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Oxidative Stress in Rat Testis and Epididymis Under Intermittent Hypobaric Hypoxia: Protective Role of Ascorbate Supplementation

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ABSTRACT: Hypobaric hypoxia (HH), an environmental condition of high altitude encountered by mountaineers, miners, and observatory, rural health, border patrol, and rural education workers, jeopardizes normal physiologic functions in humans. The present study was conducted to evaluate the effects of intermittent HH (IHH; equivalent to 4600 m above mean sea level) on oxidative stress and the protective role of dietary ascorbic acid on rat testis and epididymis. Ten-week-old male Wistar rats were assigned to 1 of 6 groups: 1) normobaric (Nx), 2) Nx + physiologic solution (Nx + PS), 3) Nx + ascorbic acid (Nx + AA), 4) IHH, 5) IHH + PS, or 6) IHH + AA. Animals subjected to IHH were exposed for 96 hours followed by normobaric conditions for 96 hours for a total of 32 days. The control groups (2 and 5) were injected with doses of PS, and the treated groups (3 and 6) were injected with doses of AA (10 mg $\times$ kg^{-1} body weight) at an interval of 96 hours. Rats were sacrificed on day 32 after initiation of the protocol. The testis and epididymis were collected to determine the activity and expression of glutathione reductase and the levels of lipid peroxide formation. An epididymal sperm count was also performed in each animal. The results of this study revealed that IHH induced lipid peroxidation, a reduction in glutathione reductase activity in testis and epididymis, and a significant decrease in epididymal sperm count. Treatment with AA prevented these changes. In conclusion, AA was capable of decreasing oxidative stress in testis and epididymis under IHH. This protection by AA of the IHH-induced lipid peroxidation can be explained in part by the preservation of glutathione reductase activity in these organs.

Key words: Lipid peroxidation, glutathione reductase, ascorbic acid.

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It has been suggested that hypobaric hypoxia (HH) reduces fertility in humans. However, epidemiologic studies of high- and low-altitude populations have not been able to support this hypothesis (Vitzthum and Wiley, 2003; Bartsch et al, 2004). Previous publications from our laboratories showed that the exposure of male rats to continuous chronic HH and intermittent chronic HH induced evident changes in testicular morphology, loss of germinal cells, arrest of spermatogenesis, and metabolic stress in the mitochondria of round spermatids, consistent with oxygen-consumption processes related to lipid peroxidation (Farias et al, 2005b). Furthermore, high-altitude exposure has been shown to induce oxidative stress (Vats et al, 2008) that is accompanied by decreased levels of reduced glutathione (GSH) and ascorbic acid (AA) and by an increase in antioxidant enzymes like glutathione reductase (GR). Thus, a likely mechanism of HH-induced inhibition of spermatogenesis and sperm production (eg, Bustos-Obregon and Olivares, 1982; Farias et al, 2005b) can be related to oxidative stress in male reproductive organs.

Lipid peroxides have been implicated in decreased organ weight, tissue damage, cell loss, and cellular aging processes (Koksal et al, 2003; Voss and Siems, 2006). Antioxidants arrest these processes (Frei, 1999; Gilgun-Sherki et al, 2002) and prevent oxidation by inactivating free radicals or reactive oxygen species (ROS). AA or vitamin C is a water-soluble, nonenzymatic antioxidant that is able to react with aqueous free radicals and ROS and has the potential to protect both cytosolic and membrane components of cells from oxidative damage (Devi et al, 2007).

The glutathione system (and enzymatic antioxidant mechanism) plays an essential role in preventing oxidative damage in cells and tissues (Meister and Anderson, 1983). The levels of GSH are maintained by the action of GR (EC 1.8.1.7), which recycles oxidized

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glutathione (GSSG) to GSH using NADPH as the electron donor (Meister and Anderson, 1983). It has been reported that GR activity is affected by ROS and that AA is capable of preventing this effect (El-Missiry, 1999).

The present study, which was designed to understand the mechanisms associated with spermatogenic and sperm production impairment by HH, examined the effects of IHH on body weight, testis and epididymis weight, epididymal sperm count, lipid peroxidation and activity and expression of GR in rat testis and epididymis in the presence and absence of AA dietary supplementation. Whereas IHH induced a decrease in body weight and testis and epididymis weight, as well as a decrease in GR expression and activity, it induced an increase in lipid peroxidation. AA treatment played a protective role in oxidative stress processes in testis and epididymis under the IHH condition and maintained GR activity at levels similar to normobaric conditions.

