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Determinants of blood oxygenation during pregnancy in Andean and European residents of high altitude

M. Vargas, E. Vargas, C. G. Julian, J. F. Armaza, A. Rodriguez, W. Tellez, S. Niermeyer, M. Wilson, E. Parra, M. Shriver and L. G. Moore

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Ancestry explains the blunted ventilatory response to sustained hypoxia and lower exercise ventilation of Quechua altitude natives

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Brutsaert, Tom D., Esteban J. Parra, Mark D. Shriver, Alfredo Gamboa, Maria Rivera-Ch, and Fabiola León-Velarde. Ancestry explains the blunted ventilatory response to sustained hypoxia and lower exercise ventilation of Quechua altitude natives. *Am J Physiol Regul Integr Comp Physiol* 289: R225–R234, 2005. First published March 31, 2005; doi:10.1152/ajpregu.00105.2005.—Andean high-altitude (HA) natives have a low (blunted) hypoxic ventilatory response (HVR), lower effective alveolar ventilation, and lower ventilation (VE) at rest and during exercise compared with acclimatized newcomers to HA. Despite blunted chemosensitivity and hypoventilation, Andeans maintain comparable arterial O₂ saturation (Sa_{O₂}). This study was designed to evaluate the influence of ancestry on these trait differences. At sea level, we measured the HVR in both acute (HVR-A) and sustained (HVR-S) hypoxia in a sample of 32 male Peruvians of mainly Quechua and Spanish origins who were born and raised at sea level. We also measured resting and exercise VE after 10–12 h of exposure to altitude at 4,338 m. Native American ancestry proportion (NAAP) was assessed for each individual using a panel of 80 ancestry-informative molecular markers (AIMs). NAAP was inversely related to HVR-S after 10 min of isocapnic hypoxia ($r = -0.36$, $P = 0.04$) but was not associated with HVR-A. In addition, NAAP was inversely related to exercise VE ($r = -0.50$, $P = 0.005$) and ventilatory equivalent (VE/ $\dot{V}O_2$, $r = -0.51$, $P = 0.004$) measured at 4,338 m. Thus Quechua ancestry may partly explain the well-known blunted HVR (10, 35, 36, 57, 62) at least to sustained hypoxia, and the relative exercise hypoventilation at altitude of Andeans compared with European controls. Lower HVR-S and exercise VE could reflect improved gas exchange and/or attenuated chemoreflex sensitivity with increasing NAAP. On the basis of these ancestry associations and on the fact that developmental effects were completely controlled by study design, we suggest both a genetic basis and an evolutionary origin for these traits in Quechua.

genetic markers; admixture; arterial saturation; Andes

THE HYPOXIC VENTILATORY RESPONSE (HVR) is the increase in ventilation (VE) that occurs when arterial blood oxygen partial pressure Po₂ decreases (60). Typically, on acute exposure to hypoxia, VE increases to a peak in the first few minutes and thereafter declines somewhat to a level higher than control depending on the level of hypoxia (13, 49). This attenuation of VE has been termed the hypoxic ventilatory decline (HVD), and it is usually assessed by protocols that give a sustained hypoxic exposure greater than ~10 min. The sustained vs. acute response has not been systematically evaluated in An-

dean highland natives, but groups from this region appear to have a lower or “blunted” acute HVR and a lower effective alveolar ventilation compared with high altitude (HA)-acclimatized control groups from the lowlands (10, 35, 36, 43, 57, 61, 62). Andeans also have lower VE during exercise at HA compared with acclimatized lowland controls (7, 32, 55, 66). These traits may be unique to Andeans, as many studies show normal HVR and higher VE in natives of the Himalayan plateau (2, 21, 25, 29, 75). However, little is known about the underlying genetic and/or environmental basis for ventilatory trait differences between populations. One possibility is that there are genes at which allele frequencies differ between populations, as both HVR and VE are under genetic control (68). The purpose of this study was to evaluate whether ventilatory traits were related to Quechua ancestry. The latter was quantified using a panel of 80 ancestry-informative genetic markers (AIMs) to give estimates of Native American ancestry proportion (NAAP), European ancestry proportion (EAP), and West African ancestry proportion (AAP) for each individual in the study. This represents a relatively new strategy to understand complex trait architecture as significant associations can arise between ancestry proportions and a specific physiological trait, even if the markers informative for ancestry are not physically linked to the trait in question (41, 47, 59). The approach has been used recently to reveal ancestry associations with some complex disease traits in admixed populations (15, 23, 41, 72), although it should be recognized that such associations reveal nothing direct about causal genetic mechanisms or evolutionary origins. Another limitation of this approach is that physiological traits can be sensitive to environmental effects, and it is well known that ventilatory control and pulmonary gas exchange systems are affected by developmental exposure to hypobaric hypoxia (17, 10, 70, 20, 63). For this reason, we studied Peruvian subjects who were born and raised at sea level (Lima, Peru). These subjects were the children of previous migrants from the highlands to lowlands, and none had ever been to HA. The influence of NAAP on the HVR was assessed by giving subjects at sea level both short (5 min) and long (20 min) protocols of isocapnic hypoxia. Thus we distinguish between the ventilatory response to both acute (HVR-A) and sustained (HVR-S) hypoxia, respectively. In addition, we evaluated the influence of ancestry on the VE at rest and during submaximal exercise after these subjects were transported to

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the city of Cerro de Pasco at 4,338 m. In this location, subjects were tested after ~10–12 h of continuous exposure to hypobaric hypoxia.

MATERIALS AND METHODS

Subjects and study design. The subjects for this study were 32 young males (18–35 yr) who gave written informed consent according to guidelines approved by the Institutional Review Boards at the University at Albany, State University of New York, and the Universidad Cayetano Heredia, Lima, Peru. Female subjects were also studied, but data are not reported here as reproductive hormone levels were not controlled for and these have large effects on ventilatory measures (44). Subjects were identified as nonsmokers and screened via a brief clinical history and medical examination for conditions contraindicating participation in the study protocols, including chronic obstructive respiratory diseases, cardiovascular disease, and renal disease. At screening, a venous blood sample was drawn from the antecubital vein for later genetic analyses. Hemoglobin [Hb] concentration, in grams per deciliter, was immediately determined by a Hemocue blood hemoglobin analyzer (Angelholm, Sweden). Subjects with [Hb] less than the sex-specific cutoff values for anemia were excluded from the study. The subjects were recruited from within a specific district of Lima, Peru, (Barrios Altos district, population ~150,000). In this district, ~10% of inhabitants are recent downmigrants from highland Peru. Individuals accepted into the study were born and raised in Lima or near sea level, and both sets of their parents and grandparents were born at an altitude >3,000 m. Thus all study subjects were second-generation down migrants, with no developmental exposure to HA. The majority of subjects described themselves as “Peruvians” but acknowledged both their Quechua and Spanish origins.

