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Exhaled nitric oxide in ethnically diverse high-altitude native populations: A comparative study

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Abstract

Objectives: Andean and Tibetan high-altitude natives exhibit a high concentration of nitric oxide (NO) in the lungs, suggesting that NO plays an adaptive role in offsetting hypobaric hypoxia. We examined the exhaled NO concentration as well as partial pressure of several additional high-altitude native populations in order to examine the possibility that this putative adaptive trait, that is, high exhaled NO, is universal.

Methods: We recruited two geographically diverse highland native populations, Tawang Monpa (TM), a Tibetan derived population in North-Eastern India ($n = 95$, sampled at an altitude of ~3,200 m), and Peruvian Quechua from the highland Andes ($n = 412$). The latter included three distinct subgroups defined as those residing at altitude (Q-HAR, $n = 110$, sampled at 4,338 m), those born and residing at sea-level (Q-BSL, $n = 152$), and those born at altitude but migrant to sea-level (Q-M, $n = 150$). In addition, we recruited a referent sample of lowland natives of European ancestry from Syracuse, New York. Fraction of exhaled NO concentrations were measured using a NIOX NIMO following the protocol of the manufacturer.

Results: Partial pressure of exhaled nitric oxide (PENO) was significantly lower ($p < .05$) in both high-altitude resident groups (TM = 6.2 ± 0.5 nmHg and Q-HAR = 5.8 ± 0.5 nmHg), as compared to the groups measured at sea level (USA = 14.6 ± 0.7 nmHg, Q-BSL = 18.9 ± 1.6 nmHg, and Q-M = 19.2 ± 1.7 nmHg). PENO was not significantly different between TM and Q-HAR ($p < .05$).

Conclusion: In contrast to previous work, we found lower PENO in populations at altitude (compared to sea-level) and no difference in PENO between Tibetan and Andean highland native populations. These results do not support the hypothesis that high nitric oxide in human lungs is a universal adaptive mechanism of highland native populations to offset hypobaric hypoxia.

KEYWORDS
Andean Quechua, exhaled nitric oxide, hypobaric hypoxia, Tawang Monpa

1 | INTRODUCTION

Nitric oxide (NO) is found in various forms and locations in the body, and is a key regulator of blood pressure and oxygen delivery

(Erusalimsky & Moncada, 2007; Knott & Bossy-Wetzel, 2010; Moncada & Higgs, 2006). In the lung, NO is produced by nitric oxide synthase (NOS) (See & Christiani, 2013; Guo, 2000) and plays a role in regulating pulmonary blood flow by dilating pulmonary blood vessels

(Hoit et al., 2005; Scherrer et al., 1996; Vaughan et al., 2003). Three NOSs (Types I, II, and III) have been identified in the human lung. Type I and III NOS are expressed primarily in neuronal and endothelial cells of the normal human lung, respectively, and are dependent on intracellular calcium for enzyme activation and NO production. Type II NOS is the major NOS protein expressed in normal human airway epithelium, which is calcium independent, and produces nanomolar levels of NO (cf. Dweik et al., 1998). The cellular level of molecular oxygen (O_2) is an essential substrate for NO synthesis by NOS and therefore NO production is generally decreased by low oxygen tension in tissues (Le Cras & Mcmurtry, 2001). More specifically, NO levels decreases as inspired oxygen levels decrease below ambient air, since molecular O_2 concentration determine the rate of NO synthesis (Dweik et al., 1998).

It has been reported that individuals breathing varying levels of inspired O_2, NO levels were notably dependent on O_2 concentration ($K_m O_2 190 \mu M$), which is well within the physiological range (0–250 μM). NOS Type II enzyme activity in vitro was similarly dependent on molecular oxygen levels ($K_m O_2 135 \mu M$), revealing a means by which O_2 concentration affects NO levels in vivo (Dweik et al., 1998). In this regard, Rengasamy and Johns (1996) have also suggested that O_2 substrate limitation may regulate NO production in conditions like hypoxia (cf. Le Cras & Mcmurtry, 2001). As a consequence, it has been observed that acute hypoxia reduced exhaled NO by 30% in animals and almost 60% in humans (Le Cras & Mcmurtry, 2001).