Materials and Methods

Experimental Design

Ten-week-old male Wistar rats (225 ± 14 g; n = 36) from the University of Valparaiso Biotery were assigned to 1 of 6 groups (6 rats in each group): 1) normobaric conditions (Nx), 2) Nx + physiologic NaCl solution (Nx + PS), 3) Nx + AA, 4) intermittent HH (IHH), 5) IHH + PS, and 6) IHH + AA. A 3 ×2-factorial experimental design was used considering 3 injection treatments (no injection, PS, and AA) and 2 environmental treatments (Nx and IHH). The animals were injected (intraperitoneally) with doses of AA (10 mg × kg^{-1} body weight) or vehicle (0.1 mL of PS) at intervals of 96 hours. The dose of AA was that described by Acharya et al. (2008) that had significantly inhibited lipid peroxidation induced by $CdCl_2$ in rat testicles. The IHH group of animals were exposed to HH for 96 hours (428 torr; PO_2 89.6 mm Hg) followed by the Nx condition for 96 hours (96 hours of hypoxia/96 hours of normoxia) for a total period of 32 days. Pressure changes in the hypobaric chamber were achieved by steps of 150 mm Hg per minute, which simulated altitude changes. The Nx animals were housed in the same room next to the IHH animals (22 ± 2°C, 15 g of food pellet per day, and 1 L of water per cage). All procedures were performed in agreement with the Principles of Laboratory Animal Care, advocated by the National Society of Medical Research, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Animal Laboratory Resources, 1996)

Hematocrit

Blood samples were obtained by cardiac puncture of the left ventricle. Hematocrit was determined by centrifugation of a capillary tube with heparinized blood in a microhematocrit centrifuge (IEC model MB; GSR Technical Sales, Edmondton, Alberta, Canada).

Determination of Thiobarbituric Acid–Reactive Substances

Body weight was determined 32 days after initiation of the protocol. The animals were then killed by cervical dislocation. The testis and epididymis were collected in phosphate-buffered saline (PBS; pH 7.2). Determination of thiobarbituric acid–reactive substances (TBARS) was undertaken as an index of lipid peroxidation (Magalhães et al, 2005). TBARS were estimated at 532 nm, and their concentrations were calculated using a molar extinction coefficient of 1.56 × 10^5 M^{-1} cm^{-1} obtained utilizing malondialdehyde (MDA; Sigma-Aldrich, St Louis, Missouri) as a standard. The results were expressed as nmol of MDA equivalents/mg tissue.

Isolation and Sperm Count

Epididymal spermatozoa were separated by cutting the caudal epididymis into segments of approximately 1 mm^3 with a sharp razor blade in 1 mL of PBS (pH 7.2). Spermatozoa from caudal regions were completely removed by vortexing gently in PBS, and the tissue debris was allowed to settle for 5 minutes.

Spermatozoa released in the buffer were aspirated, centrifuged at 800 × *g* for 15 minutes, and used for biochemical determinations. All of these procedures were completed at 4°C. The number of sperm in the suspension was counted using a Neubauer chamber.

Preparation of Tissue Homogenates and Protein Assay

Testis and epididymis tissues were homogenized in 0.5 mL of extraction buffer (Tris 50 mM, NaCl 100 mM, EDTA 1 mM, EGTA 2.5 mM, Tween-20 0.1% [pH 7.4], phenylmethylsulfonyl fluoride [PMSF] 100 µg/mL; Sigma-Aldrich) with a Potter homogenizer (Glass-Col K4424; Glas-Col, Terre Haute, Indiana) at .050301 × *g*. The samples were then centrifuged at 7820 × *g* for 30 minutes at 4°C. Protein concentrations were determined using the Coomassie blue method (Bradford, 1976).

