ORIGINAL RESEARCH

Predictive Value of Basal Exhaled Nitric Oxide and Carbon Monoxide for Acute Mountain Sickness

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Objective.—The purpose of this study was to examine the relationship between acute mountain sickness (AMS) and the fraction of exhaled nitric oxide (FE_{NO}) and carbon monoxide (FE_{CO}) before ascent to high altitude and to evaluate their predictive value for AMS.

Methods.—A total of 314 healthy young male recruits were voluntarily enrolled. Before ascent to an elevation of 4300 m, their FE_{NO} and FE_{CO} values, demographic factors, drinking and smoking history, vital capacity, and forced vital capacity were obtained. The investigators followed the subjects in the first exposure week to obtain their Lake Louise Score (LLS) each day. Subjects with LLS > 4, headache, and at least 1 other symptom were diagnosed with AMS, and the highest LLS of each individual during 7 days was considered the final LLS score.

Results.—The AMS group had lower FE_{NO} ($P = .003$) and FE_{CO} ($P < .001$) values, and a lower smoking rate ($P < .001$) than the non-AMS group. Mean FE_{NO} and FE_{CO} values were 11.03 ppb (95% CI, 9.07 to 12.98) and 4.39 ppm (95% CI, 3.76 to 5.02), respectively, in the AMS group, and 14.74 ppb (95% CI, 13.25 to 16.23) and 6.10 ppm (95% CI, 5.49 to 6.72), respectively, in the non-AMS group ($P < .0001$). Using linear regression, both FE_{NO} and FE_{CO} were found to be significantly associated with the group's maximal LLS. Using logistic regression, FE_{NO} and FE_{CO} were also found to be significantly associated with AMS.

Conclusions.—Basal FE_{NO} and FE_{CO} are significantly negatively correlated with AMS development. However, the gases have only modest predictive value for the development of AMS.

Key words: acute mountain sickness, susceptibility, exhaled nitric oxide, exhaled carbon monoxide

Introduction

Acute mountain sickness (AMS) is a common illness affecting people entering high altitudes, causing adverse health effects that seriously affect physical performance. In the field of high altitude medical research, the prediction of AMS susceptibility in individuals going to high altitudes is still a thriving area of study. Researchers have been looking for the factors to predict AMS. So far, data from studies on the factors to predict AMS are inconsistent. Some studies have shown that poor hypoxic ventilatory response,^{1,2} age,^{3–5} and body mass index (BMI)⁵

are associated with AMS development; however, other studies have indicated that these factors are not associated with AMS.^{6–10} Macinnis et al¹¹ reviewed the genetics of altitude illness in 2010 and reported that no reliable genetic factors to predict AMS have yet been identified. In addition, the methods for measuring some factors cannot be adopted for a large population, as these methods are not noninvasive, simple, quick, convenient, and relatively low-cost. Thus, we are searching for some simple, quick, convenient, and relatively low-cost methods to predict AMS risk.

The pathophysiological mechanisms of AMS development are still unclear; however, it is reported that vascular constriction during hypoxia, especially cerebrovascular and pulmonary vascular constriction, plays a role in AMS.¹² Nitric oxide (NO) is a gaseous signaling molecule with a large variety of physiological functions,

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including airway and vascular smooth muscle relaxation and the regulation of ventilation–perfusion matching.¹³ Nitric oxide is produced endogenously within the upper and lower respiratory tract and can be measured in exhaled gas.¹⁴ Studies have revealed that during hypoxic breathing, the exhaled NO from the respiratory tract of high altitude pulmonary edema (HAPE) –susceptible subjects was lower than that from the control subjects at both altitude and sea level and that a negative relationship existed between the exhaled NO and the pulmonary artery pressure.¹⁵ Exhaled NO is associated with AMS susceptibility during exposure to normobaric hypoxia.¹⁶ The defect in pulmonary endothelial cell and respiratory epithelial cell NO synthesis is one of the mechanisms contributing to exaggerated hypoxic pulmonary hypertension during short-term high altitude exposure.¹⁷ These findings indicate that exhaled NO might be associated with AMS susceptibility during exposure to high altitude.

