Antioxidant defense and oxidative damage vary widely among high-altitude residents

Allison J. Janocha1 | Suzy A. A. Comhair1 | Buddha Basnyat2
Maniraj Neupane3 | Amha Gebremedhin4 | Anam Khan1 | Kristin S. Ricci1
Renliang Zhang5 | Serpil C. Erzurum1,6 | Cynthia M. Beall7

1 Department of Pathobiology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195
2 Oxford University Clinical Research Unit-Nepal, Kathmandu, Nepal
3 Mountain Medicine Society of Nepal, Kathmandu, Nepal
4 Department of Internal Medicine, Addis Ababa University, Addis Ababa, Ethiopia
5 Research Core Services, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195
6 Respiratory Institute, Cleveland Clinic, Cleveland, Ohio 44195
7 Department of Anthropology, Case Western Reserve University, Cleveland, Ohio 44106

Correspondence
Cynthia M. Beall, PhD, Case Western Reserve University, Department of Anthropology, 238 Mather Memorial Building, 11220 Bellflower Road, Cleveland, OH 44106-7125.
Email: cmb2@case.edu

Funding information
HL60917; HL115008; NSF 0452326, 0924726

Abstract

Objectives: People living at high altitude experience unavoidable low oxygen levels (hypoxia). While acute hypoxia causes an increase in oxidative stress and damage despite higher antioxidant activity, the consequences of chronic hypoxia are poorly understood. The aim of the present study is to assess antioxidant activity and oxidative damage in high-altitude natives and upward migrants.

Methods: Individuals from two indigenous high-altitude populations (Amhara, \(n = 39\)), (Sherpa, \(n = 34\)), one multigenerational high-altitude population (Oromo, \(n = 42\)), one upward migrant population (Nepali, \(n = 12\)), and two low-altitude reference populations (Amhara, \(n = 29\); Oromo, \(n = 18\)) provided plasma for measurement of superoxide dismutase (SOD) activity as a marker of antioxidant capacity, and urine for measurement of 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a marker of DNA oxidative damage.

Results: High-altitude Amhara and Sherpa had the highest SOD activity, while highland Oromo and Nepalis had the lowest among high-altitude populations. High-altitude Oromo and Nepalis had the lowest among high-altitude populations. High-altitude Amhara had the lowest DNA damage, Sherpa intermediate levels, and high-altitude Oromo had the highest.

Conclusions: High-altitude residence alone does not associate with high antioxidant defenses; residence length appears to be influential. The single-generation upward migrant sample had the lowest defense and nearly the highest DNA damage. The two high-altitude resident samples with millennia of residence had higher defenses than the two with multiple or single generations of residence.

1 INTRODUCTION

Acute hypoxia (less than the normal amount of oxygen) causes an increase in oxidative stress and damage. Oxygen serves as the terminal electron acceptor for the mitochondrial electron transport chain that produces CO₂, H₂O, and heat. Relative oxygen scarcity results in a partial reduction to superoxide and hydrogen peroxide. Reactive oxygen species (ROS) can damage intracellular biomolecules, including DNA (Cash, Pan, & Simon, 2007; Klimova & Chandel, 2008; Moller, Risom, Lundby, Mikkelsen, & Loft, 2008; Schumacker, 2011); however, they are necessary for initiating transcription of loci underlying adaptive responses such as erythropoietin synthesis (Simon, 2006). Cells defend against ROS by upregulating antioxidants, primarily the powerful superoxide dismutases (SOD) (Comhair & Erzurum, 2010).

Chronic hypoxia is less studied. Reports of healthy highlanders on the Tibetan and Andean Plateaus suggest modest
long-term elevation of some, although not all, ROS and antioxidants, and thus imply a parallel elevation of oxidative damage. However, their long-term residence suggests the hypothesis of an evolved antioxidant response. Long-term indigenous human populations at altitudes above 2500 m include Tibetans and the Sherpas of Nepal, who have lived at altitude for perhaps more than 30,000 years; and the Amhara of Ethiopia, who have lived at altitude for ~5000 years or more. Shorter-term, multigenerational populations include the Oromo, also in Ethiopia, who have lived at altitude for ~500 years (Beall, 2014). Our understanding of the antioxidant response in high-altitude populations is incomplete due to limitations in samples and measures selected, and variation in the genetic background of populations, altitudes, and length of exposure (Moller et al., 2008).

We hypothesized that indigenous high-altitude populations have higher levels of SOD to counteract the increased oxidative stress resulting from hypoxia and prevent a rise in DNA damage. To test this, we evaluated plasma SOD activity as a biomarker of defense against ROS (Comhair & Erzurum, 2010) and urine 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a biomarker of oxidative DNA damage (Wu, Chiu, Chang, & Wu, 2004) in two indigenous and one multigenerational samples (Sherpa, Amhara, and Oromo), one upward migrant sample (Nepali), and two low-altitude, paired comparison samples (Amhara and Oromo).

