Hypoxia Impairs Systemic Endothelial Function in Individuals Prone to High-Altitude Pulmonary Edema

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Rationale: High-altitude pulmonary edema (HAPE) is characterized by excessive pulmonary vasoconstriction and is associated with decreased concentrations of nitric oxide (NO) in the lung. Objectives: We hypothesized that individuals susceptible to HAPE (HAPE-S) would also have dysfunction of the vascular NO vasodilator pathway during hypoxia in the systemic vasculature. Methods: During normoxia (FiO2 = 0.21) and 4 hours of normobaric hypoxia (FiO2 = 0.12, corresponding to an altitude of 4,500 m above sea level) endothelium-dependent and endothelium-independent vasodilator responses to intraarterial infusion of acetylcholine (ACh) and sodium nitroprusside, respectively, were measured by forearm venous occlusion plethysmography in nine HAPE-S subjects and in nine HAPE-resistant control subjects. Main Results: Pulmonary artery systolic pressure increased from 22 ± 3 to 33 ± 6 mm Hg (p < 0.001) during hypoxia in control subjects, and from 25 ± 4 to 50 ± 9 mm Hg in HAPE-S subjects (p < 0.001). Despite similar responses during normoxia in both groups, ACh-induced changes in forearm blood flow markedly decreased during hypoxia in HAPE-S subjects (p = 0.01) but not in control subjects. The attenuated vascular response to ACh infusion during hypoxia inversely correlated with increased pulmonary artery systolic pressure (p = 0.04) and decreased plasma nitrite correlated with attenuated ACh-induced vasodilation in HAPE-S subjects (p = 0.02). Conclusions: Hypoxia markedly impairs vascular endothelial function in the systemic circulation in HAPE-S subjects due to a decreased bioavailability of NO. Impairment of the NO pathway could contribute to the enhanced hypoxic pulmonary vasoconstriction that is central to the pathogenesis of HAPE.

Keywords: edema; endothelin; endothelium; hypoxia; nitric oxide

High-altitude pulmonary edema (HAPE) is a life-threatening form of noncardiogenic pulmonary edema that is characterized by pulmonary hypertension and elevated capillary pressure in the pulmonary circulation (1, 2). The increase in intravascular hydrostatic pressure augments fluid filtration across the alveolar capillary barrier into the alveolar space and is believed to be a key factor in the pathogenesis of HAPE (3). Individuals susceptible to HAPE (HAPE-S) have both an augmented pulmonary vasoconstrictor response to hypoxia (4) and an enhanced increase of pulmonary artery systolic pressure (PASP) during exercise in normoxia (5). The underlying mechanisms predisposing to exaggerated pulmonary vasoconstriction in HAPE-S individuals are not known, but several studies suggest that susceptibility to HAPE may be associated with reduced pulmonary synthesis of the endothelium-derived vasodilator nitric oxide (NO) and an impairment of NO-mediated pulmonary vasodilation. Indeed, constitutively released NO mediates relaxation of the pulmonary vascular bed in healthy individuals both during normoxia and acute hypoxia (6). Moreover, exhaled NO is decreased in HAPE-S individuals during exposure to normobaric hypoxia (7) and also in patients with established HAPE (8). In both studies, a negative correlation between exhaled NO and the extent of hypoxic pulmonary vascular responses was shown. In another study, decreased concentrations of the NO metabolites nitrate and nitrate were observed in bronchoalveolar lavage fluid in HAPE-S subjects (9), supporting the hypothesis of an impairment of NO formation or metabolism. Although these findings all point to a possible impairment of NO production by the lung vascular endothelium during hypoxia, they offer no direct proof-of-concept because measurements of exhaled NO and alveolar fluid NO metabolites only imperfectly reflect pulmonary vascular NO production (10, 11).

We hypothesized that susceptibility to HAPE is related to a generalized hypoxia-induced vascular endothelial dysfunction with subsequent reduction of NO bioavailability. Therefore, we directly assessed by blood flow measurements the effect of hypoxia on the function of the systemic vascular endothelium of HAPE-S and HAPE-resistant control subjects in a single-blind, randomized trial.

Some of the results of these studies have been previously reported in the form of an abstract (12).