Ventilatory control studies were conducted in Lima only. Further exercise studies were conducted within 2 wk at 4,338 m in the town of Cerro de Pasco, Peru. Cerro de Pasco is a 8- to 10-h bus ride from Lima on a paved road. The first ~4–6 h of the trip involves a steady gain in altitude to a high mountain pass (~4,800 m). The road then descends to the Peruvian Altiplano (3,600–4,300 m) for the next 3–4 h of the trip. Subjects arrived in Cerro de Pasco from Lima in groups of 4–5 per day over a 2-wk period, and rested in the laboratory for 2–4 h before studies were initiated. Thus studies in Cerro de Pasco were conducted after 10–12 h of acute exposure to hypobaric hypoxia. One of the 32 subjects did not make the trip to Cerro de Pasco for personal reasons. One subject complained of headache and nausea, and resting measures only were obtained before the subject was returned to Lima.

Anthropometry and pulmonary function. Standard anthropometry was performed on each subject by the same investigator. Measurements included height, weight, and skinfolds at subscapular, suprailliac, biceps, and triceps sites. Body density was calculated according to age- and sex-specific equations given by Durnin and Womersley (12). Kashiwazaki et al. (30) have tested the validity of a number of reference equations against doubly labeled water measures of body composition in Bolivian Aymara and conclude that the reference equations above are the best available for use in Andean native populations. Percent body fat was calculated from the Siri equation. Forced vital capacity (FVC, ml BTPS) was assessed on each subject in Cerro de Pasco using a VS400 Volumetric Spirometer (Puritan-Bennett, Mallinckrodt, Hazelwood, MO) calibrated daily with a three liter calibration syringe. Each subject performed a maximal inspiration, followed immediately by a forced maximal expiration while in a standing position. FVC was determined based on the highest of at least two efforts, and values were corrected for BTPS.

Genetic markers. Individual admixture proportions were estimated using a panel of 80 AIMs. Initially, we genotyped 24 markers (MID-575, FY-null, F13B, TSC1102055, WI-11153, GC, SGC30610, WI-17163, WI-9231, WI-4019, LPL, WI-11909, D11S429, TYR-192, DRD2 TaqD, DRD2 BclI, OCA2, WI-14319, CYP19, PV92, WI-

7423, CKM, MID-161, and MID-93), which we have already described in previous publications (3, 59). To increase the precision of the individual admixture estimates, we characterized 56 additional AIMs in the sample National Center for Biotechnology Information dbSNP rs# identifiers for single nucleotide polymorphisms: rs2225251, rs725667, rs963170, rs723822, rs725416, rs1506069, rs1861498, rs1063, rs1435090, rs1350462, rs1344870, rs768324, rs1465648, rs2317212, rs1316579, rs719776, rs951784, rs1112828, rs1403454, rs1461227, rs2077681, rs1935946, rs1881826, rs2396676, rs2341823, rs1320892, rs983271, rs1373302, rs1808089, rs1987956, rs1928415, rs1980888, rs1327805, rs951308, rs1594335, rs2207782, rs1891760, rs2366882, rs714857, rs1487214, rs726391, rs708156, rs717091, rs1900099, rs1152537, rs724729, rs1153849, rs2351254, rs764679, rs1074075, rs717962, rs1369290, rs386569, rs718092, rs718387 and rs878825). These markers were selected from the Affymetrix GeneChip Mapping 10K Array (58). The 80 AIMs included in the final panel are characterized by high-frequency differences between populations from West Africa, Europe, and the Americas (Native Americans), and are extremely informative for determining ancestry proportions at the population or individual level. Of the 80 markers, 58 have frequency differences higher than 30% between Native American and European populations, 61 have frequency differences higher than 30% between Native American and West African populations, and 47 show differences in frequency higher than 30% between European and West African populations. This panel comprises 79 diallelic markers (75 single nucleotide polymorphisms (SNPs), 3 small insertion/deletions and one Alu polymorphism) and one triallelic marker (GC). The markers are distributed along all autosomal chromosomes (chromosomes 1 to 22). Within each chromosome, the physical distances between markers are quite variable, but with the exception of the markers DRD2 Taq D-DRD2 BclI (located at a distance of 4.6 Kb on chromosome 11) and the markers rs386569 and CKM (located at a distance of 23 Kb on chromosome 19), the intermarker distances are higher than 0.5 Mb.

Genotyping. For the initial panel of 24 AIMs, SNPs, and insertion/deletion markers were scored by a melting curve assay (McSNP), in which the target sequence containing the SNP or insertion/deletion polymorphism is amplified by PCR using a mismatched primer where necessary to create an artificial restriction site polymorphism. PCR products were digested with a restriction enzyme and the resulting restriction fragment length polymorphisms were scored by their melting curves in a Hybaid DASH machine (ThermoHybaid, UK). Further details about this genotyping method are provided by Akey et al. (1). PCR products of the PV92 Alu insertion polymorphism were scored by conventional 2% agarose gel electrophoresis. Genotyping of the additional panel of 56 AIMs was outsourced to the company KBiosciences (Herts, UK).

Estimation of individual admixture proportions. Usually, to estimate ancestry proportions in admixed individuals, samples representing the contributing parental populations are used as a contrasting reference. In this case, although there is information on the allele frequencies for the panel of 80 AIMs in several European and West African populations, no such information is available for the Quechua parental population. Thus, to estimate admixture we have used a strategy in which information on all parental frequencies is not required. We have used the program STRUCTURE v. 2.1, developed by Jonathan Pritchard (14, 50), to infer admixture proportions in the study sample. This program has been designed to infer the presence of genetic structure and to estimate the admixture proportions in samples with unknown genetic structure. STRUCTURE can be used to estimate the number of populations present in a sample and to assign individuals to each of those populations, including estimates of individual admixture from each population. To estimate admixture in our sample, we prepared an input file that included the genotype data for the samples from Lima and 45 additional samples from Spain (not reported here). We then ran the STRUCTURE program using the linkage model with $K = 2$ as the predefined setting for the number of