2 METHODS

2.1 Study samples

Data and samples were collected over the course of several field studies over a ten-year period. The Sherpa (Nepal), Amhara, and Oromo (both Ethiopia) samples have been previously described (Alkorta-Aranburu et al., 2012; Hoit et al., 2011; Jeong et al., 2014). Low-altitude native Nepalis of South and East Asian ancestry had migrated to high altitude and lived in the same locations as the Sherpa sample. Approximately half (N = 7) of the Nepalis had been living at high altitude for ~1.5 years, with the remainder between 8 months and 8 years. The Institutional Review Boards at Case Western Reserve University, the Cleveland Clinic, the Addis Ababa University Faculty of Medicine, the Ethiopian Science and Technology Committee, the Nepal Health Research Council, and OxTREC approved the studies. All participants provided written informed consent.

No individuals from high-altitude samples had traveled to altitudes below 2500 m in the previous 6 months, while no individuals from low-altitude samples had traveled to altitudes above 2500 m in the same timeframe. All study volunteers were healthy (by physician exam or self-report), non-pregnant (by self-report), normotensive, non-anemic, and non-smoking.

To exclude potential confounding by inflammation or infection, we measured C-reactive protein by ELISA (R&D Systems, Minneapolis, MN) and tested red cells from the Ethiopian samples to detect all four human malarial parasites (Hoit et al., 2011). Six individuals with CRP levels above the upper detection limit of the assay were excluded along with seven individuals with confirmed malaria infection. Blood pressure measurements are the average of three measurements taken while participants rested in a seated position. Pulse and percent oxygen saturation were measured via pulse oximetry (Criticare Model 503 and SpO2; Criticare Systems, Waukesha, WI), and values reported are the average of six readings taken 10 seconds apart.

2.2 Sample collection

Venous blood was collected via peripheral venipuncture into heparinized tubes. Hemoglobin concentration was measured immediately in duplicate in whole blood using the cyanmethemoglobin method (Hemocue, Angelholm, Sweden). Blood samples were first centrifuged, and spot urine samples were aliquoted immediately. The aliquots were stored in liquid nitrogen, shipped frozen to Cleveland, and stored at −80°C until analysis.

2.3 SOD activity in plasma

SOD activity was measured in plasma as previously described (Nebot et al., 1993). Briefly, activity was determined by the rate of reduction of ferri-cytochrome c, with one unit of SOD activity defined as the amount of SOD required to inhibit the rate of cytochrome c reduction by 50% in the presence of a superoxide-generating system. Plasma SOD activity is comprised of the total activity of the three isoforms of SOD (CuZnSOD, MnSOD, and EC-SOD). SOD activity measured at the same time in a reference Cleveland population was similar (24.6 ± 3.7 U/ml, N = 10) to previous reports from our lab, confirming the accuracy of the assay.

2.4 Oxidative DNA damage in urine

Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 2′ deoxyguanosine (dG) were measured by liquid chromatography/tandem mass spectrometry as previously described (Teichert et al., 2009). Concentrations of 8-OHdG were normalized by calculating the ratio of oxidized base (8-OHdG) to non-oxidized base (dG).

2.5 Statistical analysis

Data are reported as mean ± SEM for normally distributed variables or median for non-normally distributed variables. Comparisons across all samples were made by ANOVA or
Kruskal–Wallis test at a 0.05 significance level. Confidence intervals for the differences in location between pairs of groups were generated based on Wilcoxon rank sum tests performed at Bonferroni-corrected individual significance levels of $0.05/6 = 0.0083$. Therefore, the individual confidence levels of the intervals were 99.17%. Statistical analyses were done in JMP v7.0.1 (SAS, Cary, NC) or R v3.3.3 (R Foundation for Statistical Computing, Vienna, Austria)(R Core Team, 2017).

