CHEST

Original Research

OCCUPATIONAL AND ENVIRONMENTAL LUNG DISEASES

Oxidative-Nitrosative Stress and Systemic Vascular Function in Highlanders With and Without Exaggerated Hypoxemia

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Background: **Acute exposure to high altitude stimulates free radical formation in lowlanders, yet whether this persists during chronic exposure in healthy, well-adapted and maladapted highlanders suffering from chronic mountain sickness (CMS) remains to be established.**

Methods: **Oxidative-nitrosative stress (as determined by the presence of the biomarkers ascorbate radical [$A^{\bullet-}$], via electron paramagnetic resonance spectroscopy, and nitrite [NO_2^-], via ozone-based chemiluminescence) was assessed in venous blood of 25 male highlanders in Bolivia living at 3,600 m with CMS (n = 13, CMS+) and without CMS (n = 12, CMS−). Twelve age- and activity-matched, healthy, male lowlanders were examined at sea level and during acute hypoxia. We also measured flow-mediated dilatation (FMD), arterial stiffness defined by augmentation index normalized for a heart rate of 75 beats/min (AIx-75), and carotid intima-media thickness (IMT).**

Results: **Compared with normoxic lowlanders, oxidative-nitrosative stress was moderately increased in the CMS− group ($P < .05$), as indicated by elevated $A^{\bullet-}$ (3,191 ± 457 arbitrary units [AU] vs 2,640 ± 445 AU) and lower NO_2^- (206 ± 55 nM vs 420 ± 128 nM), whereas vascular function remained preserved. This was comparable to that observed during acute hypoxia in lowlanders in whom vascular dysfunction is typically observed. In contrast, this response was markedly exaggerated in CMS+ group ($A^{\bullet-}$, 3,765 ± 429 AU; NO_2^-, 148 ± 50 nM) compared with both the CMS− group and lowlanders ($P < .05$). This was associated with systemic vascular dysfunction as indicated by lower ($P < .05$ vs CMS−) FMD (4.2% ± 0.7% vs 7.6% ± 1.7%) and increased AIx-75 (23% ± 8% vs 12% ± 7%) and carotid IMT (714 ± 127 μM vs 588 ± 94 μM).**

Conclusions: **Healthy highlanders display a moderate, sustained elevation in oxidative-nitrosative stress that, unlike the equivalent increase evoked by acute hypoxia in healthy lowlanders, failed to affect vascular function. Its more marked elevation in patients with CMS may contribute to systemic vascular dysfunction.**

Trial registry: **ClinicalTrials.gov; No.: NCT01182792; URL: www.clinicaltrials.gov**

CHEST 2013; 143(2):444–451

Abbreviations: AIx-75 = augmentation index normalized for a heart rate of 75 beats/min; $A^{\bullet-}$ = ascorbate radical; CMS = chronic mountain sickness; CV = coefficients of variation; EPR = electron paramagnetic resonance; FMD = flow-mediated dilatation; IMT = intima-media thickness; NO = nitric oxide; NO_2^- = nitrite; ONS = oxidative-nitrosative stress; PBN = α-phenyl-N-tert-butylnitrone; PBN-$LO^{\bullet}$ = lipid-derived alkoxyl radical; RSNO = *S*-nitrosothiol

Emerging evidence suggests that at physiologic, albeit undefined, concentrations, free radicals are involved in the adaptive response to hypoxia, given their capacity as signaling molecules to activate oxygen-sensitive genes through stabilization of the transcription factor, hypoxia-inducible factor-1α.[1] In excess, however, they are equally capable of causing structural cell-membrane damage, vascular endothelial dysfunction, and promoting atherogenesis.[2] Accordingly, acute exposure to high altitude has been shown to cause oxidative-nitrosative stress (ONS) in healthy human lowlanders, as indicated by an increase in free radical formation and corresponding reduction in vascular nitric oxide (NO) bioavailability.[3-5] However,

there are no studies, to our knowledge, that have examined this response during lifelong exposure to high altitude in healthy, well-adapted and maladapted highlanders.

