

Original Article

Activated corticosterone synthetic pathway is involved in poor responses to re-oxygenation after prolonged hypoxia

Chang Liu^{1,2,3}, Jian Chen^{1,2,3}, Bao Liu^{1,2,3}, Wen-Ting Liao^{1,2,3}, Jie Liu^{1,2,3}, Gang Xu^{1,2,3}, Bing-Da Sun^{1,2,3}, Er-Long Zhang^{1,2,3}, Yu-Qi Gao^{1,2,3}

¹Institute of Medicine and Hygienic Equipment for High Altitude Region, College of High Altitude Military Medicine, Third Military Medical University, Chongqing, China; ²Key Laboratory of High Altitude Environmental Medicine, Third Military Medical University, Ministry of Education, Chongqing, China; ³Key Laboratory of High Altitude Medicine, PLA, Chongqing, China

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Abstract: Diverse response patterns to re-oxygenation lead to various physiological or pathological phenotypes, but now lack of systematic research models in vivo. High-altitude de-acclimatization syndrome (HADAS) describes systematic alterations of re-oxygenation returning to plain after a long living in high altitude. In this study, we aim at employing a comprehensive metabolomics to explore the mechanisms for different reactions to re-oxygenation based on systematic quantitation scoring methods of HADAS model. Plasma samples were collected from 22 subjects when they finished their stay in high altitude for 1 year (5300 m), returning plain for 30th day and 180th day. These participants were divided into HADAS-S or HADAS-R group based on HADAS model on the 30th day after their reaching. Metabolic profiling was performed by ultra-performance liquid chromatography-quadrupole time-of-light mass spectrometry (UPLC-QTOFMS) in conjunction with univariate and multivariate statistical analysis. A total of 20 differential metabolites were identified by the comparison between HADAS-S and HADAS-R group. Pathway analysis suggested that the most potential disturbed pathway is sterol synthesis pathway, especially corticosterone synthetic sub-pathway. These molecules detected in this pathway are detailed that they showed a rapid and significant increasing manner in HADAS-S subjects comparing to HADAS-R group in the process of re-oxygenation. In conclusion, we identified that excessive stress responses to re-oxygenation might contribute to the distinctions between HADAS-S and HADAS-R group. These findings provide novel insights for further understanding of the pathogenesis for metabolic abnormalities in re-oxygenation after prolonged hypoxia.

Keywords: Re-oxygenation, hypoxia, UPLC-QTOF/MS, metabolic profiling, human plasma

Introduction

Impairment caused by re-oxygenation after hypoxia is widely encountered in various physiological or pathological processes, such as transplantation, pregnancy, wounds and even cancer [1]. High-altitude de-acclimatization syndrome (HADAS) describes a series of clinical symptoms and physical discomfort featured by insomnia, unresponsiveness, memory loss, fidgetiness, headache, which are suffered by the individuals who have acclimated to high altitudes and returned lower altitudes recently [2]. Undergoing the similar process of oxygen desaturation and subsequent re-oxygenation,

HADAS is regarded to share similar physiological procedures of these preceding anoxic conditions [3].

Through large-scale population based clinic experiments and multi-centers studies, HADAS model has proposed an in vivo systematical quantitative evaluation system to divided diverse responses to re-oxygenation into mild or severe reactions, namely reacted well (HADAS-R) or poor responders (HADAS-S). Symptoms of HADAS-S are significantly worse than others in HADAS-R group. Based on its scoring system, HADAS model provided a comprehensive understanding of the mechanisms of re-oxygenation

ation, and facilitated the resolutions to re-oxygenation associated clinical sickness [4]. More realistically, along with the development of tourism and economic construction in high altitude, actual personnel flowing between plain area and high altitude district is increasing enormously. Regarded as a major threat to life quality of the personnel who have adapted to plateau environment and departed from the plateau recently, the poor performance to HADAS has been a public health issue in these highlands country. A more comprehensive understanding of HADAS is requested urgently.

HADAS related alterations have been documented in explorers, and working staff in high-altitude since 1908 [3]. Previous studies have explored the onset and progression mechanisms of HADAS. He et al. reported the elevated LDH in the conversion from HADAS-R to HADAS-S after prolonged re-oxygenation [4]. The increasing in inflammation molecules of IL-17, IL-10 and TNF- α are also detected in the serum of subjects with poor response to HADAS [5]. However, lacks a comprehensive understanding of diverse reactions to HADAS, none effective therapeutic strategies for this symptom could be offered in clinical practice.