METHODS

Study Population

We enrolled 18 healthy mountaineers, whose susceptibility or resistance to HAPE was known from their participation in previous studies at high altitude (3, 9, 13). All participants were nonsmokers living at low altitude. Volunteers with hyperhomocysteinemia, diabetes, or cardiac or pulmonary diseases, including airway infections, were excluded from the study. In terms of other known conditions associated with endothelial dysfunction (hyperlipidemia, arterial hypertension), the groups were matched (Table 1). In each group, there was one subject with mild hypertension, treated with 25 mg metoprolol daily.

The study was conducted in accordance with the Declaration of Helsinki and its current amendments, and was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg. Before the study, all participants provided written, informed consent.

General Procedures

The study was performed as a single-blind, randomized trial, in which the participants were blinded with respect to the FiO2, and the investigators were blinded with respect to the participants’ history of HAPE. The participants were studied in a normobaric hypoxia room that provided a constant FiO2 level of 0.21 (normoxia) or 0.12 (hypoxia) by admixture of N2-enriched air. Each participant was exposed to 4 hours of normoxia and 4 hours of normobaric hypoxia in randomized order while comfortably

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resting in a supine position. After an equilibration period of 90 minutes, blood samples were taken for determination of plasma nitrite and plasma endothelin 1 (ET-1). Thereafter, endothelium-dependent and endothelium-independent vasodilator responses and PASP were assessed. Afterwards, the FIO2 was switched accordingly to 0.21 or 0.12. After another equilibration period of 90 minutes, all measurements were repeated in the same order as in the morning session.

In the preceding 12 hours and during the study, the participants were not allowed to perform any physical exercise or consume methylxanthine-containing food and beverages. Fruits and salads were also not provided to minimize dietary nitrite intake.

Assessment of Endothelium-dependent and Endothelium-independent Vasodilator Responses
To measure drug-induced changes of forearm blood flow (FBF), venous occlusion plethysmography with strain gauges placed around the forearms was performed as described previously (14, 15). FBF was measured by the rate of increase in forearm volume during periods of venous occlusion achieved by rapid inflation of an upper arm cuff to 40 mm Hg. During FBF measurements, the hand was excluded from the circulation by inflating a wrist cuff to suprasystolic pressures. Changes in vascular reactivity were assessed observing flow responses to different doses of vasoactive agents. Under local anesthesia, a catheter was inserted into the brachial artery. After resting FBF levels had been established, the following compounds were administered intraarterially: (1) sodium nitroprusside (SNP), to assess endothelium-independent vasodilation, and (2) acetylcholine (ACh), to stimulate endothelium-dependent vasodilation. Both substances were infused in randomized order in six increasing dose rates (SNP: 0.006 – 0.03 – 0.075 – 0.15 – 0.3 – 0.6 μg/min/100 ml forearm volume; ACh: 0.01 – 0.05 – 0.2 – 1 – 4 – 16 μg/min/100 ml forearm volume) for a period of 5 minutes each, with SNP and ACh infusions separated by a wash-out phase of at least 30 minutes. Area under the dose–response curves was calculated from each individual’s dose–response curve plotted in linear scaling. Arterial blood pressure, ECG, and oxygen saturation were continuously monitored.

Echocardiography
PASP was estimated from peak tricuspid regurgitation jet velocities according to the following equation: \[ \text{PASP} = 4V^2 + 5 \text{ mm Hg}, \] with \( V \) being the peak velocity (in m/second) of tricuspid regurgitation jet, and 5 mm Hg being the estimated right atrial pressure (16).

Measurement of Plasma Nitrite, Nitrate, and ET-1
Plasma nitrite concentrations were determined by a flow injection analysis technique based on the Griess reaction (17). Nitrate was determined after reduction to nitrite by preincubation with vanadium chloride (18) and subsequent detection by the flow injection analysis system. Plasma ET-1 was measured by radioimmunoassay as described previously (19).

Statistics
Local hemodynamic responses to infusion of study drugs (normoxia vs. hypoxia) were compared by using two-way repeated measures analysis of variance. Two-tailed paired and unpaired \( t \) tests were used for comparisons within and between the groups, respectively. Pearson correlation was performed to analyze the influence of selected factors on vasodilator responses, and \( p \) values less than 0.05 were considered statistically significant. Unless otherwise indicated, data are expressed as mean values \( \pm \) SD.

RESULTS
Baseline clinical characteristics of the study population were similar between HAPE-S and control subjects (Table 1), and in each group, one participant with hypertension was treated with metoprolol.