Thus, the aim of the present study was to determine the magnitude of the ONS response in healthy, well-adapted highlanders and in patients suffering from the maladaptive syndrome of chronic mountain sickness (CMS). To place the magnitude of the ONS response in humans permanently living at high altitude into clearer perspective, we also examined healthy lowlanders at sea level during acute exposure to simulated high altitude and during maximal exercise at low altitude, interventions known to stimulate (sub)maximal activation of ONS.[6,7] We hypothesized that systemic ONS would be as follows: (1) permanently elevated in highlanders compared with lowlander control subjects, (2) further exaggerated in patients suffering from CMS, and, in turn, (3) associated with impaired vascular function.

Materials and Methods

Ethical Approval

The experimental protocol was approved by the Institutional Review Boards for Human Investigation at the University of San Andres, La Paz, Bolivia (CNB number 52/04), University of Lausanne, Switzerland (numbers 89/06 and 94/10), and University of Glamorgan, Wales (number 4/07), and subsequently registered (clinicaltrials.gov; Identifier: NCT01182792).[8]

Manuscript received March 30, 2012; revision accepted July 1, 2012.

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Funding/Support: This study was funded by the Swiss National Science Foundation, the Cloëtta Foundation, the Eagle Foundation, the Leenaards Foundation, the Placide Nicod Foundation (Drs Scherrer and Sartori), The Physiological Society, and SRIF-III (Dr Bailey).

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DOI: 10.1378/chest.12-0728

Participants

Highlanders: Written informed consent was obtained from 13 male patients with primary CMS (CMS+ group) and 12 healthy age-matched control subjects without CMS (CMS− group) native to La Paz (Table 1). These participants were recruited as part of a wider collaborative research study that used a larger sample size (CMS+, n = 23 vs CMS−, n = 27) with a similar focus on vascular function.[9] Symptoms of CMS were scored and clinical diagnosis confirmed by an excessive erythrocytosis (hemoglobin >20 g/dL) in the presence of normal pulmonary function and no history of working in the mining industry.[9] All participants had a typical Aymara surname, were self identified as Aymaras, and were from similar socioeconomic backgrounds, having been born and bred in La Paz. None of the participants was a smoker or taking medication, and all were considered as physically active but not exercise trained. Participants were encouraged to follow a low nitrate/nitrite diet prior to and throughout the duration of the study with specific instructions to avoid fruits, salads, and cured meats.[10] All studies on the high-altitude dwellers were performed at the Instituto Boliviano de Biologia de Altura in La Paz, Bolivia (altitude, 3,600 m).

Lowlanders: We also recruited 12 age-, sex-, and physical activity-matched healthy white subjects born and bred close to sea level (approximate altitude, 80 m) within the United Kingdom (Table 1). This group was included not only as a normoxic comparator but also as a means to determine the independent impact of acute (simulated) high-altitude exposure and maximal normoxic exercise on oxidative stress with measurements confined exclusively to the ascorbate radical ($A^{\bullet-}$). Lowlanders were examined at low altitude (normoxia). They also were examined following 6-h, passive exposure to normobaric hypoxia (12% oxygen equivalent to approximately 4,600 m simulated altitude) and after an incremental cycling test to volitional exhaustion in normoxia on a semirecumbent cycle ergometer according to an established protocol previously described in a separate study.[6] These additional interventions were used strategically because they are known to stimulate, respectively, moderate and maximal activation of ONS,[6,7] and, thus, they help place the magnitude of the highlanders' response into clearer perspective.

Metabolic Assessment

Blood Sampling: All subjects provided a 12-h, overnight-fasting, resting, blood sample from a catheter located in a forearm antecubital vein. Additional samples were obtained on two separate occasions from the lowlanders only, following 6-h, passive exposure to 12% oxygen and maximal (normoxic) exercise. Samples were immediately centrifuged at 600*g* (4°C) for 10 min; the supernatant was snap-frozen without delay and stored under liquid nitrogen prior to transport (Cryopak CP100; Taylor-Wharton International) to Wales for analysis.

