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Changes in cerebrovascular reactivity in healthy adults after acute exposure to high altitude

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Abstract. – OBJECTIVE: To study changes in the cerebrovascular reactivity (CVR) at different altitude area in healthy adults.

SUBJECTS AND METHODS: CVR was tested using transcranial Doppler combined with CO2 inhalation, near-infrared spectroscopy (NIRS) was used to detect the regional cerebral oxygen saturation (rScO2). Blood samples were collected, and the vasoactive substances in serum were detected using the enzyme-linked immunosorbent assay. In this study, 59 healthy adults were divided into 3 groups: low altitude group, medium altitude group and high altitude group. All the indicators in low altitude group were tested at 24h before departure and after arrival from Beijing (at an altitude of 44.4 m) to Xining (at a medium altitude of 2200 m). Then, after resting for 48h, all the indicators were tested at 24h and 48h after arrival from Xining (at a medium altitude of 2200 m) to Yushu Jiegu town (at a high altitude of 3700 m) together with those at the medium altitude. Intergroup comparisons were made for the subjects in the three altitudes.

RESULTS: There was an increase in the CVR in low altitude group after acute exposure to high altitude, and the difference was significant (CVR: 1.94re was vs. 0.91±0.53, $p<0.001$); the CVR index was increased, and the difference was significant [cerebrovascular reserve index (CVRI): 3.65he CVR vs. 1.37e CVR, $p<0.001$]; the rScO2 level was decreased with the increase of altitude, and the difference was significant [(66.78±4.61)% vs. (70.29±4.52)%, $p<0.001$]. The levels of vasoactive substances in low altitude group were decreased after acute exposure to high altitude compared with those before exposure: NO: [(79.14±9.54) μmol/L vs. (58.01±9.93) μmol/L, $p<0.001$]; serum eNOS level was increased, and the difference was significant [(77.23±6.20) pg/ml vs. (65.07±9.82) pg/ml, $p<0.001$]; EPO: [(84.68±13.16) pg/ml vs. (65.01±5.92) pg/ml, $p<0.001$]; VEGF: [(71.91±11.62) pg/ml vs. (54.92±11.86) pg/ml, $p<0.001$]; sFlt: [(384.18±42.73) pg/ml vs. (320.62±78.96) pg/ml, $p<0.001$].

There was also an increase in CVR in medium altitude group after acute exposure to high altitude, and the difference was significant [CVR: 2.00±0.79 vs. 0.91±0.66, $p<0.001$]; the difference of CVRI was significant [3.83±0.67 vs. 1.67±0.87, $p<0.001$]; rScO2 was slightly decreased with the increase of altitude, and the difference was not statistically significant [(67.53±4.61) % vs. (69.63±5.59) %, $p<0.001$]. Before exposure to high altitude area, the levels of NO, NOS, EPO, VEGF, and sFlt in low and medium altitude groups were higher than those in high altitude group. CVR level of subjects at different altitudes was negatively related to the ScO2 (r=-0.91) but positively related to NO and NOS levels (rs=0.89, r=0.75); CVR was moderately related to VEGF and EPO (rs=0.45, r=0.42). rScO2 was positively related to RBC, HB and VEGF levels (r=0.89, r=0.75, rs=0.86), but had a moderately negative correlation with NO and NOS levels (rs=-0.52, r=-0.57).

CONCLUSIONS: After subjects at a low altitude are exposed to high altitude rapidly, CVR is increased, RBC and vasoactive substances in serum, such as NO, eNOS, and EPO, are dramatically increased, VEGF is increased first and then decreased, sFlt-1 level is increased gradually, and rScO2 level is gradually decreased with the increase of altitude, indicating the local brain anoxia of subjects at a high altitude.

Key Words:
Acute exposure to high altitude, Cerebrovascular reactivity, Regional cerebral oxygen saturation, Vasoactive substances.

Abbreviations

CVR = cerebrovascular reactivity; NIRS = near infrared spectroscopy; regional cerebral oxygen saturation ($rScO_2$); CVRI = cerebrovascular reserve index; NO = nitric oxide; VEGF = vascular endothelial growth factor; EPO = erythropoietin; sFlt-1 = soluble fms-like tyrosine kinase 1; (sFlt-1); eNOS = endothelial nitric oxide synthase; HIF-1 = hypoxia-inducible factor 1; CBF = cerebral blood flow; MCA = middle cerebral arteries; ACA, anterior cerebral artery; PCA = posterior cerebral artery; BA = basilar artery; $ETCO_2$ = end-tidal CO_2.

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Introduction

There are about 0.14 billion people in the world living at high altitude areas above more than 2500 m. Qinghai-Tibet plateau is the highest plateau in China and even in the world, which is known as the Roof of the World[1]. With the development of construction and tourism, increasingly more people step into the plateau. However, the oxygen partial pressure in most of Qinghai-Tibet plateau is lower than 60% of that in the sea level. To adapt to the plateau after the acute exposure to the low pressure and oxygen environment, the body will produce a series of compensatory adjustment mechanism, such as increasing the pulmonary ventilation, changing the balance of arterial blood gas, speeding up the heart rate and increasing the pulmonary artery pressure, thus increasing the cerebral blood flow (CBF), keeping the brain oxygen and energy supply, producing a series of cytokines and increasing the brain blood flow rate[2]. Currently, the comprehensive and systematic data about heart artery, pulmonary vascular and blood vessel of the brain after acute exposure to high altitude are still needed. Several differences exist among different research results, and these are probably related to the different altitudes, duration of subjects at the high-altitude area and research methods[3]. Medical researchers have recognized that the medical hypoxia can be improved through learning from the research on high altitude hypoxia[4]. Therefore, our study tended to research the cerebral blood flow, blood indicators and related physiological indicators involved in the brain tissue oxygen supply and metabolism of healthy adults at different altitudes rapidly exposed to the high altitude, to investigate the adjustment features and mechanism of blood vessel of brain in hypoxia environment after acute exposure to high altitude and provide theoretical basis for improving the health and productivity of people rapidly exposed to high altitude or those who live there forever.

Subjects and Methods

Subjects

Inclusion criteria: 59 healthy adults participated in our research [age: 23-56 (30.57±6.27) years old; height: 153-184 (167.21±7.39) cm; weight: 45-100 (63.73±12.22) kg]. Low altitude group (n=17) was from Beijing at an altitude of 44.4 m, medium altitude group (n=22) was from Xining at an altitude of 2200 m, and high altitude group (n=20) was from Yushu at an altitude of 3700 m. All these 59 cases have been living in the area for more than 5 years, and are young adults of the Han nationality in either gender with no hypertension, diabetes mellitus, high cholesterol, heart disease and cerebrovascular disease.

Exclusion criteria: Patients diagnosed as hypertension, diabetes, or diseases in heart, lung or brain.

This research was approved by Ethics Committee of Qinghai People's Hospital. The research objectives and methods had been informed to subjects, and they signed the informed consent.

