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Nitric oxide and cardiopulmonary hemodynamics in Tibetan highlanders

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Hoit, Brian D., Nancy D. Dalton, Serpil C. Erzurum, Daniel Laskowski, Kingman P. Strohl, and Cynthia M. Beall. Nitric oxide and cardiopulmonary hemodynamics in Tibetan highlanders. *J Appl Physiol* 99: 1796–1801, 2005. First published July 14, 2005; doi:10.1152/japplphysiol.00205.2005.—When O_2 availability is reduced unavoidably, as it is at high altitude, a potential mechanism to improve O_2 delivery to tissues is an increase in blood flow. Nitric oxide (NO) regulates blood vessel diameter and can influence blood flow. This field study of intrapopulation variation at high altitude tested the hypothesis that the level of exhaled NO (a summary measure of pulmonary synthesis, consumption, and transfer from cells in the airway) is directly proportional to pulmonary, and thus systemic, blood flow. Twenty Tibetan male and 37 female healthy, nonsmoking, native residents at 4,200 m (13,900 ft), with an average O_2 saturation of hemoglobin of 85%, participated in the study. The geometric mean partial pressure of NO exhaled at a flow of 17 ml/s was 23.4 nmHg, significantly lower than that of a sea-level reference group. However, the rate of NO transfer out of the airway wall was seven times higher than at sea level, which implied the potential for vasodilation of the pulmonary blood vessels. Mean pulmonary blood flow (measured by cardiac index) was 2.7 ± 0.1 (SE) l/min, and mean pulmonary artery systolic pressure was 31.4 ± 0.9 (SE) mmHg. Higher exhaled NO was associated with higher pulmonary blood flow; yet there was no associated increase in pulmonary artery systolic pressure. The results suggest that NO in the lung may play a key beneficial role in allowing Tibetans at 4,200 m to compensate for ambient hypoxia with higher pulmonary blood flow and O_2 delivery without the consequences of higher pulmonary arterial pressure.

high altitude; oxygen delivery; oxygen availability; hypoxia

WHEN OXYGEN AVAILABILITY is reduced, as it is at high altitude, an increased blood flow could potentially improve O_2 delivery to tissues. However, the pulmonary vasoconstriction response to hypoxia decreases blood flow in the lung, the first point of contact with the circulation. This response probably evolved at sea level to maintain gas exchange by redistribution of blood flow from temporarily small, poorly oxygenated to better-oxygenated areas of the lung (17, 32). At high altitude, the entire lung is always hypoxic, and the resulting general vasoconstriction does not redistribute blood flow; instead, it increases pulmonary arterial pressure, sometimes causing pathological remodeling of the heart and lungs (30). However, many people live at high altitude without pulmonary hypertension or cardiac hypertrophy, which suggests that another factor may intervene to maintain blood flow when the blood carries less O_2 and the usual vasoconstriction response increases pulmonary resistance. That factor may be nitric oxide (NO), a vasodilator found in high concentrations in the lungs of high-altitude natives, particularly among Tibetans (5).

A wealth of studies support a key role for NO in determining basal pulmonary vascular tone at sea level and in effecting the hypoxic vasoconstriction response. An animal study using NO synthase gene transfer to the airway demonstrates elegantly that increasing NO decreases hypoxic pulmonary vasoconstriction (7). Studies of humans are consistent and have demonstrated that *1*) NO is critical in regulating basal pulmonary vascular tone (10, 27), *2*) inhibiting NO synthesis exacerbates hypoxic pulmonary vasoconstriction, and *3*) inhaling gas mixtures with high concentrations of NO diminishes hypoxic pulmonary vasoconstriction at sea level (6, 15) and lowers pulmonary artery systolic pressure at high altitude (23). Moreover, sea-level natives exposed to acute hypoxia (and who have relatively high levels of exhaled NO) have less hypoxic pulmonary vasoconstriction, as measured by pulmonary artery systolic pressure (8, 11). This physiological evidence justifies an examination of the relation between NO and pulmonary blood flow in the normal range of variation among healthy high-altitude natives. Accordingly, we tested the hypothesis that the level of exhaled NO, a summary measure of pulmonary synthesis, consumption, and transfer of NO, is directly related to pulmonary blood flow measured by cardiac index in a sample of healthy Tibetans, native residents at 4,200 m.

