Cerebral Vasodilatation to Exogenous NO Is a Measure of Fitness for Life at Altitude

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Background and Purpose—Andean highlanders, unlike Ethiopians, develop chronic mountain sickness (CMS), a maladaptation to their native land. Ambient hypoxia induces NO-mediated vasodilatation. Fitness for life at altitude might be revealed by cerebrovascular responses to NO.

Methods—Nine altitude-native men were examined at 3622 and 794 m in Ethiopia and compared with 9 altitude-native Andean men tested at 4338 and 150 m in Peru. We assessed CMS scores, hematocrits, end-tidal pressure of carbon dioxide ($P_{ET}CO_2$), oxygen saturations, and cerebral blood flow velocity (CBV). We evaluated fitness for life at altitude from the cerebrovascular response to an exogenous NO donor.

Results—At high altitude, CMS scores and hematocrits were higher in Andeans, and they had lower oxygen saturations. Ethiopians had higher $P_{ET}CO_2$ at all study sites. At low altitude, saturations were similar in both groups. Responsiveness of the cerebral circulation to NO was minimal in Ethiopians at low altitude, whereas Andeans had a large response. In contrast, at high altitude, Ethiopians showed large responses, and Peruvians had minimal responses.

Conclusions—By our measure, high altitude–native Peruvians were well-adapted lowlanders, whereas Ethiopian highlanders were well adapted to altitude life. Environmental pressures were sufficient for human adaptation to chronic hypoxia in Africa but not South America. The mechanisms underlying these differences are unknown, although studies of neurovascular diseases suggest that this may be related to a NO receptor polymorphism. **(*Stroke*. 2006;37:1754-1758.)**

Key Words: altitude ■ nitric oxide ■ cerebral hypoxia ■ cerebrovascular circulation ■ evolution

Altitude is an inhospitable environment for humans, and yet permanent settlements are found at 5100 m.[1] The fall in barometric pressure with increasing altitude in these habitats reduces the availability of oxygen compared with sea level. Therefore, hypoxia in the mountains is inescapable. Ethiopian highlanders are, by some measures, the best adapted for life at altitude,[2] showing no evidence of chronic mountain sickness (CMS), a failure to adapt to altitude that is commonly seen in the high Andes.[3,4] The severity of CMS is inversely related to fitness for survival in the Andes.

A key measure of survival in the mountains is the maintenance of optimal oxygen concentrations in all tissues. An adequate blood flow to the brain is essential for maintenance of a proper oxygen supply, and blood flow, in turn, is profoundly affected by changes in oxygen availability. In response to hypoxia, blood vessels, including those in the cerebral circulation, undergo NO-mediated vasodilatation. Inappropriate release of NO with hypoxia has been implicated in some maladaptation syndromes in sojourners to altitude.[5] We hypothesized that cerebrovascular responsiveness to NO donors would predict the ability for adaptation to life at high altitude. To test this proposal, we compared cerebral blood flow velocity (CBV) responses to NO donors in 2 populations: Peruvian native highlanders, who frequently have CMS,[6] and Ethiopian altitude natives, in whom maladaptation to altitude has not been reported. Furthermore, we asked whether using baseline CBV at low and high altitudes in the 2 populations as an expression of the "trait of interest" and the response of the cerebral vessels to an exogenous NO donor as an indicator of "fitness" for survival(s) in the mountains could predict the better adaptation to altitude life of Ethiopian highlanders.

Methods

For additional information on Methods, see the online supplement, available at http://stroke.ahajournals.org.

Nine Ethiopian men were examined at their resident altitude of 3622 m in the Simen Mountain National Park and again within 24 hours of arrival at the Tekeze River Gorge (794 m). We compared these results with those reported previously from 9 native men of Cerro de Pasco, Peru, at 4338 m and from the same men within 24 hours of arrival at Lima, Peru (150 m).[7]The study was approved by

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the Ethiopian government and local research ethics committees. All studies were performed in accordance with the Declaration of Helsinki (2002) of the World Medical Association. Subjects gave written informed consent in Amharic. We excluded women and subjects with a clinical history suggestive of diabetes, alcoholism, or chronic infections, including HIV. Four men from our Peruvian study were diagnosed with CMS, reflecting the prevalence of the condition in that locality. We thought they might offer a clue, most likely genetically determined, for failure of adaptation, especially of the cerebral circulation, to chronic hypoxia. We did not include CMS patients in Ethiopia because neither we, nor others before us, could find them in the highlands of East Africa (see online supplement).