Free Radicals: Electron paramagnetic resonance (EPR) spectroscopy was used for the direct detection of free radicals. Spectral acquisition (magnetic field resolution, 4,096 points) was initiated 60 s after complete thawing of the sample (at 21°C) using an X-band (about 9.8 GHz) EMX spectrometer (Bruker Biospin) operating at 100 kHz modulation frequency equipped with a TM_{110} cavity.

Ascorbate Radical: This biomarker was used to assess global, free radical flux.[11] Potassium-ethylenediaminetetraacetic acid plasma (1 mL) was injected into a high-sensitivity, multiple-bore, sample cell (AquaX; Bruker Instruments Inc) and the characteristic $A^{\bullet-}$-EPR doublet was recorded by signal averaging three scans with the following instrument parameters: resolution, 1,024 points; microwave power, 20 mW; modulation amplitude, 0.65 G; receiver gain,

Table 1—*Baseline Data*

Characteristics	Lowlanders (Altitude: About 80 m) Control Subjects (n = 12)	Highlanders (Altitude: About 3,600 m) CMS− (n = 12)	Highlanders (Altitude: About 3,600 m) CMS+ (n = 13)
Age, y	55 ± 9	52 ± 9	57 ± 7
Hemoglobin, g/dL	15.0 ± 1.3	17.3 ± 0.9[a]	20.9 ± 0.9[a,b]
Hematocrit, %	44 ± 4	52 ± 3[a]	65 ± 4[a,b]
Arterial oxyhemoglobin saturation, %	98 ± 1	90 ± 3[a]	81 ± 4[a,b]
CMS score, points	0 ± 0	1 ± 2	7 ± 3[a,b]
Body mass, kg	83 ± 11	82 ± 11	79 ± 10
Height, cm	174 ± 6	170 ± 8	164 ± 7[a]
BMI, units	27.3 ± 3.2	28.3 ± 3.4	29.1 ± 3.7
Systolic BP, mm Hg	119 ± 16	125 ± 12	129 ± 13
Diastolic BP, mm Hg	79 ± 9	82 ± 9	81 ± 9

Values are mean ± SD unless otherwise indicated. CMS = chronic mountain sickness; CMS− = without chronic mountain sickness; CMS+ = with chronic mountain sickness.

[a]Significantly different vs lowlander control subjects ($P < .05$).

[b]Significantly different vs CMS− ($P < .05$).

2×10^5 arbitrary units; time constant, 40.96 milliseconds; scan rate, 0.25 G/s for scan width, 15 G.[4] The intraassay and interassay coefficients of variation (CV) were both < 10%.

N-tert-butyl-α-phenylnitrone Adducts: Ex vivo spin trapping was used for the specific detection of lipid-derived free radicals.[3] Aliquots (4.5 mL) of whole blood were added to a 6-mL glass vacutainer (SST; Becton, Dickinson and Company) primed with 1.5 mL N-tert-butyl-α-phenylnitrone (PBN) dissolved in physiologic saline (50 mM final concentration). The vacutainer was gently mixed, then placed in the dark for 10 min for the blood to clot. Following centrifugation, 1 mL serum adduct was added to a borosilicate glass tube containing 1 mL spectroscopic-grade toluene, and vortex mixed for 10 s. The sample was centrifuged at 600*g* for another 10 min, and 200 μL of the organic supernatant was added to a nitrogen-flushed, precision-bore, quartz EPR tube and vacuum degassed to remove oxygen. Blocks of 10 incremental EPR scans were recorded using the following parameters: resolution, 2,048 points; microwave power, 20 mW; modulation amplitude, 0.50 G; receiver gain, 1×10^5; time constant, 82 milliseconds; scan rate, 0.4 G/s for scan width, 50 G. The intraassay and interassay CV were both < 10%.

Signal Quantification: EPR spectra were filtered identically using Bruker WinEPR version 2.11 software and simulated using Bruker SimFonia or SimEPR32 (Bruker Biospin).[12] Relative free radical concentrations were calculated by double integration using Origin 5.0 software (Microcal Inc).