Methods

Research Programs

As has been shown in Figure 1, the subjects took flight from Beijing at a low altitude of 44.4 m to Xining at a medium altitude of 2200 m, and all the indicators were tested at 24 h before departure and after arrival. After resting for 48 h, they flew from Xining to Yushu Jiegu Town at an altitude of 3700 m together with those in medium-altitude area (Xining 2200 m), and all indicators were tested at 24 and 48 h after arrival, respectively; indicators of subjects in medium-altitude areas were tested before departure from Xining, and indicators of subjects in high-altitude areas were tested at 48 h after arrival to Yushu together with those in low- and medium-altitude areas.

Detection of Cerebral Oxygen Saturation via Near-Infrared Spectroscopy

Cerebral oxygen saturation was detected using near-infrared spectroscopy (NIRS, YuYue, Zhengjiang, China)[5], a novel method. It can monitor the regional cerebral oxygen saturation ($rScO_2$) in a non-invasive and real-time way[6], and evaluate brain oxygen metabolism balance, so that abnormal blood flow and imbalance of oxygen supply and demand in brain tissue can be observed in advance.

Subjects were observed in an indoor quite environment with only testers and subjects present. They sat with no movement of the head, the detection probes were fixed every 3-4 cm spacing in subjects' forehead, and the detection probes were adjusted, followed by testing immediately after all display signals were connected. The forehead mark included the bridge of the nose, eyebrows, and hairline[7], used to make sure that the detection

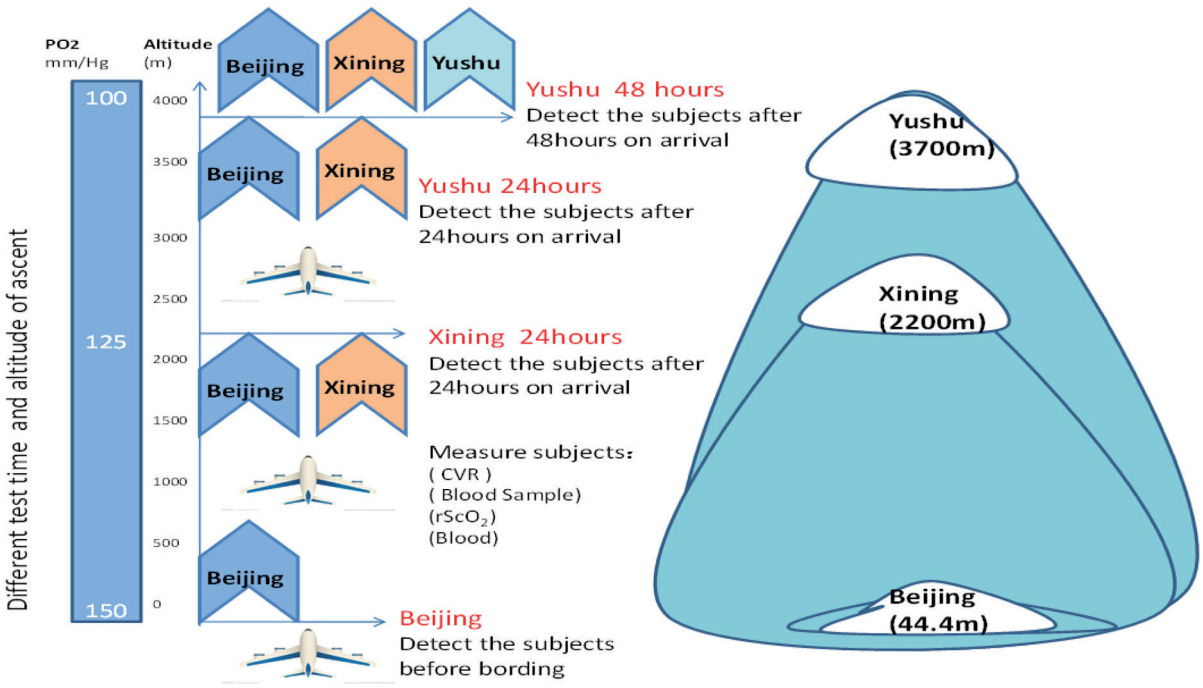

Figure 1. Research protocol.

probe holders were fixed in the same position, and the detection data can be compared at different times.

Detection of Cerebrovascular Reactivity via Transcranial Doppler (TCD) Combined with CO_2 Inhalation

TCD (YuYue, Zhengjiang, China) was used to detect middle cerebral arteries (MCA), anterior cerebral artery (ACA), posterior cerebral artery (PCA), basilar artery (BA) and vertebral artery through a temporal window. Peak systolic velocity and end-diastolic velocity were monitored.

Subjects were monitored using Multi-DopX TCD (DopX DWL, Germany), and the specific operation was as follows[8]: subjects lay on the examination bed under a quiet condition (respiratory rate of 16-20/min), and two 2 MHz ultrasonic probes were fixed on the bilateral temporal window of subjects using the head frame. With the detection depth of 55-65 mm, the artery speed, direction, and spectrum on both sides of the brain were detected. After the probe was fixed, signals were acquired from approximately the same depth on both sides. Subjects were required to wear masks and cooperate in the test procedures as follows: record after eupnea for 2 min → overventilation (deep breath) for 2 min → eupnea for 2 min → autologous inhalation of CO_2 a (2 m-long extension tube connected after the mask) → (2 m-long ex min m-breath-holding (as much as possible) after the mask) 2 min. Mean artery flow velocity (V baseline) and mean end-tidal CO_2 ($ETCO_2$) content of the brain in eupnea before and after CO_2 inhalation were recorded. Cerebrovascular reactivity (CVR) and cerebrovascular reserve index (CVRI) were calculated by measuring the artery blood flow parameters and CO_2 partial pressure on both sides of the brain. After acute exposure to high altitude, CVR value was measured at 72 h. The calculation formulas of CVR and CVRI are as follows[9].

In the above calculation formulas, the CVR is its absolute slope, which indicates that the changes in CO_2 partial pressure every mmHg corresponds to the absolute change value of blood flow velocity, and it is significantly affected by individual different of baseline blood flow velocity; CVRI refers to the relative slope, which means that the changes in CO_2 partial pressure every mmHg corresponds to the absolute change value of percentage of blood flow velocity, providing an index reflecting the changes in CBF velocity with the CO_2 partial pressure. The hypoxic ventilatory response test was performed to test the sensitivity of subjects to the hypoxic ventilatory response.