Metabolomics, as a systematically biologic approach, plays an essential role in detecting and identifying the distinguishing endogenous metabolites among experimental samples in diverse biological phenotypes [6]. Based on the illustration of activated metabolic pathways, this modern analytical technique could afford a systematic description of pathophysiological mechanisms associated with disease [7]. Recent researches has utilized metabolic profiling technologies to analyze metabolic variations in high altitude related disorders [8]. These results revealed significantly perturbed expression of amino acids, fatty acids, energy metabolism, bile acid metabolism and heme metabolism during the process of hypobaric hypoxia [9-11]. Therefore, we explored plasma metabolomics based on the highly sensitive ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTO-FMS) to describe the metabolic profiling of distinct responses to re-oxygenation after the process of chronic hypoxia.

In this study, we identified a panel of differential plasma metabolites and described meta-

bolic mechanisms which are involved in phenotypic variations among HADAS-R and HADAS-S subjects. Additionally, metabolic profiling in this research details the expressions of molecules related to steroid synthesis pathway, highlighting unfavorable functions of activated corticosterone synthesis sub-pathway in HADAS-S individuals. Taken together, our study provides new insights into the mechanisms for diverse responses of re-oxygenation or HADAS, and suggests potential biomarkers for these poor responders.

Material and methods

Subjects

22 healthy men of ethnic Han (22.8 ± 1.8 years old) with normal body weight and body mass index (height 171 ± 1 cm, body mass 61.5 ± 2.7 kg), were enrolled in this study. These participants have been dwelling in the altitude of 5,300 m for 12 months. All of them were lowlanders born, without any prior exposure to high altitude. To avoid acute stimuli from sudden changes in environment and reduce the interference of accidental error from long-time re-oxygenation, we focused on these subjects on the 30th day when they came back to Ye City (1,100 m) simultaneously instead of evaluating their longitudinal alterations on 180th day [4].

The participants were required to obey the identical daily schedule, food arrangement and stay same level of labor intensity throughout the experiment. Informed consents were written by all enrolled subjects in this study, which was approved by the medical ethical committee of Third Military Medical University. The entire experiment was complied with the principles of the Declaration of Helsinki.

Diagnostic scoring criteria for high altitude de-acclimatization syndrome

Since proposing the concepts of HADAS in High Altitude Medicine Conference held by Third Chinese National Symposium in September 1995, the diagnostic and scoring criteria for HADAS based on epidemiological study data have been extensively researched and are listed as follows briefly [4, 12]: (1) Adults less than 60 years old; (2) Immigrants (Han Chinese) who settled down in an altitude of more than 3000 m, returned to lower altitude (below 3000 m)

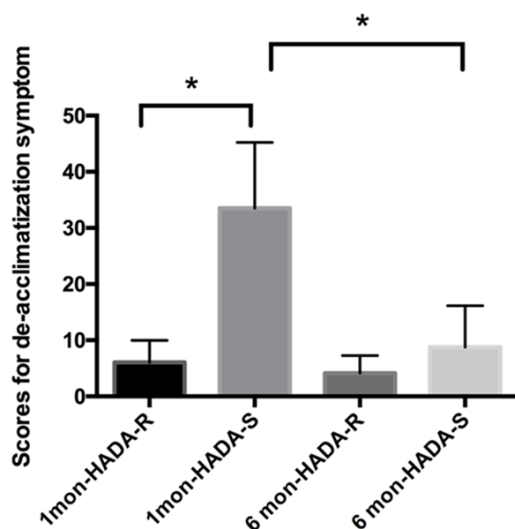


Figure 1. Symptom scores of HADA-S and HADA-R subjects. The average score of HADA-S subjects are significantly higher than that of HADA-R group after returning plain for one month (* p -value < 0.05, p -value was calculated by student's t -test). None significant clinical features could be detected in these two groups half a year later.

recently; (3) Symptoms of mental disorders and physical degeneration; (4) Any participants suffering primary diseases affecting the system of respiratory, cardiovascular, nervous, urinary, and hematological were excluded.

Two independent physicians evaluated the symptoms of enrolled subjects based on the diagnostic and scoring criteria of HADAS separately. If there existed discrepancies between these two physicians, a final consensus was reached after discussion with another physician. Individuals with serious symptoms (scores larger than 16) were enrolled in HADA-S group while the ones with lower than 15 score were included in HADA-R group.

