Plasma Metabolomic Profiling of Individuals Susceptible to High Altitude through Gas Chromatograph- mass Spectrometry

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Abstract

Objective We aimed to characterize metabolic alterations of people ascending to high altitude and susceptible to acute mountain sickness (AMS).

Methods Peripheral blood samples were collected from 36 healthy volunteers on the 3rd day ascending to high altitude (4300m). AMS status was assessed using the Lake Louise Questionnaire. Plasma samples were characterized by gas chromatography-mass spectrometry (GC-MS) and principal component analysis (PCA) was used to discriminate the metabolite changes between sea level and high altitude status and between AMS group and non-AMS group.

Results High altitude hypobaric hypoxia caused significant and comprehensive metabolic changes in plasma, including 18 metabolites between sea level and high altitude, 6 metabolites between AMS group and non-AMS group. By using MetaboAnalyst 3.0, several key metabolic pathways were found to be involved, including cysteine and methionine metabolism, alanine, aspartate and glutamate metabolism.

Conclusion The GC-MS profiling was a useful approach to analyze metabolites variances and provides potential targets for further investigation to understand the pathophysiological mechanism of hypobaric hypoxia and susceptibility to high altitude.

Introduction

In high-altitude plateau environments, the main physiological challenge is hypoxia. Upon rapid ascent to altitude above 3000 m, non-acclimatized healthy individuals are highly prone to contracting acute mountain sickness (AMS), high-altitude cerebral edema (HACE), or high-altitude pulmonary edema (HAPE) due to hypoxia ^{1,2}. With increasing number of people entering the plateau, AMS has become a health-related issue not only impairs human activity but also enhances the costs of health care. AMS is a transient condition and the routine diagnosis method is Lake Louise Score, which evaluates symptoms such as headache, weakness, sleeplessness, etc ³. Despite the detailed clinical characterization, the pathophysiology of AMS remains unclear and this scoring system may not be suitable for early diagnosis, thus leading to the delay of treatment and worsening of disease ⁴. Therefore, accurate and prompt biomarkers are urgently required to identify people who are sensitive to hypoxia at high altitude, but none of which are currently available.

Metabolomics platform is a newly developed tool for system biology, which can identify and quantify all small molecular metabolites within a biological system ⁵. Metabolic profiling is used to define metabolic changes in response to genetic differences, environmental influences and disease or drug perturbations at global scale ⁶. Equipped with advanced high throughput platforms, bio statistical analysis software and bioinformatics tool, metabolomics has become an irreplaceable approach to obtain and compare the full complement of metabolites in tissue samples between groups to discover slight changes to external stimulation ⁷. It has developed rapidly and become an effective tool for disease diagnosis, biomarker

screening, and characterization of biological pathways. Nuclear magnetic resonance (NMR), liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are currently prevailing techniques used to explore metabolic changes in urine or plasma⁷. Single analytical approach of NMR or LC-MS may not be able to provide full coverage of even the simplest metabolites with diverse concentration and mass range⁸. GC-MS, on the other hand, has been increasingly applied in laboratories because of its easier metabolites identification approach based on by comparing their mass spectrum and retention index with authentic reference standards or commercial libraries⁹. So far, studies focusing on using “omics” have provided large amount of background information about high-altitude illness¹⁰, among which metabolomics is a newly developed one. Johnson CH came up with the idea that proteomic variation can cause metabolomics variation¹¹ and this furthers the use of metabolomics in the research of altitude illness.

Since some individuals are more susceptible to high altitude diseases than others, the incidence of high-altitude diseases is variable among different population. Given that the pathophysiology of the susceptibility of AMS remains unresolved, the broader view provided by metabolomics may provide novel insights into the etiology of AMS and identify novel biomarkers for the prompt diagnosis of the condition. Herein, a metabolomics method based on GC-MS and random forest (RF) models were applied to analyze the serum samples of people rapidly ascending to high altitude and to comprehensively investigate the metabolic profiling changes of AMS.