Antioxidants: Exactly 900 μL of 5% metaphosphoric acid was added to 100 μL potassium-ethylenediaminetetraacetic acid plasma. Ascorbic acid was subsequently assayed by fluorimetry based on the condensation of dehydroascorbic acid with 1,2-phenylenediamine.[13] Lipid-soluble antioxidants were determined using the simultaneous high-performance liquid chromatography method.[14,15] The intraassay and interassay CV were both < 5%.

NO Metabolites: Plasma NO metabolites were measured using ozone-based chemiluminescence.[3,4] Samples (20 μL) were analyzed for the total concentration of NO (nitrate [NO_3^-] plus nitrite [NO_2^-] plus *S*-nitrosothiols [RSNO]) by vanadium (III) reduction.[16] A separate sample (200 μL) was injected into modified triiodide reagent[17,18] for the measurement of NO_2^- plus RSNO and 5% acidified sulfanilamide added and left to incubate in the dark at 21°C for 15 min to remove NO_2^- for the measurement of RSNO in a (third) parallel sample. Plasma NO_2^- was calculated as total NO − (NO_2^- plus RSNO). All calculations were performed using Origin/Peak Analysis software (OriginLab Corp). The intraassay and interassay CV were 7% and 10%, respectively.

Vascular Assessment

Endothelial Function: Systemic, conduit artery, endothelial function was assessed by determining the increase of the brachial artery diameter evoked during reactive hyperemia, using high-resolution ultrasound and automatic wall-tracking software according to international guidelines[19] and as previously described.[9,20] Briefly, using high-resolution ultrasound (Acuson Sequoia C512; Siemens AG or Esaote MyLab30 Gold; Esasote SpA) and a high frequency (7-10 MHz) linear array probe, the brachial artery was identified approximately 5 cm above the antecubital fossa. The ultrasound probe was then fixed in a stereotactic clamp (AMC Vascular Imaging), and the Doppler flow was recorded continuously throughout the study. After 1 min of baseline measurements, a pressure cuff placed around the forearm was inflated to 250 mm Hg for 5 min. Following cuff deflation, the hyperemia-induced changes of brachial artery diameter and flow were continuously monitored for 3 min. Ultrasound images (B-mode) were analyzed with a validated system for automatic real-time measurement of the brachial artery diameter (FMD Studio; Computer Vision Group).[21] Flow-mediated dilation (FMD) was expressed as the maximal percentage change in vessel diameter from baseline. Endothelium-independent dilation of the brachial artery was assessed by measuring the increase of the brachial artery diameter to an oral dose (250 μg) of glyceryl trinitrate (UCB-Pharma SA). The intraobserver CV was < 5%.

Arterial Stiffness: Pulse wave analysis was used to derive a normalized augmentation index (AIx) using the SphygmoCor system (AtCor Medical Pty Ltd).[22] The radial artery pressure waveform was recorded at the wrist using applanation tonometry with a high-fidelity micromanometer (Millar Instruments Inc) and the central (ascending aortic) pressure waveform derived using a validated transfer function.[23] AIx was calculated as the difference between the first and second peaks of the central arterial waveform, expressed as a percentage of the pulse pressure and by convention normalized relative to a heart rate of 75 beats/min (AIx-75).[24] The intraobserver CV was < 5%.

Carotid Artery Intima-Media Thickness: Segments of the right and left common carotid artery 1 to 2 cm proximal to the carotid

bulb were scanned to identify the optimal angle of incidence.[25,26] Carotid intima-media thickness (IMT) was measured using radiofrequency signals with a 21 μm resolution (RF QIMT; Esaote SpA).[27] After scanning the vessel, each radiofrequency line was automatically analyzed forward and backward in real time by the echo device. The means of three measures from the right carotid artery and three from the left common carotid artery were recorded. The intraobserver CV was < 5%.

Statistical Analysis

Shapiro-Wilk W tests confirmed that all data sets were normally distributed. Baseline and metabolic data were analyzed using a combination of one-way/one factor repeated measures analysis of variance and post hoc, Bonferroni-corrected, independent/paired samples *t* tests. Vascular data were assessed using independent samples *t* tests and relationships were determined with Pearson product moment correlations. Significance was established at $P < .05$, and data were expressed as mean ± SD.

