



Arachidonic Acid Metabolism Pathway Is Not Only Dominant in Metabolic Modulation but Associated With Phenotypic Variation After Acute Hypoxia Exposure

Chang Liu^{1,2,3}, Bao Liu^{1,2,3,4}, Lu Liu^{1,2,3}, Er-Long Zhang^{1,2,3}, Bind-da Sun^{1,2,3}, Gang Xu^{1,2,3}, Jian Chen^{1,2,3*} and Yu-qi Gao^{1,2,3*}

¹ Institute of Medicine and Hygienic Equipment for High Altitude Region, College of High Altitude Military Medicine, Army Medical University, Third Military Medical University, Chongqing, China, ² Key Laboratory of High Altitude Environmental Medicine, Army Medical University, Third Military Medical University, Ministry of Education, Chongqing, China, ³ Key Laboratory of High Altitude Medicine, People's Liberation Army, Chongqing, China, ⁴ The 12th Hospital of Chinese People's Liberation Army, Kashi, China

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*Correspondence:

Jian Chen
jchenone@163.com
Yu-qi Gao
gaoy66@yahoo.com

Specialty section:

This article was submitted to
Vascular Physiology,
a section of the journal
Frontiers in Physiology

Received: 19 September 2017

Accepted: 02 March 2018

Published: 16 March 2018

Citation:

Liu C, Liu B, Liu L, Zhang E-L, Sun B,
Xu G, Chen J and Gao Y (2018)
Arachidonic Acid Metabolism Pathway
Is Not Only Dominant in Metabolic
Modulation but Associated With
Phenotypic Variation After Acute
Hypoxia Exposure.
Front. Physiol. 9:236.
doi: 10.3389/fphys.2018.00236

Background: The modulation of arachidonic acid (AA) metabolism pathway is identified in metabolic alterations after hypoxia exposure, but its biological function is controversial. We aimed at integrating plasma metabolomic and transcriptomic approaches to systematically explore the roles of the AA metabolism pathway in response to acute hypoxia using an acute mountain sickness (AMS) model.

Methods: Blood samples were obtained from 53 enrolled subjects before and after exposure to high altitude. Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry and RNA sequencing were separately performed for metabolomic and transcriptomic profiling, respectively. Influential modules comprising essential metabolites and genes were identified by weighted gene co-expression network analysis (WGCNA) after integrating metabolic information with phenotypic and transcriptomic datasets, respectively.

Results: Enrolled subjects exhibited diverse response manners to hypoxia. Combined with obviously altered heart rate, oxygen saturation, hemoglobin, and Lake Louise Score (LLS), metabolomic profiling detected that 36 metabolites were highly related to clinical features in hypoxia responses, out of which 27 were upregulated and nine were downregulated, and could be mapped to AA metabolism pathway significantly. Integrated analysis of metabolomic and transcriptomic data revealed that these dominant molecules showed remarkable association with genes in gas transport incapacitation and disorders of hemoglobin metabolism pathways, such as ALAS2, HEMGN. After detailed description of AA metabolism pathway, we found that the molecules of 15-d-PGJ2, PGA2, PGE2, 12-O-3-OH-LTB4, LTD4, LTE4 were significantly up-regulated after hypoxia stimuli, and increased in those with poor response manner to hypoxia particularly. Further analysis in another cohort

showed that genes in AA metabolism pathway such as PTGES, PTGS1, GGT1, TBAS1 et al. were excessively elevated in subjects in maladaptation to hypoxia.

Conclusion: This is the first study to construct the map of AA metabolism pathway in response to hypoxia and reveal the crosstalk between phenotypic variation under hypoxia and the AA metabolism pathway. These findings may improve our understanding of the advanced pathophysiological mechanisms in acute hypoxic diseases and provide new insights into critical roles of the AA metabolism pathway in the development and prevention of these diseases.

Keywords: metabolomics, transcriptomics, WGCNA, hypoxia, arachidonic acid

INTRODUCTION

Hypoxia is a common phenomenon associated with multiple pathophysiological processes, including inflammation, necrosis, and apoptosis, and observed in various clinic conditions such as myocardial ischemia and reperfusion as well as stroke (Michiels, 2004). The arachidonic acid (AA) metabolism pathway was shown to exhibit remarkable metabolic alterations after hypoxia exposure, but its role is controversial (Liao et al., 2016). As a part of the innate immune response, AA and its metabolites are known to induce or suppress inflammation (Bennett and Gilroy, 2016). In addition, the products of the AA cascade are shown to display multidirectional and often conflicting roles in endothelial and nerve cells, such as inhibition or promotion of cell proliferation and aggravation or alleviation of oxidative stress, in response to stimuli (Bogatcheva et al., 2005; Rink and Khanna, 2011). Although the partial function of this pathway was identified, the mechanism underlying the expression of the AA metabolism pathway and its role under acute hypoxic conditions are questionable.

Acute hypobaric hypoxia, one of the most common hypoxia types, occurs in response to the exposure to high altitude and initiates systematic pathophysiological alterations. Acute hypobaric hypoxia may serve as a principal etiological factor for acute mountain sickness (AMS) (Sun et al., 2017). Therefore, AMS is considered as a potent channel and an *in vivo* model to improve our understanding of the various acute hypoxic diseases with high-quality samples from self-controlled studies (Subudhi et al., 2010). In addition, mal-adaptation to hypoxia is characterized by symptoms such as headache, nausea, and vomiting that may progress into high altitude pulmonary edema or high altitude cerebral edema—the leading lethal diseases for mountaineers (Johnson and Luks, 2016). With ~15 million people traveling to areas of high altitude (>2,500 m) annually in China alone (Gonggalanzi et al., 2016), it is important to unravel the mechanisms underlying AMS.