METHODS

Population and sample. The research described here adheres to the principles of the Declaration of Helsinki and Title 45 of the US Code of Federal Regulations, Part 46, Protection of Human Subjects. The Institutional Review Board of Case Western Reserve University approved the protocol, and informed consent was obtained.

Data for this field study were collected from June to August 2002 in Panam Xiang, a rural agropastoral district of Xigatse Prefecture, Tibet Autonomous Region, at 4,200 m. From a total of 98 volunteers, 88 normotensive, nonanemic, nonsmoking, healthy (by self-report), nonpregnant (by self-report) high-altitude native 18- to 55-yr-old Tibetans with normal pulmonary function provided demographic information along with measures of exhaled NO. Eighty individuals (91%) volunteered for an echocardiographic examination 3–5 wk later. Of these 80 volunteers, 3 had heart disease (mild pulmonic stenosis, mild mitral stenosis, or small secundum atrial septal defect) and 12 did not have a tricuspid regurgitant jet adequate for estimation of pulmonary artery systolic pressure. Thus 65 (81%) individuals were

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Table 1. *Characteristics of high-altitude native Tibetan subjects*

	Men (n = 20)	Women (n = 37)
Age, yr	33±2	29±2
Height, m	1.64±0.01	1.54±0.01
Weight, kg	47.9±1.2	44.1±0.7
Hb concn, g/dl	16.4±0.33	14.6±0.13
O_2 saturation, %	83.3±0.8	85.5±0.6
Arterial O_2 content, ml/dl	19.0±0.4	17.3±0.2
Systolic blood pressure, mmHg	113±2	115±1
Diastolic blood pressure, mmHg	77±2	76±1
Forced vital capacity, liters	4.8±0.2	3.6±0.1

Values are means ± SE; *n,* number of Tibetan residents at 4,200 m (13,900 ft) in Panam Xiang, Tibet Autonomous Region.

free of any abnormalities on echo-Doppler examination and had a tricuspid regurgitant jet adequate for pulmonary hemodynamic estimates. Four determinations of exhaled NO were lost because of machine error, and four subjects did not provide technically acceptable efforts. Thus 57 (87% of those with an adequate echocardiogram) had technically acceptable measurements of exhaled NO and comprise the study sample described in Table 1. The reported sample of 57 and the larger samples of 80 with echocardiography and 88 healthy individuals differed by <1% in the mean values of the cardiopulmonary and exhaled NO variables reported here.

The reference group comprised 20 (7 men and 13 women) healthy (by self-report), nonsmoking, nonasthmatic sea-level residents. For men, the average age was 47 ± 15 yr, height 1.76 ± 0.07 m, and weight 80.4 ± 12.2 kg; for women, the average age was 39 ± 13 yr, height 1.66 ± 0.08 m, and weight 68.6 ± 12.3 kg.

Measurements. The fraction of NO in exhaled breath was measured using an online method in parts per billion with an NO analyzer (Sievers NOA 280i, Ionics Instruments, Boulder, CO) that has a sensitivity of <1 ppb and uses a chemiluminescent technique. Nasal NO contamination was minimized by a standardized procedure in which the subject exhales against an expiratory resistance (10–20 cmH_2O), which causes velum closure (3, 16) and generates a plateau level of NO. The exhaled NO reported is the average of three exhalations at a given flow rate. If the three values were not within 2 ppb of each other, then a fourth measurement was collected and the three most similar values were averaged. Two-point calibrations were performed once or twice a day using a Zero Air Filter (Ionics Instruments) to provide an air sample free of NO and a gas with a known concentration of NO [45 ppm (Ionics Instruments) or 9.4 ppm (Praxair, Cleveland, OH)]. The ambient conditions at the time of morning calibrations were as follows: 14.4°C average temperature, 52% relative humidity, 1.3 ppb NO in ambient air, and 464 Torr barometric pressure. To take into account the lower barometric pressure for comparisons with sea-level data, NO measured in parts per billion (the fractional concentration) was converted to NO in nanometers Hg [i.e., partial pressure (P_{NO})] by multiplying by barometric pressure minus 47 mmHg (i.e., water vapor pressure at 37°C in the lung) and dividing by 1,000. Variability between NO measurements over time is 6.6 ± 1.4% (13).

