

Original Article

Metabolomics investigations in chronic mountain sickness and offspring rat model groups

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Abstract: Objective: In this study, we aim to study the effect of the plateau hypobaric and hypoxia environments on plasma metabolic content in the chronic mountain sickness (CMS) and plateau offspring rat model groups using Nuclear Magnetic Resonance (NMR) technology to establish the potential differences between the two groups and the mechanism regulating these differences. Methods: We established the plain control group (PN), CMS model group (PU) and plateau offspring model group (PO). The ¹H-NMR data on plasma metabolic changes were evaluated in the PN, PU and PO groups using OPLS-DA analysis. Results: During the process of CMS, enhanced anaerobic glycolysis and fat mobilization as well as increased glucose glycolysis and reduced glucose levels may be closely associated with a long-term stay in a hypobaric hypoxia environment. In addition, the energy metabolism in the PO group has changed, as shown by the elevated glucose levels and reduced lipid-related metabolites. Finally, the cardiac pathologic state was alleviated in the PO group compared to the PU group. Conclusion: These results indicated that the plateau offspring rats have a certain ability to adapt to the plateau environment compared to the CMS rats.

Keywords: Chronic mountain sickness, nuclear magnetic resonance, pathological section, plateau offspring

Introduction

Although the occurrence of chronic mountain sickness (CMS) is directly linked to environmental factors such as altitude, hypoxia, and low pressure, its underlying mechanism is still not fully understood [1]. The main pathological features of CMS are represented by excessive red blood cell hyperplasia and severe hypoxemia. Hypoxia affects the blood and energy supply of the body, causing changes in the hemodynamic and cardiac pump functions. This, in turn, leads to the occurrence of CMS and disorders of various systems such as respiratory, cardiovascular, and blood circulation [2]. In addition, the abnormal modification of various indicators in the body can eventually lead to the death of many important organs, such as the heart and the brain [3], as a result of ischemia and hypoxia [4]. Theoretical, experimental, and clinical approaches have been combined by Chinese researchers to study the

physiological adaptive mechanism in high-altitude populations and native animals, establishing a unique theoretical system for the medical studies of the plateau in China [5, 6]. However, reports on the plateau offspring rat models are limited at home and abroad. Furthermore, NMR-based plasma metabolic studies on CMS and plateau offspring are even less available.

Therefore, this study aimed to investigate the effect of plateau hypobaric and hypoxia environments in the CMS and plateau offspring rat model groups, exploring and analyzing the metabolic networks in both groups. The plateau offspring model was obtained based on the CMS rat model established under a simulated altitude of 5000 m, following the mating of rats with CMS. The metabolic profile of each rat group was analyzed to study the mechanisms underlying the possible changes in the metabolic pathways and metabolic status of rats with CMS. Furthermore, the cardiac mor-

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physiological changes in both groups were analyzed. The results provided an experimental and theoretical basis for further studies on the plateau offspring.

Materials

Reagents and instruments

The main reagents included NaCl, K_2HPO_4 , NaH_2PO_4 (Tianjin Guangfu Fine Chemical Research Institute, China), distilled water, normal saline, and Deuterium oxide (Cambridge Isotope Laboratories, USA).

5-mm NMR tubes, -80°C ultralow-temperature refrigerator (Thermo, USA), super-speed low-temperature centrifuges (Microfuge 20R, Beckman Corporation, USA), 600 MHz nuclear magnetic resonance spectrometer (INOVA 600, Varian, USA), TOPSPIN 2.0 software (Bruker, Germany), SIMCA-P+14 software (Umetrics, Sweden), LEICA RM 2016 Microtomes (Shanghai Wuxiang Instrumentation Co., Ltd, China), and BHR-REL-T2 fluorescence microscope (Olympus, Japan).

ELISA kits for the following were used: rat interleukin-6 (IL-6, Semel Feischer Technology Co., Ltd., Finland; Batch number: 74350026); rat nitric oxide (NO, Hermes Criterion Biotechnology, Canada; Batch number: NH163CS) rat homocysteine (Hcy, Hermes Criterion Biotechnology, Canada; Batch number: NH056CS) rat C-reactive protein (CRP, Shanghai Xitang Biotechnology Co., Ltd., China; Batch number: 3400630); rat vascular endothelial growth factor (VEGF, Shanghai Xitang Biotechnology Co., Ltd., China; Batch number: 3203040); and rat endothelial-1 (ET-1, Shanghai Xitang Biotechnology Co., Ltd., China; Batch number: 8264255).

Methods

Animal models and plasma samples

A total of 90 healthy Sprague-Dawley (SD) rats (30 male and 60 female; body weight, 160-200 g) were provided by the Laboratory Animal Center of Xinjiang Medical University [Urumqi, China; license number: SCXK (xin) 2011-0004]. The experiments using the Simulated Cabin for Special Environment of Northwest of China were completed in the Key Laboratory of Special Environmental Medicine of Xinjiang locat-

ed in the General Hospital of Xinjiang Military Region of PLA, Urumqi.

The present study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (approval number: IACUC-20130217063).

The rats were divided into three groups and treated as follows:

Plain control (PN) group: A total of 20 healthy Sprague-Dawley rats (same number of males and females) were included in this group. The experimental conditions for these rats were as follows: simulated altitude of 720 m, 18°C - 26°C temperature, 40-60% humidity, 93.2 kPa pressure, and 19.54 kPa oxygen partial pressure. The rats were fed ad libitum for 30 days without drug intervention.