SDS-PAGE and Western Blot Analysis

Protein samples were subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to Hybond-C (Amersham Pharmacia, Piscataway, New Jersey) under semidry conditions by means of a Trans-Blot SD Semi-Dry Transfer Cell (Bio-Rad, Tokyo, Japan). The membranes were then blocked by incubation with 5% skimmed milk in PBS (pH 7.2) for 1 hour at room temperature. Subsequently, the membranes were incubated with rabbit anti–rat GR Ig (1:500 dilution) (Santa Cruz Biotechnology, Santa Cruz, California) for 12 hours at 4°C. After washing with PBS (pH 7.2) containing 0.05% Tween-20, the membranes were incubated with 1:1000 diluted peroxidase-conjugated goat anti–rabbit IgG Ig (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania) for 2 hours. After washing, peroxidase activity was detected by a chemiluminescence method using an ECL Plus kit (Amersham Pharmacia). An anti–β-tubulin antibody was used as a protein-loading control in Western blots. The immunoblots were analyzed with ImageJ software (http://rsb.info.nih.gov/ij).

Enzyme Assay

GR activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm. The reaction mixture consisted of 0.2 M potassium phosphate (pH 7.3; Merck KGaA, Darmstadt, Germany), 3.9 mM NADPH (Sigma-Aldrich), 20 mM GSSG (Merck KGaA), and tissue homogenates. The decrease in absorbance at 340 nm at 21°C was recorded as a function of time. The control reaction mixture contained buffer instead of NADPH. One unit of GR activity was defined as the amount of enzyme that catalyzed the oxidation of 1 μmol of NADPH per minute (Carlberg and Mannervik, 1985). All assays were performed in triplicate, and means ± SE are reported.

Statistical Analysis

The results were analyzed using a 2-way analysis of variance (ANOVA) test followed by Bonferroni analysis. Two-way ANOVA was performed to determine the presence of a significant interaction between injection (AA) and environment because this would indicate a different effect of AA under IHH in comparison to Nx. The Kruskal-Wallis test (nonparametric) was used to analyze hematocrit results. The level of statistical significance was set at $P < .05$ for all tests. The data were analyzed using the GraphPad Prism software version 4.0 (GraphPad Software, San Diego, California). The results are presented as means ± SE.

Results

Body, Testicular, and Caudal Epididymis Weight in IHH

According to the 2-way ANOVA on body-weight values (Figure 1), the interaction between environment and injection (AA) was significant ($P < .001$) (ie, AA had a different effect on body weight in the IHH condition compared with the Nx condition). Also, IHH significantly affected the body-weight results ($P < .0001$).There was a significant difference ($P < .01$) between IHH + AA with respect to IHH + PS and IHH, and there were no significant differences in the rest of the comparisons within hypobaric and Nx groups. Complementary analysis (1-way ANOVA; data not shown) showed that there was a significant difference ($P < .05$) in body weight between the Nx vs IHH groups (no injection) and no significant difference ($P > .05$) between the IHH + AA group vs all Nx groups. Treatment with AA abolished the effects of decreased rat body weight induced by hypoxia. The loss in body weight of hypoxic rats observed in this study (approximately 1.5 g/d in hypoxic animals without AA treatment) was not expected to give rise to significant reproductive organ weight loss in adult animals (eg, Lanning et al, 2002; Fleeman et al, 2005). However, because of a possible correlation between both parameters, the data for testis and epididymis weight were normalized to body weight for each animal (Table 1).

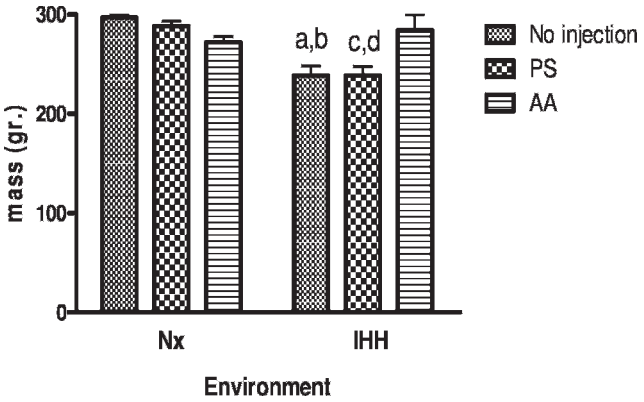

Figure 1. Effect of IHH and AA on body weight. Statistical analysis: 2-way analysis of variance followed by Bonferroni analysis. Nx indicates normobaric conditions; PS, physiologic NaCl solution; AA, ascorbic acid; IHH, intermittent hypobaric hypoxia; a, $P < .05$, IHH vs Nx; b, $P < .05$, IHH vs IHH + AA; c, $P < .05$, IHH + PS vs Nx; d, $P < .05$, IHH + PS vs IHH + AA.

