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Expression and functional activity of pro-oxidants and antioxidants in murine heart exposed to acute hypobaric hypoxia

Jayashree Karar[a,b,1], Karamjit S. Dolt[a,c,1], Manoj K. Mishra[a], Ehtesham Arif[a], Saleem Javed[c], M.A. Qadar Pasha[a,b,c,*]

[a] *Institute of Genomics and Integrative Biology, Mall Road, Delhi 110 007, India*
[b] *Department of Biotechnology, University of Pune, Pune, India*
[c] *Department of Biochemistry, Jamia Hamdard, New Delhi, India*

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Abstract Under hypobaric hypoxia, antioxidant defenses of the heart are stressed by the enhanced production of ROS. Mammalian heart acclimatizes to hypoxia through altered gene expression, which we studied in murine heart exposed to 10 h of acute hypobaric hypoxia (AHH), equivalent to 15000 ft, using cDNA arrays. Functional classification of genes with a ⩾2-fold change revealed a number of pro-oxidants like Cyba, Xdh, Txnip, Ppp1r15b and antioxidants like Cat, Gpx1, Mt1, Mgst1. Interestingly, the protein level of Cyba, a subunit of NADPH oxidase, was markedly decreased in AHH exposed heart, suggesting the involvement of some stress response pathways. The AHH exposure also caused a significant reduction (50%) in the level of GSH ($P < 0.05$). The present study provides a retrospective insight on the cellular antioxidant defense mechanisms under AHH.
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Keywords: High-altitude; Acute hypobaric hypoxia; Reactive oxygen species; Oxidative stress; Lipid peroxidation

1. Introduction

The environment at high-altitude (HA) is extreme, characterized by hypobaric hypoxia. At HA, less oxygen is available to terminally accept electrons from oxidative phosphorylation. Hypoxia partially inhibits mitochondrial electron transport, producing redox changes in the electron carriers and increase the generation of reactive oxygen species (ROS) at mitochondrial complex III [1,2]. ROS are oxygen molecules in different states of oxidation or reduction, as well as, compounds of oxygen with hydrogen and nitrogen. Oxygen is central to the generation of ROS, several of which play vital roles in vascular physiology and pathophysiology; the most important of which are nitric oxide ($NO^{\bullet}$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^{\bullet-}$) [3,4]. ROS-linked injury is mainly due to hydroxyl radical ($OH^{\bullet-}$), produced by the Fenton reaction, i.e. $H_2O_2 + Fe^{2+} \rightarrow OH^{\bullet-} + OH^- + Fe^{3+}$ [5]. Under aerobic conditions, most of free iron ions are in the oxidized form (Fe^{3+}). However, under anaerobic conditions Fe^{2+} autoxidation ceases and Fe^{3+} is immediately reduced to Fe^{2+}. The appearance of Fe^{2+} becomes dangerous as re-oxygenation activates the Fenton reaction and results in $OH^{\bullet-}$ formation [6].

In acute HA exposure, current evidence from diverse studies of both human and animal models indicates that hypoxia defenses are initiated by several oxygen-sensing and signal transduction pathways [7]. Mammals have evolved a sophisticated physiological network to maintain oxygen homeostasis at the tissue level that involves the capture, binding, transport and delivery of molecular oxygen [8]. Since the mammalian heart is an obligate aerobic organ, a constant supply of oxygen is indispensable to sustain cardiac function and viability [9]. When the supply fails to match myocardial demand cardiac contractile dysfunction occurs, and prolongation of this mismatch may lead to apoptosis and necrosis [10].

Gene expression studies of mouse heart after chronic constant or intermittent hypoxia have been explored [11]; however, effects of acute hypobaric hypoxia (AHH) on murine heart have been explored for the first time in this study. The changes in gene expression in heart of mice exposed to 10 h of hypobaric hypoxia were evaluated by cDNA arrays and validated by real time PCR. The outcome was further confirmed at post-translational level by immunoblot. The specific activity of antioxidant enzymes, lipid peroxidation (LPO) and reduced glutathione (GSH) levels were also assayed in the hypoxic heart. Our results revealed the effect of AHH on the transcript and protein levels of pro-oxidants and antioxidants. The level of reduced glutathione was found to be significantly decreased which may be the consequence of reduced synthesis along with its increased utilization.

