ELSEVIER

Comparative Biochemistry and Physiology, Part A 140 (2005) 349–362

CBP

www.elsevier.com/locate/cbpa

The acute hypoxic ventilatory response: Testing the adaptive significance in human populations

John S. Terblanche[a,*], Krystal A. Tolley[b], Andreas Fahlman[a], Kathryn H. Myburgh[a], Sue Jackson[a]

[a]*Department of Physiological Sciences, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
[b]*Centre for Invasion Biology, University of Stellenbosch, Stellenbosch, South Africa*

Received 8 July 2004; received in revised form 23 January 2005; accepted 24 January 2005

Abstract

The acute Hypoxic Ventilatory Response (HVR) is an important component of human hypoxia tolerance, hence presumably physiological adaptation to high altitude. We measured the isocapnic HVR (L min^{-1} $\%^{-1}$) in two genetically divergent low altitude southern African populations. The HVR does not differ between African Xhosas (X) and Caucasians (C) (X:−0.34±0.36; C:−0.42±0.33; $P>0.34$), but breathing patterns do. Among all Xhosa subjects, size-independent tidal volume was smaller (X: 0.75±0.20; C: 1.11±0.32 L; $P<0.01$), breathing frequency higher (X: 22.2±5.7; C: 14.3±4.2 breaths min^{-1}; $P<0.01$) and hypoxic oxygen saturation lower than among Caucasians (X: 78.4±4.7%; C: 81.7±4.7%; $P<0.05$). The results remained significant if subjects from Xhosa and Caucasian groups were matched for gender, body mass index and menstrual cycle phase in the case of females. The latter also employed distinct breathing patterns between populations in normoxia. High repeatability (intra-class correlation coefficient) of the HVR in both populations (0.77–0.87) demonstrates that one of the prerequisites for natural selection, consistent between-individual variation, is met. Finally, we explore possible relationships between inter-population genetic distances and HVR differences among Xhosa, European, Aymara Amerindians, Tibetan and Chinese populations. Inter-population differences in the HVR are not attributable to genetic distance (Mantel Z-test, $P=0.59$). The results of this study add novel support for the hypothesis that differences in the HVR, should they be found between other human populations, may reflect adaptation to hypoxia rather than genetic divergence through time.

Keywords: Evolution; Hypoxia tolerance; Low-altitude population; Repeatability; Variation; Ventilatory adaptation

1. Introduction

Inter-population comparisons of human ventilatory responses to hypoxia help elucidate the history of our species' adaptation to high altitude (HA). For example, many studies indicate that Tibetans ventilate more (volume per unit time) than Andean high-altitude natives residing at similar altitudes (see reviews by Beall, 2000; Moore, 2000; Hochachka and Monge, 2000; Hochachka and Somero, 2002), and the ventilation response to acute hypoxia (HVR) may be significantly greater among Himalayan than among Andean residents at ~4200 m (Beall et al., 1997a; Moore, 2000). Comparisons between low and high altitude populations (e.g. Lahiri et al., 1970; Zhuang et al., 1993; Curran et al., 1997) have added support to the possibility that between-population variation in the HVR reflects adaptation to local environmental (hypoxic) conditions (but see Vargas et al., 1998). However, the generality of such differences may not apply when all available studies are collectively analysed. For example, Moore (2000) regards the HVR differences between Tibetan than Andean high-altitude natives as unresolved.

Demonstrations that phenotypic differences in ventilatory sensitivity among populations arise from natural selection, reflecting genotypic adaptations to hypoxia, are generally a

* Corresponding author. S.P.A.C.E. Group, Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. Tel.: +27 21 808 2605; fax: +27 21 808 2405.
E-mail address: jst@sun.ac.za (J.S. Terblanche).

1095-6433/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2005.01.016

result of laboratory selection experiments in mouse and rat strains (see reviews by Soutiere and Tankersley, 2001; Powell, 2003; and see Garland, 2001; Bennett, 2003). The prerequisites for a physiological trait to be responsive to natural selection, defined as differential reproduction and survival, are 1) consistent between-individual variation, 2) heritability, and 3) a link between fitness and the trait of interest (Endler, 1986), and are seldom demonstrated, particularly in human populations. Where these prerequisites are not met, conclusions about the mechanisms of human adaptation to hypoxia should remain tentative.

1.1. Human hypoxia responses

Human adaptations to high altitude occur at a variety of spatial and temporal scales (Hochachka and Monge, 2000; Hochachka and Somero, 2002; Lahiri et al., 2002). Upon transfer from low to high altitude, the physiological responses to hypoxia that occur at various sub-cellular, cellular, and whole-organism levels (e.g. Sarkar et al., 2003) all potentially contribute to overall phenotypic flexibility *sensu* Piersma and Drent (2003). Such mechanisms include direct stimulation of oxygen sensors in the carotid bodies (Lahiri, 2000), that increase ventilation over short and longer time scales (acute versus chronic HVR) and is often followed by a gradual decline to higher than baseline levels (ventilatory acclimatization; see Powell et al., 1998). An important recent advance in human biology has been the recognition of tissue-level hypoxia sensing, and hence of the multi-dimensional nature of hypoxic responses incorporating both central and peripheral sensors (Lahiri, 2000; Cherniack, 2004).

Such responses may also include haematological changes, such as a reduction in the hypoxic pulmonary vasoconstrictor response that is mediated by pulmonary vasculature oxygen sensors (Archer et al., 2000; Semenza et al., 1991); increased erythropoietin secretion stimulating red blood cell production (Samaja, 1997); increased expression of vascular endothelial growth factor 1, angiogenesis, and subsequent expansion of blood volume (Winslow and Monge, 1997). Conversely, hypoxia may elicit decreased body water content (Westerterp et al., 1996) and consequently a reduction in blood volume and increased blood viscosity. Changes in ventilation also influence blood pH (Reeves et al., 1993; Sato et al., 1996; but see Clement et al., 1995), possibly a major source of variation between individuals (Sahn et al., 1977). Increased use of oxygen-sparing glucose rather than fatty acids as metabolic fuel in heart muscle has been advanced as a long-term adaptation to altitude (Hochachka et al., 1996; Hochachka and Somero, 2002).

Inter-population comparisons have identified four phenotypic traits that are potentially responsive to lifelong exposure to hypoxia, showing different trends in different high-altitude populations. Haemoglobin concentration is higher than that of sea level residents in some Andean groups, but not among Tibetans or Ethiopians (Beall et al., 1998; Beall et al., 2002). Resting minute ventilation is higher among Tibetans than among the Aymara (Beall et al., 1997a), as is the HVR (Beall, 2000). Finally, arterial oxygen saturation (SaO_2) appears higher among Andean natives than among Tibetans below 4000 m, but this trend is reversed above 4300 m (Beall et al., 1997b; Beall, 2000). Both Tibetan and Andean residents show lower SaO_2 distributions than do Ethiopians measured at 3530 m, whose values correspond to sea level SaO_2 values for US residents (Beall et al., 2002). Our knowledge of ventilatory adaptations has arisen from a substantial literature on high-altitude residents in three primary regions (Moore, 2000): the Himalaya (Sherpas and Himalayans), the Andes (Amerindians), and European descendents living in the North American Rockies. Although East Africans are recognised as one of the three main HA populations in the world (Hochachka et al., 1999; Beall et al., 2002; Hochachka and Somero, 2002), ventilatory adaptations to hypoxia among all Africans have been poorly studied (Niermayer et al., 2001; but see Cristosomo et al., 1998). A recent study (Beall et al., 2002) was the first to examine haematology in Ethiopians resident at the highest inhabited altitudes on this continent.

