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Exhaled nitric oxide changes during acclimatization to high altitude: A descriptive study.

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Short Title: PeNO changes during acclimatization

ABSTRACT

Aims: This study describes differences in the partial pressures of exhaled nitric oxide (PeNO) between subjects fully acclimatized to 5,300 m and those who have just arrived to high altitude.

Methods: PeNO was determined in eight subjects newly exposed and non-acclimatized (Non-ACC) to high altitude and compared to nine subjects who had acclimatized (ACC) to high altitude for one month. Additionally, systolic pulmonary artery pressure (sPAP) and arterial oxygen saturation (SaO_2) were measured in all participants. These measurements were repeated in the Non-ACC group 5 and 9 days later.

Results: PeNO levels on day 1 were significantly higher in the Non-ACC vs. ACC cohort (8.7 ± 3.5 vs. 3.9 ± 2.2 nmHg, p = 0.004). As the Non-ACC group remained at altitude, PeNO levels fell and were not different when compared to the ACC group by day 9 (5.9 ± 2.4 vs. 3.9 ± 2.2 nmHg, p = 0.095). Higher sPAP was correlated with lower PeNO levels in all participants (R = -0.50, p = 0.043). PeNO levels were not correlated with SaO_2.

Conclusion: As individuals acclimatized to high-altitude PeNO levels decreased. Even after acclimatization, PeNO levels continued to play a role in pulmonary vascular tone.

Keywords: exhaled nitric oxide, high altitude populations, acclimatization, systolic pulmonary artery pressure

INTRODUCTION

In the respiratory system, endogenously produced nitric oxide (NO) plays a major role in the regulation of bronchiolar and pulmonary vascular tone, with NO dysregulation seen in many disease states (Barnes and Belvisi, 1993; Gaston et al., 1994; Ricciardolo, 2003). During states of inflammation such as asthma, NO levels, as measured by exhaled nitric oxide (eNO), have been shown to increase (Kharitonov et al., 1994; Persson et al., 1994). Conversely, low levels of eNO have been observed in patients with pulmonary hypertension, and in subjects who are prone to high altitude pulmonary edema (HAPE) during acute (within 22 hours) exposure to hypobaric hypoxia (Beall et al., 2012; Duplain et al., 2000). Additional evidence for the importance of endogenously produced NO within the airways is seen by the use of exogenous NO as a therapeutic agent. In physiologic states with low NO conditions or where pulmonary vasodilation is required, such as acute cor pulmonale from a massive pulmonary embolism (Summerfield et al., 2012) or high altitude pulmonary edema (HAPE) (Scherrer et al., 1996), the administration of exogenous NO can be therapeutic. Regardless, both exogenously administered as well as endogenously produced NO are rapidly inactivated within the pulmonary vasculature through nitrosylation during binding to hemoglobin. In this way, the effects of NO are thought to be relatively pulmonary selective (Rimar and Gillis, 1993).

Pulmonary vasculature production of NO is catalyzed by nitric oxide synthases, both endothelial eNOS and inducible iNOS. These enzymes oxidize L-arginine to NO and L-citrulline. Mechanistically, NO induces soluble cyclic guanosine monophosphate (cGMP) which in turn decreases intracellular calcium concentrations. Additionally, release of intracellular calcium from the sarcoplasmic reticulum is inhibited and the sensitivity of myosin to calcium is reduced.

In smooth muscle these overall changes lead to smooth muscle relaxation and pulmonary vasodilation (Murad, 1986; Palmer et al., 1988).

Prior groups have found that HAPE-susceptible subjects who sojourn to high-altitude show lower eNO levels and higher systolic pulmonary artery pressures (sPAP) as compared to controls (Duplain et al., 2000). Furthermore, higher sPAP and lower eNO was associated with the formation of pulmonary edema in a subset of HAPE-susceptible subjects, potentially suggesting increased hypoxic pulmonary vasoconstriction in these individuals (Duplain et al., 2000). The reduced level of eNO seen in HAPE-prone individuals is the basis for treatment of HAPE with inhaled NO (iNO), which has been shown to improve decreased oxygen saturation associated with HAPE (Scherrer et al., 1996). Interestingly, the administration of exogenous iNO decreased pulmonary artery pressures and increased arterial oxygen saturations only in HAPE-prone individuals exposed to hypobaric hypoxia. In non-HAPE prone subjects it actually worsened oxygenation (Scherrer et al., 1996).