CMS model (PU) group: This group comprised 20 healthy Sprague-Dawley rats (same number of males and females). The rats were fed in the Simulated Cabin for Special Environment of Northwest of China for 30 days. The experimental conditions for these rats were as follows: simulated altitude of 5000 m, 18°C - 26°C temperature, 40-60% humidity, 54.1 kPa pressure, and 10.84 kPa oxygen partial pressure.

Plateau offspring model (PO) group: This group comprised 50 healthy SD rats (40 male and 10 female). The rats continued feeding in the Simulated Cabin for Special Environment of Northwest of China for 30 days, naturally mated in the Simulated Cabin for Special Environment of Northwest of China, and gave birth to 10 live offspring (4 female and 6 male). Additional 20 rat pups were born, but they died instantly due to the high-altitude environment. The 8 offspring rats were used (4 female and 4 male, randomly selected) in this study, which were allowed to continue feeding in the Simulated Cabin for Special Environment of Northwest of China for 30 days.

Determination of pulmonary artery pressure in the rats of each group

After anesthesia, the skin of the rats was cut along the midline of the neck, and the subcutaneous and muscular tissues were obtusely separated to expose the trachea. The endotracheal tube was connected to the ventilator, whose frequency, tidal volume, and respiratory

ratio were adjusted to 60 times/min, 6 mL/kg, and 3:2, respectively. The chest was cut opened along the median line of the sternum to completely expose the lungs and heart. Pulmonary artery pressure (PAP) was measured by inserting a needle filled with heparin saline into the pulmonary artery.

Examination of oxygen saturation and partial blood pressure in the rats of each group

A portable blood gas analyzer (Abbott i-STA-300) was used to measure blood oxygen saturation and partial pressure. The plain group was tested in a plain environment, while the other groups were tested in a low-pressure chamber simulating a plateau environment.

Examination of serological indices in rats

After anesthesia, blood was collected from the abdominal aorta, placed at 20°C for 0.5 hours, and centrifuged at 3000 r/min for 20 min. Serum was obtained, and CRP, ET-1, VEGF, Hcy, NO, and IL-6 contents were measured using their corresponding kits in accordance with the manufacturers' instructions.

Plasma ¹H-NMR tests

The experimental conditions for these offspring rats were the same as for the PU group. Once the rat models were established, blood samples from the abdominal aorta of each anesthetized rat were collected in blood collection tubes containing separating gels. The blood samples were centrifuged at 3000 r/min for 15 min at 4°C, and the plasma was collected into EP tubes and stored at -80°C for further use.

Plasma (200 µL) was collected from each SD rat, and 400 µL of phosphate buffer prepared with deuterioxide was added to each sample. The mixture was placed at 25°C for 10 min and subsequently centrifuged at 10,000 r/min for 10 min. Then, 550 µL of the supernatant was transferred to a 5-mm NMR tube. The hydrogen spectrums were determined by the NMR spectrometer using the following parameters: 599.73 MHz frequency, NOESYPRESAT-1D (RD-90°-TM-90°-ACQ) pulse sequence, 64 cumulative scans, 32,768 sample data points, 10,000 Hz spectral width, 2 s sample delay, 1.64 s average scanning time, 25°C test temperature, and the use of the presaturation method for water peak suppression.

Statistical analysis

All ¹H-NMR were manually baseline and phase adjusted by Mest Renova software. The original ¹H-NMR spectrum data of rat plasma were calibrated by the standard of the proton signals of α-glucose chemical displacement at 5.233 ppm. The chemical displacement range of 10-0.05 ppm with 0.03 ppm as the integration unit was integrated and divided into 2688 segments. The integral values were normalized (each integral value was divided by the sum of all integral values) to eliminate the effects of water intake on metabolite concentration. The SMICA-P+14 software was used to perform partial least-square discriminant analysis (PLS-DA) for statistical analysis and orthogonal partial least-square discriminant analysis (OPLS-DA). PLS-DA was mainly used to observe the differences among groups, while OPLS-DA was used to determine the integral differences between groups. The metabolite correlation coefficient (*r*) represented by each integral score obtained from OPLS-DA was used to determine the differences in plasma metabolic components between male and female rats, and the inspection standard was set at *P* = 0.05. According to the significance test of the Pearson product-moment correlation coefficients, the metabolites in this study denoted by $|r| > 0.666$ (*n* = 8) represented the threshold for metabolites with statistically significant differences (*P* < 0.05). A larger $|r|$ value represented larger differences, while smaller values represented smaller differences.

Results were analyzed using SPSS17.0. Measurement data were tested for the normality and homogeneity of variance, and data subjected to normal distribution were expressed as mean and standard deviation. The test level was set at $\alpha = 0.05$.

Cardiac pathological examinations

Preparation of heart paraffin blocks: The hearts of the PN, PU, and PO group rats were collected, fixed with 10% formaldehyde, and sequentially placed into 70% EtOH for 3 h, 80% for 2 h, 90% for 1.5 h, 95% for 2 h, and 100% EtOH for 1 h. Next, the samples were cleared in xylene for 30 min, embedded in molten paraffin with a low melting point at 54°C, and stored at room temperature until the paraffin solidified.