The technical platforms of system biology allow researchers to explore systematic alterations under hypoxia exposure. Metabolomic studies have demonstrated that metabolic reprogramming with dramatic turbulence in various metabolic pathways, including linoleic acid metabolism, purinergic signaling, and glycolysis, was implied in response to acute hypoxia (D'Alessandro et al., 2016; Liao et al., 2016). In addition, a recent study on acute hypoxia stimuli based on RNA-seq

approach indicated that the disturbance in the inflammation response presented by the reduction in interleukin (IL)-10 was highly related to AMS incidences (Liu et al., 2017a). However, studies based on a single set of omics technology failed to extensively explain the alterations occurring at different levels during physiologic or pathophysiological responses, such as disabilities in interpreting metabolic regulatory processes in metabolomics (Kan et al., 2017). The development of efficient analysis methods and algorithms allows better insights, beyond those available from individual strategies through the integration of diverse omics (Li et al., 2016; Sun and Hu, 2016). In particular, the method of weighted gene co-expression network analysis (WGCNA), which could exhibit multiple network-level interactions, is effective for the identification of gene-to-metabolite relations and construction of the underlying complex regulatory networks (Acharjee et al., 2016; Liu et al., 2017b).

Here, we aimed to integrate clinical features, metabolomic datasets, and transcriptome profiling for the construction of the co-expression network to provide a better understanding of the AA metabolism pathway after acute hypoxia exposure. Our results identified that the AA metabolism pathway, a highly regulated metabolic pathway, was essential in response to acute hypoxia. Furthermore, the AA metabolism pathway may account for variations in response patterns after acute hypoxia stimuli. Our results may improve the understanding of AMS and provide new clues to counteract the effects of hypoxic diseases.

MATERIALS AND METHODS

Enrolled Subjects and Experimental Procedures

We enrolled 53 male subjects with intact clinical phenotype data of heart rate (HR), oxygen saturation (SpO₂), Lake Louise score (LLS), systolic blood pressure (SBP), diastolic blood pressure (DBP), and hemoglobin (HB) before and after high altitude exposure (5,300 m). These subjects were covered in our previous study (Liao et al., 2016). Physical and physiological characteristics [mean ± standard deviation (SD)] for enrolled subjects were as follows: age, 21.6 ± 2.0 years; height, 172 ± 1 cm; and body weight, 64.9 ± 2.3 kg. Exclusion criteria included prior exposure to high altitude,

presence of cardiovascular pathologies or other diseases, and history of drug prescribed for high altitude. These volunteers traveled to an area of 5,300 m from the starting point of 1,400 m within 4 days, as illustrated in our previous article (Liao et al., 2016). Their clinical symptoms of HR, SpO₂, LLS, SBP, DBP, HB were monitored before and after hypoxia stimuli. The local ethics committee of the Third Military Medical University approved the experimental design, methods of clinical data collection, and strategies of data evaluation. These procedures were performed in accordance with the approved guidelines. Written informed consents were obtained from all participants.

Metabolomics Analysis

Metabolic profiling was performed, and metabolic data were obtained as previously described (Liao et al., 2016). Briefly, blood was drawn from a peripheral vein in the morning soon after waking before ascending to high altitudes and after 3 days high-altitude exposures. Blood samples were centrifuged to separate serum at 14,000 × g for 15 min at 4°C. Then, these plasma samples were delivered to Chongqing for further metabolomics analysis in the courier filed with dry ice.

Metabolomics analysis was conducted on an Agilent 1290 Infinity LC system (Agilent, Santa Clara, CA, USA). Chromatographic separations were performed on an ACQUITY UHPLC HSS T3 C18 column (2.1 × 100 mm, 1.8 μm; Waters, Milford, Ireland) at 45°C. The metabolomics detection was performed totally in accordance to the manufacturers' protocols of all devices. Raw LC-MS data were converted to mzData formats via Agilent MassHunter Qualitative software (Agilent, Santa Clara, CA, USA). The program XCMS (version 1.40.0) (<https://xcmsonline.scripps.edu/>) was used to preprocess the raw data, including peak detection, peak matching, matched filtration, and nonlinear alignment of data, with the default parameters (and snthresh, 5; bw, 10; fwhm, 10).

The resulting matrix from 53 subjects comprising retention time, mass-to-charge ratio (m/z), and normalized ion intensities was introduced into the subsequent analysis based on R platform (<https://www.r-project.org>). The model of partial squares discriminant analysis (OPLS-DA) was carried out to separate pre- and post-hypoxia in metabolic profiling. Variable importance project (VIP) was used to reveal discriminatory metabolites which were enrolled in the classification effects of hypoxia was calculated. In addition, adjusted *p*-value from the paired *t*-test and fold-change value (FC) were executed to discovery dominant metabolites. Pathway analysis was established by MetaboAnalyst platform (<http://www.metaboanalyst.ca>).