Lung NO can be measured noninvasively as the fractional concentration of exhaled NO (FENO), a clinical measurement which is common in the diagnosis of airway inflammation and/or asthma (Alving, Weitzberg, & Lundberg, 1993; Franklin & Stick, 2008; Gustafsson, Leone, Presson, Wilkund, & Moncada, 1991; Olin, Bake, & Torén, 2007; Travers et al., 2007). With respect to normobaric hypoxia, FENO decreases by ~20% in humans on acute laboratory exposure to normobaric hypoxia (inspired FiO_2 = 0.05; time = 15–30 s) (Dweik et al., 1998).

High-altitude native populations, such as those in the Andes and Himalayas, are chronically exposed to hypobaric hypoxia and are the descendants of populations who may have experienced past natural selection in response to hypobaric hypoxia (Baker & Little, 1976; Beall, 2006; Bigham et al., 2010; Monge, 1978; Moore, 2001). A study by Beall et al. (2001) compared the FENO of Tibetan and Bolivian high-altitude natives measured at altitude to a low-altitude reference population from the United States. Tibetans had significantly higher FENO than the Bolivians (Tibetans = 18.6 ppb and Aymara Bolivians = 9.5 ppb), and both altitude-native groups showed higher FENO than the lowland referent group from USA (7.4 ppb). The higher FENO in Tibetans was also significantly associated with higher pulmonary (Hoit et al., 2005) and forearm blood flow (Erzurum et al., 2007). These findings support the hypothesis that high FENO is related to the population adaptive response of Tibetans at altitude and perhaps other altitude native populations as well. However, several more recent studies have complicated this narrative. For example, it also has been shown that Tibetan FENO decreases as altitude increases, against the expectations of the NO adaptive hypothesis

(Ren et al., 2014). Likewise, in the Andes, several studies confuse the overall conclusion that high pulmonary NO is an adaptive feature of highland native populations. A study by Schwab et al found that exhaled NO levels were similar between Bolivian high-altitude natives and well-acclimatized individuals of European ancestry (Schwab et al., 2008). Similarly, exhaled NO was found to be comparable between Bolivian altitude-native and children of European ancestry born and permanently residing at high altitude, and lower for both groups compared to low-altitude children resident at sea-level (Stuber et al., 2008).

Thus, a number of questions remain unanswered including: (a) Is high exhaled NO concentration, relative to lowland groups, expressed as a universal trait in all high-altitude native populations?; (b) Are there meaningful quantitative differences in exhaled NO concentrations between different highland native groups, for example, are exhaled NO values higher in Tibetans versus other native groups?; and (c) What role is played by developmental exposure to hypoxia versus high-altitude genetic ancestry? In other words, does growth and development at altitude affect the expression of exhaled NO concentration as a putative altitude-adaptive trait? Here we report on exhaled NO data collected by the same investigators using the same measurement protocol in two widely dispersed highland native populations residing at altitude (Tibetan Monpa and Peruvian Quechua) and compare these to referent data collected on lowland resident populations, including a group from Syracuse, USA, and two groups of Peruvian Quechua residing in Lima, Peru, at sea-level. The latter Peruvian groups differ by where they were born and raised (sea-level versus altitude), which allows some interrogation of the developmental question posed above.

2 | MATERIALS AND METHODS

2.1 | Study participants

Participants were healthy nonsmokers between the ages of 18–35. Every effort was made to recruit equal numbers of healthy males and females (nonpregnant, nonlactating) from each of the study groups described below. Further, we did not include any anemic or polycythemic participant in our study. All the participants gave written informed consent according to the guidelines established by the institutional review boards of three collaborating institutions, including North-Eastern Hill University (NEHU, Meghalaya, India), Syracuse University (Syracuse, NY), and the Universidad Peruana Cayetano Heredia (Lima, Peru). The study was also approved by the University of Michigan (Ann Arbor, MI). The data that support the findings of this study are available from the corresponding author upon reasonable request.