Subjects were clinically examined, and an internationally accepted CMS score (CMS-sc)[8] was assessed. CMS was diagnosed if the CMS-sc was >12. In Ethiopia, hematocrit was calculated from the packed cell ratio, in quadruplicate, from a peripheral venous sample. Blood samples were drawn in the resting supine position with the arm supported at heart level. End-tidal pressure of carbon dioxide ($P_{ET}CO_2$) was measured via a small nasal catheter using an infrared analyzer (Binos-1; Leybold-Haraeus Limited). Arterial oxygen saturation was measured using pulse oximetry (Hewlett Packard 78325C). In Peru, these parameters were assessed as reported previously.[7] We used transcranial Doppler ultrasound applied to the middle cerebral artery (MCA) to assess resting CBV (the adaptive trait of interest) for 10 minutes. We then administered a NO donor, sublingual 5 mg isosorbide dinitrate (in Peru) or 0.4 mg sublingual nitroglycerin (in Ethiopia), and measured the response of the cerebral circulation (the "fitness" for life at altitude). We recorded CBV for 7 minutes after administration of the drug. Flow velocities were adjusted for hematocrit and oxygen saturation using regression coefficients and for $P_{ET}CO_2$ using the formula of Markwalder et al[9] before analysis. Statistical analysis was performed using analysis of covariance (ANCOVA), *t* tests, and multiple regressions where appropriate. Data are reported as mean±SD. Significance was assumed when $P<0.05$.

Results

There were no significant differences in age between Ethiopians and Peruvians (35.6±2.7 and 36.9±2.8 years). At high altitude, CMS-scs and hematocrits were higher in Peruvians than in Ethiopians (Table), reflecting the higher CMS-sc and hematocrits of 4 Peruvians with CMS.[7] We found no evidence for CMS in Ethiopia, nor did we find a report of its occurrence in the Ethiopian highlands. At high altitude, Peruvians had lower oxygen saturations, and Ethiopians had higher $P_{ET}CO_2$ (Table). At low altitude, there were no differences in oxygen saturation between the groups. $P_{ET}CO_2$ remained higher in Ethiopians at low altitude.

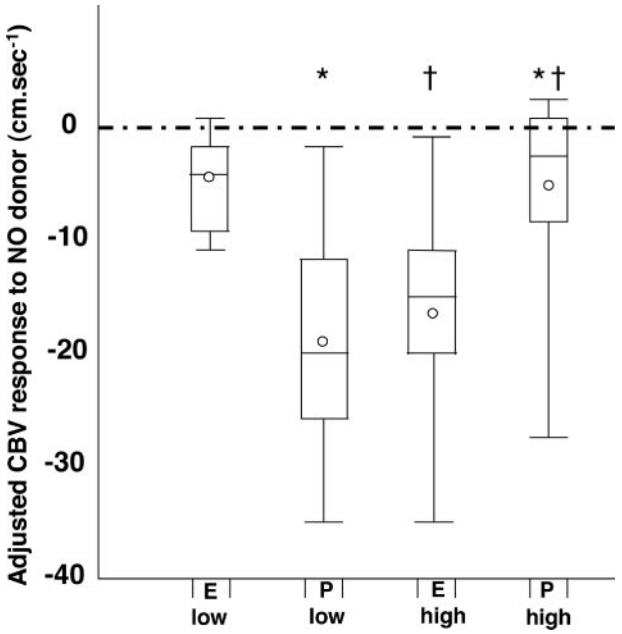

Figure 1. Box plots showing median, interquartile range and absence of outliers, and responses to NO donors at low altitude (low) and high altitude (high) in Ethiopians (E) and Peruvians (P; modified from Markwalder et al[9]) using adjusted CBV (adjusted for $P_{ET}CO_2$, hematocrit, and oxygen saturation). At low altitude, the response was minimal in Ethiopians, whereas that of Peruvians was very large. Conversely, at high altitude, the response of Ethiopians was very large, whereas that of Peruvians was negligible. *$P<0.001$ compared with Ethiopians; †$P<0.001$ compared with low altitude.