Results

Metabolic Data

Free Radicals: Baseline $A^{\bullet-}$ was chronically elevated in the CMS− group relative to normoxic lowlander control subjects (Table 2) and equivalent to the concentration observed when lowlanders were exposed to a more severe hypoxic stimulus equivalent to a simulated altitude of about 4,600 m (Fig 1). In the CMS+ group, the sustained elevation in $A^{\bullet-}$ was significantly more severe than in the CMS− group and was similar in magnitude to that evoked by maximal exercise in the lowlanders. PBN-adduct concentration was also selectively elevated in the CMS+ group, with hyperfine coupling constants (nitrogen: $a_N = 13.6$ G and hydrogen: $a_H^{\beta} = 1.9$ G) generally consistent with the trapping of lipid-derived alkoxyl radicals (PBN-$LO^{\bullet}$).[6]

Antioxidants: Ascorbate and α/β-carotene were depressed in the highlanders compared with the lowlanders, with the lowest concentration of ascorbate recorded in the CMS+ group (Table 2). In contrast, both γ- and α-tocopherol were elevated in the highlanders.

NO Metabolites: Plasma NO_2^- was lower in the highlanders compared with lowlanders, with the lowest concentration observed in the CMS+ group. No differences were observed in any of the remaining metabolites (Table 2).

Vascular Data

Baseline measurements of heart rate, brachial artery diameter, and central systolic/diastolic BP were comparable between CMS+ and CMS− groups (Table 3). Consistent with prior observations,[9] FMD was lower in CMS+, whereas glyceryl trinitrate-induced, endothelium-independent dilation was comparable. Equally, AIx-75 and carotid IMT were greater in the CMS+ groups compared with CMS− group.

Correlations

Free radicals (pooled CMS+ and CMS− data) correlated positively ($P < .05$) against AIx-75 ($A^{\bullet-}$, $r = 0.53$; PBN-$LO^{\bullet}$, $r = 0.57$) and carotid IMT ($r = 0.48$/$r = 0.49$).

Table 2—*Metabolic Data*

Metabolites	Lowlanders (Altitude: About 80 m) Control Subjects (n = 12)	Highlanders (Altitude: About 3,600 m) CMS− (n = 12)	Highlanders (Altitude: About 3,600 m) CMS+ (n = 13)
Free radicals, AU			
Ascorbate radical	2,640 ± 445	3,191 ± 457[a]	3,765 ± 429[a,b]
Hydrogen coupling constant	1.78 ± 0.02	1.79 ± 0.03	1.79 ± 0.03
Alkoxyl radical	Not assessed	16,985 ± 2,452	21,729 ± 2,526[b]
Hydrogen coupling constant	Not assessed	1.86 ± 0.02	1.87 ± 0.02
Nitrogen coupling constant	Not assessed	13.58 ± 0.02	13.59 ± 0.02
Antioxidants, μM			
Ascorbate	50.1 ± 11.9	40.4 ± 10.1[a]	23.1 ± 11.5[a,b]
α-Carotene	0.119 ± 0.079	0.045 ± 0.018[a]	0.061 ± 0.028[a]
β-Carotene	0.439 ± 0.310	0.164 ± 0.079[a]	0.214 ± 0.104[a]
α-Tocopherol	26.9 ± 8.9	41.8 ± 6.8[a]	37.8 ± 13.0[a]
γ-Tocopherol	2.4 ± 1.1	4.7 ± 1.4[a]	4.4 ± 1.9[a]
Nitric oxide metabolites,			
Total nitric oxide, μM	32.4 ± 9.8	36.6 ± 13.0	33.9 ± 10.7
Nitrate, μM	32.0 ± 9.8	36.3 ± 13.1	33.7 ± 10.7
Nitrite, nM	420 ± 128	206 ± 55[a]	148 ± 50[a]
S-nitrosothiols, nM	10 ± 6	7 ± 5	6 ± 5

Values are mean ± SD unless otherwise indicated. AU = arbitrary units. See Table 1 legend for expansion of other abbreviations.
[a]Significantly different vs lowlander control subjects ($P < .05$).
[b]Significantly different vs CMS− ($P < .05$).