Testis and epididymis relative weight analysis (Table 1) showed that the interaction between environment and injection was not significant ($P > .05$; ie, the way that AA affects Nx and IHH conditions is similar); interestingly, the environment significantly affected only the testis results ($P < .05$; ie, there was a testicular mass loss induced by IHH). There was no significant difference ($P > .05$) between hypobaric and Nx groups.

Rat Hematocrit in IHH and Nx Groups

The hematocrit was significantly greater in all of the HH groups in comparison with the Nx groups ($P < .05$) after 32 days of IHH. There were no significant differences between groups treated with AA and their respective IHH and control groups ($P > .05$) (Table 2), indicating that the IHH condition effectively triggered the characteristic hypoxia-induced increase in red blood cell production and that AA did not affect this physiologic response to hypoxia.

Oxidative Stress in Testis and Epididymis

The analysis of TBARS formation in testis and epididymis (Figure 2) showed that the interaction between environment and injection was significant ($P < .05$) in both testis and epididymis; this indicates that AA has a different effect on lipoperoxidation under IHH conditions compared with Nx conditions. Also, IHH significantly affected the results ($P < .0001$). In testis and epididymis, there was a significant difference between IHH + AA with respect to IHH + PS and IHH ($P < .01$ and $P < .01$, respectively), and there were no significant differences in the rest of the comparisons within the hypobaric and Nx groups. Complementary

Table 1. *Effect of IHH and AA on testis and epididymis*[a]

Group	Right testis, %	Left testis, %	Right epididymis, %	Left epididymis, %
Nx	.55 ± .00	.54 ± .00	.10 ± .00	.11 ± .01
Nx + PS	.51 ± .02	.52 ± .01	.09 ± .01	.09 ± .00
Nx + AA	.54 ± .02	.54 ± .03	.09 ± .01	.09 ± .01
IHH	.50 ± .02	.50 ± .02	.08 ± .01	.09 ± .01
IHH + PS	.47 ± .01	.47 ± .02	.08 ± .01	.09 ± .01
IHH + AA	.50 ± .01	.50 ± .02	.09 ± .01	.09 ± .00

Abbreviations: AA, ascorbic acid; IHH, intermittent hypobaric hypoxia; Nx, normobaric conditions; PS, physiologic NaCl solution.
[a] Values indicate the mean ± SE of N = 6. Testis and epididymis mass values are expressed as percentages. Statistical analysis: 2-way analysis of variance followed by Bonferroni analysis. No significant difference was found in the normobaric and hypobaric group comparisons.

analysis (1-way ANOVA; data not shown) showed that there was a significant difference ($P < .05$) in lipid peroxide formation between the Nx vs IHH (no injection) groups and no significant difference ($P > .05$) between IHH + AA vs all Nx groups. These results strongly suggest a protective role of AA in animal epididymis lipid peroxidation that results from exposure to IHH (Figure 2B). Thus, in a pattern that resembles the results observed in body weight and testicular and epididymal weight, AA treatment decreased TBARS content in both testis and epididymis. Figure 2 indicates that under both Nx and IHH, the levels of lipid peroxidation were higher in testis than in epididymis. Only under IHH did the lipid peroxidation reach values that were not significantly different in both organs. However, the significance level was against a possible interpretation that saline injection affected the levels of lipid peroxidation in any of these 2 organs.

Caudal Epididymis Sperm Count

Sperm count values analysis (Figure 3) showed that the interaction between environment and injection was not significant ($P > .05$), indicating that under Nx and IHH conditions, AA affects the sperm count similarly. Also, IHH significantly affected the sperm count values ($P < .0001$). There was a significant difference between IHH + AA with respect to IHH + PS ($P < .01$) and IHH ($P < .001$), and there were no significant differences in the rest of the comparisons within hypobaric and Nx groups. Complementary analysis (1-way ANOVA; data not shown) showed that there was a significant difference ($P < .05$) between the Nx vs IHH (no injection) groups and no significant difference ($P > .05$) between IHH + AA groups vs all Nx groups. Hence, IHH did not produce any significant decline in the sperm count in IHH animals treated with AA compared with Nx groups.