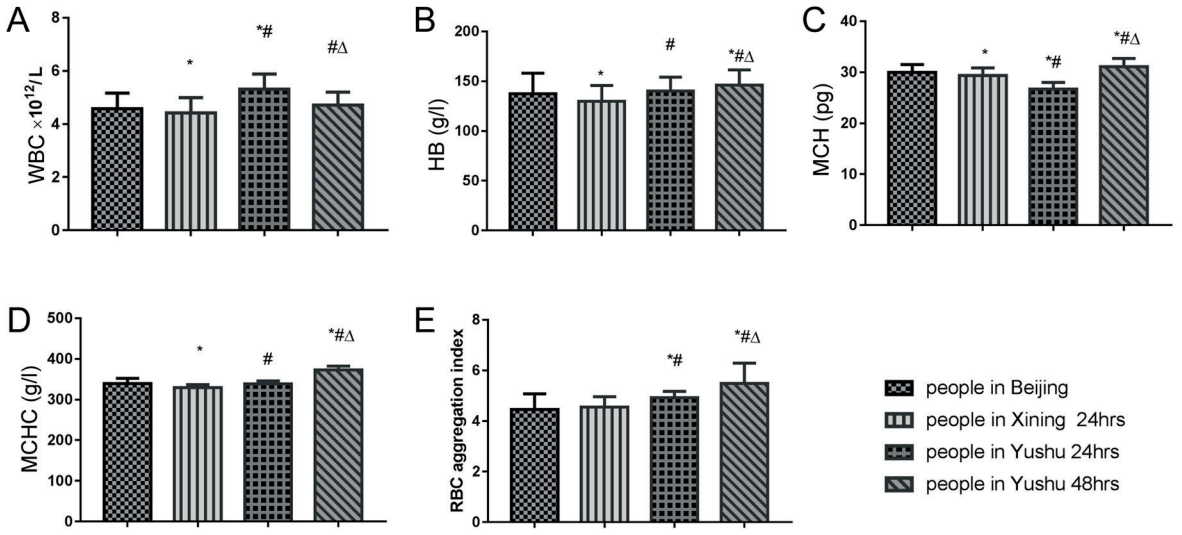

Figure 2. Changes in RBC, HB, MCH, MCHC and RBC aggregation index in subjects from Beijing.

Lab test

Fasting venous blood was taken for blood rheology test for the level of serum vasoactive substances, such as NO, VEGF, EPO, sFlt-1, and eNOS, via ELISA.

Statistical Analysis

SPSS19.0 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA) was used for data analysis; measurement data were presented as mean ± standard deviation, the repeated measurement analysis of variance and paired *t*-test were used for intergroup comparison. And linear correlation was applied for correlation analysis. Test level: α=0.05.

Results

RBC, RBC Aggregation Index, HB, MCH, and MCHC in Low Altitude Group Before and After Acute Exposure to high Altitude

After acute exposure to high altitude, some indicators, like RBC, RBC aggregation index, HB, MCH and MCHC, were increased with the rise of altitude, and the differences were statistically significant [RBC: 5.31±0.57 *vs.* 4.58 *vs.* 31, p<0.001]; [HB: 146.18±15.26 *vs.* 137.63±20.47, p<0.001]; [MCH: 31.10±1.61 *vs.* 29.99±1.51, p<0.001]; [MCHC: 373.00±9.12 *vs.* 339.63±12.61, p<0.001]; [RBC aggregation index: 5.48±0.80 *vs.* 4.45±0.62, p<0.001] (Figure 2).

Shear Rate and Casson Viscosity in Low Altitude Group

After acute exposure to high altitude, the whole blood viscosity and Casson viscosity of subjects in low-altitude areas were continuously increased with the rise of altitude, and the differences were statistically significant [shear rate 1: (23.08±4.18) *vs.* (14.98±3.13) mPa/s, p<0.001]; [shear rate 5: (9.89±1.46) *vs.* (7.05±1.14) mPa/s, p<0.001]; [shear rate 50: (5.17±0.68) *vs.* (3.94±0.49) mPa/s, p<0.001]; [shear rate 100: (4.58±0.61) *vs.* (3.58±0.42) mPa/s, p<0.001]; [shear rate 200: (4.22±0.58) *vs.* (3.35±0.39) mPa/s, p<0.001]; Casson viscosity [Casson viscosity: (3.37±0.53) *vs.* (2.81±0.31) mPa/s, p<0.001] (Figure 3).

RBC, RBC Aggregation Index, HB, MCH, and MCHC in Medium Altitude Group

The results showed that no change in RBC was found in medium altitude group and the difference was not statistically significant [HB: 165.00±21.32 *vs.* 157.09±20.26, p<0.001]; [MCH: 31.50±1.98 *vs.* 29.97±1.75, p<0.001]; [MCHC: 378.18±11.50 *vs.* 339.00±8.92, p<0.001]; [RBC aggregation index: 5.06±0.33 *vs.* 4.73±0.26, p<0.001] (Figure 4).

Shear Rate and Casson Viscosity in Medium Altitude Group

Hemorrheology examination revealed that the blood viscosity of subjects from medium-altitude area was increased with the rise in altitude, so the whole blood and Casson viscosity were progressively increased, and the differences were si-

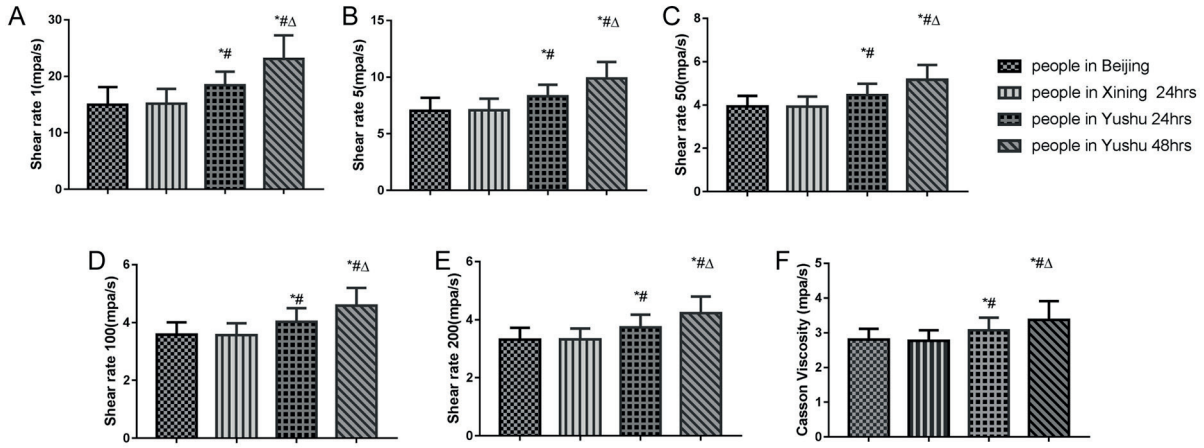

Figure 3. Change in blood viscosity of subjects from Beijing.

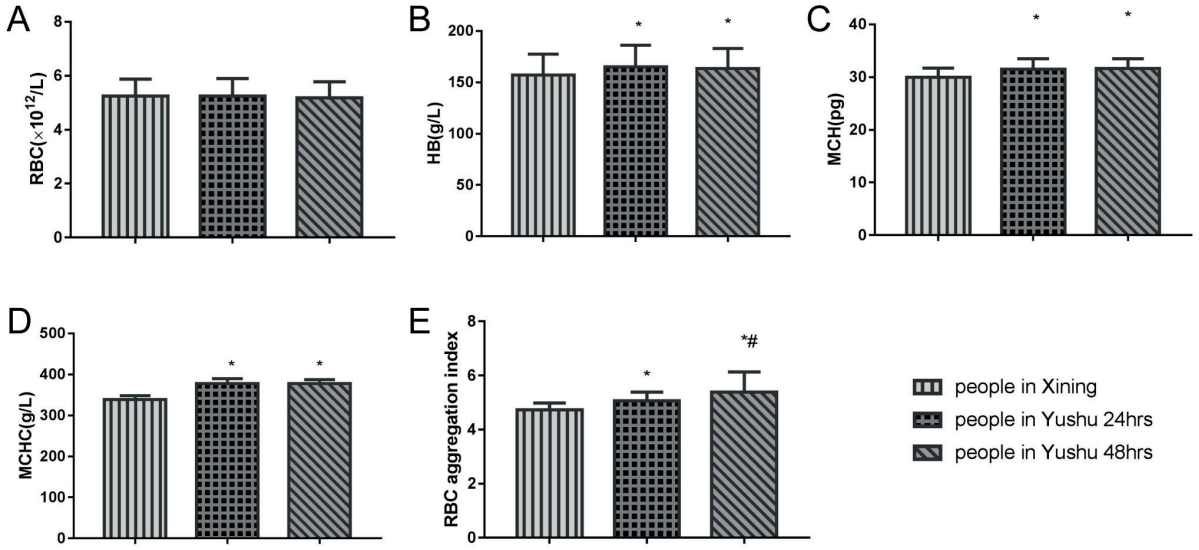

Figure 4. Change in RBC, HB, MCH, MCHC and RBC aggregation index in subjects from Xining.