2. Materials and methods

2.1. Exposure of mice to hypobaric hypoxia and RNA isolation

The study was carried out on male Swiss albino mice (8-week-old) housed in clean cages with food and water available *ad libitum*. Five mice were exposed for a period of 10 h to a simulated altitude of 4570 m (∼15000 ft) by maintaining the pressure in the decompression chamber at 426 mm Hg. Equal numbers of control mice were maintained at normal atmospheric pressure (∼760 mm Hg) for 10 h in the

*Corresponding author. Address: Institute of Genomics and Integrative Biology, Mall Road, Delhi 110 007, India. Fax: +91 011 27667471. *E-mail address:* qpasha@igib.res.in (M.A. Qadar Pasha).

[1] These authors made equal contributions.

Abbreviations: HA, high-altitude; ROS, reactive oxygen species; AHH, acute hypobaric hypoxia; LPO, lipid peroxidation; MDA, malonaldehyde

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same laboratory. The temperature for both the groups was maintained at 25 ± 2 °C. At the end of the exposure the mice were sacrificed by cervical dislocation. The hearts were removed and parts of it were processed immediately for total RNA isolation using TRIzol reagent (Invitrogen, USA). The animal ethics committee of the institute approved all the animal procedures.

2.2. Microarray experiments

Equal amount of total RNA was pooled from the five mice to give a total of 6 μg for each group. Pooled total RNA was labeled using the Micromax NEN TSA labeling kit (Perkin-Elmer Life Sciences, USA). Fluorescein and Biotin labeled cDNA probes were synthesized and purified using isopropyl alcohol precipitation. The cDNA probes were mixed and simultaneously hybridized to 15 k mouse cDNA arrays (The Microarray Center, Clinical Genomics Center, University Health Network, Toronto, Canada) in an overnight incubation at 65 °C. The 15 k double-spotted array contained 15264 sequence-verified mouse ESTs procured from the National Institute of Aging (NIA). The probes were detected using streptavidin and anti-fluorescein antibody conjugated to horse-radish peroxidase that catalyzes the deposition of Cy5 and Cy3 labeled tyramide, respectively. The microarrays were scanned and image analysis was performed using GenePix Professional 4200A scanner. GenePix result files (.gpr) were then transferred to TIGR Express Converter to generate TIGR MultiExperiment Viewer file (.mev) as output. Data normalization (lowess), flagging, filtering and flip dye consistency check was performed using TIGR MIDAS. Normalized and filtered expression files were analysed using TIGR MeV [12]. Genes showing consistency between dye swap experiments and upregulated or downregulated at and more than twofold were selected. Onto-Express [13] was used to functionally classify the selected genes according to the following Gene-Ontology (GO) categories: biological process; cellular role; and molecular function.

2.3. Real time PCR

Total RNA as pooled above was reverse transcribed to yield single strand cDNA using First-Strand cDNA Synthesis kit (Amersham Biosciences, UK). Primers for real time PCR were designed for the selected genes using the Primer Express™ software (Perkin-Elmer Applied Biosystems) and are listed in Supplementary Table S1. Real time PCR was performed on ABI Prism 7300 Sequence Detection System (Applied Biosystems, USA) using SYBR Green PCR Master Mix (Applied Biosystems) as described previously [14]. The PCR reactions were performed in triplicate for each gene. Relative transcript quantities were calculated using the $\Delta\Delta C_t$ method with β-actin (ACTB) as the endogenous reference gene amplified from the samples.

2.4. Biochemical characterization of AHH exposed heart

2.4.1. Preparation of tissue samples. Portions of hearts were rinsed in ice cold PBS (pH 7.4), weighed and a 10% (w/v) tissue homogenate was prepared in ice-cold phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged and the resulting supernatant was used for all the bioassays. Protein content was quantified by Bradford assay (Bio-Rad Laboratories, CA) with BSA as the standard. All the assays were performed on SpectraMax Plus 384 spectrophotometer (Molecular Devices, USA). Intra- and inter-assay coefficients of variation were less than 10%.