Expression of GR in Testis and Epididymis

The interaction between environment and injection in testis was not significant ($P > .05$), and environmental treatment did not significantly affect the relative expression values ($P > .05$). No significant differences were found in testis among the different groups ($P > .05$; Figure 4A). In epididymis, the interaction between environment and injection was significant ($P < .05$), and environmental treatment significantly affected the values ($P < .05$), indicating an activating effect of IHH on epididymis GR expression. Also, there was a significant difference ($P < .01$) between IHH + AA with respect to IHH, strongly suggesting that AA was able to inhibit the IHH-induced GR expression in epididymis ($P < .01$; Figure 4B). No significant differences ($P > .05$) were observed in the rest of the comparisons within hypobaric and Nx groups. Complementary analysis (1-way ANOVA; data not shown) showed that there was a significant difference ($P < .05$) between the Nx vs IHH (no injection) and no significant difference ($P > .05$) between IHH + AA vs all Nx groups.

Activity of GR in Testis and Epididymis

The interaction between environment and injection in testis and epididymis was not significant ($P > .05$), but environmental treatment significantly affected the relative expression values ($P < .05$). AA groups were significantly different with respect to PS and no injection groups (Figure 5A and B) in both the Nx and IHH treatment groups. Complementary analysis (1-way ANOVA; data not shown) showed that there was a

Table 2. *Effect of IHH and AA on hematocrit*[a]

Groups	Hematocrit, %
Nx	36 ± 2
Nx + PS	38 ± 1
Nx + AA	37 ± 2
IHH	50 ± 2[b]
IHH + PS	49 ± 2[b]
IHH + AA	51 ± 3[b]

Abbreviations: AA, ascorbic acid; IHH, intermittent hypobaric hypoxia; Nx, normobaric conditions; PS, physiologic NaCl solution.
[a] Values indicate the mean ± SD of N = 6. Statistical analysis: Kruskal-Wallis test (nonparametric) was used to analyze hematocrit values.
[b] $P < .05$ vs Nx.

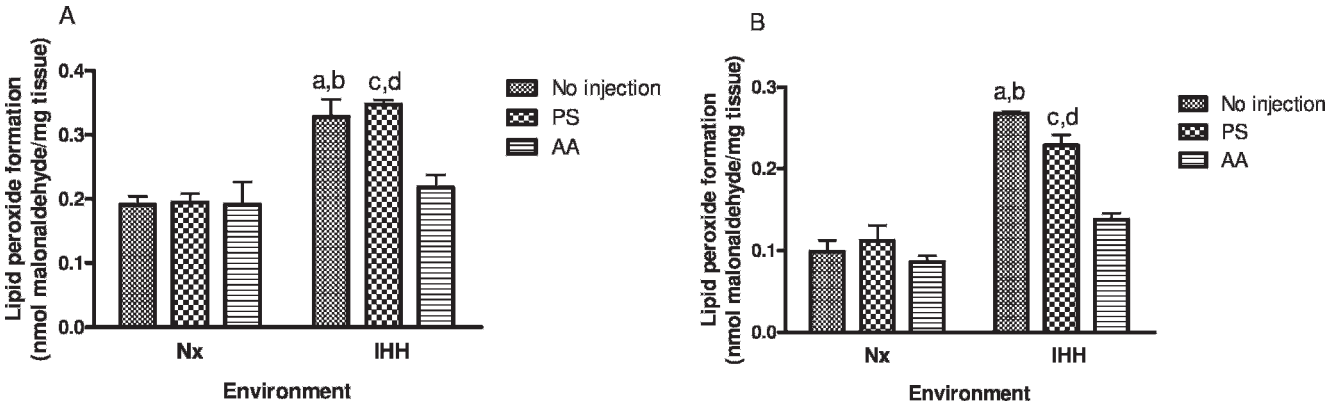

Figure 2. Effect of IHH and AA on thiobarbituric acid–reactive substances formation (nmol of malondialdehyde/mg of tissues). **(A)** Testis. **(B)** Epididymis. The bars indicate the mean ± SD of N = 6. Statistical analysis: 2-way analysis of variance followed by Bonferroni test. Nx indicates normobaric conditions; PS, physiologic NaCl solution; AA, ascorbic acid; IHH, intermittent hypobaric hypoxia; a, $P < .05$, IHH vs Nx; b, $P < .05$, IHH vs IHH + AA; c, $P < .05$, IHH + PS vs Nx; d, $P < .05$, IHH + PS vs IHH + AA.

significant difference ($P < .05$) between the Nx vs IHH (no injection) groups. The GR activity under IHH + AA conditions in testis and epididymis suggests a protective role of AA in animals exposed to IHH through the maintenance of GR activity and lipid peroxidation formation levels similar to those in the Nx condition.