Transcriptomic Profiling Detection

To improve the reliability of the integrated analysis to uncover interactions at metabolic and transcriptional level, 11 individuals were randomly selected and subjected to metabolomic and RNA-seq detection (Li et al., 2016). RNA sequencing was performed as previously described (Liu et al., 2017a) with minor modifications. Briefly, after extraction, total RNA was

quantified by spectrophotometer (NanoDrop 200, Thermo Scientific, Delaware, USA). mRNA-seq libraries were constructed according to the TruSeq RNA Sample Prep Kit v2 (Illumina) and sequenced on an Illumina HiSeq 2000 sequencer, following the manufacturer's instructions. After filtered, the clean reads were aligned to human genome hg19 based on HISAT and assembled by Stringtie (Li et al., 2014). The expression value of each transcript was subsequently calculated based on methods of Fragments Per Kilobase of exon model per Million mapped fragments (FPKM). In addition, GSE52209 datasets of blood samples from individuals suffering from adaptation or mal-adaptation to high altitude exposure were downloaded from Gene Expression Omnibus (GEO) datasets. After annotation, limma package was employed to normalize and detect the significantly differential genes involved in diverse response manner to hypoxia (Ritchie et al., 2015). ClusterProfiler package was used to determine enriched Gene Ontology (GO) terms in differentially expressed gene sets (Yu et al., 2012).

Weighted Gene Co-expression Network Analysis (WGCNA)

WGCNA, a robust tool for the integrative network analysis, is widely used in complex network construction (Liu et al., 2017b). According to the WGCNA protocol, we constructed clusters of genes firstly based on the matrix of pairwise correlations calculated across the selected samples. Then, we performed the network construction and module detection based on an appropriate soft-thresholding power. The default minimum cluster merge height of 0.25 was retained. These clusters identified by WGCNA are called modules, which represented a group of highly interconnected genes with similar expression profiles across the enrolled subjects. Further, we determined correlations among expression modules and clinical traits for all subjects. Hub genes were identified based on the connectivity among all nodes. Network was illustrated by Cytoscape software (Smoot et al., 2011).