2.4.2. Lipid peroxidation assay. LPO was assayed based on the method of Wright et al. [15]. The amount of malonaldehyde (MDA) formed in each of the samples was measured in the supernatant at 535 nm. The amount of MDA formed was expressed as nmol MDA formed/h/g tissue at 37 °C.

2.4.3. Estimation of enzyme activities. Superoxide dismutase (Sod) activity was assayed according to the method of Marklund and Marklund [16]. Changes in the absorbance were recorded at 420 nm. In brief, the inhibition of autoxidation of pyrogallol was measured as a function of time. The amount of enzyme required to obtain 50% inhibition was considered equivalent to one unit of Sod activity. Catalase (Cat) activity was assayed by the method of Claiborne [17]. Change in absorbance was recorded at 240 nm and enzyme activity was calculated in terms of μmol H_2O_2 consumed/min/mg protein. Glutathione peroxidase (Gpx) activity was assayed based on the method of Mohandas et al. [18]. Oxidation of NADPH was recorded at 340 nm at 30 s intervals for 3 min and the enzyme activity was expressed as nmol NADPH oxidized/min/mg protein. Glutathione reductase (Gsr) activity was quantitated by measuring the disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/min/mg protein [18]. Glutathione *S*-transferase activity was assayed according to the method of Habig et al. [19]. The changes in absorbance were recorded at 340 nm and enzyme activity was calculated as nmol CDNB conjugate formed/min/mg protein.

2.4.4. Reduced glutathione and total thiol estimation. GSH content was measured by the method of Jollow et al. [20]. The absorbance was measured at 412 nm. GSH content was calculated and expressed as nmoles of GSH/ mg of sample protein. The total thiol (TSH) content in the tissue homogenate was determined using the method of Sedlak and Lindsay [21] and expressed as μmol of thiol/mg of protein.

2.5. Western blotting

Tissues were ground in liquid nitrogen, sonicated in lysis buffer (7 M urea, 2 M thiourea, 2% DTT, 4% CHAPS and protease inhibitor cocktail) and centrifuged to obtain supernatant. The protein content in the supernatant was determined by Bradford assay (Bio-Rad Laboratories, CA). 50 μg of each protein sample was loaded and resolved on 15% SDS–PAGE and transferred to Hybond ECL membrane (Amersham Bioscience). The membrane was blocked with 5% (w/v) nonfat milk at room temperature for 2 h and incubated with rabbit polyclonal antibody against Cyba (1:1000, Santa Cruz Biotechnology Inc., USA), Sod1 (1:5000, Santa Cruz Biotechnology Inc.), Hmox1 (1:2000, Stressgen, USA) and mouse polyclonal antibody against α-tubulin (1:1000, Santa Cruz Biotechnology Inc.) at room temperature for 1 h. After washing with phosphate buffered saline with 0.1% Tween 20 (PBS-T), the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit antibody (1:20000, Sigma, USA) and anti-mouse antibody (1:5000, Sigma) at room temperature for 1 h. After washing in PBS-T, the bands were visualized with ECL-Plus kit (Amersham Bioscience) and 4-chloro-1-naphthol (for α-tubulin) in accordance with the manufacturer's instructions.

2.6. Statistical analysis

Statistical analysis was carried out using one-way ANOVA and P values less than 0.05 were regarded as significant.

3. Results and discussion

3.1. Differential expression analysis

The expression profile of the murine heart exposed to 10 h of hypobaric hypoxia revealed a large number of differentially regulated genes. However, only those genes that passed the quality control criteria and showed consistency in the experiments were selected. Further, using 2-fold change as a threshold we found 268 upregulated genes and 292 downregulated genes. We used ontology-based tools to assign functional category to the entire list of 560 genes (Supplementary Table S2). Interestingly, the Onto-Express output revealed a number of genes involved in oxidative stress to be differentially regulated under the hypoxic conditions. We have also found a 2.21-fold increase in the transcription of hypoxia inducible factor 1, alpha subunit (Hif1a), the oxygen-regulated subunit of Hif1 transcription factor that regulates the transcription of genes involved in oxygen homeostasis in response to hypoxia. Additionally, genes involved in methionine metabolism and taurine metabolism too were noticeably elevated. Few of the microarray results were validated by real time PCR (Table 1). The trend of expression profile of the genes was same as those obtained by microarray analysis, although, the amplitude of change differed between the two methods. The role of the analyzed genes in ROS generation and its utilization has been constructed with the help of GenMAPP [22] software (Fig. 1). Based on our findings an attempt has been made to segregate the genes according to their function and compare with the available literature.