Discussion

Previous results showed that high-altitude exposure induced a decrease in testicular function that appeared to be related to both direct early effects on spermatogenesis and later effects on the hypophysis-gonad hormonal axis (Farias et al, 2005a, 2008). The molecular mechanisms associated with these changes are not known. Hypoxia in general, and specifically HH, is known to induce oxidative stress in animal models and humans (Radak et al, 1994; Joanny et al, 2001, Askew, 2002; Jefferson et al, 2004; Magalhães et al, 2004; Behn et al, 2007; Vats et al, 2008), suggesting that ROS can be involved in the changes in testicular function and spermatogenesis observed in rats under HH conditions.

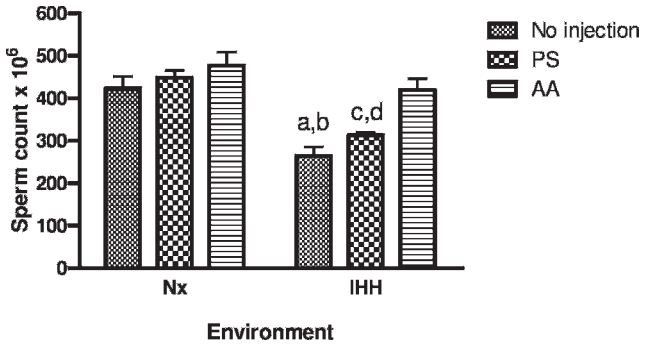

Figure 3. Effect of IHH and AA on caudal epididymis sperm count. The bars indicate the mean ± SD of N = 6. Statistical analysis: 2-way analysis of variance followed by Bonferroni analysis. Nx indicates normobaric conditions; PS, Nx physiologic NaCl solution; AA, ascorbic acid; IHH, intermittent hypobaric hypoxia; a, $P < .05$, IHH vs Nx; b, $P < .05$, IHH vs IHH + AA; c, $P < .05$, IHH + PS vs Nx; d, $P < .05$, IHH + PS vs IHH + AA.

In this study, we showed that AA did not affect the polycythemic response to HH, indicating that the systemic hypoxic condition was present in all of the IHH groups. Our results also showed that the body weight was significantly lower in the intermittent IHH groups than in Nx groups after 32 days of IHH, whereas the body weight of IHH groups treated with AA were similar to control rats after 32 days. Hence, considering that body and organ weights are the result of both anabolic and catabolic processes, it can be argued that AA was able to counteract some of the effects of hypoxia on metabolic balance in rats.

As previously described for sperm count from human ejaculates (Bustos-Obregon E and Olivares. 1982), there was a decrease in the number of epididymal sperm in the animals subjected to IHH in comparison with Nx groups. This effect of IHH on epididymal sperm count was counteracted by AA, which normalized the sperm count levels to those of Nx animals.

Our results indicate that IHH induced testicle and epididymis lipid peroxidation (TBARS production). Furthermore, such organs had higher lipid peroxidation values under IHH than those of rats treated with AA in the same hypoxic conditions, indicating that AA was effectively acting by increasing antioxidant mechanisms in these organs and IHH animals.

In the present study, we found different levels of GR expression among the IHH groups, and the level of GR expression was higher in the epididymis than in the testis. There were no significant differences in GR protein levels among the different groups (Nx, Nx + PS,