gnificant [shear rate 1: 21.30±3.56 *vs.* 18.52±3.34, $p<0.001$]; [shear rate 5: 9.53±1.39 *vs.* 8.52±1.40, $p<0.001$]; [shear rate 50: 5.03±0.63 *vs.* 4.63±0.69, $p<0.001$]; [shear rate 100: 4.53±0.55 *vs.* 4.20±0.61, $p<0.001$]; [shear rate 200: 4.19±0.50 *vs.* 3.90±0.56, $p<0.001$]; Casson viscosity [Casson viscosity: 3.43±0.38 *vs.* 3.23±0.45, $p<0.001$] (Figure 5).

Regional Cerebral Oxygen Saturation

Regional cerebral oxygen saturation ($rScO_2$) of healthy subjects from low-altitude area was decreased after acute exposure to high altitude, and the difference was significant ($rScO_2$: 66.35±4.09 *vs.* 70.29±4.52, $p<0.001$). $rScO_2$ of healthy subjects from the medium-altitude area did not change much after acute exposure to high altitude, and the difference was not statistically significant [rScO2: 68.82±5.88 *vs.* 68.73±4.60, $p>0.05$]. $rScO_2$ of healthy subjects from the high-altitude area was the highest ($rScO_2$: 72.89±4.70) (Figure 6).

CVR and CVRI Results

CVR difference was statistically significant in subjects in low-altitude area after acute exposure to high altitude [CVR: 2.36±0.33 *vs.* 0.91±0.53, $p<0.001$]; at 48 h after acute exposure to high altitude [CVRI: 2.61±0.46 *vs.* 1.32±0.87, $p<0.001$]; CVR and CVRI in subjects in medium-altitude area after acute exposure to high altitude [CVR: 2.00±2.40 *vs.* 0.91±0.43, $p<0.001$]; [CVRI:

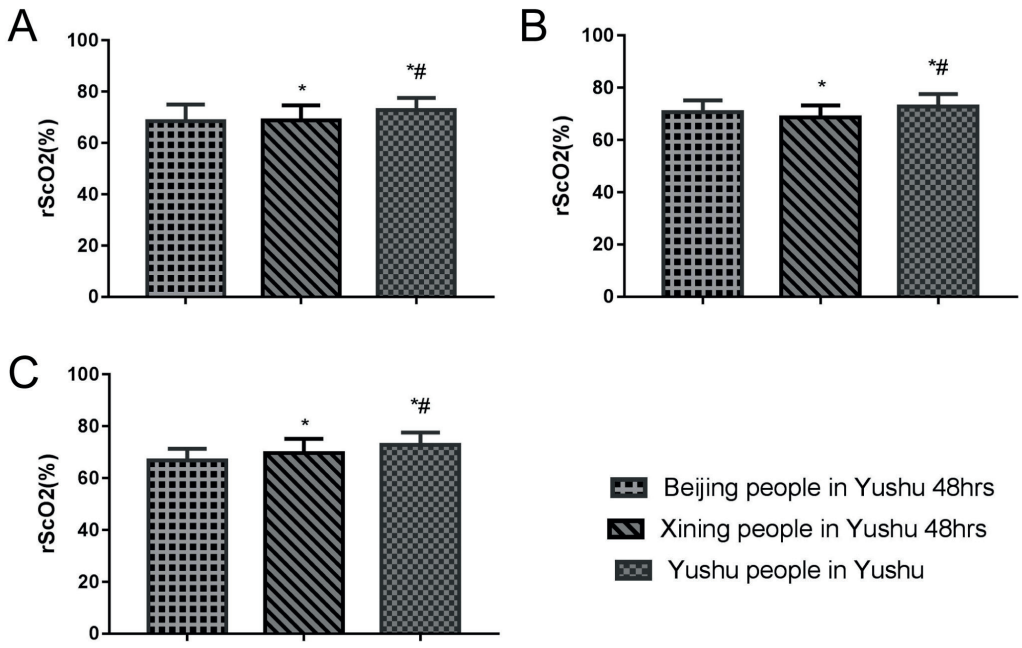

Figure 5. Change in blood viscosity of subjects from Xining.

3.83±0.67 *vs.* 1.67±0.73, $p<0.001$] healthy subjects in high-altitude area, CVR: 0.78±0.46; CVRI: 1.44±0.46 (Figure 7).

Test Results of Vasoactive Substances in Serum

As can be seen from Figure VIII, NO, NOS, and EPO levels in low altitude group were increased dramatically, VEGF level was increased first and then decreased, sFlt level was increased continuously. NO: [(79.14±9.54) μmol/L *vs.* (58.01±9.93) μmol/L, $p<0.001$]; eNOS level in serum was increased, and the difference was significant [(77.23±6.20) pg/ml vs (65.07±9.82) pg/ml, $p<0.001$], EPO [(84.68±13.16) pg/ml *vs.* (65.01±5.92) pg/ml, $p<0.001$], VEGF [(71.91±11.62) pg/ml *vs.* (54.92±11.86) pg/ml, $p<0.001$], sFlt [(384.18±42.73) pg/ml *vs.* (320.62±78.96) pg/ml, $p<0.001$] (Figure 8).

NO, NOS, EPO, sFlt, and VEGF expression levels of healthy people in medium-altitude areas were lower than those in people in low-altitude and high-altitude areas; NO level in plasma was increased and the difference was significant [(185.50±134.64) μmol/L *vs.* (183.69±2.86) μmol/L, $p<0.001$]; the eNOS level in plasma was increased and the difference was significant [(117.20±3.68) pg/ml *vs.* (62.49±4.14) pg/ml, $p<0.001$], EPO [(56.15±1.63) pg/ml *vs.* (62.71±5.52) pg/ml, $p<0.001$], VEGF [(63.42±2.67) pg/ml *vs.* (49.45±2.35) pg/ml, $p<0.001$], sFlt [(258.71±3.68) pg/ml *vs.* (209.45±5.52) pg/ml, $p<0.001$] (Figure 9)

Correlation of Different Indicators with CVR and $rScO_2$

Our research found that there was no relationship between CVR in different altitude groups and other vasoactive substances, such as RBC, HB, and sFlt (r=0.35, r=0.02, r=0.17); CVR was positively related to NO and NOS (r=0.55, r=0.50), but moderately correlated to VEGF and EPO (r=0.52, r=0.65); $rScO_2$ was positively related to RBC and HB (r=0.56, r=0.49), but moderately and negatively related to NO and NOS (r=-0.47, r=-0.50); moreover, it also had a moderate correlation with EPO (r=0.55) but there was no relationship between $rScO_2$ and CVR; VEGF and sFlt had a negative correlation (r=-0.55) (Table I).