Materials And Methods

Subject recruitment

Subjects recruited for analysis were male health volunteers living at sea level. Inclusion criteria were as follows: absence of acute infection during the past 6 months, absence of chronic inflammatory diseases, with no organic diseases, with the age >18 years, and >6 months stay at sea level. Volunteers were excluded if they had any health problems, abnormal complete blood count, chemistry panel or liver function results or those who had been to high altitude during the past 6 months. All subjects' general health status eligibility were assessed and determined prior to participation including medical history, physical check, and standard blood and urine analysis. They all ascended by train to Golmud district (altitude 4300m) in Qinghai, China. All subjects signed informed consent to all the risks involved in this study and they did not receive any preventative drugs before or during ascending to high altitude. Our study has been approved by the Ethics Committee of the Chinese PLA General Hospital.

AMS assessment and physiological examination

To evaluate AMS status, subjects recruited for this clinical study completed the self-reported questionnaire on the 3rd day arriving at high altitude. The diagnosis of AMS was based on the Lake Louise Score (LLS) system^{3,12}. Five symptoms including headache, gastrointestinal symptoms,

fatigue/weakness, dizziness/light-headedness, and difficulty in sleeping were assessed. Participants scored the severity of each symptom from 0 (no symptom) to 3 (maximal severity). Individuals whose total score exceeded 3 points and were accompanied with headache were diagnosed as AMS. And subjects whose LLS scores were lower than 3 points were selected as control group. Blood pressure, heart rate and oxygen saturation were also measured both at sea level and on the 3rd morning after ascending to high altitude. Arm blood pressure (BP) monitor (KD-5903; Andon Health Co., Ltd. Tianjin) was used to determine brachial artery BP of each volunteer for three times and average value was acquired. SpO₂ of fingertip and pulse rate were measured with pulse oximeter (YX301; Yuwell Medical Equipment & Supply Co., Ltd., Jiangsu), both of which were performed on right index fingers in the morning before breakfast.

Blood sample collection, conservation and preparation

Venous blood (4ml) was collected in EDTA-K₂ tubes from each overnight fasting individual in the morning at sea level and on the third day after ascending to Golmud. Plasma was separated through centrifugation at 3000rpm for 5 minutes, stored at -20°C and then transported to Beijing and stored at -80°C until further analysis.

The preprocessing procedures before GC-MS analysis included deproteinization, drying and chemically derivatization. Plasma was thawed at 4°C for 20 min and centrifuged for 15 min (15,800 rpm, 4°C). To the 100 µl supernatant, 400 µl methanol was added for deproteinization and centrifuged for another 15 min. Then ribitol was added as internal standards at the concentration of 1 mg/ml and mixed on a vortex for 15s. The above samples were put on ice in ventilated cabinet. After methanol was volatilized, they were put at -70°C for over 4h until dried. One microliter of each supernatant was then transferred to the sample for the following GC-MS procures. The remaining was combined to generate a pooled quality control (QC) sample. Then dried samples were derivatized using freshly prepared methoxylamine/pyridine (50 mg/ml) and incubated for 90min (30°C). Next, 80 µl of N-methyl-N- (trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) was added to the samples which were then incubated for 2h at 37°C.

Gas chromatography-mass spectrometry conditions

Samples were analyzed on the Agilent 7890A gas chromatograph with a 5975C time-of-flight mass spectrometer (Agilent Technologies, America) that equipped with an auto-sampler 7693 and electron ionization (EI) source. A 30m×0.25mm×0.25 µm deactivated fused silica capillary column (Agilent Technologies) was used for the GC-MS analysis. The primary temperature was maintained at 60°C for 4 min, programmed to 180°C at a rate of 10°C/ min, and slowed down to the rate of 3°C/min to 260°C, then rise the rate again at 10°C/min until 300°C and held for 10 min. The temperatures of the front injection port, interface and ion source were set at 270°C, 280°C and 230°C, respectively. Helium was used as the carrier gas with a flow rate of 1.0 ml/min. One microliter of sample was injected in the 10:1 split mode.

The MS quadrupole temperature was 150°C. The mass spectrometer was operated under electron impact (EI) mode at ionization energy of 70 eV, with a scanning extent of 85-500m/z and was set for five scans per second.