A total number of 670 individuals were recruited from three ethnically and geographically diverse populations in India, Peru, and the USA. The Peruvian participants were from a larger genetic study conducted from 2012 to 2016 and these were individuals who self-identified as Quechua high-altitude native. Quechua are one of the principle Andean high-altitude ethnic groups with ancestry in the region dating to at least 8,000–10,000 years before present

(Rademaker et al., 2014). Three separate subgroups of Quechua participants were recruited based on the goals of our previous study, and these included the following: Quechua born and residing at sea level (Q-BSL, n = 152), who were recruited and tested in Lima, Peru (100 m, barometric pressure 761 mmHg, partial pressure of oxygen [PO_2] = 159.81 mmHg) and who had spent the entirety or majority (>95%) of their lives living at sea level; Quechua-Migrants (Q-M, n = 150), who were recruited and tested in Lima, but who were born and raised above 3,000 m in highland Peru before migrating permanently to Lima at some point during their lives; Quechua-High Altitude Residents (Q-HAR, n = 110), who were recruited and tested in Cerro de Pasco, Peru at 4,338 m above sea level (barometric pressure 462 mmHg, PO_2 = 97.02 mmHg) and who had been born and raised at altitudes above 3,000 m. The Indian participants self-identified as Tawang Monpa (TM, n = 95), an indigenous Tibetan derived population from Arunachal Pradesh, India, in the eastern most part of the Himalaya. TM ancestry in the region is dated to ~1,000 years before present following a migration from Southern Tibet (Elwin, 1959). All TM participants were born and raised above 3,000 m, and mostly engaged in sedentary urban lifestyle like cab driving. They were recruited and tested in Tawang Town, India at 3,200 m above sea level (barometric pressure 535 mmHg, PO_2 = 112.35 mmHg). The USA participants (USA, n = 163) were from Syracuse, New York, (SYR) and self-identified as Non-Hispanic White, born and raised at low altitude. They were residents of Syracuse, NY, and students at Syracuse University. They were recruited from undergraduate and graduate courses at Syracuse University and tested in Syracuse, NY at 100 m (barometric pressure 758 mmHg, PO_2 = 159.18 mmHg).

2.2 | Anthropometry, hematology, and arterial saturation measurements

For each participant, we measured height and weight and calculated the body mass index (BMI, kg/m^2). Transcutaneous arterial oxygen saturation (SaO$_2$) was measured at rest using a fingertip pulse oximeter in India (CMS50EW, Neclife India) and a Nellcor N-560 bedside pulse oximeter in the USA and Peru (Nellcor, Puritan Bennett, Pleasantown, CA). Hemoglobin concentration was measured from a fingertip blood drop using a Hemocue portable hemoglobin analyzer (Hemocue®Angelholm, Sweden).

2.3 | Measurement of the exhaled nitric oxide

FENO was measured in exhaled air at an expiratory flow rate of 50 mL/s using the NIOX MINO®, portable, hand-held nitric oxide analyzer (Aerocrine AB, Solna, Sweden). This device relies on electrochemical sensor technology to detect exhaled NO values from 5 to 300 ppb (Alving et al., 2006). Values below 5 ppb are considered below the lower limit of detection (LOD) of the instrument and values above 300 ppb are above its upper detection limit. We recorded no values above the upper limit, but when values fell below the lower limit they were entered as 2.5 ppb for statistical analysis. Only 12 of 670 individuals, that is, 1.8% fell below the detection limit.

Importantly, results were similar whether these individuals were treated as missing values or given a value of 2.5 ppb. The standard exhalation time, as specified by the manufacturer, was 10s for persons at or above 130 cm in height.

It has been observed that the mass flow through the flow regulator falls with altitude and as a result apparently constant exhaled NO readings in ppb across altitudes failed to reflect the much lower partial pressures of exhaled NO at altitude (Hemmingsson, Horn, & Linnarsson, 2009). Thus as a corrective measure, fractional exhaled NO concentration (FENO) was converted to partial pressure of exhaled NO (PENO) by using following formula: PENO = FENO × (barometric pressure − 47 mmHg)/1,000 (Beall, Strohl, Laskowski, Hutte, & Erzurum, 2010; Hoit et al., 2005; Laskowski et al., 2010).