Which of these four phenotypic traits is demonstrably subject to natural selection? Among humans, family and twin studies show that the HVR is strongly influenced by genetic factors (e.g. Scoggin et al., 1978; Kawakami et al., 1981; Nishimura et al., 1991; Akiyama et al., 1991; Thomas et al., 1993; Kobayashi et al., 1993; Weil, 2003; Powell, 2003), although identification of candidate genes and their environmental interactions is difficult (Weil, 2003). It has been suggested that the HVR is phylogenetically constrained (Hochachka and Somero, 2002), and it is significantly heritable in Tibetan and Aymara populations (Beall et al., 1997a). Among mouse and rat strains, variation in minute ventilation's two components (tidal volume and breathing frequency) in normoxia, hypoxia and hypercapnia suggests different genetic bases of ventilation control (Han and Strohl, 2000; Fagan and Weil, 2001; Tankersley, 2003). It may, therefore, be argued that the HVR is significantly heritable.

Assessing the remaining prerequisites for natural selection, namely linking the HVR to evolutionary fitness, and demonstrating consistent between-individual variation in the HVR is difficult, particularly for the former pre-requisite, and both have not been well examined in humans. Two other phenotypic traits potentially link hypoxia adaptation with fitness: intrauterine growth restriction (Moore et al., 2001; Moore, 2003), and the prevalence of Chronic Mountain Sickness in high altitude residents (Monge et al., 2001). However, there may be long-standing difficulties associated with resolving the link to fitness in human populations, and study design for inter-population comparisons of physiological adaptation to hypoxic environments is generally complex (see Brutsaert, 2001). High within-

individual variability in the HVR (Sahn et al., 1977; Sato et al., 1996; Zhang and Robbins, 2000; Terblanche et al., 2004) may outweigh consistent between-individual variation, thereby excluding the possibility that natural selection is responsible for the origin and maintenance of inter-population variation in the HVR. Therefore, determining repeatability of the HVR is a worthwhile goal since it is relatively simple and may provide valuable insight into the adaptive potential of this trait.

1.2. Aims

By measuring the acute isocapnic hypoxic ventilatory (HVR) and cardiovascular responses, we aimed to determine non-invasively whether two sedentary South African populations, both residing at similar low altitudes and living under similar environmental conditions, differ in their ventilatory and cardiovascular hypoxic sensitivity. These two low-altitude populations will allow us to establish baseline values for subsequent comparisons with high-altitude groups in East Africa. Second, we aimed to estimate repeatability of the HVR in these two populations, thereby testing the prerequisite of consistent between-individual variation in a trait, for adaptations resulting from natural selection. Third, we provide an assessment of the phylogenetic dependence of the HVR, investigating whether inter-population differences in this trait are purely correlated with genetic distance. For this component of our study, we extracted data on genetic distances and HVR from the literature for three populations: Han Chinese (Zhuang et al., 1993), Tibetans (Zhuang et al., 1993; Beall et al., 1997a), and Andeans (Beall et al., 1997a) and compared these with literature values on genetic distance with the HVR measured for Europeans and Xhosa Africans.

2. Methods

2.1. Subjects

As part of a larger family study, 63 individuals from 20 South African families (10 Caucasian, and 10 Xhosa) participated voluntarily in the study. All test procedures were fully explained to each person, verbally and in written form, before he or she signed a consent form. Under-age subjects signed a consent form in the presence of their parent. Ethical approval for all procedures was granted by the Sub-Committee C of the Research Committee of the University of Stellenbosch, which conforms to the internationally accepted ethical guidelines detailed in the Declaration of Helsinki.

2.2. Family Criteria

Families invited to participate in the study had a minimum of two children and one parent. The oldest parent was younger than 70 years and the youngest of the children was 15 years old. All individuals had low activity lifestyles, had never participated in national or international sports, and lived at sea level in the same town (~50 m asl). For Xhosa-speaking families, the family for at least two previous generations was of only Xhosa origin. No families were included if they were not both Xhosa-speaking and self-declared to be of Xhosa origin. Both autosomal and Y-chromosome DNA studies reveal that in general, but especially in the Xhosa-speaking people, South African Bantu-speaking groups cluster according to their linguistic groupings (Lane et al., 2002), thereby supporting our use of language as an indicator of genetic distinctness between populations. Matching of subjects' height and mass was not possible between populations due to differences in population anthropometry and morphology (see e.g. van de Wal et al., 1970; Johnston et al., 1987) particularly in the case of females, and instead, statistical approaches which account for such differences were incorporated specifically to handle this aspect of the study (see statistical analyses below).

2.3. Questionnaires

Each subject completed a questionnaire (assisted by a translator when necessary). No subjects were classified as having an altitude history (having been born at an altitude of greater than 3000 m), although some Caucasian subjects (n=3) had prior exposure to acute hypoxia (as may be seen in mountaineers or pilots). All subjects were born at altitudes less than 1500 m and had lived at sea level in Stellenbosch for more than 11 years. The previous occurrence of respiratory (e.g. asthma) or haematological/cardiovascular (such as anaemia) disorders was noted, and whether these were treated or untreated. Smokers and non-smokers were recorded. The phase of the menstrual cycle (follicular or luteal) was noted for female subjects, as was the absence of menstruation in subjects who were post-menopausal, breastfeeding or using injectable contraceptives (e.g. Depo-provera® Pharmacia and Upjohn, Kalamazoo, MI, USA).

2.4. Protocol

During exposure to hypoxia, isocapnic eucapnia was maintained during hyperventilation using the non-rebreathing method described in Fahlman et al. (2002) and Terblanche et al. (2004). All experimental recordings were performed by a single investigator in order to reduce variation as a result of observational differences between investigators.

Before the study, the subjects each completed one or two preliminary experiments involving normoxic and hypoxic exposures identical to those in the actual experiments, for familiarisation with the breathing circuit and the study protocol. Data from preliminary experiments were used only to calculate coefficients of variation, not in any other

analyses. Experiments were conducted on each subject three to five times, including familiarisation tests, and were separated by at least 60 min. Subjects were asked to refrain from drinking alcohol and caffeine-containing beverages from the evening before the experiments, as these factors are known to affect ventilatory chemosensitivity (D'Urzo et al., 1990). During each test the HVR (L min^{-1} $\%^{-1}$) was measured using a previously-described protocol (Fahlman et al., 2002), but with a single hypoxic exposure per test (see Terblanche et al., 2004).