Plasma sample preparation and analysis by UPLC-QTOFMS

The methods of plasma sample preparation and experimental procedures of UPLC-QTOFMS were performed based on our published report with minor modifications [8]. Briefly, morning fasting venous blood (100 μ L) was collected (with EDTA as an anticoagulant) and centrifuged at 14000 \times g for 15 min at 4°C to separate serum. After fast-frozen, these samples were delivered to Chongqing in a courier filled with dry ice for further metabolomics analysis. Pro-

cessing procedures were totally performed in accordance to the manufacturers' protocols of all devices in this study. As part of the system conditioning and quality control (QC) process, a pooled QC samples were prepared by mixing equal amounts of each serum sample.

LC-MS analysis was performed on an Agilent 1290 Infinity LC system coupled to Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) mass detector (Agilent, Santa Clara, CA, USA). Chromatographic separations were performed on an ACQUITY UHPLC HSS T3 C18 column (2.1 mm \times 100 mm, 1.8 μ m, Waters, Milford, Ireland) maintained at 45°C. The flow rate was 400 μ l/min and the injection volume was 4 μ l. The mobile phase A was 0.1% formic acid and mobile phase B was ACN. The gradient was used as follows: 4% B for 0-3 min, 2%-95% B at 3-20 min, 95% B at 20-22 min and followed by re-equilibrated step of 5 min. An electrospray ionization source interface was also used in the optimized conditions after exploration in the prior study, and set in both positive and negative modes to monitor as many ions as possible.

Data preprocessing and metabolomics analysis are identical to our previous article [8]. Briefly, raw LC-MS data were converted to mzData formats via Agilent Mass Hunter Qualitative software. The internal standards were removed after the employment of these molecules in data quality control (reproducibility) and data normalization. The variables that did not present in at least of 80% groups were filtered. The resulting three-dimensional matrix, including retention time and m/z pairs, sample names and normalized ion intensities, was introduced to later differential analysis.

Statistical analysis

Statistical analysis was performed on Matlab platform (MathWorks, Natick, MA), except for PCA and OPLS-DA, which were executed on SIMCA-P (Umetrics AB, Umeå, Sweden). Principal component analysis (PCA) and Orthogonal Partial least squares discriminant analysis (OPLS-DA) methods were employed to reveal the global metabolic alterations between disparate phenotypes of HADAS. The R2X, R2Y and Q2 (cum) parameters were used for OPLS-DA model evaluation. Correspondingly, the variable importance in the projection (VIP) score

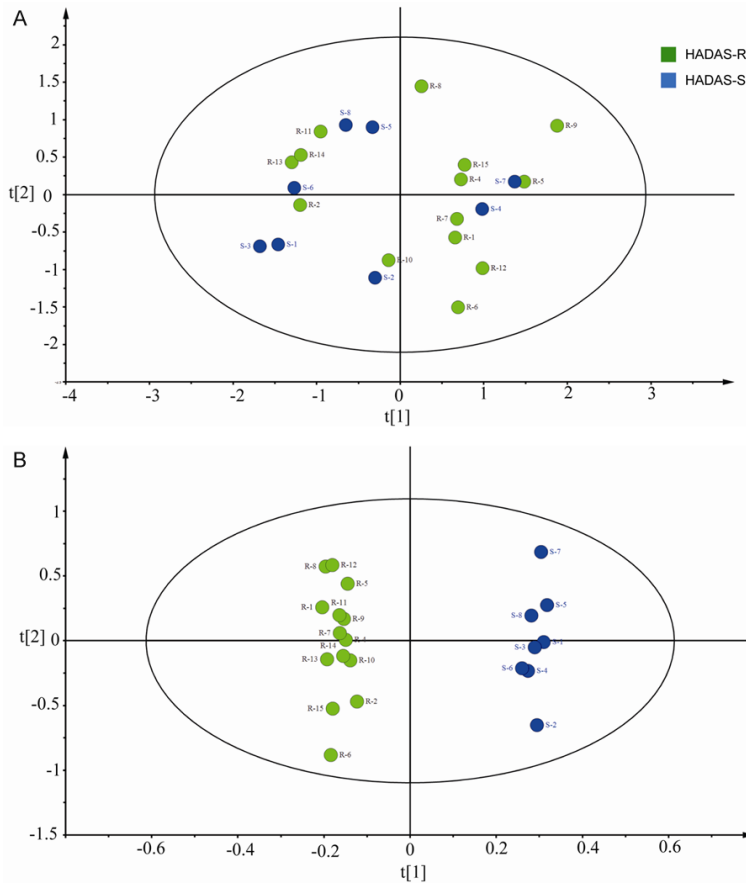


Figure 2. PCA and OPLS-DA score plots of 22 subjects with HADAS-S (green) and HADAS-R (blue) based on plasma spectral data of (A) UPLC-QTOFMS positive ion mode (B) UPLC-QTOFMS negative ion mode.

which reflects the metabolites' influence on the classification of the phenotypes was evaluated. Fold change (FC) value was calculated between HADAS-S and HADAS-R groups to filter the features. Additionally, the Mann-Whitney U test was also employed to measure the significance of each metabolite in separating HADAS-R from HADAS-S. The features with p -value < 0.05 , $VIP > 1.2$ and FC value increased or decreased metabolites that changed by 1.5 folds were recognized as the significantly altered molecules. Data of the general characteristics in these participants are presented as mean \pm SDs.