Table 1
Comparison of fold change in AHH versus control mice obtained from microarray and real time PCR experiments

Gene symbol	Gene name	Fold change	
		Microarray	Real time[b]
Cyba	Cytochrome *b*-245, alpha polypeptide	−2.2	−1.2
Hmox1	Heme oxygenase 1	–[a]	1.9
Cat	Catalase	4.1	1.8
Sod1	Superoxide dismutase 1	1.7	1.2
Mat1a	Methionine adenosyltransferase I, alpha	26.2	139.3
Cdo1	Cysteine dioxygenase 1	12.0	3.7

[a]Flagged off in microarray analysis, i.e. spot data was excluded in the array data analysis.
[b]Real time PCR data represent the mean fold change.

3.2. Cellular pro-oxidants and ROS generators

3.2.1. Cyba, Xdh/XO and Ppp1r15b. The AHH exposure led to >2-fold increase in the transcript levels of protein phosphatase 1, regulatory (inhibitor) subunit 15b (Ppp1r15b) and xanthine dehydrogenase (Xdh) and ∼2-fold decrease in the transcript levels of cytochrome *b*-245, alpha polypeptide (Cyba), as revealed by the microarray results.

Cardiac tissues are rich source of ROS and the enzymatic sources capable of generating $O_2^{\cdot-}$ in the vascular tissues include NADPH oxidase and xanthine oxidase (XO). Cyba is an essential subunit of NADPH oxidase, an enzyme considered to be a major source of $O_2^{\cdot-}$ in cardiac cells catalyzing the one-electron reduction of oxygen using NADH or NADPH as the electron donor. Like the phagocytic NADPH oxidases, the vascular NADPH oxidases are multicomponent enzymes composed of two membrane-associated proteins (Nox homologues and Cyba) and three cytosolic subunits [23]. Although real time PCR analysis of Cyba showed no change (1.25-fold) in the transcript levels (Table 1), we observed marked decrease in the protein levels of Cyba in the AHH exposed heart (Fig. 2). A study has shown that induction of heme oxygenase 1 (Hmox1), the rate-limiting enzyme of heme degradation leads to decreased protein expression of cytochrome *b*-245,