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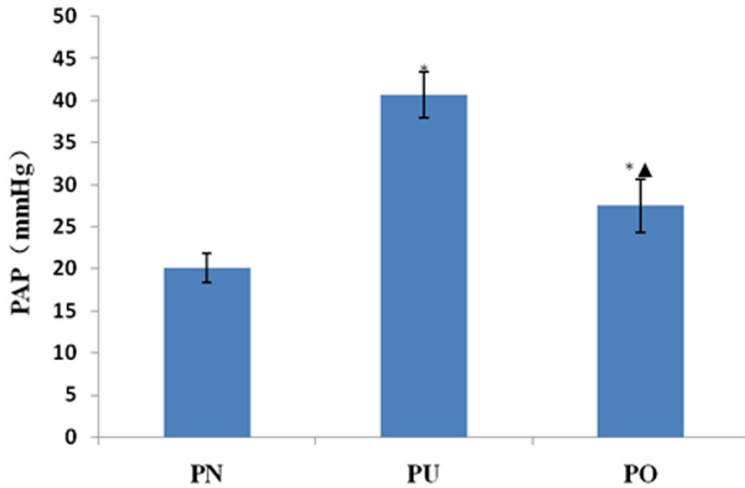


Figure 1. The results of PAP determination in each group. Note: Compared with PN, * $P < 0.05$; Compared with PU, ▲ $P < 0.05$.

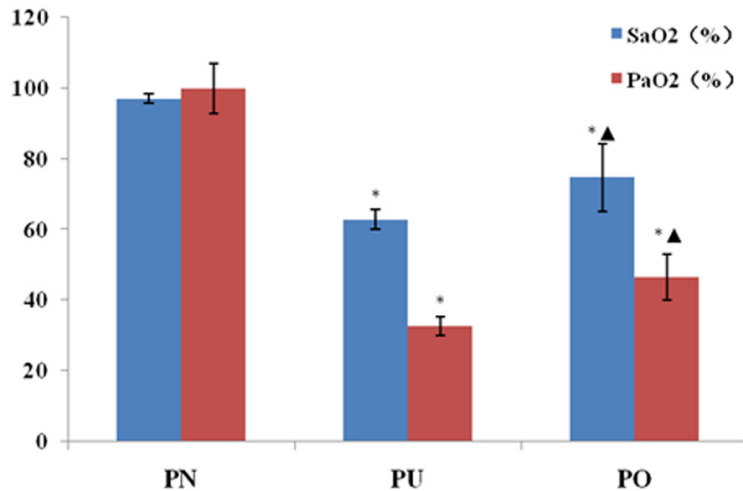


Figure 2. The results of SaO₂ and PaO₂ measurement in each group of rats. Note: Compared with PN, * $P < 0.05$; Compared with PU, ▲ $P < 0.05$.

Preparation of cardiac tissue sections: The embedded tissue was cut into 4- μ m-thick sections and immersed in 30-40% EtOH and 38°C water. The sections were removed, mounted onto gelatin-coated slides and dried in a 37°C incubator for 12 h. The slides were stored at room temperature until staining.

Hematoxylin and eosin staining

The paraffin sections were immersed in xylene three times, 15 min each time, to dewax, and then rinsed twice in 100%, 95%, and 80% EtOH, for 2 min each, followed by a distilled water rinse. After staining in hematoxylin for 6 min, the sections were rinsed with distilled

water, followed by differentiation in 0.1% hydrochloric acid ethanol for a few seconds and rinsing with distilled water. The sections were blued in 0.5% ammonia water, rinsed in distilled water three times, counterstained in eosin solution for a few seconds, and rinsed with distilled water. Finally, the slides were dehydrated in four changes of ethanol: 80%, 95%, and 100% for several seconds, and 100% for 2 min. After 10-min drying in a 64°C incubator, a drop of neutral gum was added, and the sections were mounted with coverslips, dried, and sealed. The morphological changes in the rat heart tissues were observed under standard optical microscopes.

Results

Determination of PAP of the rats of each group

In **Figure 1**, the PAP of PU and PO rats significantly increased compared with that of PN rats ($P < 0.05$). The PAP of PO rats significantly decreased compared with that of PU ($P < 0.05$).

Examination of partial blood pressure and oxygen saturation in rats of each group

In **Figure 2**, PaO₂ and SaO₂ of PU rats significantly reduced compared with those of PN rats ($P < 0.05$). PaO₂ and SaO₂ of PO rats significantly increased compared with those of PU rats ($P < 0.05$), but they were still significantly lower than those of PN rats ($P < 0.05$).

Examination of serological indices in rats

In **Figure 3**, the serum levels of IL-6, CRP, and VEGF of PU and PO rats significantly increased compared with those of PN rats ($P < 0.05$). The contents of IL-6, CRP, and VEGF in the serum of the offspring of the plateau model rats significantly reduced compared with those of PU rats ($P < 0.05$).