populations, using 30,000 iterations for the burn-in period and 70,000 additional iterations to obtain parameter estimates. The output file provides an estimate of the NAAP (Quechua) and EAP (Spanish) for each individual in the sample and also the 90% confidence interval of the ancestry estimates. The model with $K = 2$ is based on the history of the Andean region, in which the main parental contributions have been from European (mainly from Spain) and Native American populations. However, in many regions in the Americas, there has also been a substantial West African contribution. For this reason, we also estimated admixture proportions using a model with three parental populations (Native Americans, Europeans, and West Africans). We prepared an input file with the genotypes of the individuals from Lima, as well as individuals from Spain and West Africa, and we ran the STRUCTURE program using a model with $K = 3$ as the number of populations. Additionally, individual ancestry proportions were also estimated using an alternative computer program, ADMIXMAP. ADMIXMAP is a general-purpose program for modeling admixture, which uses genotype data on a sample of individuals from an admixed population. The program is extremely flexible: It can handle linked or unlinked markers and the program can be run without specifying the allele frequencies of the parental populations or alternatively with ancestry-specific allele frequencies specified either as fixed or as random variables. Additional information about this program can be found in Hoggart et al. (27) and at <http://www.ucd.ie/conway/Uploads/admix2.pdf>. As with STRUCTURE, we ran ADMIXMAP using a model with two parental populations, using 5,000 iterations for the burn-in period and 45,000 iterations to obtain parameter estimates. Ancestry-specific allele frequencies for the European parental population were specified as random variables (0.5 plus allele counts of each allele in the Spanish sample). Given that information on parental frequencies is not available for the Quechua population, we specified prior parameters of 0.5 for each allele ("reference prior"). We also ran the program using a model with three parental populations, in which we specified European and West African ancestry-specific allele frequencies as random variables and Quechua ancestry-specific allele frequencies as a reference prior. All models yield continuous variable estimates of ancestry proportion, which range from 0 to 1, reflecting the contribution of two (or three) different population histories to the genetic makeup of an individual.

Ventilatory control studies. Ventilatory control studies were conducted in Lima only. In a preliminary procedure, the subjects' end-tidal carbon dioxide partial pressure (P_{ETCO_2}) and end-tidal oxygen partial pressure (P_{ETO_2}) were determined using a fine nasal catheter, so as to disturb the subject as little as possible. An instantaneous value for the respiratory quotient was calculated to ensure that the subject was not hyperventilating due to anxiety with the protocol. After the preliminary procedures had been completed, the subjects undertook two protocols administered at least 1 h apart, both of which involved imposing certain profiles for P_{ETCO_2} and P_{ETO_2} using an end-tidal forcing system (see below). *Protocol 1* was administered first and was designed to assess the HVR-A by a short, progressive, stepwise induction of hypoxia. This protocol was devised by Mou et al. (45) and validated further by Zhang and Robbins (74). It is sufficiently brief so as to minimize the confounding effects of HVD on the HVR-A. Throughout the protocol the P_{ETCO_2} was held at ~ 2 Torr above the subjects' natural resting value. A settling period of 5 min was used during which the P_{ETCO_2} was held at 100 Torr. After this settling period, P_{ETO_2} was lowered stepwise in a series of seven steps from 100 to 45 Torr, with each step lasting for 50 s. Values for P_{ETO_2} for the five intervening steps had been calculated so as to provide approximately even reductions in SaO_2 between steps and, consequently, approximately even increases in VE between steps. *Protocol 2* was designed to assess the ventilatory response to sustained (20 min) hypoxia (HVR-S) and the HVD. P_{ETO_2} was again held at 100 Torr for the first 10 min (euoxia), then dropped to 50 Torr for the next 20 min (hypoxia), and finally returned to 100 Torr again for a final 10

min. The first minutes of hypoxia during *protocol 2* also provide an alternate measure of the HVR-A.

Apparatus and techniques. In both *protocols 1* and *2*, the technique of end-tidal forcing was used to generate the desired profiles in P_{ETCO_2} and P_{ETO_2} . In this technique, a computational model of the cardiorespiratory system and gas stores is first used to calculate the profiles for inspiratory PCO_2 and PO_2 that are likely to generate the desired P_{ETCO_2} and P_{ETO_2} . Once these values have been calculated, a computer connected to a fast gas-mixing system is used to generate the inspiratory gas mixtures. The experiment starts with the computer mixing the predicted inspiratory gas mixtures. In general, these predicted inspiratory gas mixtures will not of themselves generate the desired end-tidal values with sufficient precision because the physiology of the individual deviates from the assumptions of the cardiorespiratory model. To overcome this, these predicted inspiratory values are modified during the course of the experiment by using breath-by-breath feedback from the measured P_{ETCO_2} and P_{ETO_2} . These measured values of P_{ETCO_2} and P_{ETO_2} are compared with the desired values, and an integral-proportional feedback control algorithm is used to calculate the actual adjustments required for the inspiratory PCO_2 and PO_2 . Details of the forcing procedure and the gas-mixing system used in Peru have been described previously (20, 53). In all experiments, the subject sat upright and breathed to and from a gas-mixing chamber via a mouthpiece while wearing a nose clip. A pulse oximeter monitored SaO_2 (Ohmeda 5740). Respiratory volumes were measured using a turbine volume-measuring device (VMM 400, Interface Associates, Laguna Niguel, CA) fixed in series with the mouthpiece. Gas was sampled continuously from the mixing chamber (100 ml) close to the mouth at a rate of 20 ml/min and analyzed using a gas analyzer (Datex Normocap 200-Oxy, Meda SA). All experimental data were recorded in real time by computer at a sampling rate of 50 Hz using real-time data acquisition software written in LabView (National Instruments, Austin, TX).

Ventilatory control data processing. In *protocol 1*, numerical values for the HVR were calculated in three different ways. For HVR-A₁, the average values for ventilation (VE in BTPS units) and P_{ETO_2} were calculated over the last 20 s for each of the seven steps. The average values for P_{ETO_2} were converted into calculated values for arterial saturation using the equation of Severinghaus (56), and a slope term (HVR-A₁, $l \cdot \text{min}^{-1} \cdot \%^{-1}$) was calculated for each individual via linear regression for the relationship between VE and desaturation. This measure of HVR is nearly identical to the calculated "A-shape parameter" described in the literature (see Ref. 71). HVR-A₂ was calculated in similar fashion, but instead of using the calculated SaO_2 values, the measured values of SaO_2 from pulse oximetry were used. Finally, the A-shape parameter, which appears frequently in the literature on HVR, was calculated from the source equation (see Ref. 71) as follows: VE (BTPS) was related to P_{ETO_2} via the equation $VE = VE^\circ + A/(P_{ETO_2} - 32)$, where VE° is the ventilation asymptote, 32 is the P_{ETO_2} asymptote, and A is the shape parameter that reflects the acute hypoxic ventilatory response.