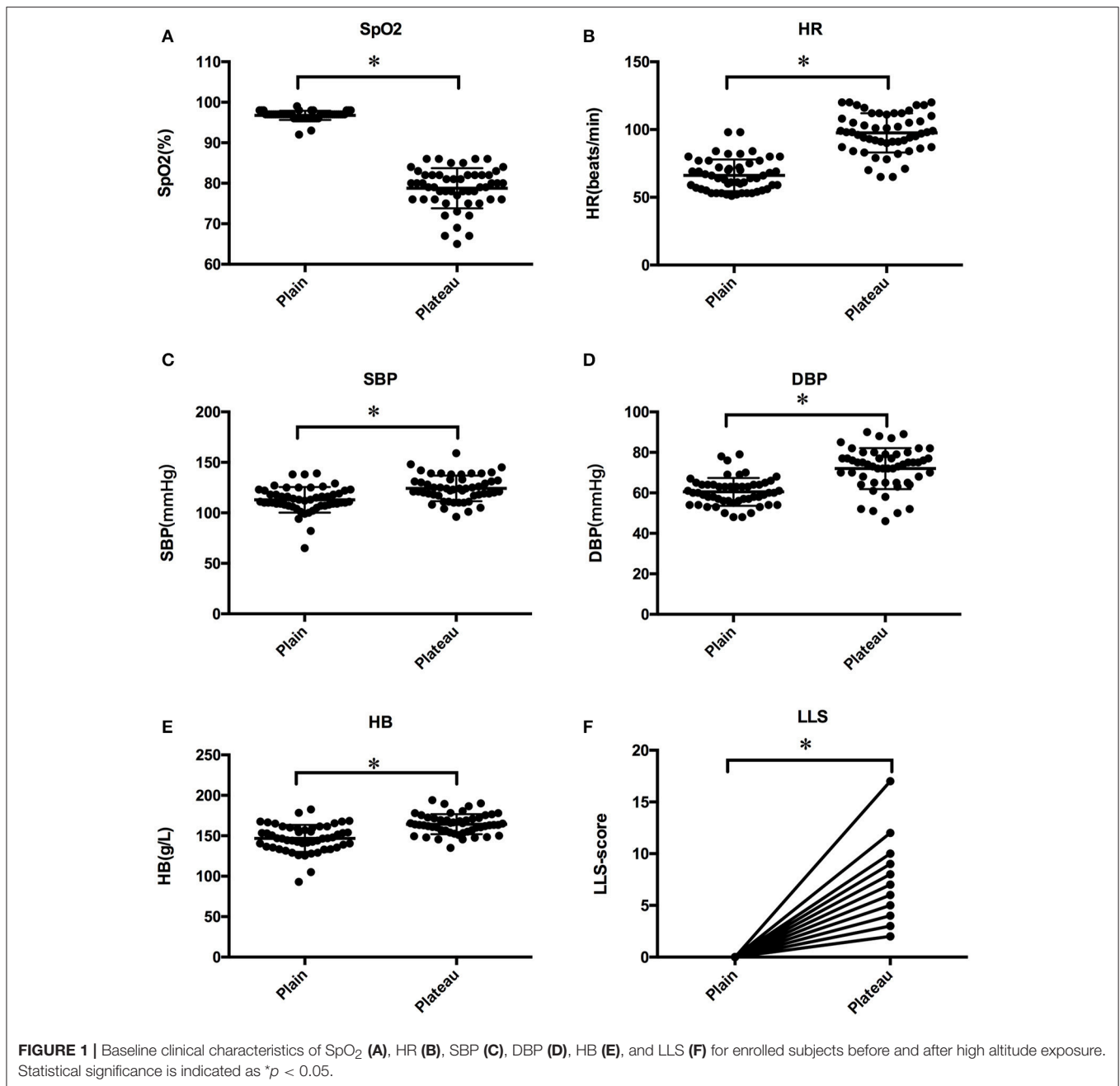
Statistical Analysis

Differences before and after hypoxia exposure were determined by paired *t*-test. The unpaired Student's *t*-test was used to compare the expression between subjects with diverse response patterns to hypoxia. Statistical analysis was performed by GraphPad software (GraphPad Prism, USA). The data in this study were presented as the mean ± SD. A two-tailed *p* value <0.05 was considered as significant, unless specifically indicated.

RESULTS

Baseline Clinical Characteristics of Enrolled Subjects

The response of subjects to acute hypobaric hypoxia was assessed by various physiological parameters. As shown in **Figure 1A**, SpO₂, reflective of the oxygen concentration in the blood, significantly declined under hypoxia environment (*p* < 0.05; Netzer et al., 2017). Among the essential compensatory



measurements, HR (Figure 1B), SBP (Figure 1C), DBP (Figure 1D), and HB (Figure 1E) increased along with hypoxia ($p < 0.05$), indicative of the comprehensive response manner to acute hypoxia stimuli. A significant increase in LLS was observed for all individuals (Figure 1F; $p < 0.05$).

Arachidonic Acid Metabolism Pathway Shows a Remarkable Correlation With Phenotypic Alterations After Hypoxia Exposure

Metabolomic analysis of blood samples from individuals before and after high altitude exposure revealed a total of 1,720

compounds. As indicated in Figure 2A, the method of WGCNA identified that four modules (MEgreen, MEdred, MEMagenta, and MEblack) showed significant correlations with all clinical features of SBP, LBP, HR, SpO₂, LLS, and HB. To further explore influential metabolites in these modules, a volcano plot for these molecules was obtained (Figure 2B). At a cutoff point of $p < 0.05$, $FC \geq 1.5$, and $VIP > 1.5$, a total of 36 metabolites showed significant alteration and comprised nine downregulated molecules and 27 elevated metabolites (Table 1). Moreover, the enrichment analysis demonstrated that AA metabolism pathway was particularly significant in the metabolic alterations in response to acute hypoxia (Figure 2C).