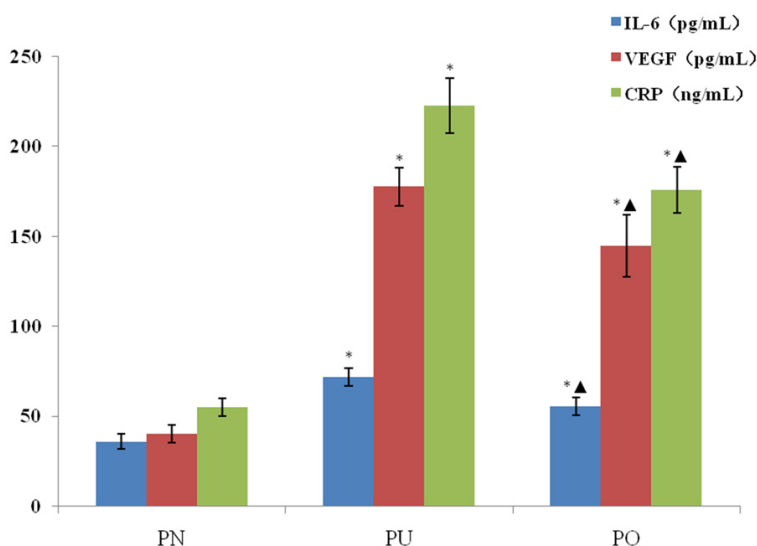


Figure 3. Determination of serum IL-6, VEGF and CRP in each group of rats. Note: Compared with PN, * $P < 0.05$; Compared with PU, ▲ $P < 0.05$.

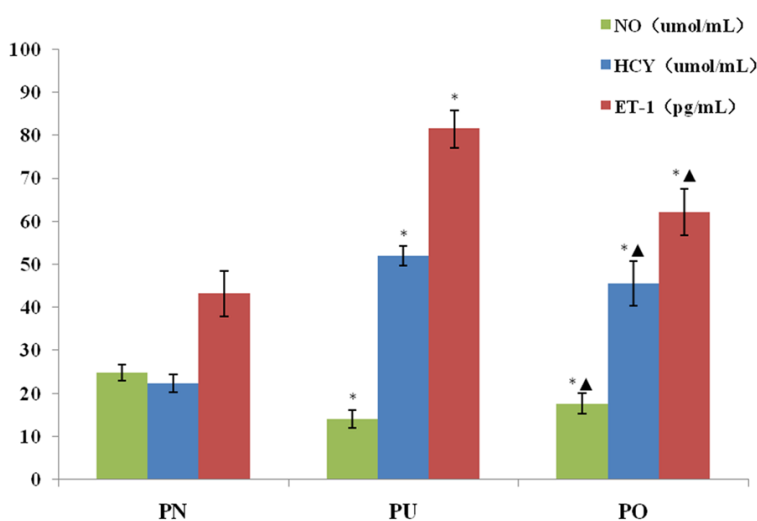


Figure 4. Determination of serum NO, HCY and ET-1 in each group of rats. Note: Compared with PN, * $P < 0.05$; Compared with PU, ▲ $P < 0.05$.

In **Figure 4**, the contents of HCY, ET-1, and NO in the serum of PU and PO rats significantly increased compared with those of PN rats ($P < 0.05$). The serum levels of HCY and et-1 of PO rats were significantly lower than those of PU rats, whereas the NO levels of the former were significantly higher than those of the latter ($P < 0.05$).

Analysis of serum $^1\text{H-NMR}$ metabolomics spectral results from each rat group

Figure 5 shows the serum $^1\text{H-NMR}$ spectrum of the PN, PU, and PO groups. PLS-DA disci-

minant analysis was used to compare the differences of metabolites in serum of rats in each group. The results were shown in **Figure 6**. This analysis showed $R^2X = 0.411$, $R^2Y = 0.832$, and $Q^2 = 0.4$. **Figure 6** shows that the distribution of each group was distinctly separated, indicating that the metabolic components in the rat serum of each group were significantly different. The degree of difference was calculated based on the correlation coefficients combined with the chemical shifts of the metabolites in every two groups obtained from the OPLS-DA analysis of the correlation coefficients. Next, the differences in plasma metabolic components of the rats in the PN, PU, and PO groups were determined using NMR (**Table 1**).

The results showed that the various plasma metabolites from each group were significantly different. The concentrations of various metabolic components in the rat serum, including leucine, valine, lactic acid, alanine, glycoprotein, tyrosine, acetone 1-methyl-histidine, VLDL, LDL, methionine, creatine, and β -hydroxybutyrate, were significantly increased, whereas the concentrations of alpha-glucose and beta-glucose were significantly lower in the PU group than in the PN group ($P < 0.05$). In addition, the metabolite levels in the PO group, such as α -glucose, β -glucose, and creatine, increased, whereas VLDL, formic acid, and β -hydroxybutyrate reduced compared with the PU group, and the differences were statistically significant ($P < 0.05$) (**Table 1**).