Pulmonary artery hemodynamics were measured noninvasively using echocardiography. Echocardiographic studies were performed with an Acuson Cypress ultrasonograph (Siemens Medical Solutions). Standard parasternal, apical, and subcostal two-dimensional views were obtained, and color flow-directed pulsed-wave Doppler measurements of transvalvular flows and continuous-wave Doppler measurements of the tricuspid regurgitant flow were obtained. A single-lead electrocardiogram was recorded on the ultrasonograph. Measures obtained using this noninvasive technique correlate closely with those obtained using cardiac catheterization (2, 18), an invasive technique that is not appropriate for a field study of healthy individuals in a rural, out-of-hospital setting.

O_2 saturation of hemoglobin was measured by pulse oximetry (model 504, Criticare Systems, Waukesha, WI). Hemoglobin concentration was determined in duplicate using the cyanmethemoglobin technique (Hemoglobinometer, Hemocue, Angelholm, Sweden) immediately after a venous blood sample was drawn. Arterial O_2 content (AOC) was calculated in milliliters O_2 per 100 ml blood (volume percent) as follows: AOC = 1.39 × (O_2 saturation × hemoglobin concentration)/100 (31). Pulmonary function was assessed by forced vital capacity and forced expiratory volume at 1 s (QRS Diagnostic Spirocard, Plymouth, MN) (4). A determination was the best of three measurements. Reported blood pressures are averages of three resting, seated determinations.

Analyses. Pulmonary artery systolic pressure (PASP) was calculated as follows

$$PASP = [4(TR_{vel})^2] + RAP \quad (1)$$

where TR_{vel} is tricuspid regurgitation jet velocity and RAP is the estimated right atrial pressure based on the respiration variation in inferior vena cava size (20).

Cardiac output (CO) was calculated as follows

$$CO = [P(D/2)^2]Ao_{vti}\,HR \quad (2)$$

where $P(D/2)^2$ is the cross-sectional area of blood flow into the aorta, Ao_{vti} is the velocity time integral through the aorta, and HR is heart rate.

In the absence of shunts and significant regurgitation, cardiac output is equal to pulmonary blood flow. To control for variation in body size, cardiac index was calculated as cardiac output divided by body surface area calculated from height and weight.

Total pulmonary resistance (PR) was measured as follows

$$PR = PASP/CO \quad (3)$$

NO exchange in the lung was analyzed using a theoretical two-compartment model of pulmonary NO that includes a distal alveolar component and an airway component (26, 29) and provides estimates of NO concentration in the airway wall (nmHg), transfer rate of NO from the airway wall ($nl \cdot s^{-1} \cdot mmHg^{-1}$), and maximum potential airway flux of NO (nl/s). In the model, exhaled NO is primarily affected by NO concentration in the airway wall and the rate of NO transfer from the wall to the airway lumen; both can be estimated from measurements of exhaled NO at two or more flow rates (i.e., 17 and 50 ml/s). A two-point linear regression is used to estimate the airway wall NO content and the NO transfer rate. For each subject, exhaled NO (ppb) at each flow rate is plotted against the elimination rate of NO, which is determined by the product of exhaled NO concentration and flow rate (17 or 50 ml/s). The slope of the line provides the transfer rate of NO, and the y-intercept provides the airway wall NO content. The amount of NO that enters the airstream from the airways per unit time during exhalation is the NO flux, which can be calculated mathematically as the product of airway wall NO content and transfer rate of NO.

Multiple linear regression models (SPSS version 11) were used to predict cardiac index, pulmonary artery systolic pressure, and total pulmonary resistance; variables included age, gender (and their interaction), exhaled NO, and O_2 saturation. Dependent and independent variables used in the multiple regression were first subjected to bivariate correlative analysis. Natural logarithmic transformation normalized the distribution of NO exhaled at 17 and 50 ml/s as well as the variables derived from these direct measures. Statistical analyses were performed on the transformed values; results are presented in the original units. Geometric means (the natural antilogarithm of the natural logarithmically transformed mean) are reported for those variables. The dispersions about the geometric means were expressed in terms of the standard deviations of the transformed means. Coef-

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Table 2. *Exhaled NO and its kinetics in Tibetan subjects and a sea-level reference group*