Smoking can cause hypoxia and is also associated with a decline in lung function. Some scholars have suggested that smoking is not significantly correlated with AMS,^{3,10,18} but others believe that it may be a risk factor for AMS.¹⁹ However, these conclusions cannot be effectively verified because of a lack of quantitative measurement of smoking behavior in these studies (the data were qualitative and collected by questionnaires) and an absence of effective measurement of the inhalation level, which is of great importance when evaluating exposure. Because of the great individual variations, the inhalation level may not be adequately evaluated by self-reported smoking behavior. The use of carbon monoxide (CO) monitors to quantify smoking has been reported, in which the level of exhaled CO has been widely used as an indication of smoking cessation.^{20,21} Carbon monoxide may also play an important role in the pathophysiology of airway diseases²² and hemoglobin survival, which may be potentially related to AMS.²³ However, more precise monitoring is required to determine the relationship between the level of exhaled CO and AMS.

As gaseous signaling molecules,^{24,25} NO and CO have the characteristics of continuous production, rapid delivery, and rapid diffusion; thus, they can be detected by exhaled gas tests.^{14,26} In practice, measuring the fraction of exhaled NO and CO (FE_{NO} and FE_{CO}) is noninvasive, simple, repeatable, quick, convenient, and relatively low-cost. As a result, these fractions have been increasingly used in clinical diagnosis. Therefore, we attempt to validate the hypothesis that FE_{NO} and FE_{CO} before ascent to high altitude are associated with AMS and to assess the predictive value of the 2 gases for AMS susceptibility.

Materials and Methods

ETHICS STATEMENT

This study was approved by the Ethical Review Board of the Third Military Medical University, Chongqing, China. There was no interaction between the study researchers and the individuals whose data were accessed. Personal information was removed from all data before retrieval and analysis.

SUBJECTS AND SETTINGS

Recruits of a highland troop in West China were selected as the study population. They were receiving training at a military base at an altitude of 1350 m. A total of 391 healthy male servicemen were voluntarily enrolled. Before the questionnaire and physical examination, all recruits attended a meeting held at the base to clarify the purpose, method, and significance of the study. Individuals interested in participating in the study signed a consent form. The consent form required the participant's signature only and excluded other personal identifying information.

One investigator then performed an examination to obtain basic demographic data, ascent history, and drinking and smoking histories. Their health records were obtained from the troop's health service authority. An individual was excluded if any of the following was found: 1) previous ascent to an altitude >2900 m, 2) being born and raised in a region with an altitude >2500 m, 3) other acute diseases during the study period, including cold and diarrhea, 4) asthma or other pulmonary diseases, 5) any other severe chronic diseases, or 6) incomplete study information.

Variables of age and BMI (calculated as weight in kilograms divided by stature in meters squared) extracted from the health record were controlled. Drinking behaviors were surveyed by questionnaire and classified into 2 categories: 1 for current drinking and 0 for no current drinking. Smoking behaviors were also surveyed by questionnaire and classified into 2 categories: 1 for current smoking and 0 for no current smoking. If smoking currently, the subject needed to provide the duration of his smoking history and the amount of his weekly consumption. Vital capacity (VC) and forced vital capacity (FVC) were tested with a pulmonary function meter (AS-505, Minato Medical Science Corp, Osaka, Japan), according to the manufacturer's instructions. Smoking and drinking were also prohibited during the operation.

FRACTION EXHALED NITRIC OXIDE AND CARBON MONOXIDE TESTS

The FE_{NO} and FE_{CO} were measured using a portable NObreath and Micro⁺ Smokerlyzer monitor (Bedfont Scientific Ltd, Maidstone, Kent, UK). The lower and upper limits of detection (LOD) of NObreath and of the Micro⁺ Smokerlyzer were 1 and 300 ppb and 1 and 250 ppm, respectively. The calibration and measurement procedures were performed according to the device operating manuals consistent with the European Respiratory Society (ERS) and American Thoracic Society (ATS) standards.²⁷

Tests for FE_{NO} and FE_{CO} were conducted in CO- and NO-free clinic rooms before subjects' ascent to high altitude, and the ambient air was tested by the devices in advance. Before formal testing, all observers were well trained, and all subjects were instructed in detail on how to use the devices and asked to avoid smoking for half a day. For the FE_{NO} test, an observer attached a mouthpiece to the device and confirmed that all connections were firm and all settings correct. The subject inhaled ambient air to near total lung capacity (not through the mouthpiece) and exhaled for 20 seconds at a constant flow rate through the mouthpiece into the device until a beep was heard; the device recorded and displayed the result in parts per billion. For the FE_{CO} test, subjects inhaled as deeply as possible and held their breath for 20 seconds, then exhaled slowly and gently into the mouthpiece until being unable to exhale further; the device recorded and displayed the result in parts per million. A subject with an FE_{CO} value > 6 was considered to be smoking. After each test was completed, the mouthpieces were replaced. There was a 15-minute rest interval between each 30-person test to reset the sensors.