Baseline CBV (when adjusted for $P_{ET}CO_2$, oxygen saturation, and hematocrit[7,9]) was higher at altitude in both Ethiopians and Peruvians ($P<0.001$) but not different between the 2 populations (Table). CBV decreased after exogenous NO administration, as seen in previous studies.[7,10] The magnitude of the responses to exogenous NO at low and high altitude in Peruvians and Ethiopians was significantly different ($P<0.001$). At low altitude, Ethiopians had a minimal response, whereas Peruvians had a very large response. At high altitude, the reactivity of the vessels to exogenous NO in the 2 groups was reversed (Figure 1), with the Ethiopians showing a large response and the Peruvians a minimal one (see supplemental Figs

Parameters Used to Evaluate the Response to NO Donor Drugs in Ethiopia and Peru

	Ethiopian LA	Peruvian LA	Ethiopian HA	Peruvian HA
Measured baseline CBV ($cm \cdot s^{-1}$)	43.7±10.6	46.2±8.4	48.9±9.5	45.8±8.5
Measured NO CBV ($cm \cdot s^{-1}$)	37.8±8.6	33.2±9.4	38.4±7.8	42.5±10.1
Adjusted baseline CBV ($cm \cdot s^{-1}$)	46.7±10.6	62.6±13.4	74.7±15.4	81.4±14.5
Adjusted NO CBV ($cm \cdot s^{-1}$)	40.4±9.6	43.7±13.3	57.9±11.0	75.1±19.4
Adjusted CBV response to NO ($cm \cdot s^{-1}$)	−6.3±4.2	−18.8±10.0	−16.8±10.4	−6.3±9.7
Oxygen saturation (%)	96.8±1.4	96.7±1.5	88.0±3.2	83.8±6.4**
$P_{ET}CO_2$ (mm Hg)	47.1±4.4	39.3±2.2**	37.1±2.3	31.8±3.5**
CMS-sc	...	...	0.3±0.5	11.6±8.5**
Hematocrit (%)	...	...	48.5±4.6	57.9±10.8*

Measured CBVs are shown at baseline and 7 minutes after administration of the NO donor drug (NO CBV). Measured CBVs were then adjusted for differences in oxygen saturation, $P_{ET}CO_2$[9] and hematocrit. Values are reported as high altitude (HA) and low altitude (LA) in both groups. CMS-scs and hematocrits were not assessed at LA. *$P<0.05$; **$P<0.01$ compared with Ethiopians.