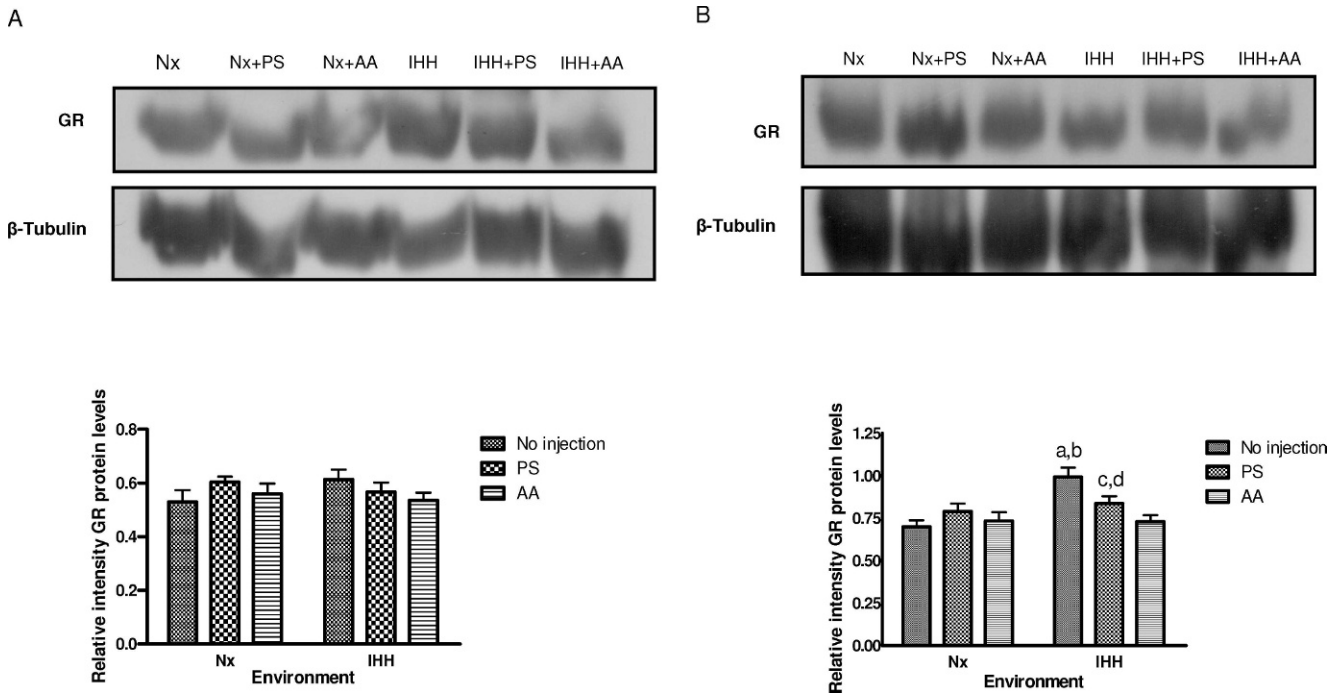

Figure 4. Effect of IHH and AA on expression of glutathione reductase (GR). (**A**) Testis. **(B)** Epididymis. The bars indicate the mean ± SD of N = 3. Statistical analysis: 2-way analysis of variance followed by Bonferroni analysis. Nx indicates normobaric conditions; PS, physiologic NaCl solution; AA, ascorbic acid; IHH, intermittent hypobaric hypoxia; a, $P < .05$, IHH vs Nx; b, $P < .05$, IHH vs IHH + AA; c, $P < .05$, IHH + PS vs Nx; d, $P < .05$, IHH + PS vs IHH + AA.

Nx + AA, IHH, IHH + PS, and IHH + AA) in the testis. In contrast, the levels of GR in the epididymis in the IHH group were significantly higher in comparison with those of the Nx group. These data suggest that in epididymis, the oxidative stress induced by IHH is capable of triggering an up-regulation of GR expression levels as a likely protective mechanism in response to increases in ROS production (Vats et al, 2008). In other organs such as brain (Maiti et al, 2006), HH appears to decrease antioxidant mechanisms. In testis, the expression level and activity of the protein were not modified by IHH.