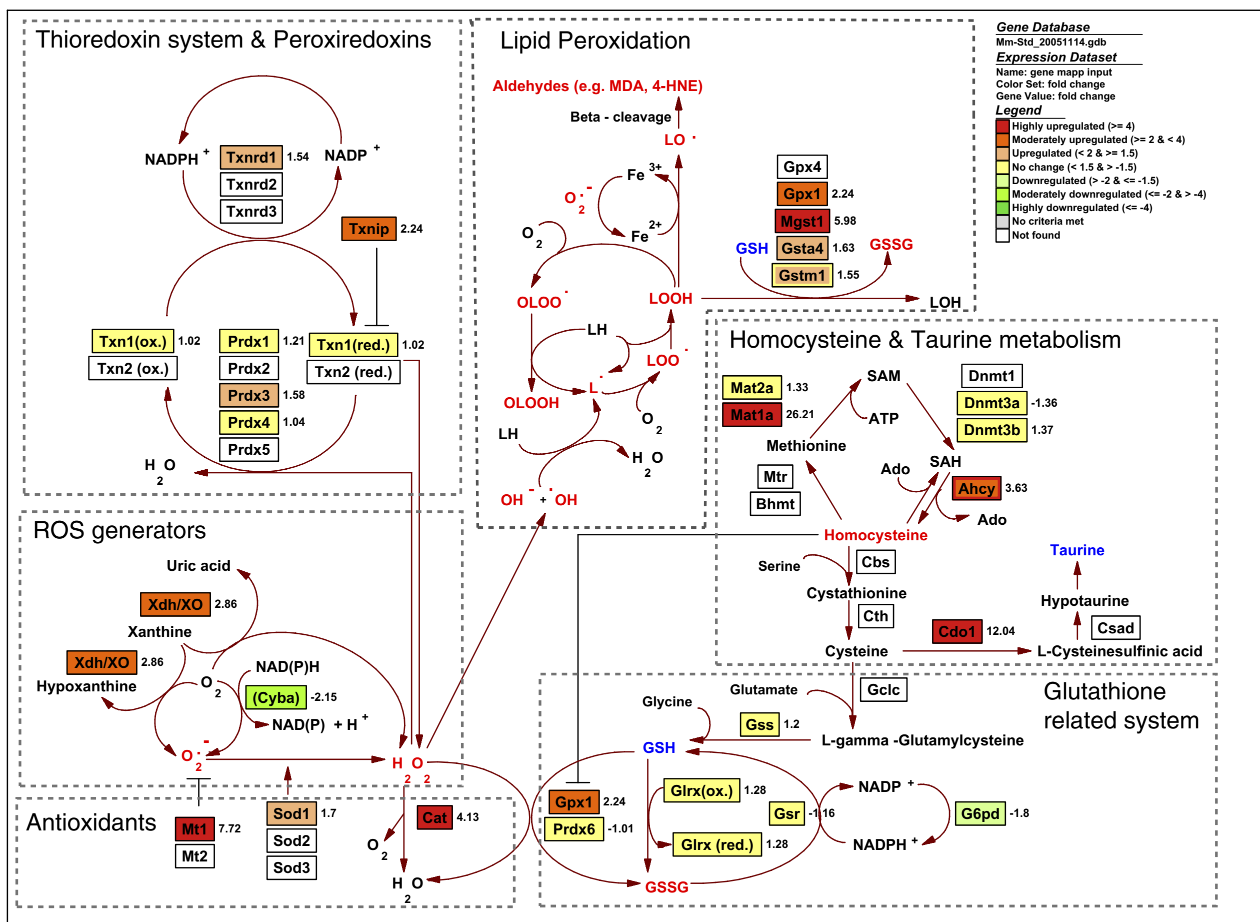

Fig. 1. Schematic pathway depicting the involvement of genes in the formation and turnover of both ROS and lipid hydroperoxides. The pathway was constructed with the help of GenMAPP software. It also depicts the fold change in expression of the genes identified by microarray analysis. LH, unsaturated lipid; L·, lipid alkyl radical; LO·, lipid alkoxyl radical; LOO·, lipid peroxyl radical; OLOO·, lipid epoxyperoxyl radical; LOOH, lipid hydroperoxide; LOH, lipid hydroxyl derivative; Sod, superoxide dismutase; Gpx, glutathione peroxidase; Cat, catalase; Mt, metallothionein; Mgst1, microsomal glutathione *S*-transferase 1; Gst, glutathione *S*-transferase; G6pd, glucose-6-phosphate dehydrogenase; Txn, thioredoxin; Txnrd, thioredoxin reductase; Txnip, thioredoxin-interacting protein; Prdx, peroxiredoxin; Cyba, cytochrome *b*-245, alpha polypeptide; Xdh/XO, xanthine dehydrogenase/xanthine oxidase; Glrx, glutaredoxin; Cbs, cystathionine β-synthase; Cth, cystathionase; Gclc, glutamate-cysteine ligase, catalytic subunit; Gss, glutathione synthetase; Gsr, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; Cdo1, cysteine dioxygenase 1; Csad, cysteine sulfinic acid decarboxylase; Sam, *S*-adenosylmethionine; Sah, *S*-adenosyl homocysteine; Mat, methionine adenosyltransferase; Dnmt, DNA methyltransferase; Ahcy, *S*-adenosylhomocysteine hydrolase; Mtr, 5-methyltetrahydrofolate-homocysteine methyltransferase.