In *protocol 2*, which lasted 40 min total, minute averages were calculated for VE, P_{ETCO_2} , P_{ETO_2} , and the SaO_2 signal from pulse oximetry. According to conventions established previously (20), four 1-min periods were used to calculate the magnitude of the rapid component of the ventilatory response with the onset of hypoxia (ON response), the magnitude of the rapid component of the ventilatory decrease when subjects were returned to euoxia (OFF-response), and the magnitude of the HVD. These included the last minute of the first euoxic period (prehypoxic VE) occurring at *minute 10*, the second minute of the hypoxic period (peak VE) occurring at *minute 12*, the last minute of the hypoxic period (depressed VE) occurring at *minute 30*, and the second minute of the second euoxic period (posthypoxic VE) occurring at *minute 32*. These points are easily visualized on Fig. 1, which is presented in the RESULTS section. The ON response was calculated as the difference in VE between *minutes 10* and *12*, the OFF-response was calculated as the difference between *minutes 30*

and 32, and the HVD was obtained as the difference between *minutes* 12 and 30.

As described, *protocol 2* was also designed to assess the HVR to sustained hypoxia. Four HVR-S measures were constructed representing the ventilatory response measured at different times during the 20-min hypoxic period. HVR-S₁ is a slope term ($l \cdot \text{min}^{-1} \cdot \%^{-1}$) that was calculated via linear regression using minute average data from the past 5 min of the first euoxic period and the first 5 min of the hypoxia period. Thus the measure captures the ventilatory response as measured from average baseline through the first 5 min of hypoxic exposure. In this regard, HVR-S₁ is an alternate measure of HVR-A, as both capture the ventilatory response to a short (~5 min) bout of hypoxia. HVR-S₂, HVR-S₃, and HVR-S₄ were calculated in similar fashion, but using data from subsequent 5-min periods of hypoxic exposure, that is, HVR-S₂ using data from *minutes* 6–10 of hypoxia, HVR-S₃ using data from *minutes* 11–15 of hypoxia, and HVR-S₄ using data from *minutes* 16–20 of hypoxia. Because of ventilatory rolloff, that is, HVD, it was expected that HVR-S₁ > HVR-S₂ > HVR-S₃ > HVR-S₄, and this was what was found (see Table 2). Multiple measures of HVR-S allow assessment of the changing slope of the VE-Sa_O₂ relationship as hypoxia continues.

Exercise studies. Exercise studies were conducted in Cerro de Pasco. Previously published maximal oxygen consumption data from Lima are also presented here to demonstrate that cardiorespiratory fitness was not a confounding factor relative to the respiratory control studies (5). Again, subjects were tested in Cerro de Pasco after 10–12 h of exposure to 4,338 m. To begin, \dot{V}_{O_2} and Sa_O₂ were measured at rest (5 min) with the subject seated. After resting measurements were taken, \dot{V}_{O_2} was measured during two steady-state workloads on a mechanically braked Monarch 818e research ergometer. Subjects started with a workload of 1.0 kg resistance and were encouraged to maintain a cadence of 60–70 rpm (*Work 1*, 60–70 W). After 3 min, the work resistance was incremented by 0.5 kg for an additional 3 min (*Work 2*, 90–100 W). *Work 1* and *Work 2* reached a mean of $53 \pm 8\%$ and $67 \pm 9\%$ of subjects measured $\dot{V}_{O_2 \text{ max}}$ in Cerro de Pasco (5).

During testing, subjects breathed through a low-resistance breathing valve (Model 2700, Hans Rudolph, Kansas City, MO). Expired ventilation (VE, converted to l/min BTPS), as well as the fractional concentrations of O₂ and CO₂ in expired air were processed by a Parvo-medics True Max metabolic measuring system (Sandy, UT). This produced 1-min interval calculations of \dot{V}_{O_2} (l/min) and carbon dioxide production (\dot{V}_{CO_2} , l/min). These data were used to calculate the ventilatory equivalent for oxygen, VE/ \dot{V}_{O_2} . Gas analyzers were calibrated with standard gases before each exercise test. The pneumotachometer (Hans Rudolph, Kansas City, MO) used to measure ventilatory flow was also calibrated before each test with a 3-liter calibration syringe. Heart rate (HR) was continuously monitored via telemetry (Polar Electric Oy, Sweden) interfaced with the metabolic measuring system. Arterial oxygen saturation by pulse oximetry (Sa_O₂) was continuously monitored by an Ohmeda 5740 pulse oximeter using a finger-tip sensor. The pulse oximetry signal was acquired by an REM/400M data acquisition system (CB Sciences, Dover, NH) and recorded every 15 s during \dot{V}_{O_2} measurements.

Statistics and variable transformations. All variables were evaluated for normality using the Kolmogorov-Smirnov test against a standard normal distribution using the Lilliefors two-tail probability. Relationships with NAAP were evaluated by linear regression and by repeated-measures ANOVA using various general linear model procedures of the SPSS statistical software package, version 10.0. Mean values are expressed as means \pm SD unless otherwise indicated. Statistical significance criteria was $P < 0.05$ for all tests.

RESULTS

Subjects. Characteristics of the study population, including ancestry estimates, are given in Table 1. Ancestry estimates from STRUCTURE and ADMIXMAP were highly correlated

Table 1. *Subject characteristics and relationships with Native American ancestry proportion*

Variable	Value	P Value*
Age, yr	25.3 \pm 4.2	0.48
Height, cm	163.8 \pm 6.1	0.96
Weight, kg	64.6 \pm 10.7	0.86
[HB], g/dl	14.9 \pm 0.9	0.38
$\dot{V}_{O_2 \text{ max}}$ at sea level, l/min	2.48 \pm 0.32	0.09
Body fat percent, %	21.8 \pm 6.2	0.69
FVC, l/min	4.85 \pm 0.50	0.91
BSA, m ²	1.70 \pm 0.15	0.89
NAAP _{ADMIXMAP}	0.85 \pm 0.10	N/A
EAP _{ADMIXMAP}	0.14 \pm 0.10	<0.001
AAP _{ADMIXMAP}	0.01 \pm 0.02	0.58
NAAP _{STRUCTURE}	0.87 \pm 0.12	<0.001
EAP _{STRUCTURE}	0.10 \pm 0.12	<0.001
AAP _{STRUCTURE}	0.02 \pm 0.04	0.41

Values are expressed as means \pm SD. *P value from the regression of a given variable on NAAP_{ADMIXMAP}; [HB], hemoglobin concentration; $\dot{V}_{O_2 \text{ max}}$, maximal oxygen consumption; FVC, forced vital capacity; BSA, body surface area; NAAP, Native American ancestry proportion; EAP, European ancestry proportion; AAP, West African ancestry proportion; N/A, not applicable. STRUCTURE and ADMIXMAP suffixes refer to the different models used to estimate ancestry proportions, as described in the text.