Quality control

A quality control (QC) sample theoretically identical to the biological samples, with a metabolic and sample matrix composition similar to those of the biological samples under study, is used in our study ¹³. A pooled QC strategy deriving from all the subject population was applied. Equal quantities were taken from every sample and mixed together as the QC sample. The serum samples and the QC samples were labeled. Before the experimental samples were injected, QC samples were used to equilibrate the GC-MS. All the samples were then analyzed in GC-MS at random in order to avoid the run order effect.

Identification and quantification of metabolites

Data from GC-MS was processed by GC/MSD Chem Station Software (Agilent) for auto-acquisition of GC total ion chromatograms (TICs) and fragmentation patterns. Then all transformed data was processed sequentially with the assistance of Qualitative Analysis B.04. Since there was a serious of split molecular ions for each compound fragmentation pattern, the National Institute of Standards and Technology (NIST) mass spectra library by the Chem Station Software was used to make the comparison. The standard mass chromatogram from NIST, which includes the mass charge ratios and the abundance of compound fragmentation, can search the following parameters including the compound's name, constitutional formula, retention time, relative molecular weight ect. For each peak, the software generated a list of similarities with those within the NIST library. Peaks with matching factor over 85% were assigned compound names, those having < 85% similarity were listed as unknown metabolites. Internal standard was used to obtain the relative quantification of these compounds. The results were exported in .cef files, which included the above parameters.

Multivariate data processing

Feature extraction and pre-processing of the obtained data were performed using R software, and then normalized and edited into two-dimensional data matrix by Excel 2010 software. Parameters including Retention time (RT), Mass-to-charge ratio (MZ), Observations (samples) and peak intensity were collected. Multiple variables statistical procedures of principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were applied to observe the plasma profiles from different groups using SIMCA-P 13.0 software (Umetrics AB, Umea, Sweden). The quality of the models was evaluated by the relevant R^2X (explainable index) and Q^2 (predictable index). PCA models were regarded as valid only if $R^2(X) > 0.4$. PLS-DA models were regarded as valid only if $Q^2 > 0.4$. All of the data from the differentially expressed compounds were used in calculating PCA models (an unsupervised

statistical procedure that uses orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components).

Significantly characteristic differential metabolites or metabolic features between sea level and high altitude groups, AMS and non-AMS groups were screened using the OPLS-DA model. The Paired t-test was selected to measure the significance of each metabolite in separating sea level from high altitude group and AMS from non-AMS groups. Fold change is calculated through the Logarithmic value (2 as the base value) of ratio between AMS group and non-AMS group. A positive value indicates a relatively higher concentration present in AMS group, whereas a negative value indicates a relatively higher concentration in non-AMS group. The differences were considered significant when $p < 0.05$.

Commercial databases, including the Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg/>) databases were used to search for metabolite pathways. More detailed analysis of the relevant pathways and networks of AMS is performed by MetaboAnalyst 3.0, which revealed that these differential metabolites are important for the organism response to high altitude or AMS and are responsible for multiple pathways. MetaboAnalyst is a free, web-based tool that combines results from a powerful pathway enrichment analysis concerning the conditions under study ¹⁴.

Results

General characteristics of enrolled subjects

In our current study, 36 volunteers met the inclusion and exclusion criteria and thus were included. Based on the Lake Louise Score System, 17 of the included subjects were divided into AMS group while the rest 19 were defined as non-AMS. The clinical characteristics of these subjects were illustrated in Table 1. On the third day after arriving at high altitude, the physiological responses could be reflected through significantly increased heart rate (66.3 ± 5.6 at sea level, 81.3 ± 11.2 for AMS group and 82.3 ± 8.2 for non-AMS group) and blood pressure (75.3 ± 5.3 mmHg at sea level, 85.4 ± 8.9 mmHg for AMS group and 82.4 ± 7.2 mmHg for non-AMS group) and significantly decreased oxygen saturation ($98.7 \pm 1.8\%$ at sea level, $86.9 \pm 6.7\%$ for AMS group and $89.3 \pm 6.5\%$ for non-AMS group). None of these subjects progressed to the more severely fatal forms of high altitude pulmonary edema or high altitude cerebral edema.