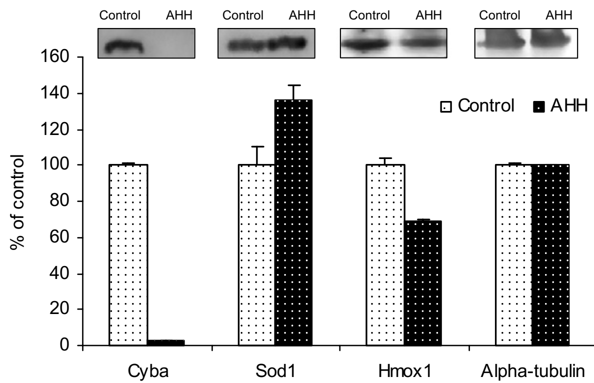

Fig. 2. Immunoblot and quantitative analysis for Cyba, Sod1, Hmox1 and Alpha-tubulin (loading control) protein levels in control and AHH exposed mice. The immunoblots were repeated thrice and the images shown are the best representation of the data. The graphical representation shows the relative integrated densitometry values (IDV) quantified and normalized by that of Alpha-tubulin signal using AlphaEaseFC software. Values are expressed as a percentage of those obtained in control group and represent means ± S.D.

beta polypeptide (Cybb/Nox2) and Cyba, without changes at the mRNA level [24]. We investigated the changes in the mRNA levels of Hmox1 by real time PCR and found 1.88-fold increase (Table 1) in its transcript levels. Our immunoblot results revealed decrease in the protein levels of Hmox1 in the AHH exposed heart (Fig. 2) which is in agreement with some of the earlier reports in certain human cell lines [25,26]. Hence the decrease in the protein levels of Cyba could be due to some regulatory factors other than Hmox1 and needs to be explored in detail.

Ppp1r15b dephosphorylates eukaryotic translation initiation factor 2α (eIF2α) and it is reported that blocking Ppp1r15b expression protects cells from oxidative stress. The 2.21-fold upregulation of Ppp1r15b, a short-lived protein whose levels depend on continuous translation of its mRNA [27] cannot be ignored. When Xdh is converted to XO, molecular oxygen accepts electrons resulting in the generation of $O_2^{\cdot-}$ or H_2O_2 [28]. It has also been reported that hypoxia increases the transcript levels as well as the activity of Xdh/XO in bovine endothelial cells [29] and similarly we also observed a 2.86-fold increase in the transcript levels of Xdh in AHH. Hence, the upregulation in the transcript levels of Ppp1r15b and Xdh are suggestive of enhanced oxidative stress in the AHH heart.

3.2.2. Lipid peroxidation. Acute hypoxia did increase the levels of MDA but not significantly (Fig. 3A). MDA, the end product of LPO indicates the presence of free radicals and LPO-induced cardiotoxicity. Formation of LPO products leads to propagation of free radical reactions as can be see in Figure 1. The extent of peroxidative injury mediated by LPO levels can determine whether a stressed cell will either survive or succumb via apoptosis or necrosis [30]. Relatively low LPO stress as observed in response to AHH might trigger cytoprotective responses like induction of antioxidants.

3.3. Cellular antioxidant defenses

3.3.1. Sod, Cat and Gpx. We observed increase in the mRNA levels of Sod1 (1.7-fold), Cat (4.13-fold) and Gpx1 (2.24-fold). The microarray results of Sod1 and Cat were validated by real time PCR (Table 1). The generation of $O_2^{\cdot-}$ results from one-electron reduction of oxygen by a variety of oxidases as can be seen in Figure 1. $O_2^{\cdot-}$ is rapidly dismutated by Sod to the more stable ROS, H_2O_2 [31]. Both Cat and Gpx catalyze the dismutation of H_2O_2 to H_2O and molecular O_2 [31]. The observed decrease in the enzyme activity of Sod, Cat and Gpx (Fig. 3B) will lead to increased oxidative stress. Hence, we speculate that the concomitant increase in the mRNA levels of antioxidants could lead to increase in the respective protein levels as observed in case of Sod1 (Fig. 2), which might ultimately help in alleviating the oxidative stress.