($r > 0.95$), and ancestry proportions from both models are given in Table 1. Regression of NAAP from ADMIXMAP (NAAP_{ADMIXMAP}) on NAAP from STRUCTURE (NAAP_{STRUCTURE}) yielded $r = 0.99$, beta (slope) 0.85, and intercept 0.10. Thus the two models do produce slightly different mean values of ancestry proportion. However, the rank order of individual NAAPs within the sample was minimally affected by model choice, and study results were similar no matter which estimates (STRUCTURE or ADMIXMAP) were used. Similarly, 2-parental vs. 3-parental models yielded highly correlated measures of both NAAP and EAP ($r > 0.98$), especially as AAP was very low in the sample, that is, mean AAP from ADMIXMAP was 0.01 ± 0.02 . Only three individuals showed some evidence of AAP, which could confound a 2-population analysis, but their inclusion/exclusion in the analyses had no effect on overall study results. Results are presented using NAAP_{ADMIXMAP} as the study-independent variable using a 3-parental model. This was the preferred variable because, as described, ADMIXMAP uses parental allele frequencies instead of genotype frequencies (for which there are more missing data), and it can handle tightly linked markers. NAAP was selected over EAP, as the former had a more clearly normal distribution. However, again, study results were similar no matter which ancestry estimate (NAAP or EAP) was used. Mean NAAP_{ADMIXMAP} (given in Table 1) was relatively high at 85% but ranged from a low of 60% to a high of 98%. NAAP_{ADMIXMAP} was not significantly related to any subject characteristic, including age, height, weight, [Hb], sea-level $\dot{V}_{O_2 \text{ max}}$, percent body fat, FVC, or body surface area (BSA).

Ventilatory response. Table 2 gives calculated ventilatory control measures from sea level testing. The mean control value for PETCO₂ for each protocol is given, and these did not differ between protocols. Mean values of HVR-A₁ and HVR-A₂ were nearly identical from *protocol 1*, and these were similar to HVR-S values from *protocol 2*. Mean minute VE \pm SE, PETO₂, and PETCO₂ during the sustained protocol are given in Fig. 1 to demonstrate the time course of the HVR and the

Table 2. Mean values of hypoxic ventilatory response measures and relationships with Native American ancestry proportion

	Value	Regression parameters*		
		β	r^2	P value
<i>Protocol 1: Acute hypoxia</i>				
PET _{CO₂} , mmHg	41.0±1.7	—	—	N/A
HVR-A ₁ , l·min ⁻¹ ·%SaO ₂ ⁻¹	1.14±0.75	—	—	0.87
HVR-A ₂ , l·min ⁻¹ ·%SaO ₂ ⁻¹	1.15±0.75	—	—	0.79
A-shape parameter, units	322±215	—	—	0.85
<i>Protocol 2: Sustained hypoxia</i>				
PET _{CO₂} , mmHg	41.3±1.7	—	—	N/A
ON-response, l/min	11.3±8.4	—	—	0.90
OFF-response, l/min	8.6±5.3	—	—	0.18
HVD, l/min	1.4±6.6	—	—	0.22
HVR-S ₁ , l·min ⁻¹ ·%SaO ₂ ⁻¹	1.36±0.97	—	—	0.43
HVR-S ₂ , l·min ⁻¹ ·%SaO ₂ ⁻¹	1.20±0.84	—	—	0.28
HVR-S ₃ , l·min ⁻¹ ·%SaO ₂ ⁻¹	1.09±0.80	—	—	0.11
HVR-S ₄ , l·min ⁻¹ ·%SaO ₂ ⁻¹	0.95±0.58	-2.04	0.13	0.04

*From the linear regression of a given variable on NAAP. β and r^2 values are given only when $P < 0.05$. PET_{CO₂}, end-tidal partial pressure for carbon dioxide. See text for details on the specific HVR measures.

maintenance of strict isocapnia. As expected, HVR-S values decreased with increasing time of hypoxia, reflecting the attenuation of VE over time. There were no significant relationships between NAAP and HVR parameters, except for parameters that reflected exposure to hypoxia longer than 10 min. That is, HVR-S₄ (minutes 16–20 of hypoxia, protocol 2) was significantly lower in subjects with high NAAP ($r = -0.36$, $P = 0.04$, Table 2 and Fig. 2), while HVR-S₃ (minutes 11–15 of hypoxia) trended lower with increasing NAAP ($P = 0.11$). Parameters for the regression of NAAP on HVR-S₄ are given in Table 2, and the significant association between these two variables persisted in multivariable models that controlled for age, height, weight, FVC, $\dot{V}O_{2\max}$, and BSA. In a repeated measures analysis across all HVR-S measures, there were no significant NAAP-by-time interaction effects.

Exercise testing at high altitude. Exercise response variables measured at 4,338 m are given in Table 3. On the basis of repeated-measures ANOVA, there were no significant relationships of NAAP with resting and exercise $\dot{V}O_2$, $\dot{V}CO_2$, HR, or SaO₂, although P reached 0.07 for SaO₂. Additionally, there were no significant NAAP-by-exercise level interactions. In contrast, both VE and VE/ $\dot{V}O_2$ were significantly lower with increasing NAAP ($P = 0.007$ and $P = 0.013$, respectively). These effects on ventilation also persisted in multivariable models that controlled for age, height, weight, FVC, $\dot{V}O_{2\max}$, and BSA. When the P value for the repeated-measures ANOVA between subjects effect (i.e., NAAP) was less than 0.05, we also conducted analyses for each exercise level individually, and regression parameters are given in Table 3 for VE and VE/ $\dot{V}O_2$. From these results, it can be seen that the association between NAAP and VE or VE/ $\dot{V}O_2$ is only significant during exercise, not at rest. The β -regression parameters given in Table 3 represent the change in exercise response per unit change in NAAP, or specifically, the decrease in exercise VE as NAAP went from 0 to 1. However, β describes a linear model, and care should be taken in extrapolating across a full range of NAAP, as our subjects spanned less than half of this range, that is, 60–98%. As shown in Fig. 3, exercise VE at work level 1 was ~12 l/min or ~20% lower in subjects with

the highest vs. lowest NAAP ($r = -0.50$, $P = 0.005$), and a similar difference was seen at work level 2 ($r = -0.43$, $P = 0.019$, not shown).