**TABLE 1** Sample description

<table>
<thead>
<tr>
<th></th>
<th>Amhara&lt;sup&gt;a&lt;/sup&gt; 1200 m</th>
<th>Oromo 1700 m</th>
<th>Nepali 3800 m</th>
<th>Oromo 4000 m</th>
<th>Amhara 3700 m</th>
<th>Sherpa 3800 m</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N (Male/Female)</strong></td>
<td>21/8</td>
<td>14/4</td>
<td>9/3</td>
<td>25/17</td>
<td>23/16</td>
<td>18/16</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>33 ± 2</td>
<td>24 ± 1</td>
<td>26 ± 1</td>
<td>27 ± 1</td>
<td>30 ± 1</td>
<td>34 ± 2</td>
</tr>
<tr>
<td><strong>Systolic BP (mm Hg)</strong></td>
<td>120 ± 1</td>
<td>114 ± 2</td>
<td>120 ± 2</td>
<td>119 ± 2</td>
<td>115 ± 2</td>
<td>114 ± 2</td>
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<tr>
<td><strong>Diastolic BP (mm Hg)</strong></td>
<td>79 ± 1</td>
<td>78 ± 1</td>
<td>80 ± 2</td>
<td>78 ± 1</td>
<td>75 ± 1</td>
<td>75 ± 2</td>
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<tr>
<td><strong>Pulse (BPM)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>78 ± 3</td>
<td>74 ± 3</td>
<td>75 ± 2</td>
<td>73 ± 2</td>
<td>72 ± 3</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Female</td>
<td>78 ± 5</td>
<td>86 ± 3</td>
<td>89 ± 7</td>
<td>90 ± 3</td>
<td>92 ± 3</td>
<td>70 ± 2</td>
</tr>
<tr>
<td><strong>Height (m):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.72 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.70 ± 0.02</td>
<td>1.66 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.69 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.66 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.66 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female</td>
<td>1.58 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64 ± 0.03</td>
<td>1.45 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.57 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.58 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.52 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Weight (kg):</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>59.2 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.3 ± 1.5</td>
<td>62.3 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.4 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.2 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.8 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Female</td>
<td>50.6 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.8 ± 0.3</td>
<td>49.4 ± 4.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.1 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.3 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.1 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;):</strong></td>
<td>20.0 ± 0.4</td>
<td>18.7 ± 0.4</td>
<td>22.6 ± 0.7</td>
<td>20.5 ± 0.4</td>
<td>18.5 ± 0.3</td>
<td>21.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male</td>
<td>20.3 ± 0.7</td>
<td>19.6 ± 0.6</td>
<td>23.5 ± 2.2</td>
<td>21.5 ± 0.5</td>
<td>18.9 ± 0.6</td>
<td>23.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female</td>
<td>20.6 ± 0.4</td>
<td>19.9 ± 0.6</td>
<td>22.5 ± 2.2</td>
<td>21.5 ± 0.6</td>
<td>19.0 ± 0.6</td>
<td>22.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Saturation (%):</strong></td>
<td></td>
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<tr>
<td>Male</td>
<td>97.0 ± 0.3</td>
<td>96.5 ± 0.3</td>
<td>90.9 ± 0.6</td>
<td>86.6 ± 0.6</td>
<td>91.6 ± 0.5</td>
<td>89.5 ± 0.5</td>
</tr>
<tr>
<td>Female</td>
<td>96.3 ± 0.6</td>
<td>96.0 ± 0.6</td>
<td>90.5 ± 0.6</td>
<td>86.5 ± 0.6</td>
<td>91.5 ± 0.5</td>
<td>89.5 ± 0.5</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dl):</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.6 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.1 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.3 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.8 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female</td>
<td>13.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.0 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.2 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.0 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.4 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.0 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM.
BP, blood pressure; BPM, beats per minute; BMI, body mass index: weight (kg)/(height (m))².
<sup>a</sup>Sample (collection year): mean barometric pressure, temperature, relative humidity (%).
Low-altitude Amhara (2005): 659 mm Hg, 22°C, 36%.
Low-altitude Oromo (2007): 635 mm Hg, 15°C, 32%.
Sherpa/Nepali (2010): 484 mm Hg, 7°C, 67%.
High-altitude Oromo (2007): 469 mm Hg, 3°C, 40.5%.
High-altitude Amhara (2005): 498 mm Hg, 6°C, 36%.
<sup>b</sup>Significant difference between men and women within a sample.
<sup>P</sup>-value across samples by ANOVA.

3 | RESULTS

Altogether, 42 high-altitude Oromo, 18 low-altitude Oromo, 39 high-altitude Amhara, 29 low-altitude Amhara, 34 Sherpa, and 12 Nepali provided plasma and urine samples. Table 1 describes the samples. Blood pressures were normal, and BMI was in the low-normal range (Table 1). As expected, percent oxygen saturation was lower in high-altitude samples than in low-altitude samples.

3.1 | Superoxide dismutase activity

Among the high-altitude samples, Amhara and Sherpa had the highest SOD activities, while multigenerational highland Oromo and upward-migrant Nepalis had the lowest among high-altitude residents (Figure 1). At both altitudes, there was a trend toward greater SOD activity in Amhara as compared to Oromo, but neither high-altitude sample differed in
SOD activity compared to its low-altitude counterpart. We considered a 5-unit difference in the mean value of SOD between groups to be meaningful. Confidence interval results for group differences demonstrate that all the intervals extended beyond 5 units on at least one side (negative or positive). Therefore, this analysis is neither able to rule out the null hypothesis of no difference (since 0 is in each interval) nor can it rule out meaningful differences (since −5 or +5 is in each interval). We conclude that a larger sample size is needed to better estimate the group differences.