2.4 | Statistical analysis

All statistical analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago, IL) and/or STATA 11.1 (Stata Corp LP, TX). Prior to analysis, FENO measures were log-transformed to account for non-normality as assessed by a Shapiro–Wilk normality test. In graphs and tables, the nontransformed FENO values are presented. Group and sex differences for most measured variables were assessed by ANOVA applying the Walter-Duncan post hoc test to account for multiple comparisons. Values are expressed as means ± standard error, unless otherwise indicated. Statistical significance criteria were set at $p \leq .05$ for all tests.

3 | RESULTS

General participant characteristics by study group are presented in Table 1. For nearly all variables there were significant sex differences ($p < .001$), including height, weight, Hb, and BMI, as expected. In general, there were also significant group differences for most measured variables, which were expected given the geographic and ethnic diversity of the participant populations. Rather than belabor these group differences, here we simply highlight several important descriptive features of the overall data set. First, the USA group was slightly younger than the other study groups because they were sampled from a US college population. The USA group was also taller, heavier, and mostly leaner than the other study groups. Among the three Quechua groups, participants born and raised at sea-level were taller and heavier, which could be due to myriad factors, but which is also consistent with the restrictive effect of hypoxia on growth and development (Beall, Baker, Baker, & Haas, 1977; Frisancho & Baker, 1970). Hemoglobin concentration is also an important variable to consider as it clearly marks hematological acclimatization to high altitude, at least among the Andean participants. That is, the Q-HAR, who were high altitude residents at 4,300 m, showed significantly higher [Hb] (17.6 ± 0.19 g/dL) compared to all other study groups in consistent with their chronic exposure to hypobaric hypoxia. The [Hb] of the TM group is also noteworthy as this group was the other high-altitude resident group sampled in the study. The TM group had a [Hb] of 14.1

TABLE 1 General characteristics of the study groups (mean ± standard error)

Variables	Quechua born at sea level (Q-BSL) Group A		Quechua-migrants (Q-M) Group B		Quechua high altitude residents (Q-HAR) Group C		Tawang Monpa (TM) Group D		USA Group E	
	Males (n = 76)	Females (n = 76)	Males (n = 70)	Females (n = 80)	Males (n = 57)	Females (n = 53)	Males (n = 48)	Females (n = 47)	Males (n = 76)	Females (n = 87)
Age (yr)	23.86 ± 0.47	25.05 ± 0.57 [E]	24.14 ± 0.54	26.65 ± 0.66 [a][E]	24.21 ± 0.69	25.91 ± 0.77 [E]	23.69 ± 0.73	25.38 ± 0.78 [E]	22.14 ± 0.50	21.25 ± 0.32 [A,B,C,D]
Height (cm)	167.85 ± 0.64	154.19 ± 0.69 [a][B,E]	164.12 ± 0.66	152.81 ± 0.59 [a][A,E]	164.84 ± 0.73	151.71 ± 0.74 [a][A,E]	164.93 ± 0.93	154.17 ± 0.95 [a][E]	178.09 ± 0.84	166.69 ± 0.75 [a][A,B,C,D]
Weight (kg)	71.13 ± 1.27	61.56 ± 1.20 [a][B,C,D]	66.50 ± 1.04	57.52 ± 0.91 [a][A,E]	62.32 ± 0.99	56.82 ± 1.03 [a][A,E]	63.21 ± 1.42	56.86 ± 1.50 [a][A,E]	77.78 ± 1.34	62.93 ± 0.98 [a][B,C,D]
Hb[b] (g/dl)	14.91 ± 0.11	12.61 ± 0.08 [a][C]	14.89 ± 0.11	12.68 ± 0.10 [a][C]	19.09 ± 0.19	16.10 ± 0.17 [a][A,B,D,E]	15.38 ± 0.17	12.87 ± 0.19 [a][C,E]	14.70 ± 0.11	12.68 ± 0.10 [a][C,D]
FENO[b] (ppb)	33.07 ± 4.00	19.59 ± 1.52 [C,D]	33.34 ± 4.29	21.00 ± 2.33 [a][C,D]	15.02 ± 1.03	12.84 ± 2.23 [A,B,E]	15.53 ± 1.46	9.70 ± 0.95 [a][A,B,E]	22.51 ± 1.72	19.66 ± 1.20 [C,D]
PENO[b] (nmHg)	23.69 ± 2.86	14.04 ± 1.09 [a][C,D]	23.89 ± 3.07	15.05 ± 1.67 [a][C,D]	6.24 ± 0.43	5.33 ± 0.92 [A,B,E]	7.59 ± 0.72	4.74 ± 0.46 [a][A,B,E]	15.71 ± 1.20	13.72 ± 0.84 [C,D]
O₂ saturation (%)	90.26 ± 0.38	89.76 ± 0.47 [C,D,E]	90.08 ± 0.41	89.96 ± 0.37 [C,D,E]	88.84 ± 0.45	89.53 ± 0.40 [A,B,D,E]	96.67 ± 0.30	96.57 ± 0.34 [A,B,C,E]	91.28 ± 0.36	91.17 ± 0.32 [A,B,C,D]
BMI (kg/m²)	25.22 ± 0.41	25.86 ± 0.45 [C,D,E]	24.67 ± 0.34	24.64 ± 0.37 [D,E]	22.94 ± 0.34	24.70 ± 0.44 [A]	23.27 ± 0.51	23.89 ± 0.56 [A,B]	24.54 ± 0.41	22.60 ± 0.27 [a][A,B]