Expired volume ($\dot{V}_E$, L min^{-1}, STPD), tidal volume, (V_T, L, STPD), and f_R (breaths min^{-1}) was sampled by a metabolic system (MetaMax™, Cortex Biophysik, Leipzig, Germany) and average values recorded every 10 s. The end-tidal CO_2 partial pressure ($P_{ET}CO_2$ at body temperature, pressure and saturation, BTPS) was sampled by a capnograph (Microstream™, Microcap, Oridion Medical, Jerusalem, Israel) and average values recorded every 5 s. All values were converted to BTPS prior to analyses and are presented as such.

For the first 5 min subjects breathed air (21% O_2, balance N_2). The resting $\dot{V}_E$ and $P_{ET}CO_2$ were averaged during the last 2 min of this period. If these two variables were not stable, the initial period was extended until values stabilized. Inspired gas was then switched instantaneously to 8.2% O_2 ($\pm$0.3%, n=3 bottles) for 120 s, then back to air (21$\pm$0.2% O_2, n=7 bottles) for another 120 s while the subject was monitored to ensure full recovery of all ventilatory and cardiovascular parameters to resting levels. The use of only one hypoxic exposure per test eliminated the possibility of acute HVD (Powell et al., 1998). Total experimental time was at least 9 min, with each test comprising an initial resting phase (N_1) of 5 min or more (of which only the last 2 min were used for analyses), 2 min of hypoxia (H), and 2 min of normoxia (N_2). Tests were at least 60 min apart. Each subject's $P_{ET}CO_2$ was maintained at normocapnic levels ($\pm$1 mmHg), established during the last 2 min of N_1.

2.5. Arterial O_2 saturation (SaO_2) and heart rate (f_H)

SaO_2 (%) and f_H (beats min^{-1}) were measured using a pulse oximeter (Nellcor N-395 Pulse Oximeter, Mallinkrodt, St Louis, MO, USA) with a forehead sensor (Nellcor RS10, Mallinkrodt, St Louis, MO, USA). The area of application of the sensor was massaged with a mild capsaicin ointment (0.25/100 g, Sloan Heat Rub, Warner-Lambert, South Africa), approximately 2 min before attachment, to promote surface blood flow. Analogue signals from the oximeter were relayed to the metabolic system, which recorded SaO_2 and f_H every 10 s.

2.6. Data processing

Resting values of $\dot{V}_E$, SaO_2, V_T, f_R and f_H for each subject were calculated as means for the final 120 s of N_1 (data points, n=22$\pm$1), except in the case of $P_{ET}CO_2$ where the last 60 s were used (data points, n=20$\pm$1). For all variables during the hypoxic exposure, a 30 s period was used (H; data points, n=7$\pm$2).

2.7. Calculations

All variables representative of normoxia and hypoxia were averaged during the last 120 s of N_1 and 30 s of H, respectively. Averages of two test values (excluding the familiarisation tests) were used, unless the coefficient of variation (CV) of the calculated HVR values between the two experiments was greater than 26% (see Sahn et al., 1977; Zhang and Robbins, 2000), in which case a third experiment was performed and the median of the three test values used to calculate population means for all variables. All estimates of HVR were performed by a single investigator in order to reduce observer-induced variation which may affect the calculation of repeatability (Krebs, 1999).

HVR and HHR were estimated as the change in $\dot{V}_E$ or f_H, respectively, per 1% change in SaO_2 from normoxia to hypoxia (HVR: $\Delta\dot{V}_E$ ΔSaO_2^{-1}, L min^{-1} $\%^{-1}$; HHR: Δf_H ΔSaO_2^{-1}, heart beats min^{-1} $\%^{-1}$).

The repeatability (R) that we estimate is not the coefficient of repeatability defined by Bland and Altman (1986), but rather a measure used in quantitative genetics, calculated as R=(s^2 between individuals)/(s^2 between individuals+s^2 within individuals) where s^2=variance (Falconer and Mackay, 1996; Krebs, 1999). R ranges between 1 and 0, and when close to 1 suggests that experimental measurements are precise and that most variation is partitioned between individuals. Because R represents inter-individual variation, traits with a high R are those most likely to respond to natural selection (Dohm, 2002), and their identification is therefore useful to evolutionary physiologists interested in adaptation to environmental conditions. Providing that repeated measures of a trait have equal variances, and that the repeated measures of a parameter assess exactly the same underlying genetic trait itself, R provides an estimate of the maximum possible heritability of a trait (Dohm, 2002). Dohm (2002) provides an excellent summary of the assumptions between heritability and repeatability, and discusses various cases in which repeatability may not set the upper limit to heritability. In brief, these are when a) measured traits are not genetically identical, b) common environmental effects work in opposition to direct genetic effects, c) the temporary environments for each trait are negatively correlated, d) significant genotype-environment interaction is present, or e) the traits are influenced by maternal effects. Thus, R offers comparative and evolutionary physiologists a conceptually simple way to identify traits that are likely to have a strong genetic basis. A low R suggests either experimental error, or a strong influence of environment on the trait in question, and/or low heritability for that trait (Falconer and Mackay, 1996; Krebs, 1999). Confidence limits (Krebs,

1999) of mean repeatability estimates were calculated for all parameters.

2.8. Statistical analysis

All data are reported as means±1 standard deviation (SD), unless otherwise specified. Categorical data from questionnaires were compared between the two populations using chi-square and two-tailed Student's *t*-tests, or Mann–Whitney *U*-tests if data proved to be non-normal (Zar, 1996). Likewise, initial paired comparisons between N_1 and H using two-tailed Student's *t*-tests or Mann–Whitney *U*-tests explored the effects of isocapnic hypoxia on respiratory variables in all subjects. For each subject, averages (n=2) or medians of data (n=3) were used to assess differences between and within the groups (Winer et al., 1991). Each subject is therefore represented by one value (n=30 for X, and n=33 for C). Misrepresentation of a subject's HVR caused by one extreme value is thus minimized. For calculation of R, data from all tests were used.

Stepwise multiple regressions isolated suitable covariates for analyses of covariance (ANCOVA), and general linear model (GLM) analyses and ANCOVA were then used to take into account the effects of body size or gender on ventilatory and other variables. We used NCSS 2000 (Kaysville, UT) for all analyses, with significance set at P<0.05 unless otherwise stated.