3.2 DNA oxidative damage

Among high-altitude samples, urine 8-OHdG/dG levels were lowest in the Amhara (P < .0001, P = .05, P = .04 compared to Oromo, Nepali, Sherpa, respectively; Figure 1). The Oromo, but not the Amhara, had an altitude-associated increase in 8-OHdG/dG. Sherpa and Nepali levels did not differ. Oromo and upward-migrant Nepalis had the highest DNA oxidation levels as measured by 8-OHdG/dG levels.

4 DISCUSSION AND CONCLUSION

These findings reject the hypothesis that chronic hypoxia elicits higher antioxidant defenses regardless of ancestry. The lack of a universal pattern of antioxidant defense and oxidative damage is consistent with findings relating to other physiological systems that report different patterns of adaptation described in Tibetans, Amhara, Aymara, and Oromo high altitude populations (Beall, 2014). Instead, we found that the Amhara and Sherpa samples with millennia of residence had higher levels of antioxidant defense than the two high-altitude resident samples with multiple or single generations of residence. The latter samples had the highest DNA damage. This suggests that natural selection acted on Sherpa and Amhara highlanders over the millennia to maintain a healthy balance of defense, damage, and hypoxia signaling.

A limitation of the present study is that a single antioxidant and measure of oxidative damage were assessed. Other antioxidants and ROS might have roles in the response to hypoxia (Simon, 2006). Additionally, environmental factors such as increased UV radiation at high altitude (Askew, 2002) and air pollution [eg, from indoor cook fires (Commodo et al., 2013)] are sources of oxidative stress at high altitude, while a relatively low intake of dietary antioxidants (owing to low intake of fresh fruits and vegetables) might contribute to insufficient defense. Other sources of defense and damage were not quantified in this study. Reproductive history and age have also been identified as contributors to oxidative damage (Ziomkiewicz et al., 2016). This study did not collect reproductive history. Four of our samples (Amhara women at both altitudes, Oromo highland women, and Oromo lowland men) showed a direct correlation with age and 8OHdG/dG. The samples with the highest and lowest damage had similar average ages (27 vs 30 years). While age and reproductive history might account for some of the within-sample variation in both oxidative DNA damage and
SOD activity, these factors are unlikely to explain the differences between samples based on the data we have.

The long-term stability of 8-OHdG in frozen urine samples has not been tested beyond 2.2 years at −80°C (Matsumoto et al., 2008) and 6 years at −20°C (Poulsen et al., 1998). Both studies found a high degree of correlation. The urine samples in the present study had been stored at −80°C between 5 and 10 years prior to measurement. The highest 8-OHdG concentrations were found in the samples stored for 7 years; overall, there was no correlation between concentration and length of storage. The paired samples were collected during the same years.

In conclusion, high altitude alone does not associate with high antioxidant defenses or increased oxidative damage, but rather defense and damage might be related to time at altitude and other environmental factors. A more complete profile of antioxidants, ROS and their signaling function, oxidative damage, and environment should be considered to explore the range of possible roles of ROS and antioxidant defenses among these populations exposed to high-altitude hypoxia.

ACKNOWLEDGMENTS
The authors are grateful to the study participants and communities for their hospitality and cooperation in their field work. We thank the officials of the Nepal Health Research Council, the Ethiopian Science and Technology Commission, the Amhara and the Oromia Regional Governments, and the staffs of the Semien Mountains and Bale Mountains National Parks for permissions and local arrangements. The Frankfurt Zoological Society generously allowed the use of the Wolf Research Camp, Ethiopia. Gezahnegn Fentahun, MD, and the late Daniel Tessema of Addis Ababa University worked in both Ethiopian field sites. Jeff Hammel provided assistance with statistical analyses.

CONFLICT OF INTEREST
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS
AJJ, MN, RZ, AK, and KSR performed research and collected data. AJJ, RZ, SAAC, SCE, and CMB analyzed data. BB, MN, AG, SCE, and CMB designed the study. AJJ, SCE, and CMB wrote the manuscript.

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**How to cite this article:** Janocha AJ, Comhair SAA, Basnyat B, et al. Antioxidant defense and oxidative damage vary widely among high-altitude residents. *Am J Hum Biol*. 2017:e23039. [https://doi.org/10.1002/ajhb.23039](https://doi.org/10.1002/ajhb.23039)