Heart pathological examinations of each rat group

A normal cardiac structure was observed in the PN group under both low- and high-power lens with no apparent pathology. The myocardial

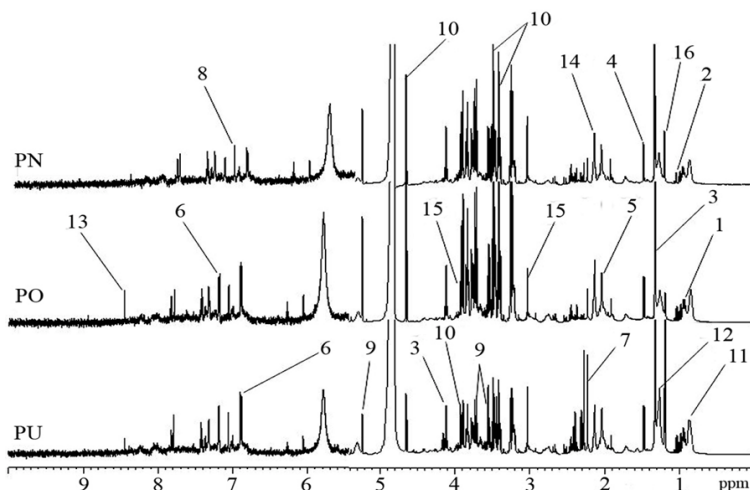


Figure 5. $^1\text{H-NMR}$ spectra of rat's serum samples. 1. Leucine; 2. Valine; 3. Lactic acid; 4. Alanine; 5. Glycoprotein; 6. Tyrosine; 7. Acetone; 8. 1-methyl-histidine; 9. α -glucose; 10. β -glucose; 11. Lipoproteins; 12. LDL; 13. Formic acid; 14. Methionine; 15. Creatine; 16. β -hydroxybutyric acid.

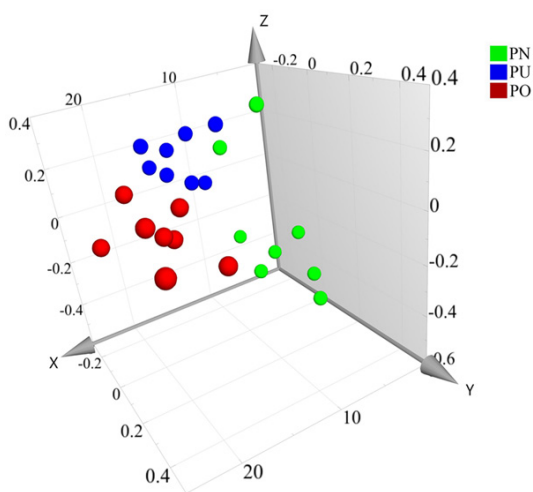


Figure 6. OPLS-DA score plots from $^1\text{H-NMR}$ spectra of rat's serum.

fibers were neatly arranged with clear staining of myocardial cells and dense nuclei.

Severe dilation and congestion of epicardial blood vessels were visible in the PU group under the low-power lens. Intermycardial blood vessels showed partial dilation and congestion with indistinguishable cross-striation. Partial myocardial swelling, cytoplasmic degeneration, some eosinophil degeneration in the myocardial fibers, and inflammatory cell infiltration were also visible under the high-power lens.

No dilation and congestion of epicardial blood vessels, partial dilation and congestion of intermyocardial blood vessels, indistinguishable cross-striation, and organized arrangement of myocardial fibers were visible in the PO group under the low-power lens. On the contrary, light myocardial swelling, myocardial eosinophil degeneration, and inflammatory cell infiltration were observed under the high-power lens (**Figure 7**).

Discussion

Chronic altitude sickness, globally known as chronic mountain sickness (CMS), refers to the clinical syndrome caused by the long-term failure of acclimation or the loss of the adaptability of a migrant population living on a plateau and characterized by pulmonary hypertension and hypoxemia [7]. Oxygen saturation (SaO_2), which is an important physiological parameter of respiratory circulation, is the percentage of oxygenated hemoglobin in the blood bound by oxygen to the total bound hemoglobin volume, i.e., the concentration of oxygen in the blood. PaO_2 , which reflects the oxygen intake status of pulmonary capillary and serves as an indicator of external respiration, is formed by the arterial blood ingestion of O_2 from the lungs.

The results of this study showed that the SaO_2 and PaO_2 of the rats in the plateau group decreased to different degrees, whereas their PAP increased, which is a typical characteristic of chronic plateau disease. Compared with plateau group, the SaO_2 and PaO_2 of the offspring of the rats in the plateau group decreased to different degrees, while the PAP increased. Though their PAP was not close to the level of the plain group, it was significantly lower than that of the plateau model group. This result indicated that the offspring of the plateau rats showed some adaptability to the plateau environment.

Low-pressure hypoxia stress enhances sexual arousal to stimulate nerve and endocrine fun-

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Table 1. Groups of rats serum ¹H-NMR spectrum after OPLS-DA differences between main metabolites and their correlation coefficients