Differential metabolites identification and metabolic pathway reconstruction

Metabolites identification was firstly conducted by matching the retention behavior, mass assignments, and isotopic pattern from web-ba-

sed resources, namely Human Metabolome Database (<http://www.hmdb.ca/>) [13] and METLIN (<http://metlin.scripps.edu/>) [14]. Commercially available standards were adopted to further confirm essential metabolites by comparing their MS/MS spectra and retention time. Pathway analysis and visualization was carried out by Metaboanalyst (<http://www.metaboanalyst.ca>) [15] to identify the biological mechanisms that are most relevant to the metabolites of interests using KEGG (www.genome.jp/kegg/) pathway database.

Results

HADAS scoring of participants

As shown in **Figure 1**, HADAS scores were evaluated on the 30th day when enrolled subjects reached low-altitude area after exposure to hypoxic conditions for a year. The average score of subjects who responded to re-oxygenation after hypoxia well was 5.87 ± 3.85 ,

while average score in the HADAS-S group was 33.5 ± 11.72 . The subjects in these two variation groups showed significantly differential clinical features (p -value < 0.05). HADAS scores for these individuals in separating groups are also evaluated after their return to plain for half a year. None significant clinical features were detected between these groups after 6 months.

Metabolic profiling analysis

Multivariate analysis was performed to investigate if groups with lower grades could be separated from phenotypes with HADAS-S group by their metabolomics profiles. As illustrated in **Figure 2A**, an overview PCA model was calculated and the resulting score plots containing both negative and positive ions were developed. It is obvious that, sharing partially identical molecular alterations, HADAS-R and HADAS-S subjects could be pooled as one category of

Metabolomics research for High-altitude de-acclimatization syndrome

Table 1. Summary of differentially expressed plasma metabolites in HADAS-S relative to HADAS-R

Metabolites	KEGG ID	p-value	Fold change	VIP
ESI+				
3-Hydroxycapric acid	HMDB02203	0.018537339	1.256960472	2.39683
N-Undecanoylglycine	HMDB13286	0.01273084	1.290963524	2.45087
Undecanoylcholine	HMDB13322	0.031559214	1.628580487	2.22788
Glutamylglutamic acid	HMDB11737	0.037370148	1.312986997	1.68734
9(10)-EpODE	HMDB10220	0.044067865	1.270596132	2.28269
Tryptamine	HMDB00303	0.031559214	1.370612292	1.91781
ESI-				
Citrulline	HMDB00904	0.001899688	1.377197985	3.11624
N-Acetylglutamic acid	HMDB01138	0.031559214	1.406539834	1.78013
5-Methoxytryptophan	HMDB02339	0.031559214	1.280015885	2.26429
N-Undecanoylglycine	HMDB13286	0.015394668	1.321485989	2.71708
17-Hydroxylinolenic acid	HMDB11108	0.031559214	1.33451222	1.83572
Deoxycorticosterone	HMDB00016	0.044067865	1.311872737	1.71451
11b-Hydroxyprogesterone	HMDB04031	0.031559214	1.325087537	1.74457
17-HDoHE	HMDB38207	0.044067865	1.430068522	1.97383
Cortisol	HMDB14879	0.044067865	1.24969953	1.54716
18-Hydroxycortisol	HMDB00418	0.037370148	1.676739902	2.15822
Cholic acid	HMDB00619	0.031559214	1.253150068	2.28189
1,25-Hydroxyvitamin D3-26,23-lactone	HMDB11590	0.031559214	0.789037806	2.30282
Lithocholytaurine	HMDB00722	0.044067865	1.438421011	1.23912
LysoPE(20:4)	HMDB11485	0.031559214	0.746126449	2.15081

symptoms of de-acclimatization after chronic hypoxia. Though partial overlap areas could be detected, these two phenotypes of HADAS-S and HADAS-R showed clear separating trends by their respective metabolic features.