Table 1
General physiological characteristics of enrolled subjects (Data are presented as mean $\pm$ SD)

Variables		Sea level	High altitude (3rd day)	
			AMS	Non-AMS
Altitude		20–60 m	4300 m	4300 m
Heart rate (bpm)		66.3 $\pm$ 5.6	81.3 $\pm$ 11.2*	82.3 $\pm$ 8.2*
Oxygen saturation (%)		98.7 $\pm$ 1.8	86.9 $\pm$ 6.7*	89.3 $\pm$ 6.5*
Blood pressure (mmHg)	Diastolic BP	75.3 $\pm$ 5.3	85.4 $\pm$ 8.9*	82.4 $\pm$ 7.2*
	Systolic BP	113.6 $\pm$ 7.8	120.3 $\pm$ 8.6*	117.6 $\pm$ 10.3*
Lake Louise Score		0	4.2 $\pm$ 0.8*	1.9 $\pm$ 0.6
* $p < 0.05$, compared with sea level status.				

Multivariate statistical analysis of metabolites

1892 features were collected in this experiment. To determine whether the metabolite fingerprints in plasma differed between non-AMS and AMS subjects, subjects at sea level and high altitude, we first evaluated separation using unsupervised PCA and supervised OPLS-DA. The obvious separation was achieved between sea level group and high altitude group (Fig. 1A). For the two groups, there were 17 principal components. The PCA model score plots were characterized by the following parameters: $R^2X = 0.894$ and $Q^2 = 0.682$. Based on the OPLS-DA model (Fig. 1B), sea level group and high altitude group were discriminated with an $R^2X = 0.508$, $R^2Y = 0.78$, $Q^2 = 0.496$. The OPLS-DA model is regarded as valid due to $Q^2 > 0.4$.

Comparison of metabolites between AMS group and non-AMS group

The variable importance in the projection (VIP) parameter reflecting the importance of variables was applied to filter the important metabolites in the model. Variables with VIP values higher than 1 were selected in this study and variables without support of its confidence interval were rejected. The qualitative analysis of differential metabolites is based on the retention time (RT), m/z and the matched metabolites were researched in the NIST library. 18 variables were identified by comparison between the sea level group and high altitude group (Table 2), among which alanine and cyclohexasiloxane significantly increased in high altitude group, while other 16 metabolites decreased compared with sea level group. In addition, 6 variables were identified between AMS group and non-AMS group, among

which uric acid decreased in AMS group, while Propanoic acid, silane, talose, D-glucose and 1-Deoxyglucose increased compared with non-AMS group (Table 3).

Table 2
Significantly different metabolites between sea level and high altitude status

No.	ID	Metabolite	VIP	m/z	RT	P value	Log ₂ Fold change (SL/HA)
1	25	Propanoic acid	1.607	43.0930	9.71	0.013	0.585
2	132	Urea	1.012	66.0003	12.38	0.026	0.229
3	1089	Pentasiloxane	1.192	249.9649	11.23	0.021	0.796
4	931	L-Tyrosine	1.054	219.0911	9.72	0.004	0.415
5	1508	Phosphoric acid	1.142	358.0740	48.35	0.002	0.419
6	862	D-Glucose	1.375	206.1377	24.54	0.012	0.114
7	97	Silamine	1.303	58.1236	7.74	0.012	0.552
8	423	Alanine	1.361	123.0981	37.89	0.000	-0.875
9	824	Pentanedioic acid	1.528	198.0938	24.56	0.006	0.237
10	536	L-Proline	1.375	143.0983	17.43	0.000	0.628
11	1079	L-Cysteine	1.434	248.1007	24.55	0.031	0.262
12	839	Hexanoic acid	1.542	201.0984	24.55	0.001	0.157
13	1126	1-Deoxyglucose	1.517	259.0339	16.45	0.000	0.456
14	586	Pyridine	1.548	153.0160	9.30	0.000	0.273
15	1292	Silanol	1.341	302.1519	24.55	0.013	0.120
16	1390	Cyclohexasiloxane	1.993	329.1422	37.86	0.000	-0.402
17	230	Aspartic acid	1.348	84.0175	16.36	0.003	0.918
18	169	D-Galactose	1.751	73.1000	24.54	0.000	0.183
ID: number of metabolite from the NIST library, VIP: Variable Importance in the Projection, RT: retention time. Fold change is calculated through the Logarithmic value (2 as the base value) of ratio between sea level group and high altitude group. Fold change with a positive value indicates a relatively higher concentration present in sea level group, whereas a negative value means a higher concentration in high altitude group. SL: sea level, HA: high altitude.							