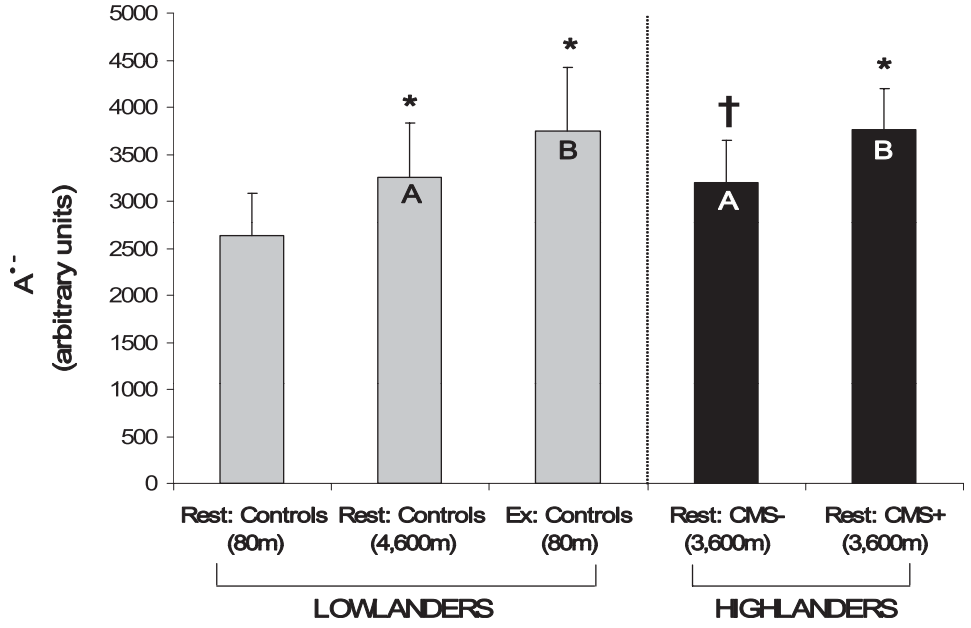

FIGURE 1. Differences in the ascorbate radical ($A^{\bullet-}$) between lowlanders and highlanders. Lowlanders were passively exposed to 12% oxygen (equivalent to a simulated altitude of about 4,600 m) for 6 h and, on a separate occasion, exercised to volitional exhaustion in normoxia. Highlanders were examined in the resting state only at their native altitude of 3,600 m. A, Baseline oxidative stress in CMS− subjects is permanently elevated and equivalent to the response observed when (healthy) lowlanders were exposed to a more severe hypoxic stimulus (simulated altitude of approximately 4,600 m vs approximately 3,600 m). B, Baseline oxidative stress in CMS+ patients was more severe and comparable to the response observed when lowlanders exercised to exhaustion in normoxia. Values are mean ± SD based on 12 healthy lowlanders and 25 high-altitude dwellers (12 without and 13 with CMS). *Significantly different ($P < .05$) vs preceding value(s) as a function of group. †Significantly different ($P < .05$) vs lowlander rest (80 m). $A^{\bullet-}$ = ascorbate radical; CMS− = without chronic mountain sickness; CMS+ = with chronic mountain sickness; Ex = exercise.

Inverse correlations ($P < .05$) were observed with arterial oxyhemoglobin saturation ($r = -0.49/r = -0.60$), NO_2^- ($r = -0.40/r = -0.56$) and FMD ($r = -0.63/r = -0.63$). In addition, NO_2^- (pooled CMS+ and CMS− data) was positively associated ($P < .05$) with FMD ($r = 0.40$) and inversely associated with AIx-75 ($r = -0.60$).