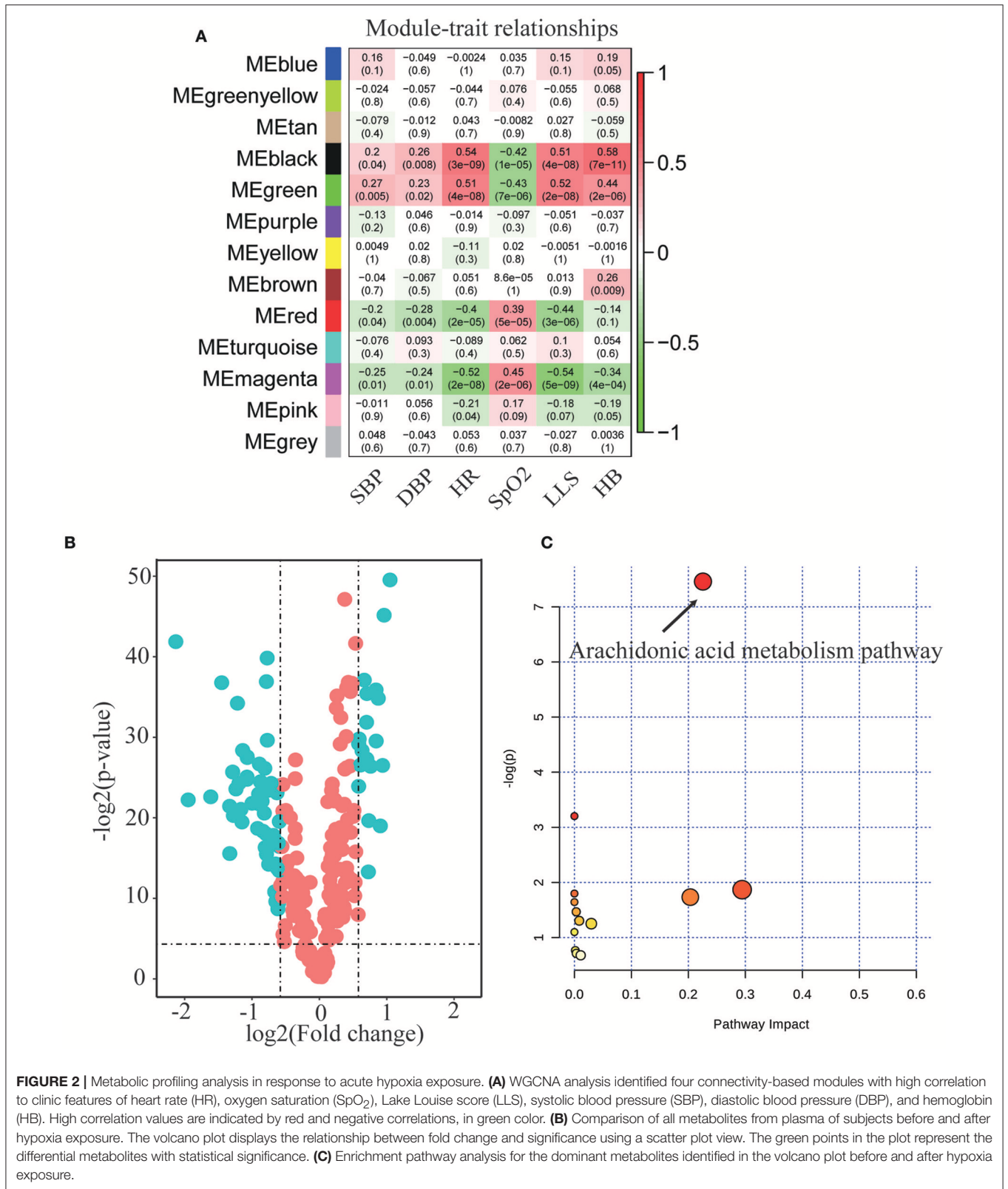


TABLE 1 | Summary of the influential metabolites in 53 subjects at high altitude relative to plain.

Metabolites	P-value	Fold change	VIP
12-Oxo-trihydroxy-leukotriene B4	2.04E-07	0.26	2.29
Bilirubin	1.57E-07	0.33	3.26
Undecanoylcarnitine	8.49E-12	0.37	1.75
15(S)-HETE	2.06E-05	0.40	1.85
Oleic acid	1.85E-08	0.41	4.47
L-Hexanoylcarnitine	8.01E-08	0.42	1.84
Ceramide (d18:1/16:0)	4.99E-11	0.43	2.20
15-Deoxy-d-12,14-PGJ2	4.84E-08	0.44	1.60
3, 5-Tetradecadienylcarnitine	1.38E-06	0.45	2.33
20-Hydroxy-leukotriene E4	2.89E-09	0.45	1.57
Pimelylcarnitine	3.49E-08	0.48	2.23
3-hydroxydecanoyl carnitine	4.50E-09	0.48	2.25
9-Hexadecenoylcarnitine	5.37E-09	0.48	2.07
Dodecanoylcarnitine	2.75E-07	0.50	2.15
Decanoylcarnitine	2.35E-06	0.53	2.60
Tetradecanoylcarnitine	1.23E-07	0.54	1.73
trans-2-Dodecenoylcarnitine	2.80E-07	0.54	2.28
Linoleic acid	4.28E-08	0.55	4.33
DG(15:0/18:0/0:0)	2.29E-07	0.55	1.90
LysoPE(14:0/0:0)	1.35E-08	0.57	2.98
Palmitic acid	1.23E-05	0.57	2.87
N-methylphenylalanine	7.75E-12	0.58	1.55
Tiglylglycine	1.02E-12	0.58	2.93
21-Hydroxypregnenolone	8.95E-08	0.59	1.54
L-Octanoylcarnitine	5.25E-05	0.59	2.00
Arachidonic acid	1.17E-07	0.65	2.61
Alpha-Linolenic acid	8.90E-05	0.66	1.55
LysoPC(20:2)	6.82E-12	1.59	3.08
LysoPC(P-18:0)	2.57E-10	1.62	2.14
LysoPC(20:0)	6.09E-09	1.63	1.79
LysoPE(0:0/22:0)	2.17E-11	1.63	2.26
Stearoylcarnitine	1.22E-06	1.66	2.26
Glycocholic acid	1.14E-08	1.69	2.62
Deoxyinosine	3.28E-11	1.83	1.74
Deoxyribose 1-phosphate	1.92E-06	1.87	1.69
LysoPC(18:2(9Z,12Z))	1.23E-15	2.07	2.346

Integrative Network-Based Analysis Revealed Mechanisms of AA Metabolism Pathway

The physiological characteristics corresponding to SpO₂, HR, HB, LLS, SBP, and LBP for subjects evaluated by metabolomics and transcriptomics are listed in Supplemental Figure 1. These subjects chose for both omics detection, posed clinical features similar to those of the whole population after hypoxia stimuli. To provide a comprehensive interaction network between metabolomic and transcriptomic data sets, WGCNA was performed to identify connectivity-based modules comprising genes closely related to the aforementioned impact metabolites. As illustrated in **Figure 3A**, gene modules labeled by a

violet frame were significantly correlated with these molecules, especially to metabolites of the AA metabolism pathway. As shown in **Figure 3B**, heme catabolism, gas transport, and porphyrin-related metabolism pathways were dominant in these gene modules after GO analysis ($p < 0.05$). To further clarify the mechanisms underlying the AA metabolism pathway after hypoxia exposure, genes with the highest connectivity from the co-expression network and a correlation coefficient >0.65 to at least one molecule among 13 metabolites in the AA metabolism pathway are circled in red in **Figure 3C**. These marked genes promoted proliferation and regulation of red blood cell metabolism. The detailed information is listed in **Figure 4**.