The supervised clustering methods of OPLS-DA model was carried out to enhance the separation obtained in PCA model. As presented in **Figure 2B**, a clear discrimination of HADAS-S from HADAS-R groups was attained in the OPLS-DA score plots. OPLS-DA models contained three latent variables with the performance values of $R^2X=0.426$, $R^2Y=0.906$, $Q^2=0.201$, which showed validity and robustness of OPLS-DA models. In combination with FC, VIP and p-value, our analysis revealed that discriminatory metabolites could contribute to the variation of HADAS-S and HADAS-R. These differential metabolites were selected as biomarker candidates and listed in **Table 1** after subsequent metabolic identification.

Metabolic network analysis

We employed pathway set enrichment analysis in elucidating the metabolic pathways affected

by metabolite distinctions between two different phenotypes of HADAS based on MetaboAnalyst platform. As shown in **Figure 3A**, our analysis revealed that steroid hormone biosynthesis pathway was the most perturbed pathways for the conditions undertaken in this study, including Cortisol, 11-Hydroxy pregnenolone and Deoxycorticosterone. The complementary metabolomic pathway of steroid hormone biosynthesis was acquired in **Figure 3B** to identify subtle and significant relationships among these metabolites. It is obvious that these three molecules are enriched to corticosterone synthetic sub-pathway.

Dynamic alterations of metabolites in corticosterone synthetic sub-pathway

To further validate the molecule alterations in of these potential biomarkers during the process of re-oxygenation, the dynamic expressions of cortisol, deoxycorticosterone and 11-Hydroxy pregnenolone are investigated and showed in **Figure 4** on the time points when they left high altitude, their reach in plain for 1 month and their arrival in Ye City for half a year.

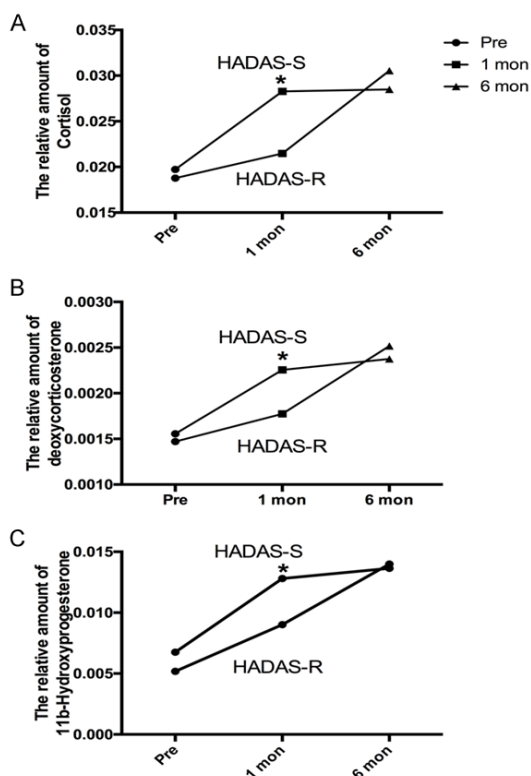


Figure 4. Dynamic alteration of potential biomarkers along with re-oxygenation. A. The level of cortisol in HADAS-S and HADAS-R groups. B. The relative content of deoxycorticosterone among HADAS-S and HADAS-R subjects. C. Level of 11-Hydroxy progesterone.

Discussion

To our best knowledge, this is the first study to compare diverse responses to re-oxygenation after hypoxia based on HADAS model. In this study, we provide a holistic presentation of metabolic changes related to HADAS in plasma based on metabolomics technology. A panel of distinguishing metabolites in steroid hormone biosynthesis pathway were identified and further depicted at time points of re-oxygenation before, re-oxygenation for a month, re-oxygenation for half a year. Our findings of these discriminatory molecules offered a conclusion that the sterol biosynthesis pathway serves as a great role in phenotypic variation, and could be potential therapeutic targets for treatment in poor responses to re-oxygenation.

By detailed analysis of sterol biosynthesis pathway, we found that all upstream molecules of corticosterone, including Deoxycorticosterone, 11b-Hydroxyprogesterone, cortisol have been

raised, which indicated the up-regulated corticosterone synthetic sub-pathway. Corticosterone is the glucocorticoid synthesis related molecule which is released by adrenal glands in a circadian manner with response to physiologic cues and stress, regulating a myriad of physiologic processes, including metabolism, immune function, skeletal growth, cardiovascular function, reproduction, and cognition [16, 17]. Our result is also identical to these findings, which showed that re-oxygenation contributes to the increasing of corticosteroid hormone by inspiring systemic stress response in a pituitary-adrenal depended manners, and thereby causes systemic metabolic disorder [18, 19]. Cortisol level is demonstrated to be involved in the response to high altitude exposure [20, 21]. Adrenal function is demonstrated to be decreased after prolonged hypoxia and experienced a recovery slowly after restoring oxygen [22, 23]. In combination with relieved clinical syndromes for 6th month, the sustaining elevated corticosteroid hormones in long term which might be contributed to the recovery function of adrenal glands. However, in this study, we did not detect any expression of corticosterone in the peripheral blood for its course of entering the nucleus to execute its roles, with little residual in serum to be detected by our technology performed in this research [24]. Further explorations about corticosterone after re-oxygenation in serum is needed.