No	Metbolite	Chemical shift (mg·L ⁻¹)	Assignment	PU vs PN		PU vs PO	
				R ² X	Q ²		
				0.75		0.93	
				0.4		0.65	
1	Leucine	0.95 (d), 0.97 (d)	δ-CH ₃ , δ-CH ₃	-0.73			
2	Valine	0.98 (d), 1.03 (d)	CH ₃ , CH ₃ , α-CH ₂	-0.74			
3	Lactic acid	1.33 (d), 4.11 (q)	CH ₃ , CH	-0.71			
4	Alanine	1.47 (d)	CH ₃	-0.67			
5	Glycoprotein	2.03 (s)	NHCO-CH ₃	-0.8			
6	Tyrosine	6.89 (d), 7.18 (d)	α-CH, H ₃ /H ₅ , H ₂ /H ₆	-0.68		-0.91	
7	Acetone	2.22 (s)	CH ₃	-0.78			
8	1-methylhistidine	7.05 (s)	H ₄ , H ₂	-0.68			
9	α-glucose	3.53 (dd), 3.72 (dd), 5.23 (d)	C-H ₂ , halfCH ₂ -CH ₆ , C-H ₁	0.94		-0.93	
10	β-glucose	3.40 (t) 3.47 (ddd), 3.90 (dd), 4.64 (d)	C-H ₄ , C-H ₅ , halfCH ₂ -CH ₆ , C-H ₁	0.98		-0.96	
11	Lipoproteins	0.85 (m), 0.88 (m)	CH ₃ (CH ₂) _n , CH ₃ CH ₂ CH ₂ C	-0.69		0.67	
12	LDL	1.26 (m)	CH ₃ CH ₂ (CH ₂) _n	-0.68			
13	Formic acid	8.44 (s)	CH			0.82	
14	Methionine	2.13 (s)	S-CH ₃	-0.69			
15	Creatine	3.03 (s), 3.93 (s)	CH ₃ CH ₂	-0.7		-0.79	
16	β-hydroxybutyric acid	1.19 (d)	γ-CH ₃	-0.81		0.83	

Note: In the "comparison between PU and PN", metabolites with negative correlation coefficients represent metabolites that were increased in the PU group and vice versa. In the "comparison between PO and PU", metabolites with negative correlation coefficients represent metabolites that were increased in the PO group and vice versa. s-single peak; d-double peaks; t-triple peaks; q-quadruple peaks; m-multiple peaks; dd-double-double peak; ddd-double-double-double peak.

ction. NO is a vasodilator and novel cellular signaling molecule [8]. ET-1 is a potent vasoconstrictor and active substance [9] that can be synthesized and released by vascular endothelial cells. NO is a vascular relaxation factor. In vivo, the ET-1 and NO are in a dynamic equilibrium relationship [10], jointly maintaining normal vascular tension. Once this balance is broken, the vasoconstriction and relaxation of blood vessels are impeded. In hypobaric hypoxia, ET-1 level significantly increases, endogenous NO synthesis decreases, pulmonary artery smooth muscle relaxation decreases, and PAP increases. In this study, the serum ET-1 level of the plateau model group was higher than that of the plain group, and the NO level of the former was lower than that of the latter. This result confirmed our conclusion, suggesting that hypoxia-induced lung tissue damage might be caused by the damage to the vascular endothelial function, which increased PAP and triggered a series of organ damage.

Hypoxia can directly initiate inflammatory responses and promote the release of various inflammatory mediators [11]. Studies have shown that [12] hypoxic conditions at a high altitude and a low pressure can increase the cir-

ulation of CRP in blood. CRP is a sensitive inflammatory marker of inflammation and tissue damage and can reflect the inflammation level in vivo. CRP and IL-6 are involved in the damage to tissue inflammatory responses in a hypoxic environment, so CRP and IL-6 can be used as indicators of tissue damage in this environment. They can also be used to indirectly evaluate the development and prognosis of diseases. The experimental results demonstrated that the serum CRP and IL-6 levels of the plateau model group significantly increased compared with those of the plain group. Therefore, the rats were in a state of chronic damage.

NMR spectroscopy was used in the present study to conduct metabolomics on the serum samples of the rats with CMS. As one of the important research fields in the postgenomic era, metabolomics is a science that quantitatively describes the overall endogenous metabolites of the organism and their responses to both internal and external changes. From a holistic perspective, metabolomics systematically studies the changes in endogenous metabolites and their developmental patterns in the organism, thus indicating the metabolic ab-