Although changes in sPAP and eNO have been well described with ascent to high altitude, they have not been monitored as subjects acclimatize. Thus, it is not known what, if any, further changes occur during adaptation to high altitude. Our aim was to investigate associations between eNO, sPAP, Hemoglobin, and SaO_2 during acclimatization to hypobaric hypoxia.

METHODS

Subjects

Seventeen healthy non-smoking adult lowlanders (2 female) with no history of cardiorespiratory or metabolic disease participated in the study. Each participant gave written informed consent after being provided a detailed description of the study requirements. The experimental

procedures were approved by the Mayo Clinic Institutional Review Board and were performed in accordance with the ethical standards of the Declaration of Helsinki. All study participants were prohibited from prophylactic administration of any medication to aid altitude acclimatization (e.g., sildenafil, acetazolamide). Moreover, no subject required emergent pharmaceutical treatment (e.g., dexamethasone) for high altitude illness.

Expedition details and experimental procedures

All experimental procedures were performed at Mount Everest base camp (5,300 m). To reach base camp, each participant travelled to Kathmandu, Nepal (1,400m) before being transported by airplane to Lukla, Nepal (2,860 m). From Lukla, the participants completed an 8- to 10-day hike at progressively increasing altitudes to reach base camp. The 17 participants were separated into two groups: Group 1 (n = 9, 2 female; acclimatized, 'ACC') arrived and stayed at base camp for ~1 month before the arrival of Group 2 (n = 8, 0 female; non-acclimatized, 'Non-ACC'). Within 24 h of arrival of the Non-ACC group to base camp, exhaled nitric oxide (eNO), systolic pulmonary artery pressure (sPAP), arterial oxygen saturation (SaO_2) (Nonin Medical Inc, Plymouth, Minnesota), and hemoglobin (Hgb) (Abbott iSTAT, Princeton, NJ) were measured. In addition, eNO and sPAP were measured on day 5 and day 9 at base camp in Non-ACC.

Exhaled nitric oxide

Exhaled nitric oxide (eNO) was measured in triplicate in parts per billion using a hand-held electrochemical detector (NIOX MINO Aerocrine, New Providence, NJ) according to standard procedures (Silkoff, 2005). Briefly, subjects inhaled NO free air to total lung capacity (TLC) before exhaling for ~10 s against a counter pressure of 5-20 cmH_2O. By allowing closure of the soft palate, this technique isolates the small airways and prevents the measurement of NO from the nasal cavity (Pietropaoli et al., 1999; Silkoff et al., 1997, 1998). At the time of the study, all subjects were non-

smokers. In addition, each participant abstained from eating nitrogen enriched foods for at least 3 h before each measure of eNO.

When reporting eNO at altitude, it is necessary to convert eNO (ppb) to partial pressure of eNO (PeNO, nmHg) at normal temperature and pressure conditions so that eNO values can be accurately compared across studies and altitudes. Therefore, the correction factor determined by Hemmingsson et al (Hemmingsson et al., 2009) for the NIOX MINO at an altitude of 5,000 m was applied, followed by conversion from ppb to nmHg, as follows:

$$PeNO\ (nmHg) = \left(\frac{eNO\ (ppb)}{1.856} * 760mmHg\right) * 1\ x\ 10^{-3}$$

For ease of comparability with other reported data, it should be noted that 1 nm Hg= 1.333 mbar.

Systolic pulmonary artery pressure

Systolic pulmonary artery pressure (sPAP) was estimated from the peak velocity of tricuspid regurgitation (TRV_{MAX}) using a modified Bernoulli equation as described previously (Yock and Popp, 1984). With participants in the left lateral supine position, the TR jet was located using 2D-color Doppler echocardiography in the four chamber view. To determine TRV_{MAX}, the continuous wave sampler was positioned within and parallel to the regurgitation jet and sPAP was computed as 4 × TRV_{MAX} + right atrial pressure (RAP). RAP was estimated from the ratio between minimum inferior vena cava (IVC) diameter during a maximal sniff maneuver and maximum IVC diameter during normal respiration (Lawrence G Rudski MD et al., 2010). TRV_{MAX} and IVC dimensions were measured in triplicate in each participant at each time point.

Statistical analyses

Independent samples t-test was used to compare absolute measures of PeNO, sPAP, and SaO_2 between ACC vs. Non-ACC on day 1 at base camp. One-way repeated measures ANOVA was used to compare absolute measures of PeNO and sPAP across time (base camp day 1 vs. day 5 vs. day 9) in the Non-ACC group. Following significant main effects, planned pairwise comparisons were made using the Bonferroni method. In addition, Pearson's product-moment correlation coefficient (r) was computed to assess the relationships between PeNO and either sPAP, SaO_2, or Hgb. The acceptable type I error was set at $P < 0.05$. Data are expressed as group means ± SD. Statistical analyses were performed using SPSS version 22.0 for Windows (IBM, Armonk, NY).