Table I. S correlation analysis between different indicators with CVR and $rScO_2$.

Indicators	NO	SFLT	EPO	VEGF	NOS	$rScO_2$
CVR	0.55	0.17	0.65	0.52	0.50	0.12
$rScO_2$	-0.47	0.46	0.55	0.01	-0.50	

*$p<0.05$

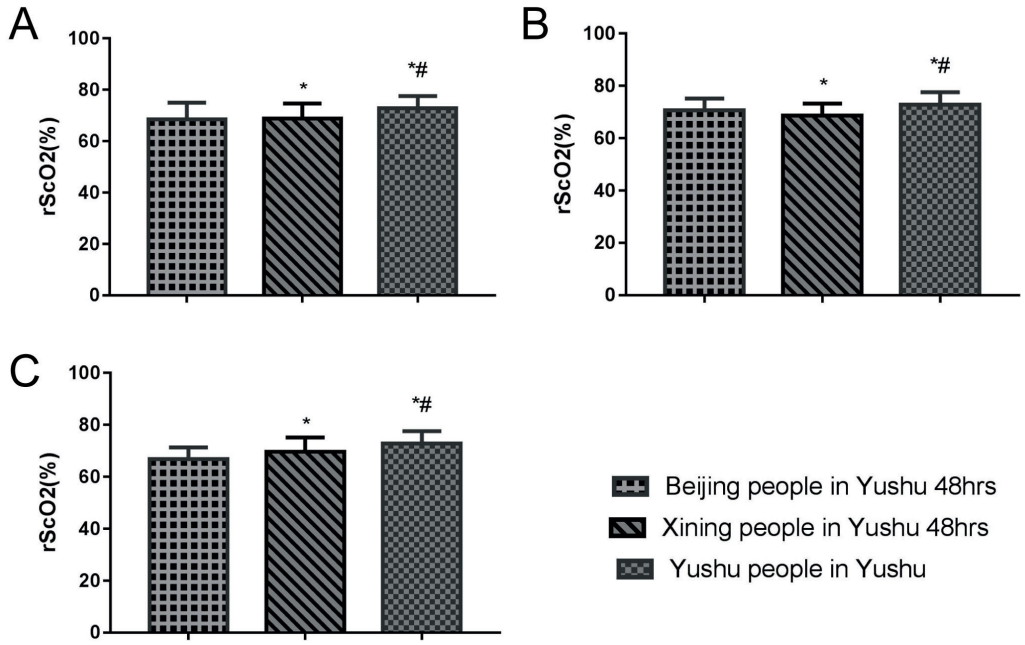

Figure 6. Comparison of $rScO_2$ between subjects from areas with different altitudes in different time.

Discussion

CVR refers to the vasomotor capacity of cerebrovascular system; in other words, the blood vessels of brain maintain normal and stable regional cerebral blood flow via self-regulation. CBF is mainly impacted by the carotid artery pressure, and the arterial pressure is between 60 and 140 mmHg, and blood vessels of the brain can maintain a stable CBF by self-regulation. CVR reflects changes in the CBF which can be demonstrated by a breath test, in which the CO_2 level in the blood is increased by respiratory depression, relaxing the cerebral arteries and increasing the CBF. CVR can be reflected by the CBF difference before and after respiratory depression, usually represented as Breath-holding index (BHI); the lower the BHI is, the worse the CVR will be. Hypoxemia itself can cause relaxation of cerebrovascular arteries[10].

Severinghaus et al[11] has found that the cerebral blood flow rate increases after acute exposure to high altitude, but this flow rate becomes normal after 1 week. Lucas et al[12] has confirmed that the average cerebral blood flow rate can be increased by 31% after acute exposure to high altitude according to the TCD research, which can restore to the cerebral blood flow rate in low-altitude area after 5-7 d. Recently, Vestergaard et al[13] has pointed out that cerebral perfusion and metabolic rate of subjects who were rapidly exposed to high altitude increase, the neuronal activity is increased and the generation of cytokine is promoted to help subjects adapt to the low oxygen content environment. The cerebral blood flow can be changed by controlling the tension of cerebrovascular muscle which is affected by many factors like neural, humoral, metabolic, and physical factors, etc. Also, it can build up a complex response network with many vascular contraction and relaxation factors like NO[14], prostaglandins, natriuretic peptide[15] and endothelin-1[16] released by endothelial cells and nerve cells. Different physiological stimulation can activate the above endogenous substances to change intracellular calcium ion concentration and adjust potassium channels, so that cerebrovascular muscle can shrink or relax. These physiological stimulations include circulating substances like $PaCO_2$, PaO_2, pH, lactate, glucose, and adenosine[17] and postganglionic neurotransmitters like NO, acetylcholine, vasoactive peptide, calcitonin gene-related peptide, and norepinephrine. The vascular dilation-systole caused by the release of these vasoactive substances may be the mechanism of brain vascular reactivity.

Our body can produce a series of compensatory reactions to adapt to the plateau when rapidly exposed to low-pressure and low-oxygen environment. This compensatory reaction series is a ho-

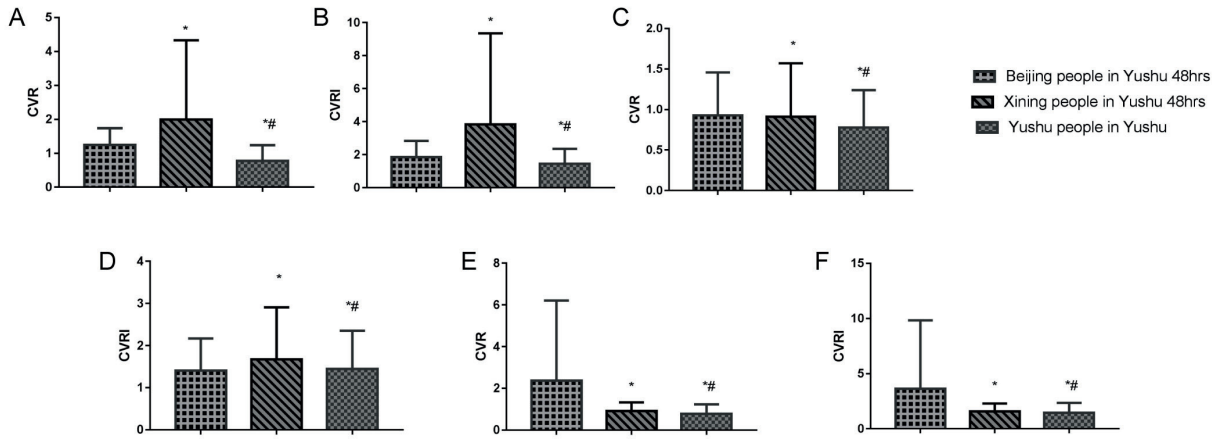

Figure 7. Comparison of CVR between subjects from areas with different altitudes in different time.

listic network reaction[18]. The test of blood system showed that after acute exposure to high altitude, RBC, HB, MCH and MCHC levels in low altitude group and medium altitude group were increased with the increase of altitude, and all the increasing trends remained the same, and the difference was statistically significant (p<0.001).