Effects of Hypoxia on Gas Exchange, Systemic Hemodynamics, and PASP
There were no differences between HAPE-S and control subjects in any of the baseline parameters during normoxia, and the changes in oxygen saturation, heart rate, and mean arterial blood pressure induced by hypoxia were similar in both groups (Table 2). In all participants, the decrease of oxygen saturation was accompanied by an increase in PASP, which was substantially higher in HAPE-S than in control subjects.

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<tr>
<th>TABLE 2. PARAMETERS OF HEMODYNAMICS, GAS EXCHANGE, AND BLOOD ANALYSIS</th>
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Definition of abbreviations: AUC = area under the dose–response curve; ET-1 = endothelin 1; FAV = forearm volume; FBF = forearm blood flow; HAPE-S = susceptible to high-altitude pulmonary edema; HR = heart rate, MAP = mean arterial blood pressure; PASP = pulmonary artery systolic pressure. 
* \( p < 0.05 \) versus normoxia in the same group. 
† \( p < 0.05 \) versus HAPE-S in hypoxia.
Baseline FBF and Vasodilator Response to ACh and SNP

Baseline FBF was similar during normoxia in both groups. Hypoxia induced an increase in FBF in control subjects but not in HAPE-S subjects (Table 2).

Neither ACh nor SNP exerted systemic vasorelaxant effects, as judged by the absence of changes in blood pressure, heart rate, and FBF in the control arm. The dose–response relationship of intraarterially administered ACh for HAPE-S and control participants is shown in Figure 1A. In HAPE-S subjects, hypoxia impaired ACh-dependent increase in FBF, whereas in control participants, endothelium-dependent relaxation was similar in normoxia and hypoxia. During hypoxia, there was a negative correlation between PASP and the area under the dose–response curve of ACh-induced FBF (Figure 2). However, SNP-induced relaxation slightly increased in control subjects during hypoxia, whereas there was no change in HAPE-S subjects (p = 0.21; Figure 1B).

Plasma Concentrations of Nitrite, Nitrate, and ET-1

Hypoxia induced a marked decrease of plasma nitrite in HAPE-S (p = 0.004) but not in control subjects, whereas plasma nitrate remained unaffected in both groups (Table 2). In HAPE-S subjects, during hypoxic exposure, a positive correlation existed between plasma nitrite and the area under the dose–response curve of ACh-induced FBF (Figure 3). Plasma ET-1 increased during hypoxia in both groups without a difference between HAPE-S and control subjects (Table 2).

**DISCUSSION**

To the best of our knowledge, this is the first study to directly measure the function of the vascular endothelium in HAPE-S individuals. The primary finding of the study is that, in individuals with previously well documented HAPE, but not in control participants, hypoxia reduced the endothelium-dependent vasodilator responsiveness of forearm resistance vessels to intraarterial infusion of ACh, a gold-standard test to assess endothelial function. The shift of the dose–response curve to ACh induced by hypoxia was comparable to that induced by cardiovascular risk factors such as hypertension (20) or smoking (21). Endothelium-independent vasodilation to SNP was unchanged, indicating that the decreased response to ACh was caused by a reduction in endothelial function rather than an attenuation of vascular smooth muscle function. Because ACh responses in the human forearm are almost exclusively mediated by NO release through activation of endothelial NO synthase (NOSIII) (22), and only to a small extent through prostaglandins (23), our findings strongly suggest that hypoxia induces endothelial dysfunction in HAPE-S individuals, resulting in decreased vasodilator responsiveness in the systemic circulation.

The potential impairment of the NO pathway in HAPE-S individuals is further supported by the finding that plasma concentrations of nitrite, an oxidative metabolite of NO, decreased in HAPE-S subjects during hypoxic exposure, and that this decrease correlated with the ACh responsiveness of the arterial bed. Because changes in plasma nitrite concentrations in the human forearm circulation accurately reflect acute changes in the activity of NOSIII (22), a constitutive enzyme that catalyzes...
endothelial NO synthesis from l-arginine (24), the reduction of plasma nitrite is a marker of decreased NO bioavailability (25).

In contrast to nitrite, plasma nitrate concentrations did not change during hypoxic exposure in HAPE-S or in control subjects, which is not surprising because only a small fraction is derived from endogenous NO and nitrate is influenced by a variety of NOSIII-independent factors (25). Moreover, the nitrate half-life of 5 to 8 hours in humans is considerably longer than that of nitrite and therefore acute changes in NOSIII activity will not readily be mirrored in its plasma concentration, further limiting its usefulness in short-term intervention studies (25).