Detailed AA Metabolism Pathway Along With Hypoxia Stimuli

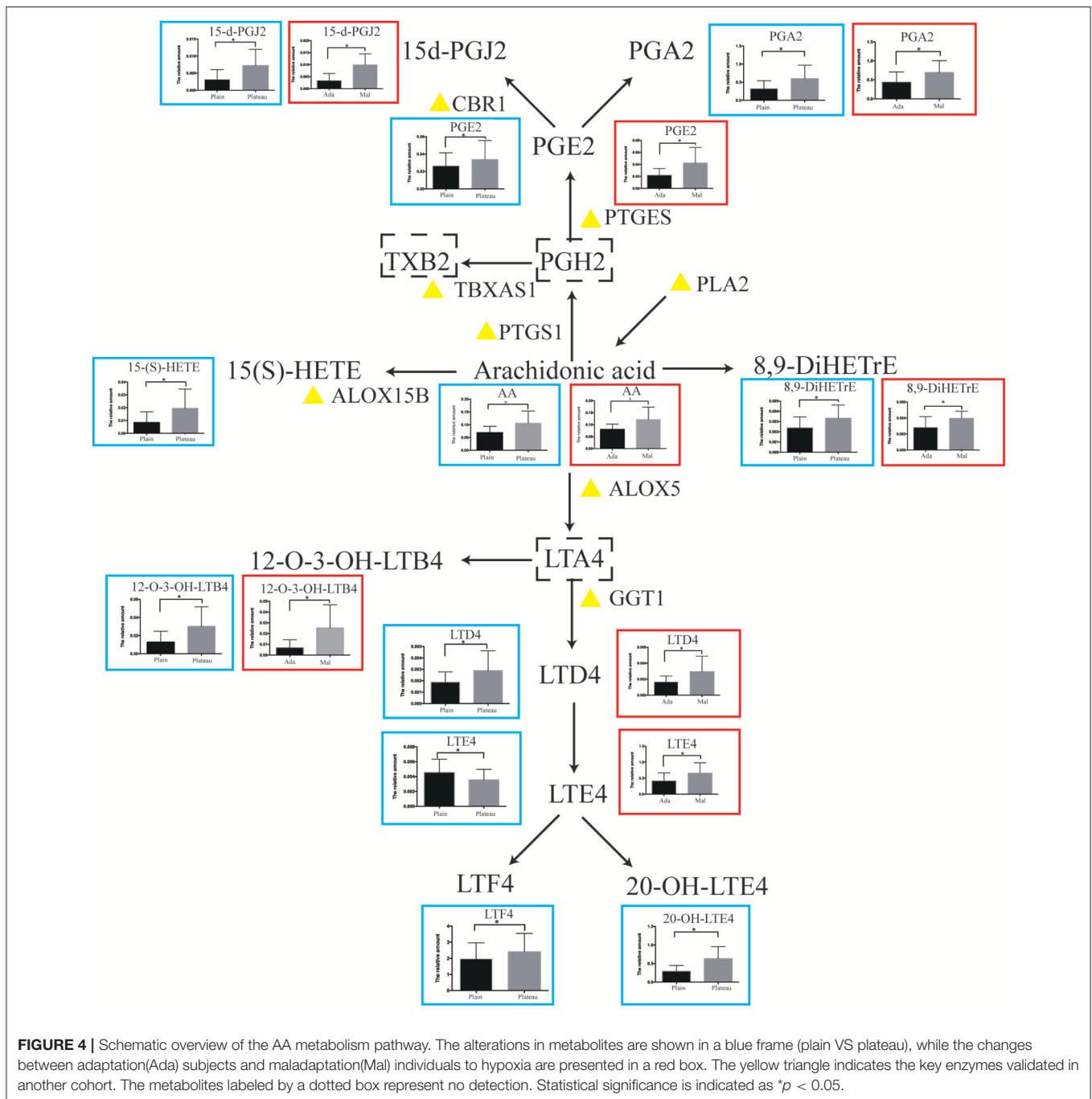
A map of metabolites in AA metabolism pathway was constructed in **Figure 4**. Alterations in these molecules before and after hypoxia exposure are listed in the blue box next to each metabolite. To elucidate the differences in subjects exhibiting diverse response patterns to hypoxia, these individuals were divided into two groups based on their LLS scores. Individuals with an LLS score lower than 4 were enrolled in the control group, while those with an LLS score higher than 9 were included in the group of mal-adaptation to hypoxia. Clinical characteristics of each group are listed in Supplemental Table 1. The metabolites exhibiting significant differences between control and mal-adjustment group are shown in the red box in **Figure 4**. As observed, the AA metabolism pathway was upregulated after hypoxia exposure and further elevated in those with poor response to hypoxia.

Validation of AA Metabolism Pathway in Another Dataset

We validated our findings in another population of GSE52209 from GEO database that comprised 14 subjects as the control group and 17 individuals suffering from mal-adaptation to high altitude. In comparison to individuals that adapted to hypoxia, those with poor response to acute hypoxia showed a significant upregulation ($p < 0.05$, **Figure 5**) in the expression of genes such as *GGT1*, *PTGES*, *PTGS1*, *CBRI*, and *ALOX12* encoding key enzymes of the AA metabolism pathway. The effects of these genes on AA metabolism pathways are labeled with a yellow triangle in **Figure 4**.