HVR-S₃ measured at sea level was positively related to resting but not exercise VE at altitude ($r = 0.40$, $P = 0.027$, Fig. 4) and VE/ $\dot{V}O_2$ ($r = 0.39$, $P = 0.030$, not shown). Additionally, both HVR-S₃ and HVR-S₄ tended to be positively related to exercise SaO₂ at 4,338 m, although these associations were weak and not significant in most comparisons. However, what these relationships do suggest is the possibility that NAAP affects VE at altitude through modulation of respiratory control. To explore this possibility, post hoc multivariable models were tested with resting and exercise altitude VE and SaO₂ as dependent variables, and HVR-S measures and NAAP as independent variables. In general, introduction of one independent variable (NAAP or HVR-S) had little or no influence on the effect (or lack of effect) of the other variable. This was true at rest and during exercise, and for different models incorporating one or both HVR-S₃ and HVR-S₄. Thus, although NAAP was associated with both lower HVR-S and lower exercise VE at altitude, multivariable analyses did not support the hypothesis that the ventilatory control system mediates the effect of ancestry to explain the maintenance of normal SaO₂ despite low VE in Quechua at rest and during exercise.

DISCUSSION

In sea-level studies with a group of admixed Peruvians who were born and raised at sea-level, we detected a significant inverse relationship between NAAP and the HVR measured after 10 min of sustained isocapnic hypoxia (i.e., HVR-S₃ (trend $P = 0.11$) and HVR-S₄ ($P = 0.04$))³ but no association of NAAP with measures of the acute HVR. In addition, we also

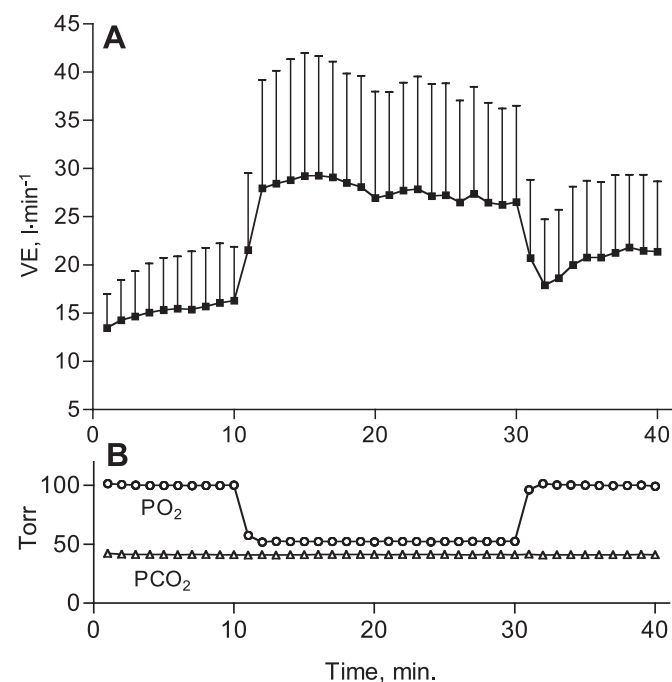


Fig. 1. Mean \pm SD of ventilation (VE, l/min; A) and end-tidal PO₂ (PET_{O₂}, torr; B) and end-tidal PCO₂ (PET_{CO₂}, Torr) during sustained hypoxia measured at sea level (i.e., protocol 2).

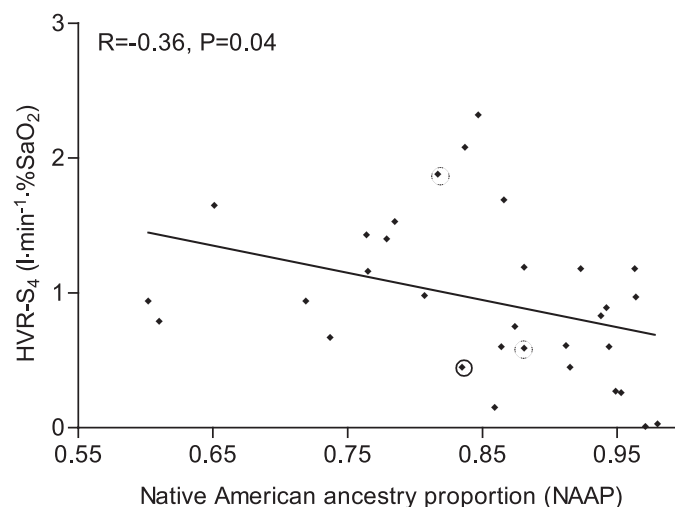


Fig. 2. Significant negative relationship between Native American ancestry proportion (NAAP) and the ventilatory response to sustained hypoxia measured between minutes 16–20 of isocapnic hypoxia (i.e., HVR-S₄, l·min⁻¹·%SaO₂⁻¹). Individuals with detectable African ancestry proportion (AAP) are identified by circles around plot symbols, but inclusion/exclusion of these subjects had little effect on the relationship shown.

detected an inverse relationship between NAAP and the exercise VE when subjects were exposed to hypobaric hypoxia at 4,338 m. Regression analyses suggest that subjects with predominantly Quechua ancestry (NAAP greater than ~0.90) have ~50% lower HVR-S₄ measured at sea level, and ~20% lower exercise VE ($P = 0.007$, Fig. 3) and $VE/\dot{V}O_2$ ($P = 0.013$) measured at altitude compared with subjects with relatively high EAP (>0.35) and low NAAP (<0.65). Thus Quechua ancestry may be partly responsible for the well-known blunted HVR (10, 35, 36, 57, 62) and the relative exercise hypoventilation at altitude (7, 32, 55, 66) of Andeans compared with European controls. From the novel study design, we infer both a population genetic basis and an evolutionary origin for these trait differences. The genetic inference depends on interpretation of the ancestry estimates, a topic that is treated more fully in the DISCUSSION. The genetic inference also depends on the fact that subjects were born and raised at sea level, effectively eliminating the possibility of developmental effects in response to hypobaric hypoxia. This is an important consideration because developmental effects are a major confounding factor in human evolutionary studies (6), and more specifically, such effects could be a major confounder vis-à-vis the well-known plasticity of the respiratory control system (39). The evolutionary inference derives from the well-documented history of exposure of past Andean populations to hypobaric hypoxia, although the direct benefit (if any) of blunted HVR and/or low VE is not immediately apparent. The latter makes it difficult to ascribe trait differences directly to natural selection, although possible scenarios are discussed below.