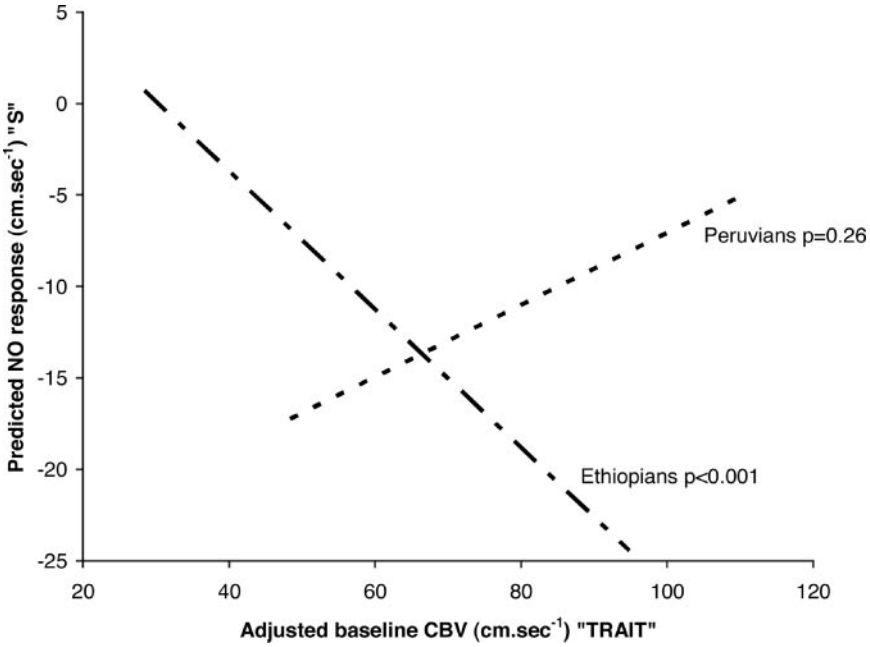

Figure 2. The relationship between baseline CBV (adjusted for $P_{ET}CO_2$, hematocrit, and oxygen saturation) and the response of the cerebral circulation to NO donors (fitness for life at altitude) at all altitudes in Ethiopians (E) and Peruvians (P). Each graph is based on model fit (ANCOVA) of actual data, showing Ethiopian and Peruvian responses as a function of baseline velocities. Differences in slopes (S) $P=0.003$. Ethiopians have large responses, indicating fitness for life at altitude. Peruvian slope=0.20, $R^2=0.08$, $P=0.26$ (modified from Markwalder et al[9]); Ethiopian slope=0.38, $R^2=0.59$, $P<0.001$.

I and II, available online at http://stroke.ahajournals.org). The individual changes in absolute CBV in Ethiopian and Peruvian subjects can be seen in the online supplement and supplemental Table I, available online at http://stroke.ahajournals.org).

We then compared the predicted response to NO, the "fitness," at all altitudes against adjusted (for $P_{ET}CO_2$, hematocrit, and oxygen saturation) baseline CBV. This comparison further underscored the robustness of the response to exogenous NO in Ethiopians and their fitness for life at altitude, whereas Peruvians appeared better adapted to low altitudes (Figure 2). Moreover, when we repeated this analysis for the Peruvian subjects after subdividing them into 2 groups, those with and without CMS, our results were even stronger. The relationship in the Peruvian controls was described by a slope=0.53, $R^2=0.48$, and $P<0.03$ (no fall in CBV), whereas in the Peruvians with CMS, the slope=0.12, $R^2=0.015$, and $P=0.77$ (no response of CBV to exogenous NO). These responses can be seen graphically in the online supplement.

When we repeated these analyses using $P_{ET}CO_2$ (for Ethiopians, slope=0.86, $r=0.55$, $P=0.02$; for Peruvians, slope=−0.90, $r=-0.37$, $P=0.13$, and interaction $P=0.009$) and oxygen saturation (for Ethiopians, slope=0.95, $r=0.51$, $P=0.04$; and for Peruvians, slope=−0.42, $r=-0.29$, $P=0.24$, and interaction $P=0.03$) as the traits of interest, the same relationship was found, whereby the Ethiopians had the more appropriate response at high altitude and the Peruvians the more appropriate response at low altitude.

Finally, because of the absence of patients with CMS (who experience profound nocturnal hypoxia) in Ethiopia, we performed an additional analysis of the results from Peru stratified according to oxygen saturation. We found that the response to NO in those with saturation <91% was -6.3 ± 9.7 cm · s^{-1}, whereas in those with saturation >91%, it was -18.9 ± 10.0 cm · s^{-1} ($P=0.02$).

Discussion

In both Ethiopians and Peruvians, altitude alone (ambient hypoxia) significantly increased CBV. Thus, hypoxia is capable of inducing vasodilatation in the cerebral circulation in both populations. In Tibetan and Andean highlanders, and maladapted newcomers to altitude, cerebrovascular responses to exogenous stimuli are deficient.[7,11] Additionally, in Andean highlanders, vasodilator responses to induced hypoxia were impaired at altitude but normal at sea level.[12] We saw marked cerebral vasodilatation to exogenous NO at altitude in Ethiopians but not in Andeans at similar heights, highlighting the adaptation of Ethiopians to their native environment. Conversely, Ethiopians showed minimal cerebral vasodilatation to NO donors at low altitude, unlike Peruvians, suggesting the Peruvians are better adapted to sea level life, whereas Ethiopians appear "maladapted" to sea level existence. In support of this interpretation, Ethiopians had robust fitness to altitude demonstrated by a significant cerebral vascular response to exogenous NO and absence of CMS at high altitude, whereas Peruvian responses remained insignificant, and they frequently experience CMS. Furthermore, when we repeated our analyses using either oxygen saturation or $P_{ET}CO_2$ as the adaptive traits of interest, instead of blood flow velocity, we found that unlike in Ethiopians, responses to exogenous NO were least at lower $P_{ET}CO_2$ levels and low oxygen saturations in Peruvians, as found at altitude, suggesting that at high altitude, the Peruvians were less well adapted. Similarly, the higher the oxygen saturation and $P_{ET}CO_2$ in Peruvian altitude dwellers, a feature at sea level, the larger the response; they were by implication sea level people. In Ethiopians, the smallest responses to NO were found at the highest oxygen saturation and $P_{ET}CO_2$ levels (at low altitude); they appeared maladapted to sea level life. These data strengthen our hypothesis that MCA CBV responses to exogenous NO are predictive of an adapted individual's oxygen saturation at altitude and signify true altitude adaptation. A progressive decrease in oxygen content in the cerebral microcirculation, as occurs with increasing altitude, elicits compensatory vasodilatation attributable to release of NO from endothelial cells,[13] and this counteracts, in part, the deleterious effects of altitude hypoxia on the brain. Because blood flow also correlates with neuronal