DISCUSSION

Here, we performed an integrated analysis of metabolomic and transcriptomic expression profiling with the whole blood of individuals that experienced acute hypoxia. These approaches revealed pivotal roles of the AA metabolism pathway in the phenomenon of metabolic reprogramming during acute hypoxia exposure. Co-expression network analysis further identified that the AA metabolism pathway showed a significant positive correlation with transcriptional alterations, particularly with genes involved in porphyrin metabolism and gas transport pathways. Taken together, this is the first study to provide



c-Jun N-terminal kinase (JNK), thereby directly promoting inflammation by increasing levels of tumor necrosis factor (TNF)- α (Saito et al., 2012). AA-derived metabolites such as leukotriene B₄ (LTB₄) and Cys-LTs from upregulated enzymes, including ALOX5 and GGT1, may operate as potent activators of and chemoattractants for leukocytes, eosinophils, and monocytes to propagate inflammation and oxidative stress (Rink and Khanna, 2011). Elevated PTGS2 may contribute to increased levels of PGE₂ and PGA₂, which activate IL-6 and nuclear factor kappa B (NF- κ B) to promote vascular inflammation

(Gomez et al., 2013). In addition to the biological effects of these metabolites, key enzymes in the AA metabolism pathway may directly trigger inflammation reactions and oxidative stress. Cyclooxygenase-2 (COX-2) contributes to the upregulation of TNF- α after hypoxia stimuli (Xing et al., 2015). The peroxidase activity of PTGS1 may serve as a source of oxygen radicals through the conversion of PGG₂ to PGH₂ by the removal of oxygen (Rink and Khanna, 2011). Moreover, phospholipase A₂ may produce free radicals during the activation of arachidonic acid cascade under hypoxia environment (Tanaka et al., 2003).

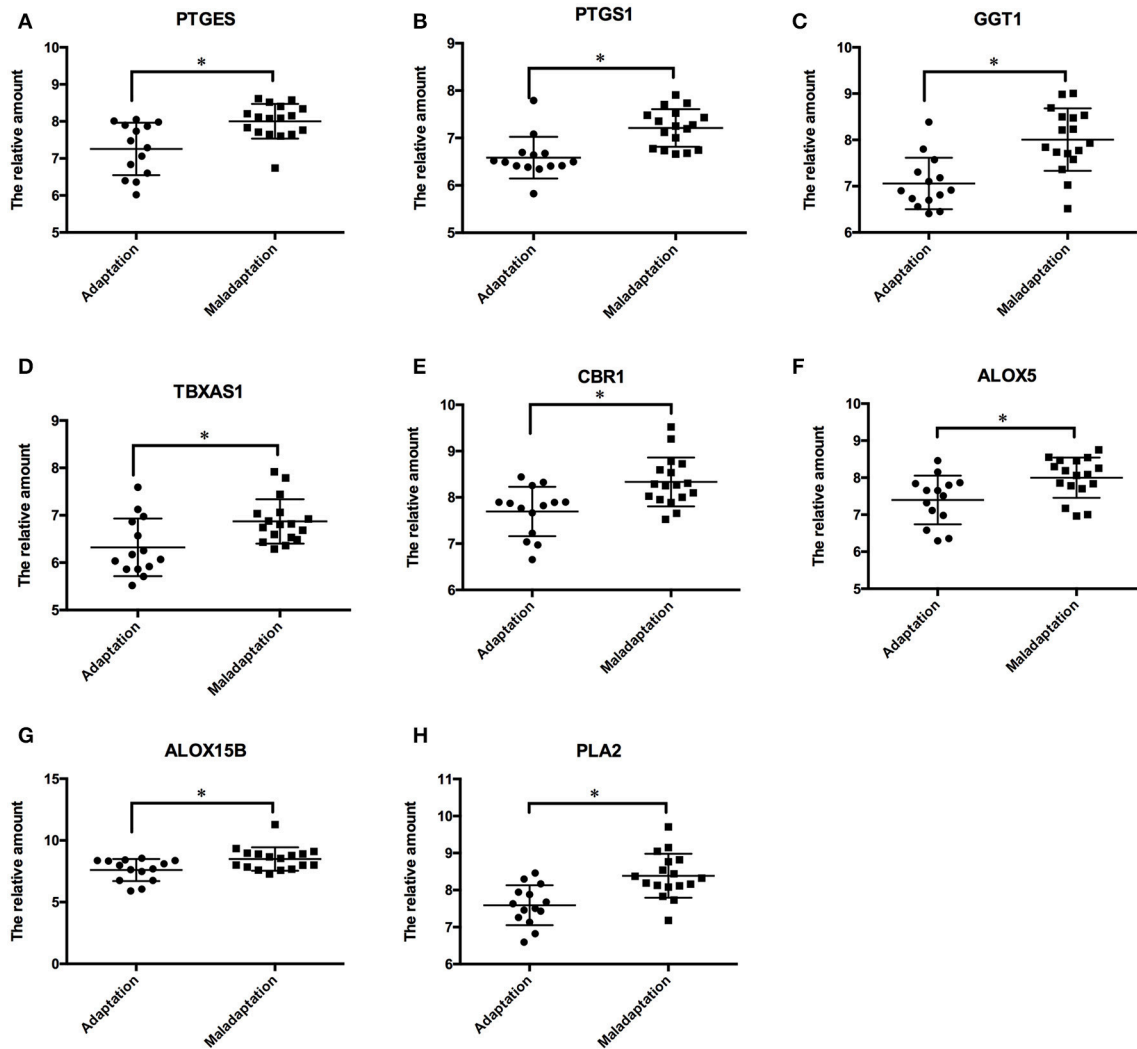


FIGURE 5 | Validation of the AA metabolism pathway in another cohort. The genes of PTGES (A), PTGS1 (B), GGT1 (C), TBXAS1 (D), CBR1 (E), ALOX5 (F), ALOX15B (G), PLA2 (H) were significantly up-regulated in those who exhibited mal-adaptation to high altitude exposure. Statistical significance is indicated as $*p < 0.05$.