By reconsidering the clinical symptoms of these individuals, we found that unfavorable clinical manifestation during the process of re-oxygenation after prolonged hypoxia, such as HADAS-S might be caused by long-term excessive stress response featured by elevated corticosteroid hormone. Previous unbeneficial clinical cases have demonstrated that acutely elevated corticosteroid hormones levels during excessive stress responses triggers depressive symptoms, aggravates ischemic neuronal damage, and induces stress injury, by binding glucocorticoid receptor to up-regulate circulating cytokines such as TNF- α , IL-1 β and IL-6; generate ROS to exaggerate cells damages [25, 26]. In addition, in the special environment of prolonged hypoxia, raising corticosteroid hormone is especially detrimental to the pulmonary vascular remodeling resulted from chronic hypoxia [27]. Corticosteroid hormones could promote the development of pulmonary hyperten-

sion, elevate risk of persistent pulmonary hypertension and worsen clinical outcome [28]. Moreover, our study also provides global metabolomics information about oxygen therapy for chronic hypoxic disease such as COPD and sleep apnea syndrome, and complement guidance for clinical application of corticosteroid hormones in the process of oxygenation therapy [29, 30]. For the subjects with unstable peripheral circulation under hypotension and hypo-perfusion, Chapados et al. has demonstrated that the administration of cortisol level could be considered as a first-intention by promoting pulmonary arterial pressure [18]. However, for the subjects with stable vital signs and none peripheral circulatory disorder, a properly lower level of corticosteroid hormones could reduce the adverse reactions of the organism during oxygenation therapy [31]. Based on our findings, we speculated that maintaining the stability of stress response to re-oxygenation could be helpful in promoting recovery after injury induced by prolonged hypoxia. Thus, we tentatively put forward that a more standardized and sophisticated managements should be built in the medical application of corticosteroid hormone during oxygen therapy in patients suffering anoxic diseases.

Our result also showed the increasing level of amino acids and their derivatives in HADA-S subjects. Cortisol is demonstrated to induce net protein breakdown, amino acid de novo synthesis from organs and increases output from organs into serum and consequently, hypercortisolemia could result in hyperaminoacidemia [32, 33]. Interestingly, among these elevated amino acids, citrulline is helpful in reducing oxidative stress reactions during re-oxygenation and relieving structural alterations induced by long-term hypoxia. Buyukuysal et al. believed that higher endogenous citrulline level may indicate an increased synthesis of NO during re-oxygenation and resultant degradation of NO to cytotoxic free radicals to reduce ischemia/Re-oxygenation induced damage [34]. A more recent study reported that citrulline increased NO production and reduced chronic hypoxia-induced pulmonary hypertension even after the onset of disease [35, 36]. Citrulline possesses therapeutic potentials which could be used as a target for structural remodeling after chronic hypoxia, and worthy of further studies.

There were several limitations should be noticed. One is the relatively small sample size in each group, which might prevent part of differential metabolites from being apparent. On account of the inaccuracy in generating diagnostic analysis with small sample size, these analyses were not executed in this study to prevent the misleading. Secondly, part of differential metabolites was not validated in other cohorts. Although a panel of homogeneous subjects were enrolled in this study, diverse populations with different ages, genders and disease categories, especially COPD patients with long-term oxygen therapy are needed to verify our results extensively. Therefore, a validation study containing larger samples, is now conducting by our group.

In conclusions, it's reasonable to consider that re-oxygenation after prolonged hypoxia could result in excessive stress response and thereby cause diverse reactions of HADAS-S or HADAS-R. These poor responders reflected a phenomenon of excessive acute reaction to stress. Precise managements and application of corticosteroid hormones in combination with re-oxygenation for the treatment of anoxic diseases are needed. Future studies are warranted to further verify the relevance of these metabolites associated with HADAS and elucidate the underlying biochemical mechanisms.

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Disclosur of conflict of interest

None.