Note. A, significantly different from Group A, *p* < .05; B, significantly different from Group B, *p* < .05; C, significantly different from Group C, *p* < .05; D, significantly different from Group D, *p* < .05; E, significantly different from Group E, *p* < .05.

[a]Results of ANOVA: Sex difference within group: *p* < .01.

[b]BMI = body mass index; FENO = fractional exhaled nitric oxide; Hb = hemoglobin; PENO = partial pressure of exhaled nitric oxide.

± 0.18 g/dL, which was not substantially higher than the sea-level resident groups (Q-BSL = 13.8 ± 0.12 g/dL; Q-M = 13.7 ± 0.12; USA = 13.6 ± 0.11 g/dL), despite living at 3,200 m altitude. This is consistent with the broader literature on Tibetans, who characteristically show a blunted hemopoietic response to high altitude (Beall et al., 1998).

Table 1 also shows the mean unadjusted FENO and PENO of the different study groups. PENO was significantly (*p* < .05) lower in both high-altitude resident groups (TM = 6.2 ± 0.5 nmHg and Q-HAR = 5.8 ± 0.5 nmHg) as compared with the groups measured at sea level (USA = 14.6 ± 0.7 nmHg, Q-BSL = 18.9 ± 1.6 nmHg, and Q-M = 19.2 ± 1.7 nmHg). PENO was not significantly different between TM and Q-HAR (*p* > .05). Similarly, unadjusted PENO values were not significantly different between the three groups measured at sea level (*p* > .05). PENO values were consistently higher in males as compared with females in all five different study groups (Figure 1).

4 | DISCUSSION

This is the second study in the literature directly comparing exhaled NO levels between two distinct high-altitude native populations (Tibetan Monpa and Peruvian Quechua) using the same measurement protocols and equipment. We found no differences in exhaled NO concentration or partial pressure between Tibetan Monpa and Peruvian Q-HAR, unlike a previous study by Beall et al. (2001) that showed substantially higher exhaled NO levels in Tibetan compared to Andean (Bolivian) highland natives. Unlike the previous study by Beall et al., our two highland populations were measured at different altitudes with different ambient PO₂ levels (97.02 mmHg in Cerro de Pasco, Peru and 112.35 mmHg in Tawang, India), but the overall significant similarity between highland groups was interestingly unexpected despite the altitude differences. Furthermore, both the highland native populations in the present study (Monpa and Quechua) showed lower exhaled NO partial pressure compared to referent populations (USA, Q-M, and Q-BSL) measured at sea-level. Thus, our results are not in agreement with previous studies which have argued that high exhaled pulmonary NO is a central feature of Tibetan adaptation. This may be due to technical/measurement differences between studies, or due to the fact that exhaled pulmonary NO is not a valid marker of the highland native adaptive response to hypobaric hypoxia.