	Tibetan Subjects	Reference Group
NO exhaled at 17 ml/s, nmHg	23.4 (17.5)	30.7 (11.9)*
NO exhaled at 50 ml/s, nmHg	12.4 (23.1)	13.7 (14.5)
NO concn in airway wall, nmHg	39.8 (21.6)	104.3 (19.6)*
NO transfer rate, nl·s^{-1}·mmHg^{-1}	0.07 (37.8)	0.01 (23.2)*
NO maximum potential flux, nl/s	2.8 (60.3)	1.2 (214.4)*

Values are geometric means, with coefficient of variation in parentheses, for 57 healthy Tibetan residents at 4,200 m and a US sea-level (282 m) reference group. *$P < 0.05$.

ficients of variation were calculated as 100 × (transformed standard deviation/transformed mean). Means ± SE are reported for other variables. Data from controls and Tibetans were compared with an unpaired *t*-test. A significance level of $P < 0.05$ is accepted.

RESULTS

Pulmonary hemodynamics. Pulmonary blood flow measured as cardiac output was 3.9 ± 0.1 (SE) l/min (range 2.6–6.5), and mean cardiac index (which takes body size into account) was 2.7 ± 0.1 (SE) l·min^{-1}·m^{-2} (range 1.9–4.6). The average pulmonary artery systolic pressure was 31.5 ± 1.0 (SE) mmHg (range 17.2–55.9). Pulmonary artery systolic pressure of 39 subjects (68%) was within the normal sea-level range of <35 mmHg (1), while the values for the remaining subjects were higher. The mean calculated total pulmonary resistance was 8.5 ± 0.4 (SE) mmHg/l (range 4.1–19.4). There were no gender differences or correlations with age among these variables.

Exhaled NO and kinetics. Exhaled NO was not associated with age or gender differences, body size, systemic blood pressure, forced vital capacity, hemoglobin concentration, or inflammation measured by the level of C-reactive protein. At exhalation of 17 ml/s, the mean P_{NO} of 23.4 nmHg among the Tibetans was lower than the mean of 30.7 nmHg for a sea-level reference sample of 20 healthy adults in the United States (Table 2). At exhalation of 50 ml/s, the mean P_{NO} of 12.4 nmHg among the Tibetans did not differ from the mean of 13.7 nmHg for the sea-level sample (Table 2). Altitude differences in P_{NO} of exhaled breath could result from differences in NO concentration in the airway wall or in the transfer rate of NO from the airway wall to the airway lumen. The mean calculated NO concentration of 39.8 nmHg in the airway wall in the Tibetan sample at 4,200 m was lower than the mean of 104.3 nmHg for the sea-level control sample (Table 2). The lower NO concentration in the airway wall may be due to the O_2 dependency of NO synthase kinetics, leading to a lower rate of synthesis (12), a greater NO consumption, or a faster rate of NO transfer out of the airway wall. The average NO transfer rate of 0.07 nl·s^{-1}·mmHg^{-1} in the Tibetan sample was seven times higher than that among the sea-level controls (Table 2). As a result, the maximum potential NO flux (product of airway wall NO content and diffusion or transfer rate of NO out of the airway wall) of 2.8 nl/s in the Tibetan sample was more than double the sea-level reference average of 1.2 ml/s, despite the lower exhaled NO and NO concentration in the airway wall (Table 2). NO flux was significantly and inversely related to pulmonary resistance ($r = -0.243$, $P = 0.041$); inasmuch as both variables were normally distributed, transformation was unnecessary. Thus the implication for blood flow and O_2 delivery is that NO, which diffuses in all directions, is not only transferred to the airway lumen at a higher rate but is probably also transferred to the pulmonary arteries (which subsequently vasodilate) at a higher rate.

Multiple regression models. To address the hypothesis that higher NO levels augmented pulmonary blood flow, Table 3 presents the bivariate correlation matrix for variables used in regression models summarized in Table 4. Heart rate was not correlated with NO ($r = 0.11$, $P = 0.39$); therefore, it was not used in the multiple regression analysis.

Higher NO was associated with higher cardiac index (Fig. 1*A*) and lower pulmonary vascular resistance (Fig. 2), consistent with the hypothesis. Age and gender alone accounted for ~20% of the variance in cardiac index; exhaled NO explained an additional 10% of the variance (Table 4). Age and gender accounted for ~5% of the variance in total pulmonary resistance; exhaled NO accounted for an additional ~8% of the variance. However, these factors did not explain variance in pulmonary artery systolic pressure.