activity,[14] enhanced vasodilatation at altitude should favor better cognition and consequently strengthen human adaptive strategies on the Ethiopian high-altitude plateau.

We performed an additional test of our hypothesis that cerebral responses to exogenous NO act as a marker of fitness for life at altitude. If the ratio (a dimensionless parameter) of the cerebral response to NO to the baseline blood velocity was always the same, at all altitudes and in both populations, the NO response might reflect some physiological constraint rather than adaptation to altitude.[15] However, if these ratios changed with altitude, the NO-induced vasodilatation could possibly be classed as an environmentally driven evolutionary feature found in adapted highlanders. We analyzed these ratios in a 2-way ANOVA (Ethiopians versus Peruvians; high altitude versus low altitude) and found that the ratios were significantly different (ANOVA interaction term $P=0.002$ and Kruskal–Wallis $P=0.01$). Thus, this additional analysis (available in the online supplement) supported our initial conclusion.

One feature of Andean life is the development of the maladaptation syndrome CMS. In their native environment, all Andean survivors to old age are thought to eventually develop this syndrome.[16] However, sea level residence of CMS Andeans eliminates all signs and symptoms of the disease.[17] This suggests that altitude natives of the Andes are not well adapted to their hypoxic environment. Conversely, Ethiopian altitude dwellers are not known to show signs of maladaptation to their native habitat. Thus, it is likely that they are physiologically better able to cope with the prevailing hypoxia at the altitude at which they live. Because CMS is frequent in the Andes, our Andean subjects included 4 men with the syndrome.[8] Oxygen saturation and hematocrit form an important part of the CMS scoring system, thus it is likely that these subjects contributed significantly, on average, to the lower oxygen saturations and higher hematocrits of our Peruvian subjects. Nevertheless, we believe that our study sample accurately reflected the condition of life at great heights in the Andes. Although hematocrit and hemoglobin are important to altitude adaptation, beyond ≈3000 m, they lose their efficiency to protect venous oxygen pressure.[18] We are not aware of epidemiologic studies to specifically assess the prevalence of CMS at any altitude in Ethiopia. Nevertheless, based on Andean results, our study site in Ethiopia was well within the range of altitudes at which CMS may occur in the Andes. We do not think the differences in altitude study sites, in the Andes and Ethiopia (≈700 m lower), could account for the absence of CMS in East Africa because Andeans living at even lower altitudes than our Ethiopian cohort (2700 m; Mantaro, Peru)[19] still had much higher CMS-scs (median CMS-sc=10 to 11) than the Ethiopians in this study.

Charles Darwin thought that "natural selection will always act very slowly, often only after long intervals of time, and generally on a very few of the inhabitants of the same region at the same time."[20] The remarkably rapid adaptation of certain organisms since the beginning of the industrial age has challenged that view[21] and heightened interest in measuring selection and adaptation in human populations. *Homo sapiens* migrated out of Africa 50 000 to 60 000 years ago, a relatively short period in Darwinian evolutionary terms. One view is that this species replaced other human species, such as *Homo erectus* in Asia, without interbreeding. A competing view proposes that modern humans from Africa spread across the globe, they interbred with archaic humans, but only African genes persisted.[22] Both theories imply that African genes conferred advantages for survival. Ethiopian altitude dwellers should be better suited for life at great heights than South Americans because humans arrived in the Americas at a later date, and their migration to high altitude was delayed by lack of population pressures. Our data support the idea that environmental pressures were sufficient to drive human adaptation in Africa over a relatively short period, in Darwinian terms. Similar studies in Tibetans could provide further support for our hypothesis. The exact mechanism of such rapid evolution remains unknown, although a polymorphic genetic mechanism, in addition to behavioral diversity, is widely postulated.[23]

The exogenous administration of NO to induce vasodilatation does not mirror hypoxia-induced cerebral vasodilatation because exogenous NO induces vasodilatation even in ambient normoxia.[10] Nevertheless, the responsiveness of the cerebral vessels in the 2 high-altitude populations studied here differed greatly. We speculate that a single polymorphism in the endothelial NO synthase gene or in the gene for an NO receptor on endothelial cells accounts for the fitness for life at altitude, as defined here, in Ethiopians.