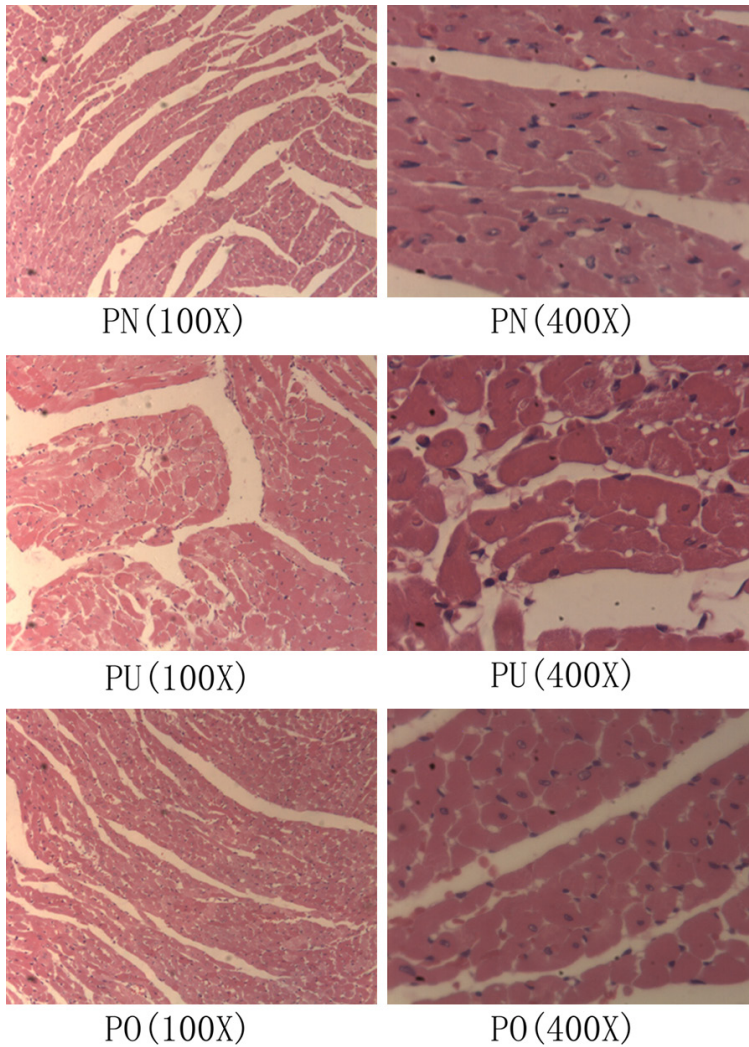


Figure 7. Heart pathology examination.

normalities in the body. Due to its noninvasive, unbiased, and reproducible characteristics, NMR spectroscopy ($^1\text{H-NMR}$) has become the main detection method in metabolomics [13-16]. At present, NMR-based metabolomics has been applied in clinical diseases, including biomarker discovery, disease diagnosis, treatment, and prognosis [17], but its most extensive application is in identifying metabolic markers for disease diagnosis and treatment [18, 19]. In this study, a pairwise OPLS-DA comparative analysis was conducted on the serum NMR of the PN, PU, and PO groups to identify differences in serum metabolites between these groups. The findings revealed that such differences might be involved in the adaptive adjustments of the body to the hypobaric and hypoxic plateau environment.

The results showed that the serum concentrations of various amino acids significantly increased, the concentrations of alpha-glucose and beta-glucose significantly reduced, and the concentration of lactic acid increased in the PU group compared with the PN group. These results indicated that glycolysis was enhanced in the PU group. A large amount of glucose was consumed in the process of glycolysis due to the hypoxic environment, resulting in an increased level of liver gluconeogenesis and increased concentration of serum amino acids, which were transported to the liver for gluconeogenesis and converted into glucose that entered into the blood. Although this process was beneficial for the energy supply under long-term energy consumption, the acquired energy might not meet the demand of the body. Hence, this might also be the potential mechanism underlying the reduced serum glucose concentration in the PU group in this study. This study demonstrated that oxygen and energy deficiency in the tissues might result in an enhanced

fat mobilization in the PU group. The serum concentrations of lipids, such as VLDL and LDL, as well as the concentrations of acetone (a component of the ketones produced by β -oxidation of fatty acids) and β -hydroxybutyric acid, increased in the PU group. The increase in ketone concentration indicated an enhanced fat mobilization in the PU group. In the hypobaric and hypoxic plateau environment, the increase in the tissue anaerobic metabolism rate and the dependence on glucose metabolism as the body's main energy supply could cause disturbances in lipid metabolism, and thus the pathology was consistent with CMS to a certain extent [20]. However, the serum concentrations of α -glucose and β -glucose significantly increased whereas the levels of VLDL and β -hydroxybutyric acid decreased in

the PO group compared with the PU group. The changes in the metabolite level reflected the metabolic state of the PO group, indicating that the energy supply in the PO group was, to a certain extent, able to meet the body's energy demand even under a hypobaric and hypoxic environment. The levels of anaerobic glycolysis and fat mobilization in the PO group were similar to those of the PN group, suggesting that the body had already adapted to the plateau environment and that the PO group had better adaptability to plateau environment than the PU group.

The hypobaric and hypoxic environment of the plateau not only affected the basal metabolism of the animals but also caused some adaptive changes in many organs, resulting in organ damages and related diseases. The heart is a vital organ for the body's circulation, and, as the energy-consuming organ with extensive muscle contraction, the changes in energy production and consumption in the myocardium under hypoxic conditions have special significance. Pathological observations of the heart in each experimental group showed that the cardiac pathological changes in the PO group were not as severe as those in the PU group. It was clear that the health conditions of the rats in the PO group were superior to those in the PU group and similar to the cardiac pathologic results of the PN group. The aforementioned results suggested that the overall adaptive capacity of the PO group was better than that of the PU group.

In conclusion, the CMS occurrence was mainly related to disturbances in energy, lipid, and amino acid metabolisms, which led to an imbalanced state of the body. On the contrary, the trend toward normal levels of energy and lipid metabolisms in the PO group indicated that the metabolic disorders were not severe and their adaptive ability to plateau environment was better than that in the PU group. The cardiac pathological observations of the rats in the PU and PO groups confirmed this conclusion.

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Disclosure of conflict of interest

None.