Table 3
Significantly different metabolites between AMS group and non-AMS group (p < 0.05)

No.	ID	Metabolite	VIP	m/z	RT	P value	Log ₂ Fold change (AMS/non-AMS)
1	250	Propanoic acid	3.428	88.8809	9.69	0.029	0.389
2	1733	Silane	3.519	438.1766	23.17	0.025	0.452
3	1121	Talose	3.031	258.0762	23.16	0.055	0.309
4	746	D-Glucose	3.328	182.1373	31.88	0.034	0.304
5	1817	Uric acid	3.913	480.4506	47.95	0.012	-0.378
6	145	1-Deoxyglucose	3.081	68.9991	23.17	0.051	0.278

Metabolic pathway analysis for differentially expressed metabolites

After searching the KEGG databases from the differential metabolites, we found 8 metabolomic pathways between AMS and non-AMS group and 27 pathways between sea level and high altitude group. The 18 differential metabolites between sea level group and high altitude status were found to be primarily involved in the aminoacyl-tRNA biosynthesis metabolism, cysteine and methionine metabolism, taurine and hypotaurine metabolism, thiamine metabolism, alanine, aspartate and glutamate metabolism, arginine and proline metabolism, pantothenate and CoA biosynthesis, nitrogen metabolism, galactose metabolism, nicotinate and nicotinamide metabolism, glycine, serine and threonine metabolism (Table 4). The 6 metabolites between AMS group and non-AMS group were found to be primarily involved in the glycolysis or gluconeogenesis metabolism, pentose phosphate pathway, propanoate metabolism, galactose metabolism, nicotinate and nicotinamide metabolism, starch and sucrose metabolism (Table 5).

Table 4

KEGG Pathways of significantly different metabolites between high altitude and sea level group

ID	Pathway Name	Compound name	KEGG ID
1	Aminoacyl-tRNA biosynthesis	L-Tyrosine	C00082
		L-Cysteine	C00097
		L-Proline	C00148
2	Thiamine metabolism	Tyrosine	C00082
		L-Cysteine	C00097
3	Galactose metabolism	D-Glucose	C00031
		D-Galactose	C00124
4	Arginine and proline metabolism	Urea	C00086
		L-Proline	C00148
5	Amino sugar and nucleotide sugar metabolism	D-Glucose	C00031
		D-Galactose	C00124
6	Cyanoamino acid metabolism	Alanine	C01401
7	Sulfur metabolism	L-Cysteine	C00097
8	Taurine and hypotaurine metabolism	L-Cysteine	C00097
9	Alanine, aspartate and glutamate metabolism	Aspartic acid	C00402
10	Pantothenate and CoA biosynthesis	L-Cysteine	C00097
11	Phenylalanine, tyrosine and tryptophan biosynthesis	Tyrosine	C00082
12	Glycolysis or Gluconeogenesis	D-Glucose	C00031
13	Pentose phosphate pathway	D-Glucose	C00031
14	Propanoate metabolism	Propanoic acid	C00163
15	Ubiquinone and other terpenoid-quinone biosynthesis	Tyrosine	C00082
16	Glutathione metabolism	L-Cysteine	C00097
17	Nitrogen metabolism	Tyrosine	C00082
18	Nicotinate and nicotinamide metabolism	Propanoic acid	C00163
19	Phenylalanine metabolism	Tyrosine	C00082
20	Lysine degradation	Pentanedioic acid	C00489
21	Glycine, serine and threonine metabolism	L-Cysteine	C00097