2.9. Inter-population comparison: global HVR analysis

Inter-population comparisons of physiological factors may be confounded by phylogenetic relationships (Harvey and Pagel, 1993). As a pre-requisite to such analyses, it is instructive to examine whether HVR variation is significantly related to genetic distance among populations. We did so using a distance matrix correlation between genetic distance and HVR "distance". The test we used has been used to assess the correspondence between metric distances in medicine, ecology, anthropology, and population genetics (e.g. Jackson and Somers, 1989; Manly, 1986; Waddle, 1994). Here, we use it to test whether or not there is a significant association between published HVR values and Nei's genetic distance, among five regional populations (Xhosa, European, Tibetan, Aymara, and Han Chinese). Pairwise genetic distances for these five populations were taken from estimates of a study of world-wide genetic relationships of 42 human populations using classical genetic markers and averaged over 128 allozyme loci (Cavalli-Sforza et al., 1994, Table 2.3.1B). This source treats Amerindians as a single, monophyletic group. Indeed, genetic differences between populations within this group, such as the Quechua and Aymara, are minor (Gene et al., 2000) relative to global differences among continental (Asians, Africans, Europeans) population groups (Zhivotovsky et al., 2003). HVR values for Andeans were measured in the Aymara (Beall et al., 1997a). Pairwise HVR "distances" were estimated by taking the differences in HVR between each pair of populations. Where multiple HVR values were available, this pairwise difference was calculated from a single median value for each population (e.g. in the case of Tibetan HVR data provided by both Beall et al., 1997a,b and Zhuang et al., 1993). We did not use studies that did not supply the information required to convert the 'A parameter' units into HVR values comparable with our own (L min^{-1} $\%^{-1}$). A permutation procedure (10,000 randomisations—for rationale see Jackson and Somers, 1989) was conducted for the two distance matrices (Mantel for Windows, M.J. Cavalcanti, 2000, http://life.bio.sunysb.edu/morph/). Departure from the null model would demonstrate a correspondence between genetic and HVR distances. A non-significant relationship would suggest that there is not necessarily an association between HVR distance and genetic distance for these regional populations. In the latter case, HVR should be independent of phylogeny. Although differences in HVR protocol may influence comparisons between studies, there are at present insufficient population studies at global scales from which to select those which only use similar methods to obtain HVR estimates. For this preliminary analysis which we hope will soon be augmented by addition of more populations, we have therefore chosen to disregard methodological differences between studies in favour of using representative samples for each population.

3. Results

3.1. Subject characteristics, anthropometry and gender

There were significantly more smokers among Caucasians than among Xhosas (61% and 13%, respectively, χ^2 test, P<0.05). Across the entire data set, both populations had similar levels of respiratory disorders (9% and 13% in Caucasians and Xhosas, respectively). For analyses of the two subsets of females and males (see next paragraph), we excluded individuals with respiratory disorders, and females on contraceptives. Anthropomorphic variables differed among populations, not unexpectedly (van de Wal et al., 1970; Johnston et al., 1987). Males were taller than females, and for both genders, Caucasians were taller than Xhosas (Table 1). Among male subjects, Caucasians were significantly heavier than Xhosas, but among females, Caucasians' BMIs were significantly lower than those of Xhosas (Table 1).

Since height differed between the two groups, and height, mass and BMI differed between genders within the two groups, these factors were tested for their influence (covariance) on all variables using correlations and comparisons of statistical significance. Across the entire data set, there were no significant interactions (GLM/ANCOVA) between gender, height, mass, BMI, or population, and

Table 1
Characteristics of Xhosa (X) and Caucasian (C) subjects

Group	Age (years)	Height (m)	Mass (kg)	BMI (kg m^{-2})
C (all) (n=33)	32.9±15.4	1.79±0.09	73.5±10.9	23.0±2.44
X (all) (n=30)	27.4±12.3	1.59±0.07**	71.5±16.8	28.6±8.30†
C (♂) (n=23)	30.5±15.2	1.83±0.06	64.8±4.90	22.7±1.83
X (♂) (n=5)	22.0±5.66	1.69±0.03**	60.5±8.51	21.3±3.51
C (♀) (n=10)	38.5±15.4	1.69±0.02	77.3±10.6	23.1±2.70
X (♀) (n=25)	28.4±13.1	1.57±0.01**	73.7±17.3	30.1±8.23†

Significant differences for each variable between Xhosa and Caucasian between subjects: **unpaired, two-tailed Student's t-test (P<0.01); or †Mann–Whitney U-test (P<0.05).

mean HVR. For analyses of the entire data set, we used mass- and/or height-independent analyses which specifically account for anthropometric differences within and among populations.

There were no significant correlations (both least squares and robust regression) between HVR values for parents, and mean HVR from the two sibling offspring of each parent. Environmental influences thus contributed substantially more to the variance in the HVR than did genetic factors. In all analyses reported below, family members were treated as separate individuals.

3.2. Inter-population variation of the hypoxic ventilatory response

Across all subjects, V_T, f_R, $\dot{V}_E$, and SaO_2 differed significantly between normoxic (N_1, N_2) and hypoxic (H) intervals, but $P_{ET}CO_2$ did not (means±S.E.: 34.1±0.51, 33.9±0.40 and 34.5±0.44 mmHg, respectively; $F_{2;413}$= 0.43; P>0.65). An acute isocapnic hypoxic ventilatory response was thus achieved for all subjects. Minute ventilation ($\dot{V}_E$) in both normoxia (N_1) and hypoxia (H) was normally distributed within both populations, and its variance did not differ between populations (Kolmogorov–Smirnov and F-tests, respectively, P>0.1). The same is true for normoxic $P_{ET}CO_2$ and $\dot{V}O_2$ (Table 2, P>0.3). Therefore, we considered both populations to be in a similar state of anxiety.

In order to balance comparisons between our two study populations as stringently as possible in terms of subject anthropometry, gender, and menstrual cycle phase, we performed separate comparisons of the HVR and its ventilatory components on a subset of data comprising five Caucasian and six Xhosa female subjects from each family, one individual per family, matched as closely as possible for body size and all in the follicular phase of the menstrual cycle (Table 3). We hereby hoped to remove the confounding effects of age, menstrual cycle phase, and lack of independence between individuals from the same family. We performed a similar analysis on a subset of five males from each population group, whom we were able to match better for BMI and for age (Table 4).

These analyses show that among both males and females (Tables 3 and 4), there is a significant positive relationship (least-squares regression) between V_T and $\dot{V}_E$, and height and mass. However, in most cases more variance in each of these dependent variables is explained by body mass index (BMI) than by height or mass separately. For example, BMI explains a significant majority of the variance in normoxic V_T and $\dot{V}_E$ in both male and female groups, and among females, BMI is the largest and significant determinant of variation in the HVR. Therefore, we considered analyses of covariance with BMI as covariate appropriate to remove the effect of subject body-size differences on the HVR and ventilatory variables. Breathing frequency (f_R) was independent of (and not linearly related to) both BMI and gender. In both hypoxia and normoxia, $\dot{V}_E$ and the HVR were similar among Xhosa and Caucasian females, but the components of the HVR differed: f_R was higher and V_T lower among Xhosa females than among Caucasians in both normoxia and hypoxia (Table 3). We were able to match Caucasian and Xhosa male subjects for both age and BMI (Table 4). Although there were not always differences during normoxia, Xhosa males showed higher f_R and lower V_T values during hypoxia than did Caucasians.

Similar trends were observed for our whole data set of 63 subjects (see Table 1 for subject characteristics), which trades a larger sample size for control of age, menstrual cycle phase and body mass index effects on ventilation and

Table 2
Absolute means (±1 S.D.) for hypoxic ventilatory (HVR) and heart rate (HHR) responses, normoxic oxygen consumption ($\dot{V}O_2$), and normoxic end-tidal PCO_2 ($P_{ET}CO_2$) in all Xhosa (X) and Caucasian (C) subjects

Group	HVR[a] (L min^{-1} $\%^{-1}$)	HHR[a] (beats min^{-1} $\%^{-1}$)	$\dot{V}O_2$[b](L min^{-1})	PET_{CO_2}[b] (mmHg)
C (n=33)	0.42±0.33	0.627±0.380	0.37±0.007	32.0±3.6
X (n=30)	0.34±0.36	0.918±0.462	0.36±0.007	31.5±4.2
P	>0.35	<0.01	>0.5	>0.5

[a] One-way ANOVA (HVR: $F_{1;\ 61}$=0.872; HHR: $F_{1;\ 61}$=7.57).
[b] Unpaired two-tailed Student's t-test.