Although this response mechanism was not observed in testis, these data are in agreement with previous reports, supporting the notion that the GR up-regulation induced by ROS to prevent oxidative damage is less important in testis than in epididymis (Ikeda et al, 1999). This difference is attributed to the fact that in the testicle, arrest of the spermatogenic cycle is triggered as

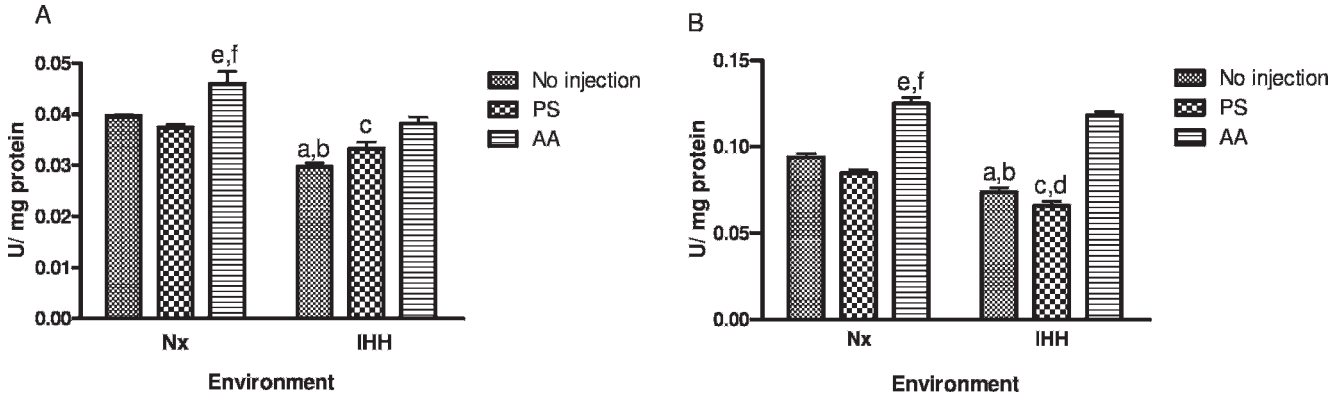

Figure 5. Effect of IHH and AA on glutathione reductase (GR) activity. **(A)** Testis. **(B)** Epididymis. Values indicate mean ± SD of N = 6. Statistical analysis: 2-way analysis of variance followed by Bonferroni analysis. Nx indicates normobaric conditions; PS, physiologic NaCl solution; AA, ascorbic acid; IHH, intermittent hypobaric hypoxia; a, $P < .05$, IHH vs Nx; b, $P < .05$, IHH vs IHH + AA; c, $P < .05$, IHH + PS vs Nx; d, $P < .05$, IHH + PS vs IHH + AA; e, $P < .05$, Nx + AA vs Nx; f, $P < .05$, Nx + AA vs Nx + PS.

an environmental protection measure, preventing the generation of defective sperm and thus, a loss of testicular mass (Table 1); in contrast, in epididymis, the antioxidant response is much more important in protecting the sperm.

Treatment with AA caused GR expression in IHH to reach similar values as Nx in epididymis, suggesting that by decreasing ROS production, AA supplementation was capable of preventing the GR up-regulation mechanism. Consistent with the results of GR expression, we observed that GR activity was significantly higher in epididymis than in testis, both under IHH and Nx conditions (Kaneko et al, 2002). Additionally, AA induced an increase in activity of GR under both Nx and IHH conditions. These results are in agreement with the notion that in testis, other antioxidant mechanisms besides those that are glutathione dependent can also be active (Ikeda et al, 1999). Our results demonstrating greater GR activity in epididymis than in testis confirmed previously described data published by Kaneko et al. (2002). Because AA does not significantly affect lipoperoxidation in the testis and epididymis under Nx conditions, the GR activity data that show activation by AA under Nx and IHH conditions strongly suggest that AA could be preventing lipoperoxidation in IHH by activating GR and that the oxidative stress by IHH is well balanced by the glutathione antioxidant system in both organs but might differ in its initiating mechanisms from those oxidative processes that give rise to Nx lipoperoxidation. No information in the literature was found on the possible mechanisms by which AA could activate GR activity in Nx testis and epididymis homogenates (El-Missiry, 1999).

Thus, our results indicated that AA alone can counteract the effects of hypoxia on body, testicular, and epididymal mass; protect against testicular and epididymal lipid peroxidation; and preserve epididymal sperm count.

These results may have important practical consequences for those people subjected to IHH, such as sea-level natives exposed to high altitude because of tourism, border patrol, mining, or rural health and education activities. Based on our results, it is expected that AA treatment could counteract the deleterious effects of HH on spermatogenesis and sperm count observed in animal models and humans (as described in this article; Gasco et al, 2003; Okumura et al, 2003; Farias et al, 2005b).

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