RESULTS

Effect of high-altitude on PeNO, sPAP, and SaO_2

Upon arrival at base camp, group mean PeNO was greater in the Non-ACC vs. the ACC group (Figure 1; Table 1). Conversely, neither sPAP nor SaO_2 was different between the Non-ACC and ACC group (Table 1). In the Non-ACC group, PeNO was not different on day 5 but significantly lower on day 9 vs. day 1 at base camp (Table 1). Indeed, after 9 days at base camp, PeNO was not different in the Non-ACC group compared to the ACC group 9 days prior (5.9 ± 2.4 vs. 3.9 ± 2.2 ppb, $p = 0.095$).

Associations between PeNO, sPAP, SaO_2, and Hgb

Previous studies have also found a correlation between sPAP and PeNO levels. Figure 2 shows this relationship for all subjects combined. Indeed, higher PeNO levels were significantly correlated with lower sPAP (Figure 2; $R = -0.50$, $p = 0.043$).

PeNO levels were also compared to resting daytime SaO_2 for both groups (Figure 3). There were no correlations between PeNO levels and daytime SaO_2, in either the Non-ACC nor ACC group, nor combined. However, individual performances were also recorded. Interestingly, it was

noted that the team member in the ACC group with the highest PeNO level and highest corresponding SaO_2 was able to subsequently summit to a height of 8,848 m without oxygen. Conversely, the member in the ACC group who had the lowest PeNO and the corresponding lowest SaO_2 was unable to successfully complete the climb.

Finally, Hgb levels were measured in all Non-ACC subjects and a subset of ACC subjects on Day 1 only. While not significant, there was a trend toward a lower PeNO being associated with a higher Hgb level (Figure 4; $R = -0.56$, $p = 0.056$).

DISCUSSION

Our results show that PeNO levels in subjects who have acclimatized to 5,300 m are significantly lower than those who have just arrived to that altitude. Additionally, the newly arrived group showed a significant decrease in PeNO levels after 9 days at high-altitude, approaching levels seen in the ACC group. Our data is also consistent with prior work done by Duplain and colleagues that showed an inverse relationship between sPAP and PeNO when subjects are exposed to high altitude (Duplain et al., 2000). Furthermore, there was no correlation between PeNO and daytime oxygen saturations in either group nor combined. Overall this data demonstrates a fall in PeNO levels during the acclimatization process, but would suggest that PeNO is still important for continued regulation of pulmonary vascular tone.

There was also a trend toward a lower PeNO level being associated with a higher Hgb level. This trend could be explained by the fact that NO is rapidly inactivated within the pulmonary vasculature through nitrosylation during binding to hemoglobin. Thus increased hemoglobin may hasten the inactivation of NO.

Previous studies have evaluated the role of pulmonary NO during acclimatization; however, these studies looked at changes across different altitudes as well as different subjects' ethnicity. Wu et al. examined both Tibetan and non-Tibetan infants born at 3,658 m and compared them to non-Tibetan infants born at 16 m. They found infants born at high altitude had increased levels of PeNO during their first week of life when compared to those born near sea level. Genetic differences were detected as well. Non-Tibetan infants born at altitude were more likely to require oxygen supplementation to improve their saturations, suggesting a genetic adaptation in those groups who permanently reside at altitude (Wu et al., 2016). In contrast to Tibetan adaptation, differences in adaptation of Andean adults may not be dependent on NO concentrations thus suggesting genetic differences in adaptation. This was demonstrated by Schwab et al. Their research, performed in Bolivia, compared PeNO and sPAP of native Bolivians to sex-matched Caucasians exposed to 3600 m. They found no differences between the two groups when PeNO and sPAP were compared (Schwab et al., 2008). Gender differences in eNO levels may also exist, as Li et al. found average fractional eNO (ppb) levels to be higher in male vs. female Tibetans (Li, 2014). Additionally, Li also found eNO levels were inversely related to altitude, with higher altitude subjects demonstrating lower eNO levels (Li, 2014).