ID	Pathway Name	Compound name	KEGG ID
22	Fatty acid metabolism	Pentanedioic acid	C00489
23	Starch and sucrose metabolism	D-Glucose	C00031
24	Cysteine and methionine metabolism	L-Cysteine	C00097
25	Pyrimidine metabolism	Urea	C00086
26	Tyrosine metabolism	Tyrosine	C00082
27	Purine metabolism	Urea	C00086

Table 5

KEGG pathways of Significantly different metabolites between AMS and non-AMS group

ID	Pathway Name	Compound name	KEGG ID
1	Glycolysis or Gluconeogenesis	D-Glucose	C00031
2	Pentose phosphate pathway	D-Glucose	C00031
3	Propanoate metabolism	Propionic acid	C00163
4	Galactose metabolism	D-Glucose	C00031
5	Nicotinate and nicotinamide metabolism	Propionic acid	C00163
6	Starch and sucrose metabolism	D-Glucose	C00031
7	Amino sugar and nucleotide sugar metabolism	D-Glucose	C00031
8	Purine metabolism	Uric acid	C00366

Consequently, potential target metabolic pathway analysis with MetaboAnalyst revealed that metabolites, important for the host response to high altitude are the metabolism of aminoacyl-tRNA biosynthesis, cysteine and methionine, taurine and hypotaurine, thiamine, alanine, aspartate and glutamate, arginine and proline, pantothenate and CoA biosynthesis, nitrogen, galactose, nicotinate and nicotinamide, glycine, serine and threonine (Fig. 2A). Metabolism of glycolysis or gluconeogenesis, pentose phosphate, propanoate, galactose, nicotinate and nicotinamide, starch and sucrose were found to be distributed in the AMS group (Fig. 2B). Detailed related metabolic pathways were constructed using the reference map by searching KEGG (Fig. 3A and 3B).

The levels of key metabolites involved in the pathways of altered metabolites

To validate the levels of

Discussion

In our current study, we performed an integrated analysis of metabolomics profiling with the whole blood of individuals that exposed to high altitude hypoxia. Traveling to elevations above 3000 m is associated with the risk of developing AMS, HACE and HAPE ¹⁵. Among these complications, AMS is a severe and common disease that occurs after a rapid ascent to high altitude. Identification of molecular markers has the potential to improve the understanding of pathophysiological mechanisms and the diagnosis and prognosis of a condition, as well as to identify the most efficacious therapy ¹⁶. To date, clinical screening for AMS mainly relies on patient interviews, physician's examination and oxygen saturation tests. Specific tests or biomarkers, which would be more reliable and accessible for early diagnosis, are under urgent requirement. The large amount of data generated by omics research may be effective for the diagnosis of high altitude illnesses and may be used to improve our understanding of the pathogenesis of illnesses ¹⁰. Among these omics methods, metabolomics studies have enormous potential to identify potential biomarkers. Gas chromatograph-mass spectrometry (GC-MS), which harvests the information from various spectra of metabolites and facilitates rapid metabolite identification and quantification, proved to be a powerful platform for identifying biomarkers and understanding biochemical pathways to improve diagnosis, prognosis, and treatment of disease ¹⁷. Thus, in our current study, we performed GC-MS to investigate metabolic biomarkers associated with the pathophysiological response and susceptibility to high altitude hypoxia. Our results showed that the plasma metabolic profiles between the sea level and high altitude status were obviously different, while the separation was not that obvious between AMS group and non-AMS group. This indicates that the metabolic profile in the plasma of subjects ascending to high altitude and of AMS patients is altered.

Metabolic pathway analysis was conducted to further understand molecular function of these plasma metabolites. The metabolic pathways are discussed in detail below. In the metabolic profiles, glucose, the predominant energy sources in most organisms, decreased in AMS group, indicating a possible up-regulation of glycolysis. During high altitude acclimation, plasma lactate and pyruvate were increased more markedly in fed rats compared to fasted rats when rapidly exposed to an 8000 m altitude ¹⁸, which utilizes blood glucose as the substrate preferentially during hypoxia. In addition, glycolysis is correlated with an increased affinity of glucose receptors for deoxyglucose, stimulated by some growth factors and cytokines ¹⁹.