The possible mechanism[19] is as follows: 1. In a low-pressure and low-oxygen environment, the systemic vascular shrink can cause a decrease of renal artery and renal blood flow and an increase of erythropoietin secretion that is related to the decrease of peripheral oxygen content. 2. The adjustment between pituitary and gland is also involved in the above reactions. 3. It is also related to the dry weather that can cause the increase of water losing, dehydration, and low drinking water, as well as blood concentrate. The rise of RBC and Hb level can enhance the oxygen-carrying capacity of blood, increase the oxygen content and capacity, and improve the symptom of hypoxia of brain tissue. However, it does not mean that the higher the RBC and Hb levels are, the stronger the oxygen-carrying capability will be[20]. The increase in Hb and the RBC levels improves the oxygen-carrying capacity of the blood to a certain extent, and the high levels of RBC and Hb lead to increased viscosity and decrease blood flow,

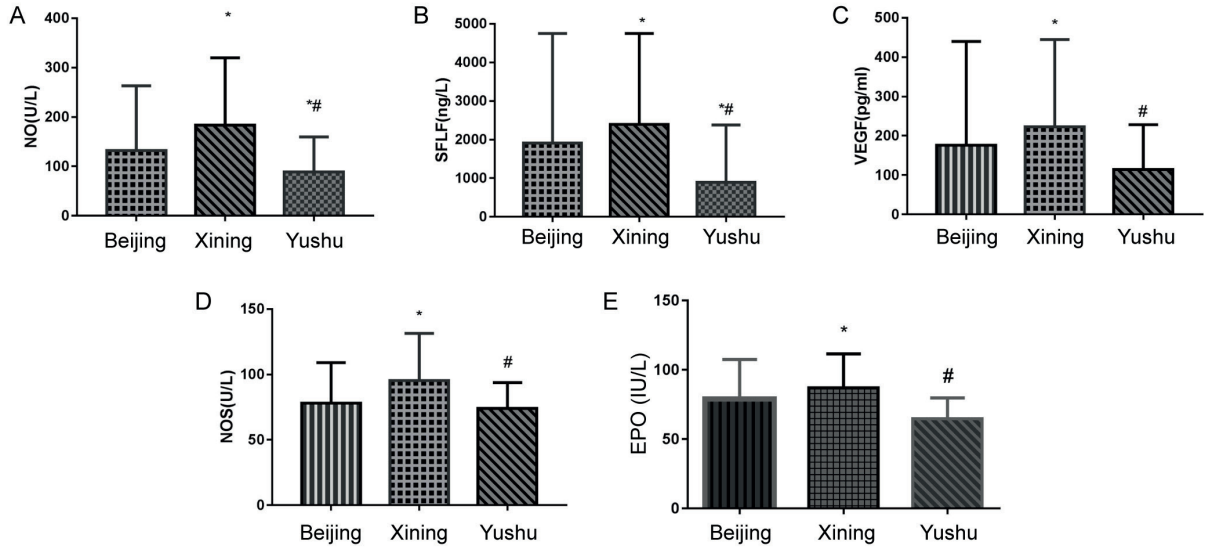

Figure 8. Comparison of vasoactive substance level in different time in Beijing group.

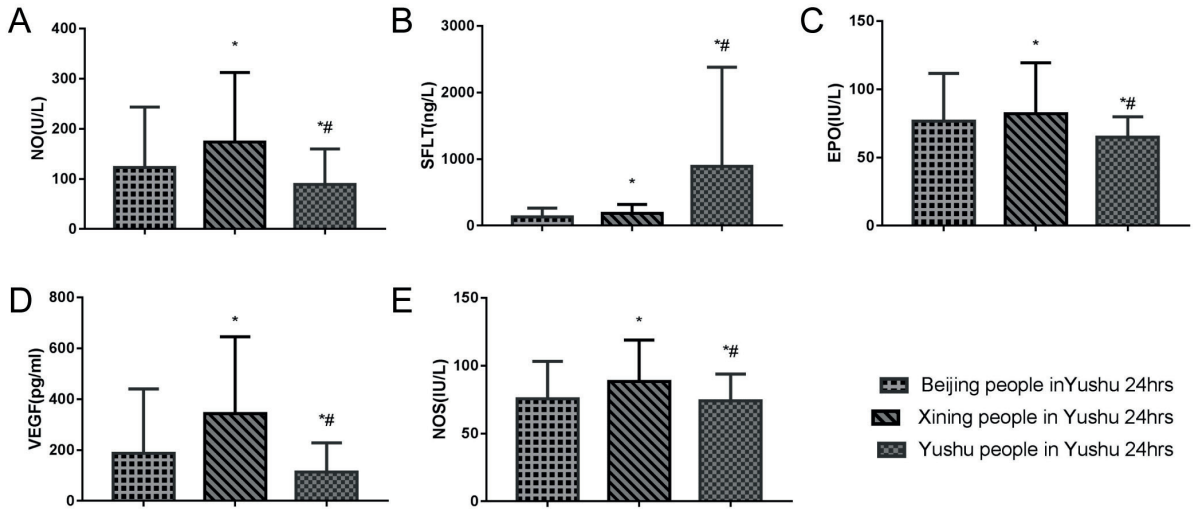

Figure 9. Comparison of vasoactive substance level in different time in Xining group.

which is detrimental to circulation. Also, there is no clear correlation between the CVR and RBC and Hb levels.

Hypoxia caused by low oxygen partial pressure after acute exposure to high altitude leads to compensatory mechanisms of hemorheology, providing effective protection and improving the body's aerobic capacity. There are other mechanisms to adapt ourselves to the low-pressure and low oxygen content environment and increase levels of immunoglobulins, which can strengthen the accumulation and weaken the deformation of RBC. All the above-stated mechanisms lead to an increase in viscosity, and the same phenomena were observed in our research: the whole blood viscosity, Casson viscosity, and RBC accumulation index were increased with the increase of altitude. After acute exposure to high altitude for 1 day, the effective blood capacity was decreased, but the blood viscosity (shear rate and Casson viscosity) was increased dramatically. With the passage of time, the effective blood capacity was increased continuously, while the blood viscosity was decreased continuously, and the difference between the fourth day and first day was statistically significant.

Hypoxia can induce the release of vasoactive substances to cause the contraction and relaxation of blood vessels, and this is probably the reactive mechanism of cerebrovascular. Some research has found that NO mediates the contraction and relaxation of cerebrovascular which is caused by hypercapnia. Endothelial NO is an important vasodilator, which is produced by nitric oxide synthase (NOS) to catalyze L-arginine in vascular endothelial cells. The latest research has found that cerebral blood flow velocity increases when exogenous NO is administrated to subjects who rapidly exposed to low altitude from high-altitude areas[21], indicating that the contraction and relaxation of cerebral blood vessels in an acute hypoxia environment are related to the release of activity factor NO. After they return from high-altitude areas, the cerebral blood flow velocity may decrease or not change when the oxygen supply is sufficient but when exogenous NO is administrated to subjects, and blood velocity will increase. Moreover, our study found that no evident increase of CVR and plasma NO content was observed after acute exposure to 3700 m-high area to 2200 m-high area. Therefore, one reason for the increase of CVR after acute exposure to high altitude is that hypoxia environment can stimulate the increase of NO level in plasma so that the contraction and relaxation ability can be improved.