ACUTE MOUNTAIN SICKNESS DIAGNOSIS

Subjects were divided into 3 groups. The groups were deployed to the destination at an altitude of 4300 m one after another by bus from the training base in April 2010. Each group traveled together with 2 observers on the 1080-km journey, in which the average altitude was 4254 ± 988.88 m (standard deviation), as shown in Figure 1. During the 4-day journey, they traveled during the day and rested for 1 night at each army service station.

The AMS diagnoses were based on the Lake Louise score (LLS), an international standard scoring system for AMS.²⁸ Before ascending, all investigators and subjects had become familiar with the LLS. The symptom investigations were performed every day after arrival at each army service station at 9 PM. The subjects were permitted

Figure 1. Changes in altitude and distance during the journey (Δ indicates the sleeping altitude each day).

a rest of 2 hours before investigations and then asked to complete an LLS self-report questionnaire (Questions 1–5: headache, gastrointestinal symptoms, fatigue and/or weakness, dizziness/lightheadedness, and sleeping difficulty) in a quiet environment. Clinical signs and symptoms (Questions 6–8: change in mental status, ataxia, and peripheral edema) were determined by observers. Each person's 4-day score during the journey was recorded. After arrival at the destination, the investigation continued for an additional 3 days. Thus, a 7-day record of the LLSs for subjects was obtained. If an individual's LLS was 5 or more (Questions 1–8) on any day, with headache and at least 1 other symptom (from Questions 1–5), that subject was considered to be suffering from AMS on that day.^{28,29} We further selected the highest LLS of each individual to build a max-LLS subset, and during 7 days the highest LLS of each individual was considered the final LLS value. Subjects were then divided into AMS (5 or greater) and non-AMS (<5) groups; the non-AMS group was further divided into 2 subgroups: 0 for no symptom and 1–4 for a few symptoms but no AMS present.

STATISTICAL ANALYSIS

The data were analyzed using SPSS 13.0 software (Chicago, IL) and a probability value of less than .05 was considered statistically significant in all analyses. Before analyses, all data were reviewed by the principal investigator for completeness.

We first performed descriptive statistics: 1) the LLSs were classified into 3 groups (0 point, 1–4 points, and 5 or more points), and the number of each category and

percentage on each day were computed; 2) the number and percentage of smoking and drinking behaviors in AMS diagnosis categories were computed; 3) the mean and standard deviation of each continuous variable (ie, age, BMI, VC, FVC, FE_{NO} , FE_{CO} , daily LLS, and max-LLS) were calculated; 4) a chart of altitude change with distance during the journey was also drawn.

A Student's t test was performed to determine the mean difference in each continuous variable between the AMS and non-AMS groups, and a χ^2 test was performed to determine drinking and smoking behavior differences between these 2 groups. Pearson's correlation test for continuous variables and Spearman's correlation test for categorical variables were used to evaluate relationships between age, BMI, VC, FVC, smoking, drinking, FE_{NO} , FE_{CO} , daily LLS, and max-LLS. To examine the normality of each continuous variable, a Kolmogorov-Smirnov test was performed, and log transformations were used for nonnormal variables.

Next, to test the relationship between the AMS score and FE_{NO} and FE_{CO} , we performed linear regression analyses using daily LLS and max-LLS as dependent variables and FE_{NO} and FE_{CO} as independent variables and controlling age, BMI, VC, and FVC. We constructed separate models to test FE_{NO} and FE_{CO} (16 separate models in total) and then put all variables into a multivariate linear model (8 integrated models in total). Categorical variables were transformed into dummy ones for analysis. The fulfillment of collinearity absence was tested by calculating variance inflation factor (VIF).

Finally, a logistic regression was used to test the predictive values of FE_{NO} and FE_{CO} for AMS susceptibility, in which AMS diagnosis was used as a dependent variable and the other processes were the same as in the linear models. The fulfillment of independence and collinearity were tested in the multiple models. The fit of the logistic regression models was evaluated by the Hosmer-Lemeshow goodness-of-fit test. The discriminative power of the significant predictive factors was visualized using receiver operating characteristic (ROC) curves. The ROC curve analyses were conducted using Med Calc 11.6 software (MedCalc Software bvba, Broekstraat, Belgium), including calculation of the area under the curve (AUC) with 95% CI, comparison of ROC curves, selection of the cutoff value, and calculation of sensitivity, specificity, positive predictive value, and negative predictive value.