Table 3
Subject characteristics, HVR (hypoxic ventilatory response) and its ventilatory components ($\dot{V}_E$=minute ventilation; f_R=breathing frequency; V_T=tidal volume) compared between a subset of young Caucasian and Xhosa females, one per family, all in the follicular phase of the menstrual cycle and matched as closely as was possible within our data set for body mass index (BMI)

	Caucasian	Xhosa	SS	*DF*	MS	*F*	*P*
HVR (L min^{-1} %$^{-1}$)[a]	−0.472±0.094	−0.350±0.086	0.040	1	0.0401	0.904	>0.37
Error			0.399	8	0.0443		
Age (years)[b]	26.0±3.5	20.1±3.3	133.05	1	133.05	2.025	>0.19
BMI (kg m^{-2})[b]	21.9±1.5	26.1±1.3	48.313	9	48.313	4.520	0.062
Hypoxia							
$\dot{V}_E$ (L min^{-1})[a]	14.0±1.3	15.1±1.3	2.842	1	2.8424	0.301	>0.60
V_T (L)[a]	0.99±0.09	0.60±0.08	0.242	1	0.2416	5.987	<0.05
Error			0.363	8	0.0404		
f_R (breaths min^{-1})[b]	12.0±1.8	21.0±1.7	303.90	1	303.90	24.27	<0.005
Normoxia							
$\dot{V}_E$ (L min^{-1})[a]	6.4±0.6	8.0±0.5	1.695	1	1.6947	0.975	>0.32
V_T (L)[a]	0.57±0.05	0.39±0.04	0.056	1	0.0562	12.55	<0.01
Error			0.089	8	0.0111		
f_R (breaths min^{-1})[b]	12.0±1.3	20.4±1.2	195.40	1	195.40	22.78	<0.01
N	5	6					

For each variable, comparisons between the two population groups are based on general linear models (or analyses of covariance, ANCOVA) with BMI as the covariate. All data are presented as means±S.E.M.
[a] ANCOVA with BMI as covariate.
[b] ANOVA.

ventilatory sensitivity. For this comparison, mean HVR did not differ between Xhosas and Caucasians ($F_{1;\ 61}$=0.872; P>0.35; Table 2). An *a posteriori* power analysis was performed to estimate the sample sizes that would be required to determine a significant difference in HVR between populations with a low probability of committing a Type II error. Using the population HVR means and associated variances that we obtained (Table 2) to calculate the effect size (0.42 vs. 0.34 L min^{-1} %$^{-1}$, effect size d=0.2285 α=0.05, and β=0.7) we found that a sample size of greater than 460 subjects would be necessary to detect any significant difference in HVR between Xhosas and Caucasians.

For the larger data set, regression analysis indicated that the BMI accounted for more variability in the HVR than did age, body mass or height successively (unpublished data, and see above). Subsequent general linear model analyses using BMI and gender as covariates also found no difference between the two populations ($F_{1;59}$=1.04, P>0.34). The HVR did not differ between Xhosa and Caucasian

Table 4
Subject characteristics, HVR (hypoxic ventilatory response) and its ventilatory components ($\dot{V}_E$=minute ventilation; f_R=breathing frequency; V_T=tidal volume) compared between a subset of young Caucasian and Xhosa males, one per family

	Caucasian	Xhosa	SS	*DF*	MS	*F*	*P*
HVR (L min^{-1} %$^{-1}$)[a]	−0.423±0.124	−0.143±0.124	0.192	1	0.192	2.514	>0.16
Error			0.533	7	0.076		
Age (years)[b]	21.4±2.1	22.0±2.2	0.900	1	0.900	0.040	>0.85
BMI (kg m^{-2})[b]	22.1±1.8	23.8±1.8	6.860	1	6.860	0.435	>0.53
Hypoxia							
$\dot{V}_E$ (L min^{-1})[a]	14.4±1.7	12.3±1.7	11.25	1	11.25	0.802	>0.40
V_T (L)[a]	1.21±0.07	0.64±0.07	0.794	1	0.794	30.465	<0.001
Error			0.182	7	0.026		
f_R (breaths min^{-1})[b]	11.9±2.5	20.2±2.5	172.09	1	172.09	5.554	<0.05
Normoxia							
$\dot{V}_E$ (L min^{-1})[a]	9.38±0.92	9.83±0.92	0.017	1	0.0173	0.003	>0.95
V_T (L)[a]	0.67±0.07	0.52±0.07	0.052	1	0.0515	1.967	>0.20
Error			0.183	7	0.026		
f_R (breaths min^{-1})[b]	14.0±2.8	18.9±2.8	58.544	1	58.544	1.495	>0.26
N	5	5					

Subjects were closely matched for body mass index (BMI) between Caucasian and Xhosa groups. All data are presented as means±S.E.M.
[a] ANCOVA with BMI as covariate.
[b] ANOVA.

males and females, nor did it differ among females tested in different phases of their menstrual cycle.

Repeatability of HVR and HHR (the intra-class correlations coefficient [or τ], Falconer and Mackay, 1996) did not differ significantly between populations (Fig. 1). For all individuals from both populations, the HVR and its primary components (SaO_2 and $\dot{V}_E$) are significantly repeatable (R=0.862, 0.633 and 0.731, respectively, P<0.05 in all cases; see Table 5). Separate calculation of R within each population and gender showed the same to be true of HVR for male Caucasians and for females in both populations (Table 6). For Xhosa males, repeatability was not significant, i.e. inter-individual variation was not significantly greater than intra-individual variation.

The Mantel test revealed no significant difference from the null model, suggesting a lack of correspondence between genetic distance and HVR distance for the five populations investigated (r=−0.143; P=0.59, Table 7). This indicates that among the human populations for which HVR values are available, there is no consistent relationship between genetic distance and the median population HVR (Fig. 2).

Baseline normoxic (N_1) SaO_2 did not differ between populations, but hypoxic SaO_2 was significantly lower among Xhosas (X: 78.4±4.7%; C: 81.7±4.7%; P<0.05). During N_2, the SaO_2 of both populations returned to levels similar to baseline N_1 (X: 99±2%; C: 98±4%, P>0.21).