NOS can be classified as neuronal, inducible, neuronal, and endothelial types. The form mainly expressed in blood vessel of brain is endothelial. Acute exposure to high altitude causes an increase of cerebral blood flow velocity and shear stress, which improves the expression of NOS, mRNA, and protein, as well as the generation of NO[22]. Beall et al[23] used a dynamic observation method to monitor NO level of subjects after acute exposure to high altitude, and he found that NO level in lung, plasma, etc., decrease in the first 2 h after exposure to high altitude. But these levels will return to the level before exposure to high altitude or slightly higher than that at 48 h, and will continuously in-

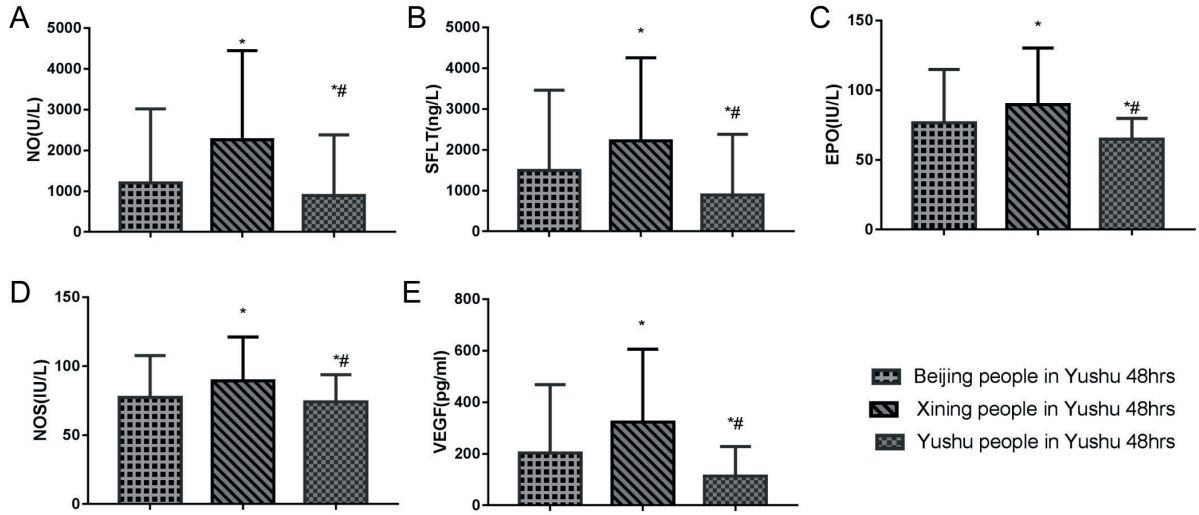

Figure 10. Comparison of vasoactive substances level between different groups in different time.

crease in the following 5 days. Our results found the similar results: subjects in low-altitude area experienced a decrease of NO level first, followed by an increase. Moreover, some scholars[24] studied the NO level using low oxygen pressure chamber model and found that NO level was increased after acute exposure to high altitude. Also, some relative studies reported that NO and NOS levels are positively related to CVR level, and NO and NOS are important vasoactive substances which can affect the blood vessel of brain.

Patitucci et al[25] found that VEGF level in the circulation blood of mountain sickness and high altitude pulmonary edema (HAPE) is increased dramatically. Hypoxia is the major factor of adjusting the expression of VEGF, the latter of which is the vascular endothelial cell that can increase pulmonary artery pressure and vascular permeability. The increased VEGF expression increases the vasodilation to enhance the blood supply for local tissue and improve the hypoxia condition. Our research results showed that the VEGF expression level of subjects from low-altitude and medium-altitude areas was increased with the increase of altitude, so that our body can adapt to the low oxygen environment in plateau. But the overexpression of VEGF can cause long-term congestion of tissue, which can change the tissue structure and make it in a pathological state, finally leading to chronic mountain sickness. Correlation analysis in our research showed that VEGF expression level was moderately correlated to CVR, indicating that VEGF influences the cerebrovascular reactivity to some extent.

Soluble vascular endothelial growth factors (sFlt-1) are produced in different tissues[26], and they can combine with one another and reduce the free VEGF level in circulation blood to inhibit its biological effects[27]. However, sFlt-1 level in most hypoxia cases increases dramatically[28]. Some research has reported that apart from VEGF, sFlt-1 can also reduce the biological activity of provascular permeability molecules like PIGF, Sema3A, and Gal-1. As an antagonist, sFlt-1 can isolate the free VEGF in circulation blood, so that the combination of VEGF with vascular endothelial cells can increase the vascular permeability effect. Neulen et al[29] found that the balance of VEGF and sFlt-1 level is the requirement to ensure that the ovary can make a correct response to pituitary gonadotropin. After acute exposure to high altitude, sFlt-1 expression level increases dramatically to prevent the overexpression of VEGF. Therefore, VEGF and sFlt can work together and both of them participate in the regulation of cerebral blood flow. Our research found that the increase of sFlt-1 was greater than that of VEGF, indicating that sFlt-1 is more sensitive to hypoxia. Correlation analysis showed that no relationship between sFlt-1 and CVR was observed, suggesting that sFlt-1 has no significant effect on cerebrovascular reactivity. However, there is a high correlation between VEGF and sFlt (rs=0.83) according to the correlation analysis. Different from the research results of Schommer et al[30], unpublished study in our group showed that sFlt-1 is more sensitive to the hypoxia and treatment than VEGF. sFlt-1 is probably a more important factor in HAPE patho-

genesis, sFlt-1 and sFlt-1/VEGF ratio can be used as potential biomarkers for studying the HAPE diagnosis and pathogenesis. Therefore, an appropriate balance exists between VEGF and sFlt-1, which can participate in the adjustment of cerebrovascular reactivity.

EPO can bind to its receptors and work on the vascular endothelial cells to make them proliferate and migrate. It has an effect in the resistance of our body tissues to hypoxia and apoptosis[31]. Some other studies[32] have found that EPO is involved in angiogenesis, and it has a similar effect with VEGF. EPO plays an important role in the angiogenesis process and can be regarded as a factor to improve the generation of vessels. When our body is exposed to high altitude, hypoxia receptors in the kidney and outside the kidney can be stimulated by hypoxia, HIF-1α expression can be induced in this process to regulate the synthesis of EPO and improve its release, thereby causing the proliferation of cells. Moreover, it is also an important factor for our body to adapt to the hypoxia environment. However, if our body is exposed to hypoxia environment for a long-term, the inducing factors of EPO will continuously exist to ensure the high expression of EPO, abnormal proliferation of RBC, and increased blood viscosity, so that a 'hypoxia-abnormal proliferation of RBC-increased blood viscosity-increased hypoxia' vicious circle is built up, causing polycythemia and other diseases. It has been already known that HIF-1α is the upstream regulation gene to VEGF and EPO, which can induce the expression levels of VEGF and EPO. EPO and VEGF are downstream genes regulated and transcribed by HIF-1α, and these genes can help tissue cells work out a series of adaptive responses including promotion of angiogenesis, erythropoiesis, and vasomotor function regulation[33,34]. However, the results of our research showed that EPO was slightly related to CVR, indicating that EPO has a small effect on changing the cerebrovascular reactivity.