Results

After filtering, 314 healthy servicemen remained in our study (mean age $\pm$ SD, 20.18, $\pm$ 1.73 years; mean

stature $\pm$ SD, 171.74, $\pm$ 6.49 cm; mean weight $\pm$ SD, 63.27, $\pm$ 7.10 kg).

The total distance of the journey was 1080 km, in which the highest altitude encountered was 5250 m and the highest sleeping altitude was 4700 m (day 3; Figure 1). The data indicated that AMS developed and increased when the subjects entered the highland. The peak appeared on day 3, after which the incidence decreased. On day 7, AMS was nearly absent in the population. There was no case of HAPE or high altitude cerebral edema.

Both the highest LLS and the largest number of individuals with an LLS > 4 appeared on day 3, when the average LLS was 3.87 ± 3.37 and 119 individuals had an LLS > 4 (37.9%; Table 1). According to our criteria, 119 (37.9%) of the 314 subjects (equal to the total number of subjects having an LLS > 4 on day 3) investigated on the total journey were diagnosed with AMS, with the highest LLS reported as 21.

The mean and standard deviation of each continuous variable in the AMS and non-AMS groups are shown in Table 2. Age, BMI, and FVC did not differ between the groups. However, FE_{NO} and FE_{CO} significantly differed between the non-AMS and AMS groups; the AMS group had lower FE_{NO} and FE_{CO} values. For VC, the probability value of the Student's t test was just below the level of significance ($P = .054$). The results also showed that the 95% CI for means of FE_{NO} value ranged from 13.25 to 16.23 in the non-AMS group and from 9.07 to 12.98 in the AMS group; the 95% CI for means of FE_{CO} value ranged from 5.49 to 6.72 in the non-AMS group and from 3.76 to 5.02 in the AMS group. As shown in Table

Table 1. The number (percentage) of subjects in different Lake Louise Score categories on each day and the mean Lake Louise Score on each day ($n = 314$)^a

	LLS category			Mean LLS $\pm$ SD
	0 <i>n</i> (%)	1–4 <i>n</i> (%)	>4 <i>n</i> (%)	
Day 1	42 (13.4)	253 (80.6)	19 (6.1)	1.73 $\pm$ 1.34
Day 2	47 (15.0)	235 (74.8)	32 (10.2)	2.19 $\pm$ 1.59
Day 3	65 (20.7)	127 (40.4)	119 (37.9)	3.87 $\pm$ 3.37
Day 4 ^a	46 (14.6)	220 (70.1)	48 (15.3)	2.46 $\pm$ 1.81
Day 5 ^a	77 (24.5)	216 (68.8)	21 (6.7)	1.81 $\pm$ 1.71
Day 6 ^a	90 (28.7)	220 (70.1)	4 (1.3)	1.07 $\pm$ 0.84
Day 7 ^a	184 (58.6)	129 (41.1)	1 (0.3)	0.49 $\pm$ 0.52
Max-LLS ^b	18 (5.7)	174 (55.4)	119 (37.9)	4.1 $\pm$ 3.17

LLS, Lake Louise Score.

^a Some subjects missed the investigation during the follow-up: 7 missed on day 4; 17 missed on day 5; 31 missed on day 6; and 44 missed on day 7.

^b Maximal LLS during the 7-day investigation.

Table 2. Mean and SD of each continuous factor in acute mountain sickness and non-acute mountain sickness groups

Factor	Mean	SD	P value ^a	95% CI for mean	
				Lower bound	Upper bound
Age (year)					
Non-AMS	20.25	1.83	.414		
AMS	20.08	1.62			
Total	20.18	1.75			
BMI (kg/m ²)					
Non-AMS	21.47	2.27	.529		
AMS	21.31	1.90			
Total	21.41	2.14			
VC (L)					
Non-AMS	3.26	0.58	.054		
AMS	3.13	0.59			
Total	3.21	0.59			
FVC (L)					
Non-AMS	2.81	0.63	.246		
AMS	2.73	0.57			
Total	2.78	0.60			
FE _{NO} (ppb)					
Non-AMS	14.74	10.53	.003	13.25	16.23
AMS	11.03	10.77		9.07	12.98
Total	13.33	10.75			
FE _{CO} (ppm)					
Non-AMS	6.10	4.34	<.001	5.49	6.72
AMS	4.39	3.46		3.76	5.02
Total	5.45	4.11			

AMS, acute mountain sickness; BMI, body mass index; FE_{CO}, fraction exhaled carbon monoxide; FE_{NO}, fraction exhaled nitric oxide; FVC, forced vital capacity; VC, vital capacity.