Greater physiological hypoxia, as measured by lower O_2 saturation of hemoglobin, was associated with higher pulmonary artery systolic pressure, consistent with the presence of varying degrees of hypoxic pulmonary vasoconstriction. O_2 saturation, as well as age, gender, and NO, explained ~13% of the variance in pulmonary artery systolic pressure compared with the model without saturation, which did not explain any variance (Table 4). Higher saturation and increased exhaled NO are associated with lower pulmonary resistance; O_2 saturation, along with age, gender, and NO, explained ~21% of the variance in pulmonary resistance (compared with the model using just age, gender, and NO, which explained ~13% of the variance). These data suggest that, although they are modest, the effects of NO and O_2 saturation on pulmonary resistance and pulmonary artery systolic pressure are additive and independent.

DISCUSSION

The main finding of this field study of hypoxic, high-altitude native Tibetans residing at 4,200 m is that higher levels of NO in the normal range of variation were associated with higher pulmonary blood flow measured by cardiac index. Moreover, these findings suggest that higher NO causes vasodilation, which allows greater pulmonary blood flow, cardiac output,

Table 3. *Bivariate correlation matrix for variables used in multivariate analysis of the effect of exhaled NO on pulmonary hemodynamics*

	Age	NO	Sat	CI	PASP
Age	1				
NO	0.063 (57)	1			
Sat	−0.278* (57)	−0.318* (57)	1		
CI	0.305* (55)	0.242† (55)	0.131 (55)	1	
PASP	−0.135 (57)	−0.067 (57)	−0.289* (57)	−0.079 (55)	1
PVR	−0.183 (54)	−0.290* (54)	−0.124 (54)	−0.301* (54)	0.470* (54)

Age, age (yr); NO, natural logarithm of nitric oxide exhaled at 17 ml/s, (ppb); Sat, O_2 saturation of arterial hemoglobin (%); CI, natural logarithm of cardiac index, (l·min^{-1}·m^{-2}); PASP, pulmonary artery systolic pressure (mmHg); PVR, total pulmonary resistance (mmHg/l). *$P < 0.05$ (2-tailed test). †$0.05 < P < 0.10$ (2-tailed test).

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Table 4. *Models with covariates age and gender (and their interaction) and exhaled NO and with covariates age, gender, and O_2 saturation and exhaled NO*

	CI				PR				PASP			
	Model with covariates only		Model with exhaled NO		Model with covariates only		Model with exhaled NO		Model with covariates only		Model with exhaled NO	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Constant		0.897		0.188		0.001		0.000		0.001		0.002
Covariate												
Age	+1.251	0.015	+1.295	0.007	−1.215	0.031	−1.255	0.021	−0.656	0.239	−0.664	0.237
Gender	+1.032	0.013	+1.117	0.004	−0.773	0.086	−0.850	0.050	−0.349	0.434	−0.365	0.419
Age * gender	−1.075	0.069	−1.087	0.049	+1.249	0.056	+1.260	0.044	+0.639	0.323	+0.641	0.325
Main effect												
lnNO			+0.345	0.005			−0.309	0.023			−0.064	0.650
Model R^2 (adjusted)	0.202		0.307		0.051		0.129		0		0	
Regression MSE (*F*)	0.184 (5.560)	0.002	0.200 (6.993)	0.000	9.225 (1.945)	0.134	12.898 (2.964)	0.029	40.565 (0.758)	0.523	33.259 (0.612)	0.656

	Model with O_2 Sat		Model with O_2 Sat and exhaled NO		Model with O_2 Sat		Model with O_2 Sat and exhaled NO		Model with O_2 Sat		Model with O_2 Sat and exhaled NO	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Constant		0.306		0.700		0.000		0.000		0.000		0.000
Covariate												
Age	+1.238	0.016	+1.290	0.008	−1.123	0.027	−1.298	0.013	−0.691	0.187	−0.722	0.164
Gender	+1.083	0.010	+1.127	0.005	−0.701	0.117	−0.756	0.068	−0.211	0.616	−0.237	0.568
Age * gender	−1.101	0.062	−1.093	0.050	+1.212	0.061	+1.202	0.044	+0.568	0.349	+0.563	0.348
Main effect												
Sat	−0.144	0.274	−0.035	0.787	−0.203	0.161	−0.336	0.019	−0.390	0.006	−0.455	0.002
lnNO			+0.334	0.009			−0.409	0.003			−0.200	0.145
Model R^2 (adjusted)	0.206		0.294		0.070		0.209		0.109		0.129	
Regression MSE (*F*)	0.148 (4.493)	0.004	0.130 (3.692)	0.006	9.277 (1.996)	0.110	15.002 (3.794)	0.006	127.845 (2.716)	0.040	122.392 (2.660)	0.033