3.3. Hypoxic heart rate response is higher among Xhosas than among Caucasians

For a comparison using our entire data set, heart rate (f_H, beats min^{-1}) was significantly higher among Xhosas during both N_1 (C: 73.1±10.2 beats min^{-1}; X: 78.2±11.1 beats min^{-1}; one-way ANOVA, $F_{1;\ 61}$=5.838; P<0.05) and H (C: 83.6±11.5 beats min^{-1}; X: 94.0±11.3 beats min^{-1}; one-way ANOVA, $F_{1;\ 61}$=25.328; P<0.01). No anthropometric variables proved to be significant covariates for this parameter. Hypoxic heart rate response (HHR, beats min^{-1} $\%^{-1}$) did not differ between genders (means±SEM, females: −0.802±0.075, males: −0.720±0.084, one-way ANOVA, $F_{1;\ 61}$=0.53, P>0.47), but was significantly lower among Xhosas (Table 2, P<0.01).

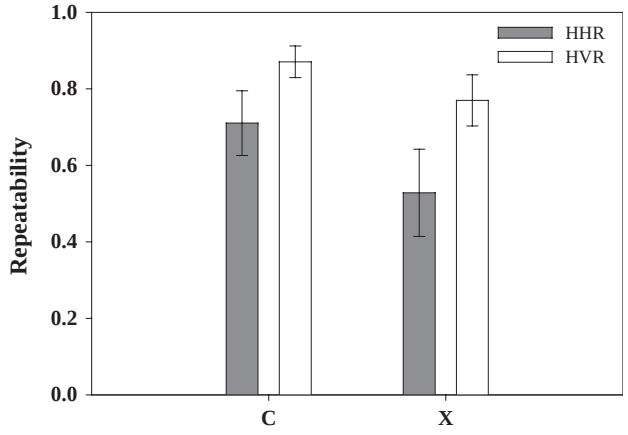

Fig. 1. Repeatability (±SE) of the HVR and HHR for Caucasian (C) and Xhosa (X) populations. Standard error of repeatability calculated according to Becker (1984).

Table 5
Analysis of variance for the HVR in Caucasian and Xhosa subjects

Source of variation	*DF*	Sums of squares	Mean squares	*F*-ratio	*P*
Among populations	1	0.407	0.407	3.23	>0.05
Within populations					
Xhosa	29	7.7414	0.2670	4.5233	<0.05
Caucasian	32	6.9820	0.2182	15.0711	<0.05

F-ratio=mean square among individuals/mean square within individuals; *DF*=degrees of freedom.

4. Discussion

4.1. Inter-population comparison of the HVR in low-altitude southern Africans

The similarity in baseline normoxic $\dot{V}O_2$ in our two study populations indicates similarity in resting metabolic rate. Normality of data distribution, equal variances and similar normoxic $\dot{V}_E$ and $P_{ET}CO_2$ were confirmed in subjects from both populations. Therefore, these populations probably have similar stimuli for ventilatory drive under sea level conditions. The HVR is influenced by higher brain inputs such as psychological factors (Shea, 1996). Conversations and behaviour before, during and after the tests indicated that subjects in both populations appeared comfortable with the test environment. Subjects were sampled randomly from within their communities and neither group contained any individuals who had participated previously in tests of hypercapnic or hypoxic ventilatory sensitivity. For the purposes of this study, we conclude that these two samples represented their respective populations adequately.

The two populations in this study presented similar levels of hypoxic sensitivity (HVR) even when specific body size-independent comparisons were performed, whether genders were separated or pooled. Our data provide us with a useful baseline for assessing hypoxic sensitivity in other African populations. Studies suggesting that high altitude (HA) natives exhibit blunted hypoxic sensitivity (e.g. Hackett et al., 1980; see also review by Weil, 2003) have not been consistently supported by more recent work (Vargas et al., 1998; Moore, 2000). Beall et al. (1997a) detected an absolute difference in HVR of ~0.41 L min^{-1} $\%^{-1}$, between two HA populations, one Himalayan and one Andean. Our populations, with ~0.08 L min^{-1} $\%^{-1}$ absolute difference in HVR, do not differ. Indeed, our HVR values appear similar

Table 6
Repeatability of the HVR calculated separately for male and female Caucasians and Xhosas

Population	Gender	Source	*DF*	SS	MS	*F*-ratio	Repeatability
Caucasian	Females	Among	9	1.2610	0.1401	40.32	0.985*
		Within	19	0.0382	0.0035		
	Males	Among	22	5.3417	0.2428	12.57	0.890*
		Within	49	0.4830	0.0193		
Xhosa	Females	Among	24	7.2356	0.3015	4.34	0.624*
		Within	60	2.4998	0.0694		
	Males	Among	4	0.0477	0.0119	0.69	0.000[a]
		Within	14	0.1559	0.0173		

[a] Negative value rounded to zero.
* $P<0.05$.

to other studies, such as in a low-altitude Han Chinese population (Zhuang et al., 1993; see also Moore, 2000).

The absence of previously published information on the HVR in any African population suggests that more work on this continent is required to test the generality of the hypothesis that long-term HA residents develop blunted HVR (Hochachka and Monge, 2000). The only published data on ventilatory sensitivity in Africans, of which we are aware, show an age-related decline in hypercapnic sensitivity among Nigerians (Elegbeleye and Femi-Pearse, 1980). Age was not significantly related to HVR in our analyses. Nevertheless, addition of HVR data from currently untested populations (e.g. residents of Pakistan, India or Australian Aborigines) to the global database (e.g. Moore, 2000), whether such populations are of high or low altitude origin, could provide valuable insight into baseline HVR and the nature of adaptive change therein.

4.2. Phylogenetic independence of the HVR confirms possibility of its adaptive potential

Estimation of inter-population variation in the HVR is complicated by a number of factors. For example, developmental effects and environmental interactions with hypoxic sensitivity may induce subtle differences in HVR in humans (Sørensen and Severinghaus, 1968) and other mammals (reviewed in Mitchell et al., 2001, and see Bavis et al., 2004). Intermittent hypoxia may be a more potent stimulus for adaptation than is continuous hypoxia (Prabhakar, 2001). In population comparisons seeking to explore the adaptive significance of hypoxic sensitivity that use subjects in their natural environment, accounting fully for such factors remains a significant challenge (Brutsaert, 2001; Hochachka and Somero, 2002; Powell, 2003).

The Mantel correlation matrix showed that HVR differences between Aymara, Tibetans, Han Chinese, Europeans and Xhosas cannot be attributed to genetic distance. This result, in conjunction with repeatability, heritability and a potential link to fitness (see Introduction), suggests that inter-population variation in the HVR may well be the result of local environmental adaptation, and is likely not merely a consequence of phylogenetic association. Reassuringly, our analysis indicates that statistical assumptions of previously published studies that regard population means as independent data points are vindicated (e.g. Beall et al., 1997a; Moore, 2000; Beall, 2000; Powell, 2003). We caution that our analyses do not replace phylogenetically independent contrasts such as those using the PDAP and PDTREE analytical packages (Garland et al., 1993). The addition of more populations to the comparative HVR database may alter our perspective of the relationship between phylogeny and HVR. A re-examination of the phylogenetic independence of HVR upon the addition of new data should shed additional light on this relationship. The inclusion of additional populations and new genetic markers (other than classical allozyme loci—see Cavalli-Sforza et al., 1994) will increase our confidence in the independence of these variables. Regardless, it is unlikely that this relationship

Table 7
Matrices representing Nei's genetic distance (upper right-hand half of matrix), (Table 2.3.1B, Cavalli-Sforza et al., 1994) and HVR difference (bottom left-hand half of matrix) among five populations investigated to date

Population	Xhosa (Bantu)	European	Tibetan	Aymara/Quechua	Han Chinese
Xhosa[a]		0.0462	0.0324	0.0573	0.0433
European[a]	−0.08		0.0142	0.0266	0.0196
Tibetan[b]	−0.36	−0.28		0.0148	0.0093
Aymara/Quechua[b]	−0.07	0.02	−0.29		0.0193
Han Chinese[c]	−0.06	0.02	0.3	0.01	

Where multiple data for a single population were available, a median value was calculated from all usable data (e.g. Tibetans).
[a] Our study.
[b] Beall et al., 1997a.
[c] Zhuang et al., 1993.