Address correspondence to: Er-Long Zhang and Yu-Qi Gao, Institute of Medicine and Hygienic Equipment for High Altitude Region, College of High Altitude Military Medicine, Third Military Medical University, Chongqing, China, Tel: 8623-68752399; Fax: 8623-68752399; E-mail: loong0810@sina.com (ELZ); gaoy66@yahoo.com (YQG)

References

- [1] Li C and Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury.

Metabolomics research for High-altitude de-acclimatization syndrome

- Am J Physiol Cell Physiol 2002; 282: C227-241.
- [2] Subudhi AW, Bourdillon N, Bucher J, Davis C, Elliott JE, Eutermoster M, Evero O, Fan JL, Jameson-Van Houten S, Julian CG, Kark J, Kark S, Kayser B, Kern JP, Kim SE, Lathan C, Laurie SS, Lovering AT, Paterson R, Polaner DM, Ryan BJ, Spira JL, Tsao JW, Wachsmuth NB and Roach RC. Altitude omics: the integrative physiology of human acclimatization to hypobaric hypoxia and its retention upon reascent. *PLoS One* 2014; 9: e92191.
- [3] Chapman RF, Laymon Stickford AS, Lundby C and Levine BD. Timing of return from altitude training for optimal sea level performance. *J Appl Physiol* (1985) 2014; 116: 837-843.
- [4] He B, Wang J, Qian G, Hu M, Qu X, Wei Z, Li J, Chen Y, Chen H, Zhou Q and Wang G. Analysis of high-altitude de-acclimatization syndrome after exposure to high altitudes: a cluster-randomized controlled trial. *PLoS One* 2013; 8: e62072.
- [5] Wu J, Sun X, Wu Q, Li H, Li L, Feng J, Zhang S, Xu L, Li K, Li X, Wang X and Chen H. Disrupted intestinal structure in a rat model of intermittent hypoxia. *Mol Med Rep* 2016; 13: 4407-4413.
- [6] Covington BC, McLean JA and Bachmann BO. Comparative mass spectrometry-based metabolomics strategies for the investigation of microbial secondary metabolites. *Nat Prod Rep* 2016; 13: 4407-13.
- [7] Wheelock CE, Wheelock AM, Kawashima S, Diez D, Kanehisa M, van Erk M, Kleemann R, Haeggstrom JZ and Goto S. Systems biology approaches and pathway tools for investigating cardiovascular disease. *Mol Biosyst* 2009; 5: 588-602.
- [8] Liao WT, Liu B, Chen J, Cui JH, Gao YX, Liu FY, Xu G, Sun BD, Zhang EL, Yuan ZB, Zhang G and Gao YQ. Metabolite modulation in human plasma in the early phase of acclimatization to hypobaric hypoxia. *Sci Rep* 2016; 6: 22589.
- [9] Guo L, Tan G, Liu P, Li H, Tang L, Huang L and Ren Q. Three plasma metabolite signatures for diagnosing high altitude pulmonary edema. *Sci Rep* 2015; 5: 15126.
- [10] Lou BS, Wu PS, Liu Y and Wang JS. Effects of acute systematic hypoxia on human urinary metabolites using LC-MS-based metabolomics. *High Alt Med Biol* 2014; 15: 192-202.
- [11] Luo Y, Zhu J and Gao Y. Metabolomic analysis of the plasma of patients with high-altitude pulmonary edema (HAPE) using ¹H NMR. *Mol Biosyst* 2012; 8: 1783-1788.
- [12] Xie SW, Liu C, Gao YX, Chen Y, Jiang CH, Chen L and Gao YQ. The study of prevalence rate, and clinical characteristics of high altitude deterioration. *Eur Rev Med Pharmacol Sci* 2015; 19: 3444-3449.
- [13] Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, Djoumbou Y, Mandal R, Aziat F, Dong E, Bouatra S, Sinelnikov I, Arndt D, Xia J, Liu P, Yallou F, Bjorn Dahl T, Perez-Pineiro R, Eisner R, Allen F, Neveu V, Greiner R and Scalbert A. HMDB 3.0—the human metabolome database in 2013. *Nucleic Acids Res* 2013; 41: D801-807.
- [14] Smith CA, O'Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R and Siuzdak G. METLIN: a metabolite mass spectral database. *Ther Drug Monit* 2005; 27: 747-751.
- [15] Xia J and Wishart DS. Using metaboanalyst 3.0 for comprehensive metabolomics data analysis. *Curr Protoc Bioinformatics* 2016; 55: 14.10.11-14.10.91.
- [16] Alfaraz DN, Wiegert O, Joels M and Krugers HJ. Corticosterone and stress reduce synaptic potentiation in mouse hippocampal slices with mild stimulation. *Neuroscience* 2002; 115: 1119-1126.
- [17] Timmermans W, Xiong H, Hoogenraad CC and Krugers HJ. Stress and excitatory synapses: from health to disease. *Neuroscience* 2013; 248: 626-636.
- [18] Chapados I, Lee TF, Chik CL and Cheung PY. Hydrocortisone administration increases pulmonary artery pressure in asphyxiated newborn piglets reoxygenated with 100% oxygen. *Eur J Pharmacol* 2011; 652: 111-116.
- [19] Gonchar OA, Nosar VI, Bratus LV, Tymchenko IN, Steshenko NN and Mankovska IN. [Energetic and antioxidant status of rat liver mitochondria during hypoxia-reoxygenation of different duration]. *Fiziol Zh* 2015; 61: 35-45.
- [20] Mackey J, Mellor A, Watchorn J, Burnett A, Boot C and Woods D. The adrenocortical response to synthetic ACTH following a trek to high altitude. *Horm Metab Res* 2016; 48: 658-663.
- [21] Woods DR, Davison A, Stacey M, Smith C, Hooper T, Neely D, Turner S, Peaston R and Mellor A. The cortisol response to hypobaric hypoxia at rest and post-exercise. *Horm Metab Res* 2012; 44: 302-305.
- [22] Chapados I, Chik CL and Cheung PY. Plasma cortisol response to ACTH challenge in hypoxic newborn piglets resuscitated with 21% and 100% oxygen. *Shock* 2010; 33: 519-525.
- [23] Poudel R, McMillen IC, Dunn SL, Zhang S and Morrison JL. Impact of chronic hypoxemia on blood flow to the brain, heart, and adrenal gland in the late-gestation IUGR sheep fetus. *Am J Physiol Regul Integr Comp Physiol* 2015; 308: R151-162.
- [24] Conway-Campbell BL, Pooley JR, Hager GL and Lightman SL. Molecular dynamics of ultradian