3.3.2. Metallothioneins. The metallothionein 1 (Mt1) gene showed 7.72-fold increase in the transcript levels. Metallothioneins are potent antioxidants and the ability of Mt1 and Mt2 to scavenge $O_2^{\cdot-}$ generated from XO/Xdh system has been demonstrated [32]. In the last few years, studies using cardiac-specific, Mt-overexpressing transgenic mouse models have produced direct evidence to support the antioxidant and protective function of Mt from oxidative injury in the heart [33,34]. In this regard the increased transcription of Mt1 may protect the AHH exposed heart from oxidative stress.

3.3.3. Thioredoxin system and peroxiredoxins. Thioredoxin reductase (Txnrd) in conjunction with thioredoxin (Txn) is a ubiquitous oxidoreductase system having antioxidant and redox regulatory roles. Although, we observed no change in the transcript levels of Txn1, there was a 1.54-fold increase in the transcript levels of Txnrd1. Thioredoxin-interacting pro-

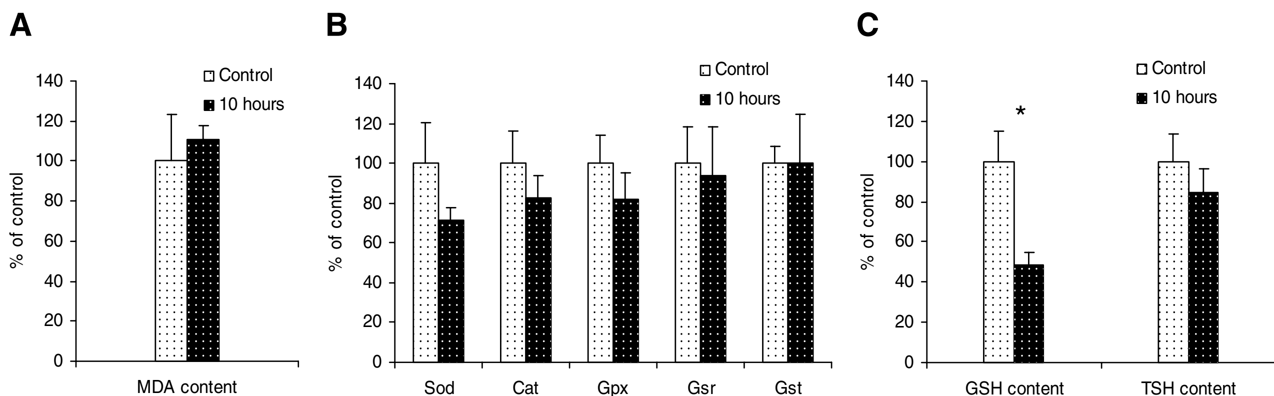

Fig. 3. Effect of AHH on the (A) Lipid peroxidation (LPO) levels, (B) Enzyme activities of Sod, Cat, Gpx, Gsr and Gst, (C) GSH and total thiol (TSH) content in murine heart. Values are expressed as a percentage of those obtained in control group and represent means ± S.D. The assays were repeated twice and the intra- and inter-assay coefficients of variation were less than 10%. *Significantly differs from controls; $F = 14.41$, $P = 0.02015$.

tein (Txnip) an endogenous inhibitor of Txn function [31,35] was found 2.24-fold upregulated and this could enhance oxidative stress in the AHH heart. The microarray results revealed no change in the transcript levels of peroxiredoxins (Prdx) like Prdx1, Prdx3, Prdx4 and Prdx6. Txn reduces the oxidized form of Prdx and the reduced Prdx scavenges ROS, such as H_2O_2 [31].