^a Student's *t* test for each factor.

3, although drinking behavior did not differ between groups, the AMS group had a significantly lower smoking rate than the non-AMS group. Table 4 shows that the

Table 3. Drinking and smoking behaviors in acute mountain sickness and non-acute mountain sickness groups

	Non-AMS	AMS	Total	P value ^a
Drinking				
No	143	83	226	.519
Yes	52	36	88	
Smoking				
No	93	83	176	<.001
Yes	102	36	138	
Total	195	119	314	

AMS, acute mountain sickness.

^a χ^2 test for each factor.

Table 4. Factors affecting fractions of exhaled nitric oxide and carbon monoxide in smoking and nonsmoking groups

Factor	Mean	SD	P value ^a
FE _{CO} (ppm)			
Non smoking	2.65	1.27	<.001
Smoking	9.03	3.69	
Total	5.45	4.11	
FE _{NO} (ppb)			
Non smoking	14.76	12.11	<.001
Smoking	11.51	8.42	
Total	13.33	10.75	

FE_{CO}, fraction exhaled carbon monoxide; FE_{NO}, fraction exhaled nitric oxide.

^a Student's *t* test for each factor.

smoking group had a significantly higher FE_{CO} value and a lower FE_{NO} value than the nonsmoking group (*P* < .001).

The correlation coefficients between LLS (daily LLS and max-LLS) and the other variables and those among the other variables were analyzed. Negative correlations with max-LLS and most-day LLSs were observed for VC, FVC, FE_{NO}, FE_{CO}, and smoking history. In addition, FE_{CO} and smoking rates negatively correlated with FE_{NO} but positively correlated with BMI. The FE_{CO} and smoking behavior, VC, and FVC were also strongly positively correlated (*r* = .831, *P* < .001 and *r* = .746, *P* < .001, respectively). Therefore, FE_{CO} was strongly correlated with the variables associated with smoking behavior. Smoking behavior was thereby excluded as a variable from further regression analyses, and FVC was excluded as well.

The separated linear regressions showed that both FE_{NO} and FE_{CO} were significantly associated with LLSs on days 1 through 6 and with the max-LLS. The controlled variables (age, BMI, and drinking behavior) were not significantly associated with AMS in either separated model (data not shown), although VC was significantly associated with AMS in most separated models. The integrated and separated model analyses showed similar results. The VIF values demonstrated an acceptable collinearity (VIF < 0.5).

In the separated and integrated logistic regression models, FE_{NO} and FE_{CO} were significantly negatively associated with LLS score, whereas VC was only significant in separated model II (FE_{CO} model). Other controlled variables were not significant in any model (Table 5). The Hosmer-Lemeshow test indicated that the models fit well. The ROC curves are depicted in Figure 2, and the results of the ROC curve analyses are shown in Table 6. The results suggested that the areas under the ROC

Table 5. Separated and integrated logistic regression models to test the predictive value of fractions of exhaled nitric oxide and carbon monoxide, adjusted for age, body mass index, vital capacity, and drinking behavior ($n = 314$)

Models	Variable ^a	β	OR (95% CI)	P value of β	P value of H-L ^b
Separated models I	FE_{NO}	-1.497	0.224 (0.113, 0.442)	<.001	.159
	VC	-0.319	0.727 (0.480, 1.100)	.131	
Separated models II	FE_{CO}	-1.705	0.182 (0.075, 0.443)	<.001	.526
	VC	-0.436	0.647 (0.427, 0.981)	.040	
Integrated model	FE_{NO}	-1.831	0.160 (0.078, 0.330)	<.001	.708
	FE_{CO}	-2.238	0.107 (0.040, 0.285)	<.001	
	VC	-0.398	0.671 (0.435, 1.036)	.072	

FE_{CO} , fraction exhaled carbon monoxide; FE_{NO} , fraction exhaled nitric oxide; OR, odds ratio; VC, vital capacity.