Metabolomics research for High-altitude de-acclimatization syndrome

- glucocorticoid receptor action. *Mol Cell Endocrinol* 2012; 348: 383-393.
- [25] Cao G, Gong S, Zhang F and Fu W. Xiao Yao San against corticosterone-induced stress injury via upregulating glucocorticoid receptor reaction element transcriptional activity. *Evid Based Complement Alternat Med* 2016; 2016: 5850739.
- [26] Miyata S, Hattori T, Shimizu S, Ito A and Tohyama M. Disturbance of oligodendrocyte function plays a key role in the pathogenesis of schizophrenia and major depressive disorder. *Biomed Res Int* 2015; 2015: 492367.
- [27] Rameh V and Kossaify A. Role of biomarkers in the diagnosis, risk assessment, and management of pulmonary hypertension. *Biomark Insights* 2016; 11: 85-89.
- [28] Rowan SC, Keane MP, Gaine S and McLoughlin P. Hypoxic pulmonary hypertension in chronic lung diseases: novel vasoconstrictor pathways. *Lancet Respir Med* 2016; 4: 225-236.
- [29] Pilcher J and Beasley R. Acute use of oxygen therapy. *Aust Prescr* 2015; 38: 98-100.
- [30] Barjaktarevic I and Cooper CB. Supplemental oxygen therapy for patients with chronic obstructive pulmonary disease. *Semin Respir Crit Care Med* 2015; 36: 552-566.
- [31] Payne RS and Schurr A. Corticosterone-aggravated ischemic neuronal damage in vitro is relieved by vanadate. *Neuroreport* 2001; 12: 1261-1263.
- [32] Cynober LA. Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance. *Nutrition* 2002; 18: 761-766.
- [33] Wu G, Jaeger LA, Bazer FW and Rhoads JM. Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. *J Nutr Biochem* 2004; 15: 442-451.
- [34] Buyukuysal RL. Ischemia and reoxygenation induced amino acid release and tissue damage in the slices of rat corpus striatum. *Amino Acids* 2004; 27: 57-67.
- [35] Fike CD, Dikalova A, Kaplowitz MR, Cunningham G, Summar M and Aschner JL. Rescue treatment with L-citrulline inhibits hypoxia-induced pulmonary hypertension in newborn pigs. *Am J Respir Cell Mol Biol* 2015; 53: 255-264.
- [36] Vadivel A, Aschner JL, Rey-Parra GJ, Magarik J, Zeng H, Summar M, Eaton F and Thebaud B. L-citrulline attenuates arrested alveolar growth and pulmonary hypertension in oxygen-induced lung injury in newborn rats. *Pediatr Res* 2010; 68: 519-525.