corresponding wild type mice (Brown-Borg, unpublished results). Therefore, the lack of GH in dwarf mice may result in higher overall levels of tissue GST and GSH, thus enhancing the detoxification ability and providing cellular protection and resistance to toxic challenges. As an added observation, GST overexpression in *C. elegans* confers increased resistance to intracellular induced oxidative stress (Leiers et al., 2003).

Glutathione may be one of the major mechanisms responsible for lower oxidative damage found in the Ames dwarf mouse. GSH levels are consistently and significantly elevated in dwarf liver tissues when compared to wild type siblings (Brown-Borg et al., 2001b). These high GSH levels may be responsible for the lower protein oxidative damage observed as GSH is involved in enzymatic restoration of cysteine sulfhydryls from disulfides (Moskovitz et al., 1999). Cells depleted of GSH are known to be more sensitive to the effects of pro-oxidants. The Ames dwarf mouse may be more resistant to oxidative stress because of the relatively large pool of total GSH due to altered activities or levels of GSH metabolites or components further upstream. As an additional note with regard to thiol-containing proteins, we have evidence demonstrating that metallothionein levels are elevated 100, 70 and 40% in dwarf kidney, liver and heart tissues, respectively, when compared to age-matched wild type mice (Meyer et al., 2003) suggesting that overall methionine metabolism is up-regulated in dwarf mice.

In stark contrast to the extended life span and enhanced oxidative stress resistance of the GH-deficient dwarf, there is significant evidence of premature aging derived from studies using transgenic mice that express pharmacological levels of plasma GH. In addition to the suppressed levels of antioxidative enzymes and increased oxidative damage (Rollo et al., 1996; Brown-Borg et al., 1999; Brown-Borg and Rakoczy, 2000; Hauck and Bartke, 2001), mice overexpressing GH have reduced replicative potential of cells in vitro (Pendergrass et al., 1993), and indications of premature central nervous system aging including reduced catecholamine turnover (Steger et al., 1993), increased astrogliosis (Miller et al., 1995) and impaired learning and memory (Meliska et al., 1997).

In summary, GH had no direct effect on GSH synthesis as detected by expression of the rate-limiting enzyme, GCS, in the liver but in kidney tissues, GH increased GCS protein levels. In addition, GST activity and hence the utilization of GSH was suppressed by GH. In heart tissue, GH suppressed both GSH synthesis (measured by GCS expression) and degradation enzymes (measured by GGT activity) resulting in a lack of hormone effects on GSH levels. In brain, GH did not affect synthesis of GSH but suppressed both the degradation and utilization of GSH which likely contributes to the higher GSH levels in brains of GH-treated mice.

The synthesis, recycling and degradation of GSH are crucial in maintaining appropriate GSH levels and GSH function. We have evidence suggesting that GH alters both MET and GSH metabolism. The methionine and glutathione pathways are intimately involved in the combat against oxidative stress. Growth hormone appears to be involved in the regulation of these pathways, and changing expression of enzymes, substrates and the redox potential of the tissues and the whole organism. Thus, we believe that GH status ultimately affects lifespan.

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