3.3.4. Total thiols and glutathione related systems. Thiol compounds are a natural reservoir of the reductive capacity of the cell. Thiols, together with non-enzymatic and enzymatic factors, regulate the intracellular metabolism defending biological structures and functions from the deleterious effect of ROS [36]. GSH, the most abundant non-protein thiol present in mammalian cells serves as a sensitive marker of oxidative stress and plays an important role in maintaining the integrity of the cell system. Though, a decrease was observed in the total thiol levels, the change was not significant (Fig. 3C). However, significant depletion (~50%) of GSH observed in the AHH heart (Fig. 3C), could promote generation of ROS and oxidative stress with cascade of effects thereby affecting the heart in response to AHH. Also as there is no simultaneous increase in the specific activity of Gsr (Fig. 3B) to replenish the GSH levels, there will be generation of oxidative stress. No significant change was observed in the transcript levels of glutathione synthetase (Gss) and Gsr which are responsible for the synthesis and regeneration of GSH (Fig. 1). Additionally, there was no change in the mRNA levels of glutaredoxin (Glrx), a class of proteins known to protect against oxidative stress by catalyzing reduction of protein mixed disulfides with GSH [31]. The ~2-fold transcriptional downregulation of glucose-6-phosphate dehydrogenase (G6pd), a rate-limiting enzyme in the pentose-phosphate pathway that generates NADPH in a reaction coupled to the de novo production of cellular ribose cannot be ignored as well. G6pd is required to maintain cytosolic GSH levels and is known to protect cardiomyocytes against ROS injury [37]. Therefore, the transcriptional downregulation of G6pd in the AHH heart could be damaging to the cardiomyocytes in the hypoxic state.

Within the alpha class of glutathione *S*-transferases (Gsts), Gsta4 which has high catalytic efficiency in the conjugation of 4-hydroxynonenal (4-HNE) and other genotoxic products of lipid peroxidation [38] has shown 1.63-fold increase in the transcript level, but no change was observed in the enzyme activity of Gst (Fig. 3B). We have observed 5.98-fold induction in the transcript levels of microsomal glutathione *S*-transferase 1 (Mgst1), which functions as a Gst as well as a peroxidase and can remove harmful products of lipid peroxidation [39]. Further, it has been shown that Mgst1 transcriptionally responds to oxidative stress and thus the upregulation in its transcript levels could play a protective role for intracellular membranes against oxidative stress in AHH heart.

3.3.5. Homocysteine and taurine metabolism: Mat1a, Mat2a, Ahcy and Cdo1. The transcript levels of methionine adenosyltransferase I, alpha (Mat1a) and *S*-adenosylhomocysteine hydrolase (Ahcy), involved in homocysteine metabolism increased significantly. In mammals there are 2 genes encoding Mats: Mat1a and Mat2a. Mat1a is expressed mostly in liver, whereas Mat2a shows a wider distribution and is responsible for *S*-adenosylmethionine synthesis in extrahepatic tissues [40]. Though, there was no significant change in the levels of Mat2a, surprisingly there was >26-fold induction in the levels of Mat1a. The microarray results for Mat1a were validated by real time PCR. Though, the amplitude of change was different between the methods, the real time PCR confirms the induction (~139-fold) of Mat1a (Table 1). Since homocysteine can exert a wide range of physiological effects in the heart, the increase in the transcription of Mat1a (26.21-fold) and Ahcy (3.63-fold) certainly holds significance with respect to oxidative stress in the AHH heart. Microarray data showed >12-fold induction of cysteine dioxygenase 1 (Cdo1), while the real time PCR indicated 4-fold induction in its transcript levels (Table 1). As it can be seen in Figure 1, Cdo1 is involved in the synthesis of taurine (2-amino ethane sulphonic acid). In the heart, taurine comprises up to 60% of the total free amino acid pool. Taurine serves several physiological and metabolic functions and is known to play a protective role in hypoxia due to its antioxidant properties [41]. Hence an increase in the transcription of Cdo1 could be beneficial to the AHH heart.

4. Concluding remarks

The study has revealed change in the transcript and protein levels of several important genes in response to AHH and provides information about the redox status of the murine heart in response to AHH as well. The effects of ROS depend not only upon the concentration of pro-oxidant molecules generated but also the tissue oxidant defense reserve, which has to combat the oxidative stress to maintain homeostasis. The present study has revealed that AHH decreases the antioxidant enzyme activities, GSH and TSH levels. We found decrease in protein levels of Cyba, a subunit of NADPH oxidase, which is a major ROS generator in the heart. It, therefore, becomes imperative to explore the mechanism involved in the downregulation of Cyba and whether it helps in decreasing the stress. This could be one of the favored mechanisms in heart to operate at low oxygen tension. In addition, investigation of these genes at different degrees of hypobaric hypoxia and multiple time points would provide valuable insights into understanding free-radical-mediated damage during HA acclimatization.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2007.08.044.

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