Cerebrovascular disease continues to kill and maim millions, especially in the developed world. Evolutionary pressures on the Ethiopian high-altitude plateau have selected for robust NO-mediated vasodilatation of the cerebral vessels, which could have implications for the pathogenesis of cerebrovascular disease at sea level in which physiological responsiveness to NO is involved. Indeed, increased inhibition of NO synthase by an endogenous inhibitor, decreasing the available NO, is a marker for stroke.[24] Furthermore, the protective effect of NO on endothelial cells is removed by promoter variants of the endothelial NO synthase 3 gene, making less NO available and, especially in young black women, increasing susceptibility to ischemic stroke.[25]

The human experiment that unfolds in the mountainous regions of the world provides great opportunities to examine the effects of evolutionary determined variations that allow survival in the face of significant ambient hypoxia and draw lessons to apply in the clinic.

Methodological Constraints

We studied our subjects in the field in 2 continents over a period of 5 years. Strict laboratory procedures were difficult to apply. Thus, there are potential limitations to this study. First, for technical reasons, we used similar but not identical drugs as NO donors. Both drugs are organic nitrates, and both delivered sufficient exogenous NO to the cerebral circulation to induce robust vasodilatation, independent of ambient hypoxia. The robustness of the responses proved to be determined by altitude and not by the type of compound. Comparison of the vasoreactivity of these drugs has been made in vitro,[26] whereby preconstricted internal thoracic artery rings relaxed more to isosorbide dinitrate than to

nitroglycerin. If a similar response occurred in vivo in the cerebral circulation, our Peruvians would have shown greater responsiveness at altitude and Ethiopians, who were given nitroglycerin, a lesser response. We found no evidence using our testing method to support the in vitro findings related to internal thoracic arteries.

The normal responses of the cerebral circulation to exogenous NO are dilatation of the MCA, which would decrease CBV, and dilatation of microvessels, which would increase CBV.[10] As in previous studies,[7,10] we saw a marked decrease in CBV after drug administration, suggesting that it is MCA diameter that is mainly affected by NO donors.[10] However, this must be inferred because we did not measure the diameter of the MCA. Furthermore, Doppler-derived blood velocities are dependent on the angle of insonation. The position of the transducer may not have been the same between or within subjects at different times. Thus, comparisons of CBV responses while the transducer was in a fixed relationship to the artery are secure. However, we also examined baseline CBV between groups as the trait of interest, although not as a feature of robust environmental adaptation, and we assumed that the number of measurements, when averaged, would be large enough to reduce errors attributable to random differences in probe position.

Finally, cerebral blood flow is affected by $P_{ET}CO_2$, hematocrit, and oxygen saturation, all of which were different at altitude in the 2 populations. Some of the differences in oxygen saturation and hematocrit could be attributed to the higher Andean habitats compared with those we found in Ethiopia. To make Ethiopians and Peruvians comparable, we adjusted CBV as follows. First, CBVs were adjusted from individual $P_{ET}CO_2$ values at altitude according to the formula taken from Markwalder et al[9] and then adjusted for individual oxygen saturation at altitude and for individual hematocrit by a regression formula given in the online supplement.

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Disclosures

O.A. and R. H. planned the project and reviewed this manuscript. V.E.C., G.G., and M.S. collected data and reviewed this manuscript. C.Q. performed data analysis and reviewed this manuscript. G.Z. reviewed this manuscript and, with A.G., obtained governmental approval and institutional review board approval from the University of Addis Ababa. They both devised the informed consent procedures used in the field and translated the International Chronic Mountain Sickness scoring system for use in Ethiopia. O.A. wrote this manuscript. The authors declare that there are no conflicts of interest relating to this manuscript.

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