It is important to understand our use of ancestry proportion as a study-independent variable. Beginning in the early 1500s, Native American, European (mostly Spanish), and West African populations came into contact in the New World. Descendant populations now people the various nations of Latin America and show a wide range of admixture depending on which populations are sampled. For example, in Mexico, the estimated mean Spanish admixture proportion is between 28

and 71%, depending on the region of the country and on socioeconomic status (22, 40). Our estimates of ancestry proportions differ from older estimates in the literature because they are based on a much larger number of AIMs, making it possible to obtain precise estimates of admixture both at the group and at the individual level (5). In a three-parental admixture model, ancestry describes the proportionate contribution of each parental group to the genetic makeup of a person. The 80 AIMs used to derive the ancestry estimate were selected because they differed greatly in the frequency of specific alleles between the three parental groups as validated by genotyping diverse samples of Native Americans, Europeans, and West Africans. With this as background, the important point is that trait associations with NAAP are to be interpreted relative to population history, not relative to the specific genetic markers used to derive the estimates. In other words, significant associations can arise between ancestry proportion and a specific trait, even if the markers informative for ancestry are not physically linked to the trait in question (41, 47, 59). This is especially true as the markers that we used represented only a small fraction of the total genome. Associating traits

Table 3. Exercise response variables for study subjects at 4,338 meters and relationships with Native American ancestry proportion

	Value	Repeated Measures P Value*	Regression Parameters†	
			β	P value
$\dot{V}O_2$, l/min				
Rest	0.38±0.05	0.645	—	—
W1	1.30±0.10	—	—	—
W2	1.65±0.10	—	—	—
$\dot{V}O_2$, l·min ⁻¹ ·kg ⁻¹				
Rest	5.9±0.8	0.783	—	—
W1	20.1±2.9	—	—	—
W2	25.6±3.5	—	—	—
$\dot{V}CO_2$, l/min				
Rest	0.29±0.05	0.499	—	—
W1	1.05±0.09	—	—	—
W2	1.43±0.10	—	—	—
VE, l/min				
BTPS				
Rest	13.5±3.1	0.007	-6.9	0.223
W1	41.4±5.9	—	-28.0	0.005
W2	57.0±7.8	—	-31.0	0.019
$VE/\dot{V}O_2$				
Rest	35.7±7.4	0.013	-19.0	0.153
W1	31.9±3.7	—	-18.0	0.004
W2	34.5±4.1	—	-15.9	0.025
HR, bpm				
Rest	87.4±12.0	0.443	—	—
W1	131.1±14.9	—	—	—
W2	151.5±16.2	—	—	—
SaO ₂ , %				
Rest	83.1±4.1	0.070	—	—
W1	79.0±4.5	—	—	—
W2	78.1±4.8	—	—	—

*Significance of NAAP effect (between subjects effect) from repeated-measures ANOVA (Rest, W1 = submaximal work level 1, and W2 = submaximal work level 2). †From the regression of a given exercise parameter on NAAP; β and P values are only presented when $P < 0.05$ from repeated-measures ANOVA, above. $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; VE, expired ventilation; $VE/\dot{V}O_2$, ventilatory equivalent for oxygen; HR, heart rate in beats per minute (bpm); SaO₂, arterial hemoglobin saturation by pulse oximetry. W1 and W2 were at 53 ± 8% and 67 ± 9%, respectively, for subjects $\dot{V}O_{2max}$ at 4,338 m.

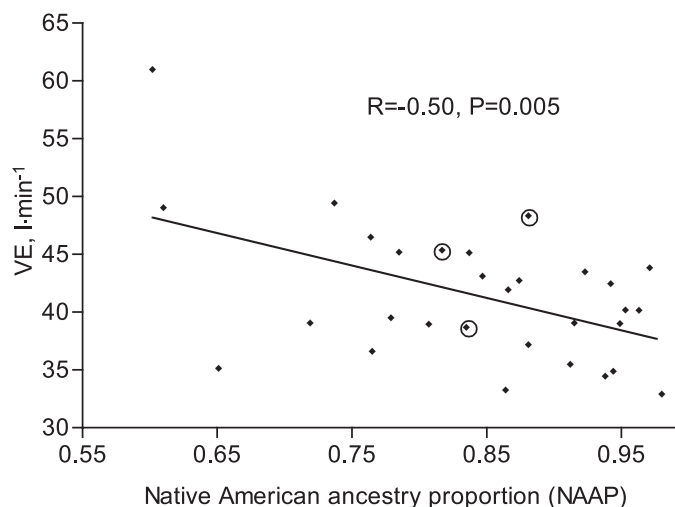


Fig. 3. Significant negative relationship between NAAP and the VE (l/min) at submaximal work level 1 (~ 70 W) when subjects exercised at 4,338 m. Individuals with detectable African ancestry proportion (AAP) are identified by circles around plot symbols, but inclusion/exclusion of these subjects had little effect on the relationship shown.

with ancestry proportion in an admixed population represents a relatively new strategy to understand complex trait architecture. This approach has been used recently to reveal ancestry associations with some complex disease traits in admixed populations (15, 23, 41, 72), as well as ancestry associations with the $\dot{V}O_{2\max}$ decrement in Quechua (5). Admixture may also be exploited in future studies to map genes involved in complex traits or diseases that have a genetic basis and are known to differ significantly between parental populations using an approach known as admixture mapping (28, 48).

Another important consideration is the methodology that we used to measure the HVR. Interpretation of HVR between studies is difficult due to variability in protocol length and CO_2 control. Both of our protocols maintained strict isocapnia on a breath-by-breath basis (see Fig. 1) and thus allowed evaluation of the ventilatory response to hypoxia as mediated by the peripheral chemoreceptors. In addition, we administered two protocols to take into account the time course of the HVR, an issue not well appreciated in earlier studies of Andeans. In adult humans, during 20–25 min of either poikilocapnic or isocapnic hypoxia, stimulation of the peripheral chemoreceptors causes VE to increase to a peak in the first few minutes (49). After the first few minutes, VE typically declines, that is, HVD, although it remains higher than control levels depending on the level of hypoxia (13). The HVD is the subject of much current investigation (49, 52), and although not well understood, one very basic issue is that the development of HVD may confound the interpretation of the acute HVR depending on the length of the protocol (45). Many previous reports describing the blunted Andean HVR administered protocols that were long enough for HVD to develop, and as a consequence, these studies may actually be describing a blunted response to a sustained rather than acute exposure to hypoxia.