DISCUSSION

These data provide, for the first time to our knowledge, information on the magnitude of the systemic ONS response during lifelong exposure to high altitude in healthy, well-adapted highlanders (CMS−), and maladapted highlanders suffering from CMS (CMS+). In the CMS− group, ONS was moderately elevated. The magnitude of this increase was similar to that evoked by acute, short-term exposure to hypoxia in healthy lowlanders. In the CMS+ group, ONS was markedly exaggerated since it was of similar magnitude to that evoked by maximal exercise in lowlanders. In healthy highlanders, this sustained increase in oxidative stress, which, when evoked acutely by short-term hypoxia in lowlanders induces vascular dysfunction, failed to exert any functional effect. Its exaggerated increase in the CMS+ group, however, was associated with vascular dysfunction, suggesting that it may play a pathogenic role.

We specifically used EPR spectroscopy because it represents the most direct, specific, and sensitive technique for the detection and molecular characterization of free radicals.[28] The increase in $A^{\bullet-}$ indicates that the global, systemic flux of free radicals was greater in the highlanders, with the highest concentration recorded in the CMS+ group. Our studies with lowlanders have helped place the magnitude of this response into clearer perspective. For example, the sustained increase of $A^{\bullet-}$ in healthy highlanders was almost identical to that observed when healthy lowlanders were acutely exposed, albeit tcelaro a more severe normobaric hypoxic stimulus (approximately 4,600 m vs approximately 3,600 m), whereas the concentration in the CMS+ group was equally comparable to that observed in lowlanders after a maximal normoxic exercise challenge, a stimulus known to evoke maximal activation of ONS in humans.[6]

In both subgroups of highlanders, the elevation in $A^{\bullet-}$ coincided with lower concentrations of ascorbate (demonstrably more marked in the CMS+ group),

Table 3—*Vascular Data*

Characteristics	Highlanders (Altitude: About 3,600 m) CMS− (n = 12)	CMS+ (n = 13)
HR, beats/min	71 ± 12	66 ± 10
Baseline brachial artery diameter, mm	4.4 ± 0.5	4.6 ± 0.6
Flow-mediated dilatation, %	7.6 ± 1.7	4.2 ± 0.7[a]
Glyceryl trinitrate, %	12.1 ± 2.4	11.5 ± 1.5
Central systolic BP, mm Hg	114 ± 11	121 ± 14
Central diastolic BP, mm Hg	83 ± 9	83 ± 10
AIx-75, %	12 ± 7	23 ± 8[a]
Carotid IMT, μM	588 ± 94	714 ± 127[a]

Values are mean ± SD unless otherwise indicated. AIx-75 = augmentation index normalized to a heart rate of 75 beats/min; HR = heart rate; IMT = intima-media thickness. See Table 1 legend for expansion of other abbreviations.

[a]Significantly different ($P < .05$).

and α/β-carotene, which likely reflects ongoing consumption during targeted repair of superoxide, hydroxyl, peroxyl, alkoxyl, and alkyl radicals. In contrast, both isomers of tocopherol, the major lipid-soluble chain-breaking antioxidant,[29] were markedly elevated, with concentrations comparable to those achieved following high-dose (400 International Units/d over 8 weeks) α-tocopherol supplementation in lowlanders (D. M. Bailey, PhD; S. F. Rimoldi, MD; Emrush Rexhaj, MD, et al, unpublished observations, June 2011). While the precise mechanism underlying the differential antioxidant response remains unclear, it was inadequate given that systemic oxidative stress ultimately prevailed.

We extended these measurements with ex vivo spin-trapping in an attempt to characterize the "individual" free radicals formed, with a specific focus on lipid-derived species, since they have previously been associated with systemic vascular dysfunction.[30] In agreement with our previous observations in hypoxic human blood,[3] we were able to detect higher concentrations of PBN-LO$^{\bullet}$ in the CMS+ relative to the CMS− group, thereby confirming a selective elevation in free radical-mediated lipid peroxidation.

Combined, these findings are the first to demonstrate that oxidative stress is permanently elevated at high altitude, which may represent a physiologic response, given that the CMS− group was healthy and vascular function was found to be normal and comparable to that observed in healthy lowlanders.[9] This interpretation adds further in vivo support to our recent suggestion that free radicals help initiate protective adaptation for the maintenance of systemic homeostasis during a chronic hypoxic challenge.[4] In contrast, the exaggerated oxidative stress response in CMS+ likely exceeded this as-yet-undefined physiologic threshold and may have contributed to the clinical manifestations of their disease.