Uric acid, the end product of purine metabolism, is generated by the action of the enzyme XO, which catalyses the last two steps of uric acid conversion: hypoxanthine to xanthine and from xanthine to uric acid ¹⁴. The increased level of uric acid in AMS group is consistent with previous reports²⁰.

The levels of a wide variety of amino acids were altered by the pathogenesis of high altitude hypobaric hypoxia. Amino acid metabolism is so complex that a large number of metabolites were involved in the process²¹. Chang Liu et al. also reported that AA metabolism pathway was one of the most pivotal alterations after acute hypoxia exposure and may account for variations in response patterns to hypoxia stimuli ²². The dysregulation of proteolysis, oxidative catabolism, and gluconeogenesis can lead to the metabolic disorder of amino acids. The decreased level of aspartic acid, a key metabolite in the pathway

of alanine, aspartate and glutamate metabolism was observed in high altitude group subjects. In addition, the level of alanine increased at high altitude, tyrosine and proline, two branched-chain amino acids, may be an important alternative energy substrate. It seems that the reduction in ATP production due to the inhibition of citrate cycle induced by the hypobaric hypoxia of high altitude could lead to the utilization of branched-chain amino acids as energy compensation²³. Branched-chain amino acids have been suggested as a useful supplementation in the treatment of lung disease and in trekking at high altitude²⁴.

The significantly decreased level of L-cysteine was also observed in high altitude group. Cysteine is known to increase the intracellular stores of glutathione there by enhancing endogenous antioxidant levels²⁵. The effects of NAC are mainly generated through cysteine and glutathione. It has been reported that NAC supplemented rats showed less free radical production in comparison with hypoxic rats²⁶.

Luo et al. studied the metabolomic variation in the plasma of HAPE patients using 1H NMR and found that HAPE patients had significant several metabolites increased while some decreased in HAPE patients compared with the control group. According to his research, the HAPE patients had significant increase in valine, lysine, leucine, isoleucine, glycerol phosphoryl choline, glycine, glutamine, glutamic acid, creatinine, citrate, and methyl histidine and decrease in α - and β -glucose, trimethylamine, and the metabolic products of lipids¹⁹.

In our current research, serum was collected from normal control volunteers before and after rapidly ascending to high altitude. In addition, a GC–MS-based metabolomics platform coupled with the machine learning method random forest models were successfully applied to explore the serum metabolic differences and changes of acute mountain sickness patients. The results showed that random forest models revealed characteristic and advantages on the discrimination between AMS and normal controls. Components that differed in levels between non-AMS group compared to AMS patients may serve as potential biomarkers for scanning acute mountain sickness at an early stage. To summarize, in AMS patients, aerobic metabolism is inhibited, whereas anaerobic glycolysis is increased. Changes in the levels of a number of related metabolites are obvious, but the specific regulatory sites and regulatory mechanisms that are involved and the biologic significance of such factors require further study.

Conclusion

Despite the limitation of sample size, this study illustrates the useful application of metabolomics analysis, based on GC–MS of blood plasma samples, for the investigation of metabolic changes in subjects rapidly ascending to high altitude and are susceptible to AMS. Moreover, this study suggests that metabolite analysis could provide a new understanding of AMS and may be useful for the pathophysiological mechanism investigation and in the diagnosis of AMS.

Abbreviations

AMS: acute mountain sickness,

HACE: high-altitude cerebral edema,

HAPE: high-altitude pulmonary edema,

GC-MS: gas chromatography-mass spectrometry,

NMR: Nuclear magnetic resonance,

LC-MS: liquid chromatography -mass spectrometry,

LLS: Lake Louise Score,

QC: quality control,

PCA: principal component analysis,

OPLS-DA: orthogonal partial least squares-discriminant analysis,

RT: Retention time,

MZ: Mass-to-charge ratio,

VIP: variable importance in the projection

Declarations

Acknowledgements

Not applicable.