In this study, NAAP was only associated with the HVR to sustained hypoxia. However, we may not have had sufficient study power to detect an association with the acute response. The HVR-A is more variable than the sustained response, and the current power to detect an effect of NAAP explaining

$\sim 10\%$ of the variability in HVR-A was only about 0.45. Further, only HVR-S was predictive of resting VE levels after ~ 12 h at 4,338 m (see Fig. 4), and no HVR measure was predictive of the exercise VE. The latter finding is not unusual, as previous studies show only weak or no correlations between HVR and measures of ventilatory and/or exercise performance at altitude (54, 60). However, NAAP was clearly associated with exercise but not resting VE, and for exercise, it is likely that gas exchange issues predominate (19, 67). Thus high NAAP may be independently associated with both attenuated chemoreflex sensitivity and improved gas exchange efficiency, the latter reflected in the lower exercise VE and $VE/\dot{V}O_2$ at altitude. This is supported by multivariable models, which failed to detect a mediating effect of HVR-S on the strong negative relationship between NAAP and the exercise VE. In addition, a large number of studies directly or indirectly support the hypothesis that Andeans have superior pulmonary gas-exchange at altitude, including studies showing larger pulmonary volumes (8, 18, 24), greater carbon monoxide diffusion capacities (51, 65), smaller alveolar-arterial partial pressure differences (33), and larger lung O_2 diffusion capacities during exercise at altitude (66). Interestingly, we did not detect an association between NAAP and FVC adjusting for body size within our sample. This included a much larger sample of highland- and lowland-born subjects reported in a previous paper (4). However, relative lung size is not a good proxy for pulmonary function, and so the hypothesis of NAAP and gas exchange remains to be directly tested.

Previous studies are uniform in suggesting the blunted chemosensitivity of Andeans at altitude (2, 10, 11, 36, 43, 57, 61), although as described, protocols vary widely. Despite this, there is no consensus on the origin of this blunting. A number (16, 70), but not all (62), early studies in Colorado showed that blunting could be acquired from lifelong exposure to hypobaric hypoxia. For Andeans specifically, one previous study suggested that blunting was acquired and became apparent after the age of 12 (35), while another study showed no increase in blunting from adolescence into old age in a large cross-

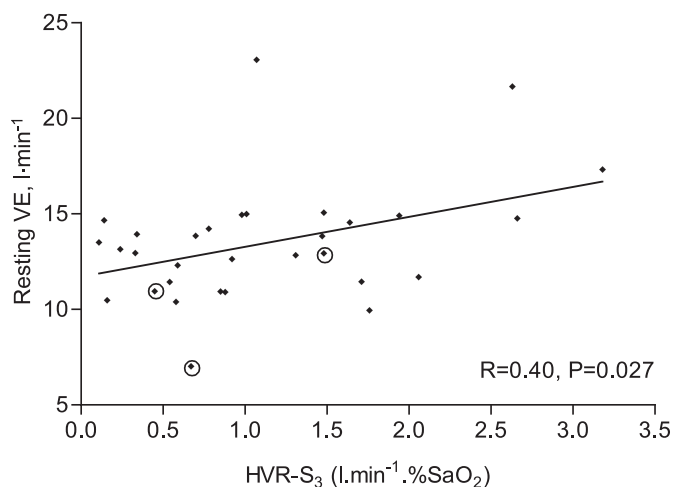


Fig. 4. Significant positive relationship between the ventilatory response to sustained hypoxia between minutes 11 and 15 of isocapnic hypoxia measured at sea level, that is, HVR-S₃ ($l \cdot min^{-1} \cdot \%SaO_2^{-1}$) and the resting VE (l/min) at 4,338 m. Individuals with detectable African ancestry proportion (AAP) are identified by circles around plot symbols, but inclusion/exclusion of these subjects had little effect on the relationship shown.

sectional sample (2). There is also conflict on the issue of the reversibility/irreversibility of blunting in adults who have returned to sea level. Older studies showed that chemosensitivity was not reversed by residence at sea level (36, 61, 64), whereas more recent studies in the Andes show at least partial reversibility of blunting in Peruvians born at HA but migrating to sea level (20, 63). Interestingly, the acute HVR may be more reversible than the HVD (20). Peruvian highlanders who migrate to sea level, compared with lowland controls, have 70% and 30% of the acute HVR and HVD, respectively. The latter suggests that the acute HVR is more labile to environmental effects compared with the sustained HVR, and this may be another reason why NAAP was only associated with the sustained response in this study.

From the studies referenced, blunting would seem to be at least partially acquired and at least partially reversible (42, 60). Thus the importance of environmental effects should not be dismissed. However, as indicated, there is no consensus in the literature on the duration and timing of exposure necessary to produce or reverse the blunting effect (9, 16, 35, 61, 70). As a consequence, there is little understanding of how phenotypic plasticity contributes to ventilatory trait variability between Andeans and other populations. In this regard, it is again worth noting that most (2, 21, 25, 29, 75), but not all (34, 37), studies of Tibetans and Sherpa show a normal HVR and high VE despite lifelong exposure to HA. Thus regional differences could arise because of differences in the developmental response to hypoxia due to underlying population genetic differences, that is, gene-environment interaction.

Given the long-term history of Quechua in the Andes, an evolutionary origin of low HVR-S and exercise VE seems a reasonable hypothesis. However, to infer natural selection as the causal mechanism requires some consideration of adaptive benefit. One possibility is that there is energetic benefit due to the reduced cost of breathing with hypoventilation. While long-term energetic benefit has not been demonstrated, there is a larger evolutionary context that makes this an interesting possibility. Faced with hypoxia, many species, for example, aquatic diving mammals, reduce total body metabolic rate (26). Weil (69) has speculated that the HVD, in particular, may represent a vestige of this diving response with a possible mediating role for the neurotransmitter GABA. GABA depresses ventilation and metabolism (31, 38, 73), and the numerous GABA-receptor polymorphisms have been widely studied in various other contexts (e.g., Ref. 46). However, we are unaware of any candidate gene studies of GABA in Andeans, and so this remains highly speculative. It should also be considered that there may be long-term costs rather than benefits of a blunted HVR-S and hypoventilation. For example, chronic mountain sickness is often associated with blunted chemosensitivity (39, 57). The alternate evolutionary explanation is that blunted HVR-S and hypoventilation are not adaptations per se, but rather that one or both of these traits result from structural/functional adaptation in another physiological system. One possibility is that evolutionary adaptations have improved pulmonary gas exchange in Andeans with secondary consequences on measured ventilatory traits. Indeed, any adaptation further down the oxygen transport chain that improves O₂ carrying capacity, O₂ delivery, O₂ extraction, and/or the metabolic efficiency of O₂ use, would in theory permit lower VE.

In summary, this study provides the first direct evidence that ventilatory traits, probably unique to Andeans, have a population genetic basis. Our quantification of ancestry as an independent variable has led us to infer both a genetic mechanism and an evolutionary origin for these traits. However, the evolutionary context is elusive in part because the full causal spectrum of gene and environment effects on these traits is unknown.

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