^a Values for FE_{NO} and FE_{CO} are log transformed.

^b Hosmer-Lemeshow test.

curve were larger than 0.5, and the 2 separated model AUCs were not significantly different. However, the integrated model AUC was significantly larger than the 2 separated models. According to Hosmer and Lemeshow's suggestion regarding AUC, the separated models had poor discrimination, whereas the integrated model had acceptable discrimination³⁰; the best cutoff value was 0.406 (positive predictive value was 59.2% and

negative predictive value was 76.2%). However, all of the model prediction values were low.

Discussion

The results of the statistical analysis suggest that higher FE_{NO} and FE_{CO} before ascent to a high altitude are protective factors for AMS. The daily LLS and the AMS

Figure 2. Receiver operating characteristic curves of the logistic regression models (a: cutoff value = 0.374, sensitivity = 60.5%, specificity = 66.2%; b: cutoff value = 0.370, sensitivity = 65.6%, specificity = 58.5%; c: cutoff value = 0.406, sensitivity = 62.2%, specificity = 73.9%). Separated model I (blue line): the logistic regression models to test the predictive value of the fraction of exhaled nitric oxide; separated model II (green line): the logistic regression models to test the predictive value of the fraction of exhaled carbon monoxide; integrated model (red line): the logistic regression models to test the predictive value of the fractions of exhaled nitric oxide and carbon monoxide).

Table 6. Receiver operating characteristic curve analyses for logistic regression models

Item	Model		
	Separated models I	Separated models II	Integrated model
Sample size	314	314	314
AUC with 95% CI	0.651 (0.587, 0.715)	0.646 (0.584, 0.707)	0.726 (0.667, 0.784)
Z statistic vs. AUC = 0.5	4.641 ($P < .001$)	4.626 ($P < .001$)	7.595 ($P < .001$)
Z statistic vs. ROC I ^a	—	0.126 ($P = .900$)	3.123 ($P = .002$)
Z statistic vs. ROC II ^b	—	—	2.826 ($P = .005$)
Cutoff value	0.374	0.370	0.406
Sensitivity (%) with 95% CI	60.5 (51.1, 69.3)	65.6 (56.3, 74.0)	62.2 (52.8, 70.9)
Specificity (%) with 95% CI	66.2 (59.0, 72.8)	58.5 (51.2, 65.5)	73.9 (67.1, 79.9)
PPV (%) with 95% CI	52.2 (43.5, 60.7)	49.1 (41.1, 57.1)	59.2 (50.1, 67.9)
NPV (%) with 95% CI	73.3 (66.1, 79.7)	73.5 (65.9, 80.3)	76.2 (69.5, 82.1)

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

^a ROC curve of separated models I.

^b ROC curve of separated models II.

diagnosis results are closely associated with the 2 exhaled gases. As for the controlling factors, age, BMI, and drinking behavior are not significantly associated with AMS, similar to the previous reports.^{3,10,18} Finally, VC is negatively correlated with AMS, which has also been previously reported.³¹

Pulmonary NO may play an important role in the physiological response to acute high altitude hypoxia.¹² Some studies have demonstrated the importance of pulmonary vascular endothelial and alveolar epithelial NO synthesis in the regulation of pulmonary vascular responsiveness to high altitude exposure,³² and that a lower FE_{NO} likely weakens airway and vascular smooth muscle relaxation and the regulation of ventilation-perfusion matching.¹³ In addition, poor gas exchange in the lungs could also contribute to AMS. Our results also suggest that LLS and the AMS diagnosis results are closely associated with the FE_{NO} before ascent to high altitude. Therefore, a lower FE_{NO} may hinder proper acclimatization to altitude, increasing the probability of AMS. Certainly, data from studies on the effect of hypoxia on exhaled NO are also inconsistent. Brown et al¹² have shown that exhaled NO had no predictive value for AMS. We think these conflicts might result from differences in procedures, experimental protocols, exposure times, and samples.

The latest FE_{NO} standard procedure to determine expired NO is defined by the ATS and the ERS, and the ATS/ERS method has primarily been designed for use at sea level or at moderate altitude.^{27,33} Some commercially available instruments for these standardized measurements have been described.³⁴ Study has also shown that FE_{NO} values measured with NObreath are reproducible and in good agreement with those obtained by NIOX

MINO and Logan, indicating that NObreath is suitable for use in practice.¹⁴ Therefore, the NObreath instrument used to measure FE_{NO} value can be adopted in our study.