With limited evidence focusing on the role of the AA metabolism pathway in the development of hypoxic diseases, our results systematically reveal the expression level of the AA metabolism pathway as well as its possible roles based on the AMS model and provide the basis for further research.

The increase in metabolites indicates the upregulation in sub-pathways activated by COX, lipoxygenase, and PLA2—the key enzymes of the AA metabolism pathway (Wong et al., 2003; Jantan et al., 2014). In line with previous studies, these proteins showed a hypoxia-regulated pattern. A recent study demonstrated that hypoxia-mediated extracellular signal-regulated kinase (ERK) activation may induce the activity of PLA2 in the erythrocyte membrane (Wu et al., 2016). In addition, as an essential oxygen-sensing molecule, heme oxygenase-1 was reported to act as an important mediator of transcription and translation of 15-lipoxygenase to promote lipoxins (Nie et al., 2013). Hypoxia-inducible factor-1 alpha (HIF-1a) was reported

to directly stimulate COX enzymes after hypoxia exposure (Huang et al., 2016). However, we failed to detect the quantitative expression of the key enzymes in the AA metabolism pathway, owing to the limited blood sample during validation. An intervention experiment with drugs targeting the AA metabolism pathway in larger population samples is now being conducted by our group to verify these findings and search for effective prevention measures.

In our study, we demonstrated a crosstalk between response manners to acute hypoxia and expression level of the AA metabolism pathway. The excessive increase in the AA metabolism pathway after hypoxia stimuli may reflect maladaptation to hypoxia stimuli, as evident from the elevated levels of HR, HB, and LLS as well as the decreased level of SpO₂. These observations suggest impaired cardiopulmonary and hemoglobin function (Fuehrer and Huecker, 2017). For cardiopulmonary performance, the addition of AA was shown to aggravate

respiration inhibition and decrease oxygen consumption at the mitochondrial level (Egorova et al., 2015), while targeting COX-2 improved hemodynamic parameters of cardiac output, left ventricular pressure, and $LVdp/dt$ (Oshima et al., 2006). AA metabolism was also reported to influence heart rates by alerting histaminergic system (Altinbas et al., 2014). In addition, the elevated level of reactive oxygen species during AA metabolism was involved in the regulation of erythroid differentiation, and the activation of phospholipase A2 was shown to induce protein kinase C to increase erythropoietin level (Luo et al., 2017) (Mason-Garcia and Beckman, 1991). Furthermore, the process of reticulocyte maturation was dependent on phospholipase A2 during the remodeling of the plasma membrane by the removal of specific proteins (Blanc et al., 2007). As an irreversible pathological process with poor clinical outcomes caused by sustained hypoxia, elevated AA metabolism pathway may decrease the apoptosis of endothelial cells, induce the proliferation of pulmonary artery smooth muscle cells, activate angiogenesis, and influence the arterial vascular tonicity via K^+ channels (Park et al., 2011; Zhang et al., 2011; Liu et al., 2012; Zhu and Ran, 2012; Pang et al., 2016). An excessive elevation in AA metabolism may result in poor response to acute hypoxia and even worse symptoms under chronic hypoxia environment.

The protective effects of anti-inflammatory molecules are increasingly recognized for the prevention of AMS. Apigenin targeting IL-1 β , IL-6, and TNF- α was demonstrated to be effective in reducing the damage caused by acute hypoxia (Du et al., 2015). Recent pilot studies reported that ibuprofen, aspirin, and dexamethasone may be effective for the prevention and treatment of AMS, especially the syndrome of headache, although the underlying mechanisms remain largely unknown (Burtscher et al., 2001; Gertsch et al., 2012; Xiong et al., 2017). In this study, our results theoretically support anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and dexamethasone targeting the AA metabolism pathway for improving clinical symptoms of AMS. In addition, targeted therapies against specific enzymes or metabolites of the AA metabolism pathway may be beneficial in AMS prevention

and demand further studies (Atluri et al., 2011). Given its close relationship with hemoglobin metabolism genes, the AA metabolism pathway may be an effective target for the prevention of hypoxia-induced erythrocytosis (Foley et al., 1978; Parise et al., 1991).

In summary, our integrated analysis of metabolomic and transcriptomic profiling revealed that the 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. The detailed description of the AA metabolism pathway may offer the basis for follow-up mechanisms and drug screening.

AVAILABILITY OF DATA

All data have been submitted to GEO under the accession GSE103940.

AUTHOR CONTRIBUTIONS

YG and JC: Conceived and designed the study; CL and BL: Oversaw laboratory analyses and contributed the statistical analysis; LL, E-LZ, and BS: Contributed to sample and physical data collections; CL: Drafted the report. All authors reviewed and approved the manuscript.

ACKNOWLEDGMENTS

This work was supported by the Key Projects in the Military Science & Technology Pillar Program during the Thirteen 5-year Plan Period (AWS14C007&AWS16J023), by National Natural Science Foundation of China (J1310001).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2018.00236/full#supplementary-material>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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