Dubowitz et al[35] used magnetic resonance imaging (MRI) for analysis and found that cerebral blood capacity and parenchymal volume of healthy adults are increased dramatically after 40 min in hypoxia environment. Therefore, they pointed out that the hypoxia of brain was the early reaction, and the mechanism of cerebral vasodilatation increase caused by hypoxia may be because our brain is sensitive to CO_2 partial pressure. Lucas et al[13] thought that the correlation between cerebrovascular reactivity and CO_2 partial pressure is the opposite, which means that the brain is more sensitive to hypocapnia than hypercapnia. Some studies have found that the brain is more sensitive to hypercapnia than hypocapnia in low-altitude area. The results in our research showed that CVR level increased significantly after acute exposure to high altitude. This is probably because the alveolar oxygen partial pressure and arterial oxygen partial pressure are decreased in hypoxia environment. The increase in CO_2 partial pressure is due to the enhancement of CVR sensitivity. Therefore, brain tissue perfusion and oxygen exchange can be increased significantly to relieve the brain tissue hypoxia.

$rScO_2$ is the value of oxygen content in brain tissue, which can be monitored continuously by NIRS. Oxygen saturation in brain microvascular level (including 25% small arteries, 70% small veins and 5% capillaries) is used to represent the brain oxygen supply and demand balance[36]. NIRS is a reliable method to monitor the self-regulation mechanism of cerebral blood flow, and timely reflect the effect of cerebral blood flow and perfusion on $rScO_2$. $rScO_2$ is affected by many factors, such as age, hemoglobin concentration, arterial oxygen saturation and end-tidal carbon dioxide[37]. No studies about the relationship between cerebrovascular reactivity and cerebral oxygen saturation after acute exposure to high altitude have been reported. Our results showed that Hb level was increased with the increase of altitude to adapt to the hypoxia environment. The $rScO_2$ level was increased as well, and the blood became thick and blood oxygen saturation was decreased after it increased to a certain level. Vasoactive substance NO was increased and dilated blood vessels, and the cerebrovascular reactivity was increased in our body to adapt to the low-pressure and low-oxygen environment. Some relative analysis indicated that cerebrovascular reactivity is negatively related to cerebral oxygen saturation, which is consistent with the results of our study. The cerebral oxygen saturation of subjects from high-altitude areas is lower than that from low-altitude area, but the cerebrovascular reactivity is better. The cerebrovascular reactivity of subjects in medium-altitude areas is better than that in subjects in low-altitude and high-altitude areas. Two groups were researched in our study, and subjects in one group were rapidly exposed to the altitude of 2200 m from altitude of 44.4 m, and after a short time, they were exposed to the altitude of 3700 m. Subjects in another group were directly exposed to the altitude of 3700 m from the altitude of 2200 m. Volunteers from the altitude of 3700 m were taken as the control group. After comparison of the

baseline between low altitude and medium altitude group, it was found that there was a certain limitation of our research. Additionally, Qinghai province is a multi-ethnic area, including Tibetan, Hui, Tu, Sala, and Mongolian nationalities. The subjects in our research were from Han nationality, and the CVR between different nationalities was not compared. Our further study will consider involving more minorities, such as Tibetan, Hui, Tu, Sala, and Mongolian nationalities to study the physiological and pathological process and role of genetic factors for people rapidly exposed to high altitude. Therefore, it can help find out more ways for the prevention and treatment of plateau diseases.

We found that the vasoactive substances level in medium altitude group was higher than that in low altitude and high altitude group. This is probably because medium altitude is a natural hypoxic preconditioning (HPC) and after a long-term exposure in the hypoxic environment, subjects can adapt to this condition easily. Hypoxic preconditioning (HPC) refers to the repeatedly light hypoxic environment in a short time for the body, which can remarkably improve the tolerance of the severe ischemia and hypoxia environment, and provide effective neuroprotection for the body[38]. Hypoxia-inducible factor-1α (HIF-1α) is the main adjustment factor depending on the oxygen content in the environment and it is very sensitive. Some scholars[39,40] observed that HIF-1α can provide neuroprotection for the adjustment of corresponded target protein. VEGF is the target protein of HIF-1α downstream code, which can participate in neurovascular remodeling to achieve neuroprotection effect[41]. It is still unclear about the neuroprotection mechanism of HPC in traumatic brain injury. HPC can improve the activation of HIF-1α and VEGF in surrounding brain tissue injury at initial stage and maintain their high expression levels. This mechanism is realized probably by the repeatedly and shortly improvement of HPC on hypoxia tolerance and activation of endothelial cell function. Our team has done some investigations about the effect of hypoxic preconditioning on brain reserve capacity in cellular and animal levels, and the results found that hypoxic preconditioning can increase the brain reserve capacity, reduce ischemic brain injury, and improve the restore of brain structure and function. This finding helps reveal the mechanism of collateral circulation and cerebral ischemia and hypoxia, providing scientific basis for prevention and treatment of stroke. Plateau research has found that subjects in high-altitude areas tend to have higher brain reserve capacity, smaller infarct size and lighter clinical symptoms than those in low-altitude area under the same vascular occlusion. Moderately and intermittently exposure to high altitude as a hypoxic preconditioning method is an effective way to protect and improve the brain reserve capacity. Based on the above research results, we can understand that the reason why the subjects in medium altitude area have the highest cerebrovascular reactivity is that their vasoactive substances increase dramatically after a long-term hypoxic preconditioning. However, subjects in high-altitude areas have been experiencing severe hypoxia condition for a long time, which causes a slow adaption to hypoxia, so that the cerebrovascular regulation ability is lower than that in medium-altitude subjects.

Conclusions

Healthy adults from low altitude (44.4 m) and high altitude (2200 m) areas after acute exposure to high altitude. RBC, RBC aggregation index, Hb, MCH, and MCHC were progressively increased. The reactivity of cerebrovascular was increased and reached the peak after 48 hours. It is considered that people can adapt to high altitude hypoxia by increasing the oxygen-carrying capacity and adjusting the cerebrovascular reactivity. NO and NOS expressions in the serum of healthy adults who are rapidly exposed to high altitude. NO and NOS expressions in low altitude and medium altitude area were increased with the increase of altitude. NO and NOS expressions in medium altitude area were increased significantly, which were higher than those in low altitude and high altitude areas (3700 m). It is considered that the increase of NO expression level in serum can improve the vasodilator capacity and cerebrovascular reactivity. sFlt and VEGF expression in healthy adults who are rapidly exposed to high altitude. sFlt and VEGF expressions in low altitude and medium altitude area were increased with the increase of altitude. sFlt and VEGF expressions in medium altitude area were increased significantly, which were higher than those in low altitude and high altitude areas. It is considered that the balance regulation between VEGF and sFlt participates in the acute hypoxic brain blood flow regulation.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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