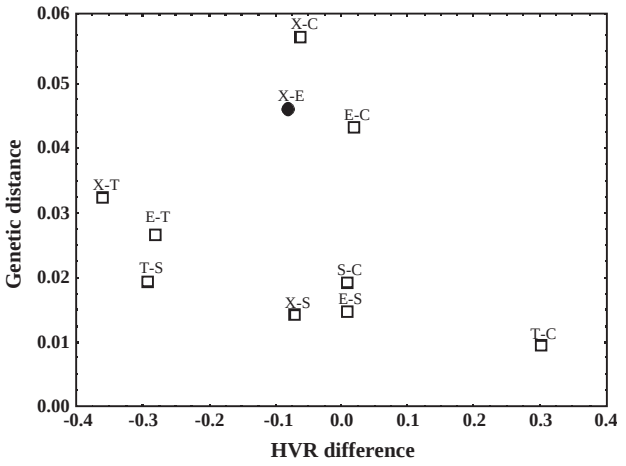

Fig. 2. Pair-wise plot demonstrating that genetic distance (raw data from Cavalli-Sforza et al., 1994) is not directly related to the magnitude of HVR difference between five main populations. X: Xhosa (Bantu, our study); C: Chinese (Zhuang et al., 1993); S: South Amerindians (Aymara; Beall et al., 1997a); T: Tibetans (Zhuang et al., 1993; Beall et al., 1997a); E: Europeans (Caucasians, our study). Closed circle: this study.

will be altered greatly since the Mantel test is robust to small sample sizes providing sufficient permutations (>1000) have been utilised (Jackson and Somers, 1989). Finally, use of HVR measures obtained by standardized methods may clarify the nature of this relationship further, but further interpretations may be premature since different data sets have been obtained using different methods in the past.

4.3. Phenotypic adaptation, variability and repeatability

Because inter-individual variability underlies differential survival and reproductive success, it can be considered as the raw material upon which natural selection acts. Repeatable differences between individuals are of great interest to evolutionary biologists, because traits showing high repeatability (inter-individual variance expressed as a fraction of summed intra- and inter-individual variance, (Falconer and Mackay, 1996; Krebs, 1999)) are likely to show high heritability (Falconer and Mackay, 1996; Dohm, 2002). Quantification of both intra- and inter-individual variation is thus critical to our understanding of physiological diversity and evolutionary adaptation (Bennett, 1987; Falconer and Mackay, 1996; Spicer and Gaston, 1999; McNab, 2003).

Physiological variability can be assessed using repeatability calculations and variance partitioning (Falconer and Mackay, 1996; Krebs, 1999). Repeatability differs considerably, depending on the nature of the character in question, the genetic properties of the population, and the extent of the influence of the local environmental conditions experienced by that population's individuals. Estimation of the variability of the HVR can provide important information for understanding the extent to which the experimental technique provides precise estimates of this parameter (see, e.g. Rebuck and Campbell, 1974; Beall et al., 1997a).

High intra-individual variability does not preclude the application of the HVR as a research tool, but without understanding the degree of variation at the respective hierarchical levels, deductions that can be made from the acquired data are severely limited, and perhaps even false. High variability in HVR within populations (Beall et al., 1997a; Hochachka et al., 1998; Hochachka and Monge, 2000; Hochachka et al., 1999) does not prevent comparison of HVR between populations, but rather necessitates care about the number of repeated measures used (see also Vizek et al., 1987; Khoo, 2001; Spengler and Shea, 2001; Terblanche et al., 2004). Such numbers should be based on good understanding of the relationship of the intra- and inter-individual variation of the HVR at both the individual and population levels.

Although repeatability (*sensu* Falconer and Mackay, 1996) has been assessed for physiological traits such as metabolic rate in mammals (e.g. McNab, 2003) and insects (e.g. Marais and Chown, 2003), and life history, behavioural and morphological traits in birds and insects (e.g. Møller, 2001; Van Dongen, 1998), this tool has not yet been used to explore the nature of the variation in hypoxic ventilatory chemosensitivity within and between human populations. Inter-individual variation in the HVR is known to be high (Sahn et al., 1977; Zhang and Robbins, 2000), and although this parameter can be considered a genotypic adaptation to the selective pressures of hypoxic environments (e.g. Beall et al., 1997a; Hochachka et al., 1999), its heritability should also be demonstrated (e.g. Strohl et al., 1997; Weil et al., 1998; Weil, 2003). Our finding that the HVR, HHR and their respective components are repeatable supports the utility of these traits as measures of human adaptation to high altitude and adds a strong evolutionary framework within which to study inter-population hypoxic ventilatory adaptation. In cases where repeated measures of the trait have equal variances, and the repeated measures assess the exact same underlying genetic trait itself, repeatability can provide an estimate of the maximum possible heritability of the trait itself providing that certain critical assumptions have not been violated (see discussion in Dohm, 2002 for further details; and see Methods).

4.4. HVR components and breathing patterns

The HVR shows significant heritability in humans and can differ between high-altitude populations (34% vs. 22% in Tibetans vs. Andeans (Beall et al., 1997a)). Family and twin studies have shown a strong genetic influence on hypoxic sensitivity (reviewed in Weil, 2003; and see Introduction). Our study contributes two potentially important findings with regard to differences in breathing patterns obtained between the Xhosa and Caucasian populations. First, hypoxic compensatory mechanisms of ventilation are indeed plastic in humans, both within (see e.g. Gozal et al., 1995) and between populations. Second, there is surprising dichotomy for variation in breathing patterns between our

study's two human populations, similar to that of inbred mouse and rat strains (Strohl et al., 1997; Tankersley et al., 1997; Tankersley, 2000; 2003; see also Powell, 2003; Weil, 2003). Phenotypic variation in hypoxic f_R is probably controlled by two major genes in mice (Tankersley, 2000), but the complexity of genetic control of the HVR among "wild-type" mouse (and human) phenotypes is likely far greater than that among inbred laboratory mouse strains, and the human genome is more heterogeneous than that of this experimental model (Tankersley, 2003). Nevertheless, investigation of the underlying genetic differences between the Xhosa and Caucasian populations may be of interest in clarifying the nature of the correspondence in breathing patterns observed between rodent models and the human populations in our study.