Other research, including ours, also demonstrated that PeNO levels decrease during exposure to altitude (Brown et al., 2006; Donnelly et al., 2011; Duplain et al., 2000). However, one must be careful in the reporting of eNO levels taken at altitude. In 2009, Hemmingsson and colleagues argued that altitude studies implementing the use of the NIOX MINO device may have errors and results should be reported as partial pressures rather than the fractional volume (parts per billion and typically termed exhaled or eNO levels) that the device detects. Their reasons for this recommendation are as follows: mass flow decreases with altitude, eNO fractional

concentrations increase with decreasing mass flow, reduced ambient pressure leads to a proportionally lower pressure of NO, and finally that the electrochemical detector is more sensitive to NO at altitude. Thus, the displayed values would not reflect the true decrease in eNO at altitude (Hemmingsson et al., 2009).

Importantly, Hemmingsson's paper details a conversion which can be used for eNO in reporting partial pressures at altitude (Hemmingsson et al., 2009). This conversion was applied to our subjects. Of note, the conversion factor depends on the study of a specific NIOX MINO device, and thus it is possible that the conversion factor may be different on the device used presently. This incorporates another limitation in that we were unable to calibrate the NIOX MINO at altitude. Thus, although the values were converted from ppb to nmHg, the values were not able to be verified while at altitude. Importantly, however, the measurements between the two cohorts were taken at the same altitude and at the same time. Thus, the differences seen between the two cohorts are comparable, even if the absolute values are not. All in all, consistency in reporting high altitude eNO measurements is crucial to the ability to compare across altitudes and studies.

The mechanism for changes in PeNO at altitude and the subsequent development of high altitude pulmonary edema in some individuals continues to be investigated. Duplain's work suggested that decreased eNO in HAPE-prone subjects may be due to a defect in pulmonary epithelial NO synthesis which may lead to increased hypoxic pulmonary vasoconstriction and subsequent pulmonary edema (Duplain et al., 2000). Pulmonary blood flow appears to have an effect on both eNO as well as plasma concentrations themselves. This was demonstrated by Tworetzky who measured eNO and plasma NO levels before and after atrial septal defects were repaired. After closure of the defect, both levels eNO and plasma levels fell within 30 minutes. This

occurred without corresponding changes in either average pulmonary or systemic artery pressures (Tworetzky et al., 2000).

During our study, PeNO levels in the ACC group were lower; this may be due in part to the length of stay at altitude leading to an increased elimination of NO, which was also proposed by Hemmingsson (Hemmingsson et al., 2009). It has also been speculated that lower bioavailability of its precursors would also lead to lower eNO levels. This was hypothesized by Schneider and colleagues. Since NO is produced during the conversion of arginine to L-citrulline they showed that after exposure to an altitude of 4,350 m L-citruline levels were decreased. L-citruline levels as well as SaO_2 levels increased with an infusion of arginine into subjects. In addition those subjects with symptoms of acute mountain sickness (AMS) demonstrated a slight decrease in AMS scores once L-arginine had been administered (Schneider et al., 2001). Further evidence to support this was described by Lundberg and colleagues who found that the inorganic anions nitrate (NO3-) and nitrites (NO2-), which were previously believed to be inert end products, can be recycled in vivo during hypoxic states and form an alternate pathway for NO production (Lundberg et al., 2008).

In this unblinded study, physical performance of each subject could also be observed. The most striking observation made was within the ACC group, between the subject with the highest PeNO level and the one with the lowest. The former also maintained the highest resting oxygen saturation with the latter achieving the lowest resting oxygen saturation. Furthermore, the latter was unable to summit to 8,848 m even on supplemental oxygen, while the former who maintained a higher PeNO level was able to summit without oxygen. This may be partially explained by work done in 2000 by Tsuchiya et al. They found that exhaled NO concentrations in lung healthy volunteers were positively correlated with PaO2 and negatively correlated with

the alveolar-arterial (A-a) oxygen gradient.(Tsuchiya et al., 2000). Larger A-a gradients would be expected to be seen in subjects with underlying diffusion problems.

Previously, MacInnis et al. has shown a correlation between altitude sickness and low baseline FENO levels (MacInnis et al., 2012). However, future research should seek to validate and use PeNO levels as a performance predictor for those traveling to high altitude, either prior to travel or during the journey itself. Conceivably an individual attempting to operate at high altitude and who might be at risk for performance degradation or the development of HAPE could be identified with falling PeNO levels before they were affected. Mitigation strategies for these individuals, such as the use of supplemental oxygen earlier or removal from the exposure through descent, could also be implemented. Additionally, in the future it may be possible to preselect individuals who are well suited for high altitude exposure. This would benefit those interested in high altitude experiences in an elective nature such as a sport climber, as well as those required to function at high altitude such as some military personnel operating in these environments.

DISCLOSURE STATEMENT

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