In addition to our observations for high-altitude resident groups, our results showed no differences in exhaled NO at sea level between two groups of Quechua who differed by where they were born and raised (sea-level versus above 3,000 m). This suggests that exhaled NO is unaffected by growth and development at high-altitude, which is a common mode of adaptive response to high altitude hypoxia (Beall et al., 1977; Frisancho & Baker, 1970; Monge, 1978; Malik & Singh, 1979). Finally, differences in exhaled NO between males and females, and differences between the US reference group and the Quechua sea-level groups, strongly suggest that much of the overall variance that we observed in exhaled NO has little to do with the adaptive response to hypobaric hypoxia, and perhaps more to do with
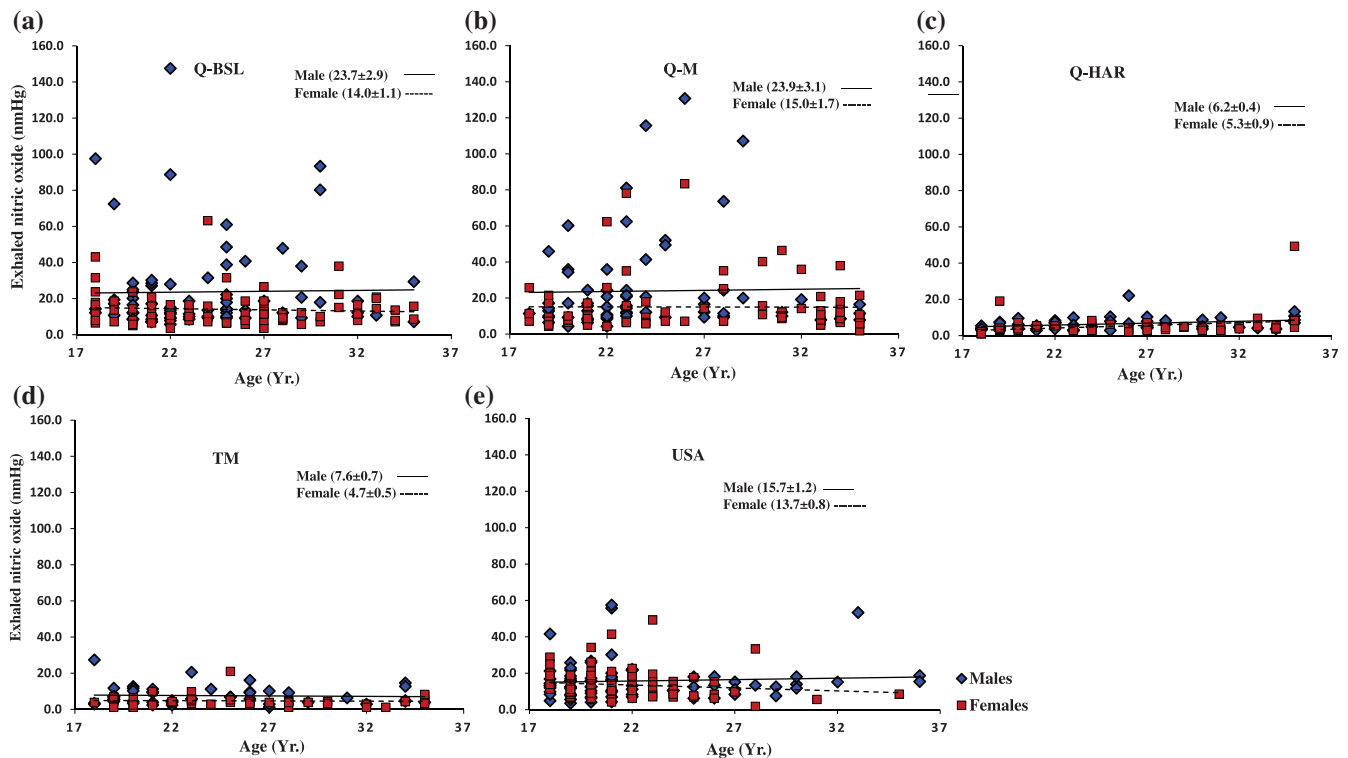
FIGURE 1   Distribution, mean and standard error for partial pressure of exhaled nitric oxide across the study groups, by sex