Regression models explain variability in cardiac index (CI), pulmonary artery systolic pressure (PASP), and pulmonary resistance (PR) of Tibetan high-altitude native residents of 4,200 m. The first category for each dependent variable indicates variability accounted for by age, gender, and their interaction. The category for each dependent variable indicates variability accounted for after addition to the regression model of the natural logarithm of exhaled NO measured at 17 ml/s. The difference in R^2 of 2 categories measures the independent contribution of exhaled NO to variability in the dependent variable. MSE, mean square error.

and O_2 delivery to the systemic circulation without elevated pulmonary artery systolic pressure. An individual in the 75th percentile of exhaled NO has ~12% higher pulmonary blood flow than an individual in the 25th percentile yet does not have higher pulmonary artery systolic pressure as a result, because pulmonary resistance is lower.

Several lines of evidence support the reasoning that elevated NO allows higher cardiac index. *1*) Exhaled NO does not increase after experimental elevation of cardiac output (12). *2*) Exhaled NO is decreased on acute exposure to high altitude, despite elevation of cardiac output (11, 23, 30). *3*) Experimental inhibition of NO synthesis causes a decrease in cardiac index at sea level (6, 27). Taken together, these findings suggest that increases in NO result in a higher cardiac output or cardiac index. An alternative interpretation of the association between NO and cardiac index in this Tibetan sample is that

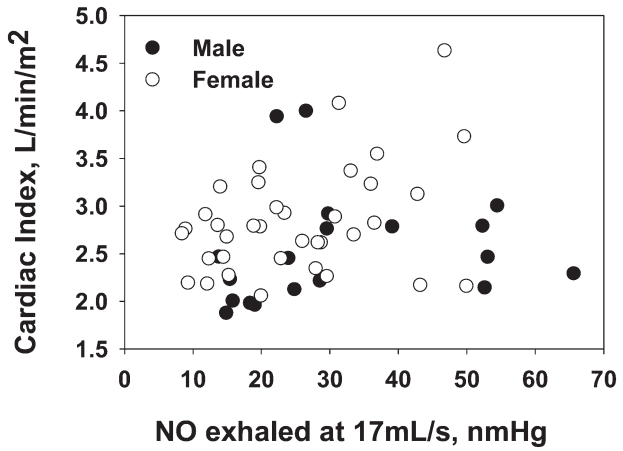

Fig. 1. Cardiac index increases with higher exhaled nitric oxide (NO) levels in the Tibetan sample at 4,200 m.

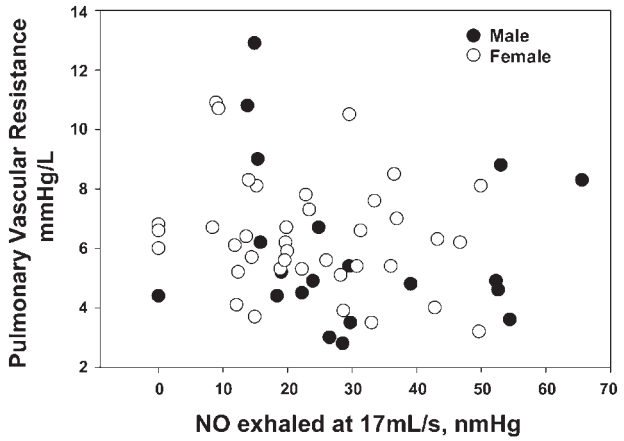

Fig. 2. Pulmonary vascular resistance decreases with higher exhaled NO levels in the Tibetan sample at 4,200 m.

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increases in cardiac output cause shear stress, which induces NO synthesis. This seems unlikely in light of the cited evidence showing that changes in NO precede changes in cardiac output or cardiac index. Other things being equal, higher cardiac output might even lower NO if it causes recruitment and perfusion of blood vessels in which NO output could be scavenged by circulating hemoglobin, instead of being exhaled. NO delivery from the circulation to the lung is discounted, because hemoglobin inactivates NO (21) and because direct measurement of bronchiolar gases showed that alveolar NO was virtually nil (12, 24).