Measured FE_{CO} is closely linked to active and passive smoking, traffic exhaust, and industrial smoke, whereas the endogenous CO production is of relatively minor importance. Considering the environment of the training base, traffic exhaust and industrial smoke can be excluded. Additionally, because the LOD of the device is 1 to 250 ppm, which is greater than the endogenous CO level (less than 1 ppm),³⁵ the FE_{CO} only represents tobacco consumption (including passive) in this study. The FE_{CO} values obtained by this method may be more accurate than those obtained from the self-report of smoking behavior.

The results indicate that smoking behavior at low elevations significantly decreases the risk of AMS when one travels to a high altitude. However, this does not mean that smoking is entirely beneficial for decreasing AMS. Some studies have shown that smokers exhale significantly less NO than do healthy volunteers.^{36,37} In our study, the FE_{NO} values of smokers are significantly lower than those of nonsmokers ($P < .001$; Table 4). Therefore, the FE_{CO} value was negatively correlated with max-LLS and most-day LLSs, but it did not indicate that the higher the FE_{CO} value is, the lower the risk of AMS would be. We think the effect of smoking is bidirectional. In some range, FE_{CO} has a protective role for AMS. Smoking during the ascent is also very harmful; indeed, smoking and drinking were forbidden in this operation.

The results of the logistic regression analysis further validate the relationship between AMS and FE_{NO} and FE_{CO} before high altitude exposure. In addition, the ROC analysis suggests that both exhaled gases have independent modest predictive value for AMS susceptibility. If they are com-

bined into one model, the significance of the predictive value and the goodness-of-fit for AMS prediction will be increased. However, the combined predictive value is still insufficient. This may be because the mechanism of AMS development is very complex and cannot be explained by 2 exhaled gases alone. This implies that studies on other variables are needed in the future to better predict AMS.

The results showed the 95% CI for the mean FE_{NO} and FE_{CO} values in non-AMS and AMS groups before the ascent to a high altitude. Whether 95% CI can be used as the criterion for diagnosing AMS susceptibility needs further validation. Because FE_{NO} and FE_{CO} values before high altitude exposure are related to AMS susceptibility, in future work we will consider researching the reference ranges of FE_{NO} and FE_{CO} for AMS susceptibility in the healthy population based on large samples and stricter tests to accurately predict AMS. We expect to achieve the prediction of AMS susceptibility by comparing the measured value and the value of FE_{NO} and FE_{CO} in the reference range.

This study is, to our knowledge, the first to focus on the relationship between AMS and exhaled gases (FE_{NO} and FE_{CO}) before a group of Chinese lowlander servicemen's ascent to a highland (>2500 m). In our study, the relationship between the exhaled gases and AMS is assessed based on a large sample size. As a prospective study, the investigation was well conducted and truly reflected AMS in detail; the observers accompanied the subjects during the full journey and recorded the daily LLS of each subject according to both self-reported and clinical signs. In addition, thanks to the nature of the military organization and routine military operation, we obtained reliable and comprehensive background information, high response rate, and full cooperation, which enhance the study quality.

LIMITATIONS

Several limitations exist in the study design and should be considered when generalizing our findings to other populations or conditions. First, in this study, occupation, diet, and physical load at a high altitude were negligible because everyone experienced the same condition. Therefore, the identifiable risk factors for AMS among such a specialized population are limited. Second, the Operating Manual of the Micro+ Smokerlyzer monitor points out that subjects with FE_{CO} values >6 are considered to be smokers, we think the FE_{CO} value can quantitatively reflect the smoking behavior of subjects, and studies also showed that smoking is positively associated with hemoglobin concentration,³⁸ so we did not consider measuring the hemoglobin concentrations. Third, because of the large number of subjects and the

routine operations, we could not collect LLS in the morning. Moreover, because AMS cases were diagnosed by different observers over the course of almost 4 days, bias is inevitable and uncontrollable. However, the sample size is large enough to at least partially limit this confounding effect.

Conclusions

In conclusion, the concentrations of FE_{NO} and FE_{CO} before exposure to a high altitude correlate with AMS and may offer a new method for predicting AMS susceptibility. However, the predictive value of measurements of FE_{NO} and FE_{CO} made before high altitude exposure still awaits validation of further studies.

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