For example, the inverse relationships observed between free radicals and both arterial oxyhemoglobin saturation and NO_2^- suggest that the hypoxia-mediated oxidative inactivation of NO may prove the unifying mechanism underlying the selective depletion of NO_2^- in CMS. Given its endocrine role as an intravascular source of bioactive NO during hypoxic vasodilatation,[31] the lower NO_2^- may have contributed to vascular dysfunction, as indicated by the blunting of FMD and stiffening of the vasculature, which are established, independent risk factors for premature cardiovascular disease.[32,33]

The selective depletion of plasma NO_2^- in the Aymaras contrasts starkly with the markedly elevated NO_3^- and NO_2^- recently documented in Tibetans[34] and lowlanders exposed to high altitude.[35] Besides potential genetic differences, other factors may have contributed to these conflicting findings. We used ozone-based chemiluminescence, arguably the most sensitive technique for the molecular detection of blood-borne NO metabolites[36], whereas previous investigators used high-performance liquid chromatography. Furthermore, unlike prior studies,[34,35] we minimized delays in sample freezing and enforced strict dietary control to avoid the potential complications associated with a NO_3^--rich diet. Further research will help reconcile these differences.

Conclusions

This study demonstrates that systemic ONS is permanently elevated in highlanders. Unlike healthy lowlanders in whom acute hypoxia-induced oxidative stress impairs vascular function, lifelong exposure to an almost identical ONS stimulus in healthy, well-adapted natives failed to exert any adverse effects. In contrast, ONS was markedly exaggerated in maladapted patients with CMS and was associated with vascular dysfunction. Although the limitations associated with a cross-sectional correlational design are acknowledged, future interventional studies incorporating targeted antioxidant prophylaxis are justified to investigate whether exaggerated ONS in patients with CMS contributes to this and/or other symptoms underlying this syndrome. From a clinical perspective, since hypoxemia was identified as a potential pro-oxidant catalyst, our findings in highlanders may have implications for lowlanders suffering from disease states associated with chronic hypoxemia.

Acknowledgments

Author contributions: Dr Bailey is the principle investigator and takes responsibility for the integrity/accuracy of the data presented. *Dr Bailey:* was involved in the clinical examinations, study design, data analysis and interpretation; wrote a first draft of the paper; and served as principal author.

Dr Rimoldi: contributed to the clinical examinations, including the assessment of vascular structure and function; study design; data analysis and interpretation; and writing and revision of the final manuscript.
Dr Rexhaj: contributed to the clinical examinations, data analysis and interpretation, and revision of the manuscript.
Dr Pratali: contributed to the clinical examinations and revision of the manuscript.
Dr Salinas Salmòn: contributed to the clinical examinations and revision of the manuscript.
Dr Villena: contributed to recruiting patients and control subjects, clinical examinations, and revision of the manuscript.
Dr McEneny: contributed to data analysis and revision of the manuscript.
Dr Young: contributed to data analysis and revision of the manuscript.
Dr Nicod: contributed to the drafting and revision of the manuscript.
Dr Allemann: contributed to design of the study, data analysis and interpretation, and writing and revision of the final manuscript.
Dr Scherrer: contributed to design of the study, data analysis and interpretation, and writing and revision of the final manuscript.
Dr Sartori: contributed to design of the study, data analysis and interpretation, and writing and revision of the final manuscript.
Financial/nonfinancial disclosures: The authors have reported to *CHEST* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.
Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.
Other contributions: We express our gratitude to Mrs Catherine Romero, BSc (Hons), and staff of the Instituto Boliviano de Biologia de Altura (La Paz, Bolivia), Philip E. James, PhD, of the Wales Heart Research Institute (Cardiff, UK). and Kathy Pogue, BSc (Hons), of Queen's University Belfast (Belfast, Northern Ireland) for technical input. We also appreciate the volunteers' enthusiasm and commitment to this study.

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