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References

- [1] Wu W, Yao H, Zhao HW, Wang J and Haddad GG. Down-regulation of inwardly rectifying K(+) currents in astrocytes derived from patients with Monge's disease. *Neuroscience* 2018; 374: 70-79.
- [2] Maignan M, Pham I, Riviera-Ch M, Privat C, Petitjean O, Leon-Velarde F and Richalet JP. Cardiac function and treatment by acetazolamide in chronic mountain sickness patients. *Fundamental & Clinical Pharmacology* 2008; 22: 28-28.
- [3] DiPasquale DM, Muza SR, Gunn AM, Li Z, Zhang Q, Harris NS and Strangman GE. Evidence for cerebral edema, cerebral perfusion, and intracranial pressure elevations in acute mountain sickness. *Brain Behav* 2016; 6: e00437.
- [4] Zhou Q, Wang D, Liu Y, Yang X, Lucas R and Fischer B. Solnatide demonstrates profound therapeutic activity in a rat model of pulmonary edema induced by acute hypobaric hypoxia and exercise. *Chest* 2017; 151: 658-667.
- [5] Buroker NE, Ning XH, Zhou ZN, Li K, Cen WJ, Wu XF, Zhu WZ, Scott CR and Chen SH. SNPs, linkage disequilibrium, and chronic mountain sickness in Tibetan Chinese. *Hypoxia (Auckl)* 2017; 5: 67-74.
- [6] Jiang C, Chen J, Liu F, Luo Y, Xu G, Shen HY, Gao Y and Gao W. Chronic mountain sickness in Chinese Han males who migrated to the Qinghai-Tibetan plateau: application and evaluation of diagnostic criteria for chronic mountain sickness. *BMC Public Health* 2014; 14: 701.
- [7] Eltzschig HK and Carmeliet P. Hypoxia and inflammation. *N Engl J Med* 2011; 364: 656-665.
- [8] Alvarez RA, Miller MP, Hahn SA, Galley JC, Bauer E, Bachman T, Hu J, Sembrat J, Goncharov D, Mora AL, Rojas M, Goncharova E and Straub AC. Targeting pulmonary endothelial hemoglo-

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- bin alpha improves nitric oxide signaling and reverses pulmonary artery endothelial dysfunction. *Am J Respir Cell Mol Biol* 2017; 57: 733-744.
- [9] Luke T, Maylor J, Udem C, Sylvester JT and Shimoda LA. Kinase-dependent activation of voltage-gated Ca²⁺ channels by ET-1 in pulmonary arterial myocytes during chronic hypoxia. *Am J Physiol Lung Cell Mol Physiol* 2012; 302: L1128-1139.
- [10] Nergui S, Fukumoto Y, Do EZ, Nakajima S, Shimizu T, Ikeda S, Elias-Al-Mamun M and Shimokawa H. Role of endothelial nitric oxide synthase and collagen metabolism in right ventricular remodeling due to pulmonary hypertension. *Circ J* 2014; 78: 1465-1474.
- [11] Colgan SP, Campbell EL and Kominsky DJ. Hypoxia and mucosal inflammation. *Annu Rev Pathol* 2016; 11: 77-100.
- [12] Suzuki Y, Ruiz-Ortega M, Lorenzo O, Ruperez M, Esteban V and Egido J. Inflammation and angiotensin II. *Int J Biochem Cell Biol* 2003; 35: 881-900.
- [13] Tajidin NE, Shaari K, Maulidiani M, Salleh NS, Ketaren BR and Mohamad M. Metabolite profiling of *andrographis paniculata* (Burm. f.) Nees. young and mature leaves at different harvest ages using ¹H NMR-based metabolomics approach. *Sci Rep* 2019; 9: 16766.
- [14] Xia JM, Hu XM, Huang CH, Yu LB, Xu RF, Tang XX and Lin DH. Metabolic profiling of cold adaptation of a deep-sea psychrotolerant *Microbacterium sediminis* to prolonged low temperature under high hydrostatic pressure. *Appl Microbiol Biotechnol* 2020; 104: 277-289.
- [15] Zhang Y, Huang MS, Liu CC, Lian LY, Shen JC, He QD, Wang YJ, Zhang LB, Liu M and Yang ZB. Dynamic observation and analysis of metabolic response to moxibustion stimulation on ethanol-induced gastric mucosal lesions (GML) rats. *Chin Med* 2019; 14: 44.
- [16] Pichler Hefti J, Sonntag D, Hefti U, Risch L, Schoch OD, Turk AJ, Hess T, Bloch KE, Maggiorini M, Merz TM, Weinberger KM and Huber AR. Oxidative stress in hypobaric hypoxia and influence on vessel-tone modifying mediators. *High Alt Med Biol* 2013; 14: 273-279.
- [17] Zubieta-Calleja GR, Paulev PE, Zubieta-Calleja L, Zubieta-Calleja N and Zubieta-Castillo G. Hypoventilation in chronic mountain sickness: a mechanism to preserve energy. *J Physiol Pharmacol* 2006; 57 Suppl 4: 425-430.
- [18] Maimaitiyimin D, Aikemu A, Kamilijiang M, Salamu A and Zhang X. Effects and mechanisms of acetyl-L-cysteine in rats with chronic mountain sickness with H1-NMR metabolomics methods. *Med Sci Monit* 2014; 20: 767-773.
- [19] Nan X, Su S, Ma K, Ma X, Wang X, Zhaxi D, Ge R, Li Z and Lu D. Bioactive fraction of *Rhodiola algida* against chronic hypoxia-induced pulmonary arterial hypertension and its anti-proliferation mechanism in rats. *J Ethnopharmacol* 2018; 216: 175-183.
- [20] Ronen R, Zhou D, Bafna V and Haddad GG. The genetic basis of chronic mountain sickness. *Physiology (Bethesda)* 2014; 29: 403-412.