Ethics approval and consent to participate

All experiments in this study were approved by the Ethical Committee of Chinese PLA General Hospital.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

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Authors' contributions

All authors conducted and contributed to the literature search. Chi Wang collected sample, analyzed data and prepared the manuscript, Hui Jiang and Jingwen Chen contribute to sample collection, Jinyan Duan contributes to the performance of GC-MS and data analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that there are no competing interests.

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Figures

Figure 1

PCA model and OPLS-DA model with corresponding values of R2X, R2Y and Q2 based on GC/MS data from sea level group and high altitude group. A. PCA score plot data of sea level groups (yellow) versus high altitude groups (green). B. OPLS-DA score plot data of sea level groups (yellow) versus high altitude groups (green). C, D. Validation plot obtained from permutation test, green triangle: R2; blue square: Q2. The green line represents the regression line for R2, and the blue line represents that for Q2.

Figure 1

PCA model and OPLS-DA model with corresponding values of R^2X , R^2Y and Q^2 based on GC/MS data from sea level group and high altitude group. A. PCA score plot data of sea level groups (yellow) versus high altitude groups (green). B. OPLS-DA score plot data of sea level groups (yellow) versus high altitude groups (green). C, D. Validation plot obtained from permutation test, green triangle: R^2 ; blue square: Q^2 . The green line represents the regression line for R^2 , and the blue line represents that for Q^2 .

Figure 2

The pathway impact of high altitude on plasma metabolites with MetaboAnalyst 3.0. A. Altered metabolic pathways between high altitude and sea level groups; a, aminoacyl-tRNA biosynthesis; b, cysteine and methionine metabolism; c, taurine and hypotaurine metabolism; d, thiamine metabolism; e, alanine, aspartate and glutamate metabolism; f, arginine and proline metabolism; g, pantothenate and CoA biosynthesis; h, nitrogen metabolism; i, galactose metabolism; j, nicotinate and nicotinamide metabolism; k, glycine, serine and threonine metabolism. B. Altered metabolic pathways between AMS group and non-AMS group; a, glycolysis or gluconeogenesis; b, pentose phosphate pathway; c, propanoate metabolism; d, galactose metabolism; e, nicotinate and nicotinamide metabolism; f, starch and sucrose metabolism.

Figure 2

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The pathway impact of high altitude on plasma metabolites with MetaboAnalyst 3.0. A. Altered metabolic pathways between high altitude and sea level groups; a, aminoacyl-tRNA biosynthesis; b, cysteine and methionine metabolism; c, taurine and hypotaurine metabolism; d, thiamine metabolism; e, alanine, aspartate and glutamate metabolism; f, arginine and proline metabolism; g, pantothenate and CoA biosynthesis; h, nitrogen metabolism; i, galactose metabolism; j, nicotinate and nicotinamide metabolism; k, glycine, serine and threonine metabolism. B. Altered metabolic pathways between AMS group and non-AMS group; a, glycolysis or gluconeogenesis; b, pentose phosphate pathway; c, propanoate metabolism; d, galactose metabolism; e, nicotinate and nicotinamide metabolism; f, starch and sucrose metabolism.

Figure 3

Schematic overview of the metabolites and major metabolic pathway changes in plasma of the 36 subjects after arriving at high altitude. A. Metabolic pathway changes between individuals at sea level and at high altitude. B. Metabolic pathway changes between AMS group and non-AMS group. The metabolites are shown in color: red represents increased metabolites or enzymes; green represents decreased metabolites or enzymes and other white ones represent metabolites not changed in our experiment.

(A)

(B)

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Schematic overview of the metabolites and major metabolic pathway changes in plasma of the 36 subjects after arriving at high altitude. A. Metabolic pathway changes between individuals at sea level and at high altitude. B. Metabolic pathway changes between AMS group and non-AMS group. The metabolites are shown in color: red represents increased metabolites or enzymes; green represents decreased metabolites or enzymes and other white ones represent metabolites not changed in our experiment.