unmeasured local environmental conditions, for example, pollution leading to lung inflammation (Dressel et al., 2008; Dweik et al., 2012; Shang et al., 2018), or population level characteristics that were not measured, for example, fitness-level (Green, Maiorana, O'Driscoll, & Taylor, 2004; Jungersten, Ambring, Wall, & Wennmalm, 1997), diet (Iijima et al., 2002; Larsen, Ekblom, Sahlin, Lundberg, & Weitzberg, 2006) and so forth.

The findings of this study only call into question the previous notion that high exhaled pulmonary NO serves as a marker of population adaptation to high-altitude. The broader "NO-highland native adaptive hypothesis", which is well elaborated in a series of papers (Beall et al., 2001; Erzurum et al., 2007; Hoit et al., 2005), is different. This hypothesis relates to systemic or circulatory NO and the clear role of NO in promoting increased blood flow, whether in the lung or in working tissues. Previous studies do suggest that NO accumulation in the bronchiolar gases is largely due to local synthesis within the lung/airway (Dweik et al., 1998), but it is unclear whether lung NO is also a measure of systemic NO. Indeed, the work by Hoit et al., shows clear associations of high concentration of exhaled NO with increased blood flow in the lungs and high concentration of systemic/circulating NO with increased blood flow in the forearm of Tibetans (Erzurum et al., 2007; Hoit et al., 2005). Together, these earlier studies do provide support for a broader adaptive hypothesis around systemic NO in Tibetans. However, our results suggest that high level of exhaled pulmonary NO is not a valid biomarker of this adaptive response. More recent studies in Andean highland natives also support the

conclusion that caution is in order when interpreting exhaled NO as a marker of the population adaptive response to high-altitude. In two studies of Bolivian adults and children, exhaled NO was similar between Aymara and well-acclimatized adults of European ancestry living at high-altitude (Schwab et al., 2008), and was lower in the Aymara children compared to low-altitude European children resident at sea-level (Stuber et al., 2008).

A major limitation of our study is the validity of the measurement system that was used to measure exhaled NO, that is, the NIOX MINO system (Aerocrine, New Providence, NJ). Generally, there are five sensor technologies that are used to detect gas-phase molecules of NO: chemiluminescence, optical spectroscopy, mass spectrometry, chromatography, and electrochemistry (Beall, Laskowski, & Erzurum, 2012). The previous study of Beall et al. (2001) on Tibetans measured exhaled NO using a chemiluminescence sensor via the Sievers NOA280i®measurement system (GE Analytical, Boulder, CO). The Sievers NOA is considered the "gold-standard" measurement system (Maniscalco et al., 2016), although the sensitivity to expired airflow and the requirement that samples be collected in balloons makes this a more technically challenging measurement system in a field setting. The NIOXMINO uses a disposable electrochemical sensor and was developed commercially for use in a clinical setting around the management of airway inflammation and asthma. The main issue with the NIOX MINO system is that it cannot be calibrated, and indeed there has been considerable debate in the literature around the external validity of NIOX MINO measurements, particularly at altitude where

only one validation study exists to support use of the device under low barometric pressure conditions (Beall et al., 2010; Gochicoa-Rangel et al., 2016; Hemmingsson et al., 2009; Laskowski et al., 2010). From this, it is clear that NIOX MINO measures are not interchangeable with Siever's measures and it is fair to question the validity of the results presented in this article.