The selective reduction of endothelial NO bioavailability in the systemic vasculature only observed in HAPE-S individuals during hypoxia fits well with recent studies demonstrating reduced NO bioavailability in the pathophysiology of HAPE: concentrations of nitrite and nitrate in bronchoalveolar lavage fluid are decreased in HAPE-S individuals after 2 days at 4,559 m, but not in control subjects (9). Congruently, the enhanced hypoxic pulmonary vascular response in HAPE-S individuals is associated with less NO in exhaled air (7, 8), and conversely, the inhalation of NO profoundly lowers PASP in HAPE-S individuals while having only mild effects in control subjects (13). Furthermore, phosphodiesterase-5 inhibitors, which slow down the degradation of cyclic guanosine monophosphate (cGMP), reduce hypoxic pulmonary vasoconstriction (26), improve performance (27, 28), and prevent HAPE (29). Although the systemic and the pulmonary circulation are regulated differently in hypoxia, it is conceivable that similar mechanisms account for the systemic endothelial dysfunction in hypoxia and contribute to the decreased bioavailability of NO in the lung of HAPE-S subjects, because we found an inverse correlation between PASP and the respective vasodilator response to ACh in the systemic circulation.

The reasons for the reduced NO bioavailability in HAPE-S subjects are unclear and may include reduced NOS substrate and cofactor availability, increased concentrations of endogenous NOS-inhibiting l-arginine analogs, differences in endothelial cell membrane proteins that alter NOS activity, downregulation of NOS, or increased NO breakdown. However, this study was not designed to answer the question of which of these parameters are primarily affected by hypoxia and responsible for the impairment of NO homeostasis in HAPE-S subjects. But the preserved vasodilator responsiveness to SNP suggests that vascular smooth muscle responses in HAPE-S individuals are well retained and that NO action itself is not critically impaired.

Hypoxic exposure was associated with increases in plasma ET-1, which is in line with previous studies (30). However, our finding of an impaired NO pathway may explain why a similar hypoxia-induced increase of plasma ET-1 is associated with a greater increase in PASP in HAPE-S subjects. ET-1 binds to two types of receptors: ET₄ and ET₅. Stimulation of ET₄ and ET₅ receptors on smooth muscle causes vasoconstriction, whereas stimulation of endothelial ET₅ receptors causes vasodilatation through release of NO and prostaglandins (31). ET₄ receptor-mediated vasoconstriction contributes to basal systemic and pulmonary vascular tone; however, acute hypoxic pulmonary vasoconstriction is not primarily mediated by stimulation of ET₅ receptors, as supported by the fact that the ET₅ receptor antagonist BQ-123 did not reduce pulmonary vascular tone in healthy individuals during acute hypoxia (32). In contrast, ET₅ receptor-mediated vasodilation may play a major physiologic role in modulating vascular tone in reaction to acute hypoxia. In an animal model, hypoxia suppresses ET₅ receptor–mediated stimulation of NO synthesis in hypoxia-induced hypertensive rat lungs and thus contributes to the development of pulmonary hypertension at least partly (33). Moreover, transgenic rats with ET₅ receptor deficiency develop exaggerated pulmonary vasoconstriction when exposed to acute hypoxia (34). Thus, ET₅ receptor–induced NO-mediated vasodilation may be impaired in HAPE-S individuals in hypoxia as part of a generalized state of vascular endothelial dysfunction and thus contributes to the exaggerated pulmonary vasoconstriction.

In conclusion, we have shown that susceptibility to HAPE is associated with hypoxia-induced endothelial dysfunction, resulting in impaired endothelium-dependent vasodilation in the systemic circulation. Because endothelium-derived NO plays a central role in pulmonary vascular regulation, reduced NO bioavailability could contribute to the enhanced hypoxic pulmonary vasoconstriction that is a causal element in the pathophysiology of HAPE. If indeed a relative lack of bioavailable NO is the key factor in the pathogenesis of HAPE, then measures increasing cGMP-induced vasodilation would be expected to favorably modulate the occurrence of HAPE. Indeed, it has recently been shown, that the phosphodiesterase-5 inhibitor tadalafil, which increases cGMP levels in lung tissue by inhibition of its degradation, lowers PASP, and prevents HAPE (29).

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References