NO concentration in exhaled breath is dependent on exhalation flow rate (19, 25). A two-compartment model explains the exhalation flow rate dependence of NO concentration. As air passes through the airways, the gas is infused, with NO diffusing from the airway wall. The lower the exhalation flow rate, the longer the time that the gases passing over the airway wall will be infused with NO from the airway wall; hence, the exhaled NO will be higher. Similar to previous studies (16, 25), the low flow of 17 ml/s is the most sensitive here, inasmuch as it reveals that Tibetans have slightly less exhaled NO than controls at sea level. [Analyses performed with the 50 ml/s flow rate revealed similar, significant correlations (data not shown).] Possible explanations for the low NO concentration among the high-altitude Tibetans include downregulation of synthesis, higher consumption, or faster rate of transfer from the airway wall. The transfer rate of 0.07 $nl \cdot s^{-1} \cdot mmHg^{-1}$ is 7–10 times higher than the range of 0.004–0.013 $nl \cdot s^{-1} \cdot mmHg^{-1}$ reported for sea-level samples, including our sea-level control (16). According to Fick's law, transfer (diffusion) is directly proportional to the P_{NO} difference between the airway wall and lumen and to the area over which diffusion takes place. Because the P_{NO} difference between the cell wall and the airway lumen is relatively small at altitude, the implication is that the higher transfer rate is achieved because transfer is taking place over a larger area. For example, NO synthesis may be extended to more airways in this population (S. Permutt, personal communication). The result is a maximum potential NO flux in this Tibetan sample that is nearly double that at sea level, which is consistent with our previous report that Tibetans accumulated more than twice as much NO as sea-level controls during a 15-s breath hold: 7.6 vs. 3.9 nmHg (5).

The initial impetus for the study was the inverse relation between pulmonary NO and pulmonary arterial pressure found in animal and human experiments (6, 7, 9, 14, 15, 22, 23, 27, 28). That relation was not found in this study, despite adequate statistical power. Different study designs may be responsible. This high-altitude field study of Tibetans examined the normal range of variation in NO, whereas the sea-level studies used experimental interventions with large effects, such as inhibition of NO synthesis or addition of large doses of inhaled NO, to markedly change pulmonary NO and, as a result, change pulmonary artery systolic pressure.

The results of this study show two independent influences on the lung of Tibetans at high altitude. Lower O_2 saturation of hemoglobin is associated with higher pulmonary artery systolic pressure (consistent with hypoxic pulmonary vasoconstriction). Higher NO allows greater pulmonary blood flow measured by cardiac index but does not increase pulmonary artery systolic pressure, presumably because NO causes dilation of pulmonary blood vessels and reduces pulmonary resistance. This independent effect of NO reduces the chance of a potential cost of higher pulmonary blood flow, i.e., higher pulmonary artery systolic pressure, which could cause pathologies including right ventricular hypertrophy and muscularization of the pulmonary arteries (30).

Several limitations merit comment. *1*) The number of Tibetan men is relatively small. The requirement for nonsmoking in the population unfortunately excluded most Tibetan adult men. A related problem is the lack of correlation between cardiac index and exhaled NO in men. The significant interaction between age and gender in the models incorporating O_2 saturation requires further study, inasmuch as there are too few men to reach a definite conclusion; indeed, the sample size lacks sufficient power to test any specific gender effect. *2*) The effect of NO is modest. However, there is clearly an additive and independent effect that is important in such a complex system. *3*) Mathematical modeling for NO exchange is not without limitations; e.g., it is possible that the model may not be valid at high altitude, even though we account for the lower barometric pressure in the comparisons of high-altitude with sea-level data. *4*) The study design and the limitations of field work do not permit distinction between genetic and/or acquired altitude-mediated mechanisms. *5*) The reference group is not a true control group. However, the reference group was included to emphasize and illustrate further the distinctiveness of the population that resides in a stressful environment.

Nevertheless, our findings support the possibility that, in the normal range of variation of exhaled NO among Tibetans residing at 4,200 m, high NO may partly compensate for ambient hypoxia and low arterial O_2 content by allowing higher pulmonary blood flow and, thus, greater O_2 delivery to working tissues.

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