In support of the NIOX device, a large number of studies have been conducted to support the use of the NIOX MINO as a clinical device at sea-level (Brody, Zhang, Kit, & Dillon, 2013; See & David, 2013; Olin et al., 2007; Travers et al., 2007). That is, the device shows high repeatability and use of calibration for the chemiluminescence analyzer (Brody et al., 2013; Choong & David, 2013; Olin et al., 2007; Travers et al., 2007). Only one validation study supports the use of the NIOX MINO at high altitude (Gochicoa-Rangel et al., 2016). The latter study compared the NIOX MINO® to the Sievers NOA 280i® and reported a high correlation (0.96, $p < .001$) between the two measurement devices (participants were healthy nonsmoking adults of Mexico City, Mexico, measured at an altitude of 2,240 m above sea level). Further, NIOX measures were highly reproducible over five consecutive days. However, the two devices showed relatively wide limits of agreement (from −8.3 to 0.2 ppb), which brings into question the external validity of the NIOX MINO when used at altitude. Despite these wide limits of agreement (i.e., low external validity of the NIOX), the Mexico study does support the use of the NIOX system as an internally valid system for measurements made at the same altitude between populations. Unfortunately, our populations were measured at three different altitudes, and there are no formal published studies to support NIOX validity between altitudes. However, our preliminary testing of the device (data not shown) revealed no significant differences in mean NIOX values when the same individual was measured serially over 2 days at sea-level ($n = 7$ repeat measures), ~3,258 ($n = 7$ measures), and 4,295 m ($n = 3$ measures). Thus, taken together, there is reasonable evidence to support use of the NIOX measurement system for the purposes put forward in this article.

The other limitation of our study is that we were unable to account for dietary NO concentrations as study confounders in the manuscript. Although these confounders are potentially important in influencing circulating NO, but previous study on Tibetans have reported that water, tea, barley beer (<15 μM nitrite and <70 μM nitrate) or foods generally contained low levels of nitrite or nitrate (<0.4 mg/kg nitrite and <125 mg/kg nitrate). Hence, the dietary NO from average daily food consumption was not at a level expected to significantly increase circulating nitrate or nitrite (Erzurum et al., 2007).

A final issue to consider with this study is our choice of a Monpa population from North-eastern India as being representative of Tibetan highland natives globally. This is especially true as Tibetan natives in the literature are more commonly represented by populations sampled in Western China (Beall et al., 2001; Erzurum et al., 2007; Hoit et al., 2005), Lhasa, Tibet (Ren et al., 2014; Moore, 2001), or Sherpa in Nepal (Faoro et al., 2014; Winslow et al., 1989). Indeed, our study of the Monpa Population, which is a relatively

isolated group in the politically sensitive district of Arunachal Pradesh, India, is perhaps the first study of the biology and physiology of this group. Culturally, the population is Buddhist and Tibetan, with the world's second largest Tibetan monastery located in Tawang Town where the study was conducted. Phenotypically, the Monpa in our study lacked a hemopoietic response to hypobaric hypoxia, that is, [Hb] levels were normal by sea-level standards, which is a trait that strongly characterizes Tibetan altitude natives (Beall & Goldstein, 1990; Moore, 2001). Finally, our unpublished genetic analysis of this population reveals that the TM are not genetically distinct from Tibetan residents of Tibet. Rather, principal component analysis (PCA) performed using ~100,000 single nucleotide polymorphisms (SNPs) reveals that these two populations cluster together and that this TM/Tibetan cluster is distinct from other South Asian and East Asian populations included in the analysis. Thus, there is abundant evidence here that Tawang Monpas, while geographically isolated, are representative of the broader range of Tibetan derived populations living in the Himalayas.

5 | CONCLUSION

Tibetans showed similar partial pressure of exhaled NO compared to Peruvian Quechua, and both highland native groups showed lower partial pressure of exhaled pulmonary NO compared to sea-level comparison groups measured at sea-level. Among the sea-level groups, concentrations of exhaled NO were not related to developmental exposure to high-altitude hypoxia. Therefore, we conclude that high partial pressure and/or concentration of exhaled NO is not a universal feature of highland natives and our results are not in agreement with the notion that high level of exhaled NO is a useful biomarker of the population level adaptive response to hypobaric hypoxia of Tibetans or Andeans.

CONFLICT OF INTEREST

Nothing to disclose.

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