The two populations in our study display different hypoxic SaO_2, a parameter that is significantly influenced by genotype (Beall et al., 1997b; Brutsaert et al., 2000). The significantly lower SaO_2 values that we report for Xhosa subjects, in conjunction with their smaller hypoxic V_T, suggest that the two patterns of respiration we describe here differ in effective oxygenation of the blood during hypoxia. It is unlikely that the measured SaO_2 differences reflected interference of skin pigmentation with the pulse oximeter (Bothma et al., 1996). The increased HHR that we report among Xhosas suggests that reduction in hypoxic SaO_2, hence total carrying capacity of the blood for oxygen, may be compensated for by increased cardiac output. Furthermore, in an analysis which removed the effect of ventilatory dead-space volume, we found that there is no difference between Xhosa and Caucasian alveolar ventilation in either normoxia or hypoxia (J.S. Terblanche and S. Jackson, unpublished data). Despite differences in body-size, therefore, effective minute ventilation in these two populations appears similar.

4.5. Potential complicating factors

Our analyses permitted us to compare two populations independently of gender ratios and body size (Aitken et al., 1986). The effect of ovarian hormones on the HVR within women is difficult to demonstrate, with some studies showing no difference within individuals between the follicular and luteal phases of the cycle (reviewed by Muza et al., 2001, see also Tarbichi et al., 2003), and equal numbers showing increased HVR in the midluteal phase, probably because elevated plasma concentrations of ovarian hormones during this phase increase sensitivity of both central and peripheral chemoreceptors (Muza et al., 2001). Our separate analyses of sub-sets of age-matched female subjects all in the follicular phase, and male subjects from each population confirmed the results of the larger comparison, showing that although the HVR is similar between the two populations we studied, Xhosas breathe more frequently but have lower V_T in both normoxia and hypoxia.

Endurance athletes have diminished HVR relative to that of mountaineers or sedentary controls (Schoene, 1982; Masuyama et al., 1986). Whilst all our subjects had non-athletic lifestyles, inter-population differences in habitual exercise levels resulting from socio-economic differences between the two populations may have affected our results. Although Caucasian subjects participated in recreational exercise more frequently than did Xhosas, the latter performed more lifestyle-related physical exertion (e.g. walking to work) than did Caucasians, thus compensating partially for this difference.

Body size influences lung size, hence ventilation and presumably anatomical dead-space. The absence of a negative correlation between height and SaO_2 in our data suggests that larger subjects were not experiencing a greater hypoxic dose as a result of potentially greater tidal volumes, hence more effective alveolar ventilation. However, V_T and SaO_2 were inversely related in our subjects. Our finding that V_T was smaller among Xhosas than among Caucasians is supported by studies showing smaller forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) in African or African-American children relative to age-matched European-American or European children (Johnston et al., 1987; Joseph et al., 2000). Similarly, adult South African Africans showed lower FEV_1 than did their compatriot Caucasians (van de Wal et al., 1970). Among Ethiopians, FEV_1 and FVC are significantly lower among people living at 1500 m than among those living at 3000 m and higher (Harrison et al., 1969; and see Wood et al., 2003). Moreover, Wood et al. (2003) found relatively greater pulmonary function (FEV_1/FVC) in high altitude Indian and Tibetan groups compared with sojourner controls.

Understanding hypoxic sensitivity in Africans is important to our understanding of the hypoxia response systems that shaped the physiology of humans, and to understanding different strategies of adaptation to hypoxia among high-altitude populations (Beall et al., 2002; Hochachka and Somero, 2002). A comparison of the HVR between East Africans from the same high-altitude population living at high and low altitude would enhance our understanding of the effects of genes and environment on the HVR (Brutsaert, 2001). Investigation of possible mechanisms compensating for lower V_T and hypoxic SaO_2 among Xhosas, such as nitric oxide-linked differences in oxygen extraction efficiency (Beall et al., 2001), would provide a basis for comparison with closely related high altitude populations in East Africa.

5. Conclusion

This is the first study of hypoxic chemosensitivity in African populations and we demonstrate that the HVR does not differ between Xhosas and Caucasians. However, its components (tidal volume, breathing frequency and hypoxic SaO_2) differed, even after body size effects have been

controlled for. This suggests two distinct breathing patterns: a) shallower, more rapid breathing among Xhosas, and b) deeper, slower breathing among Caucasians. These differences were apparent in normoxia and likely resulted in the lower arterial oxygen saturation during hypoxia among the Xhosa subjects. Their less effective oxygenation of the blood was possibly compensated for by the higher HHR demonstrated among these subjects. The high and significant repeatability for the HVR and HHR in both populations demonstrates that one of the prerequisites for natural selection, and hence adaptation, is met. Indeed, the phylogenetic independence of HVR values suggested by our inter-population analyses, in conjunction with consistent between-individual variation (repeatability), heritability and potential links to fitness, add strong statistical support to the theory that the magnitude of the HVR likely reflects an adaptation to local hypoxic environmental conditions, and is not merely the result of genetic divergence between different high altitude populations.

Acknowledgements

We are sincerely grateful to our subjects for their participation in this study. We thank Bill Louw for his patience during discussions of quantitative genetics. Steven Chown, Jaco Klok, Brent Sinclair and Elrike Marais provided valuable discussion and comments. Saskia Goldberg and various anonymous reviewers provided useful comments on an earlier version of this manuscript. The staff of National Hyperbarics assisted with gas mixing and provided air cylinders. This research was supported by the National Research Foundation of South Africa (Project No: NRF 1031).

References

Aitken, M.L., Franklin, J.L., Pierson, D.J., Schoene, R.B., 1986. Influence of body size and gender on control of ventilation. J. Appl. Physiol. 60, 1894–1899.

Archer, S.L., Weir, E.K., Reeve, H.L., Michelakis, E., 2000. Molecular identification of O_2 sensors and O_2-sensitive potassium channels in the pulmonary circulation. Adv. Exp. Med. Biol. 475, 219–240.

Akiyama, Y., Nishimura, M., Suzuki, A., Yamamoto, M., Kawakami, Y., 1991. Peripheral chemosensitivity assessed by the modified transient O_2 test in female twins. Chest 100, 102–105.

Bavis, R.W., Olson Jr., E.B., Vidruk, E.H., Fuller, D.D., Mitchell, G.S., 2004. Developmental plasticity of the hypoxic ventilatory response in rats induced by neonatal hypoxia. J. Physiol. 557, 645–660.

Beall, C.M., 2000. Tibetan and Andean patterns of adaptation to high-altitude hypoxia. Hum. Biol. 72, 201–228.

Beall, C.M., Strohl, K.P., Blangero, J., Williams-Blangero, S., Almasy, L.A., Decker, M.J., Worthman, C.M., Goldstein, M.C., Vargas, E., Villena, M., Soria, R., Alarcon, A.M., Gonzales, C., 1997a. Ventilation and hypoxic ventilatory response of Tibetan and Aymara high altitude natives. Am. J. Phys. Anthropol. 104, 427–447.

Beall, C.M., Strohl, K.P., Blangero, J., Williams-Blangero, S., Decker, M.J., Brittenham, G.M., Goldstein, M.C., 1997b. Quantitative genetic analysis of arterial oxygen saturation in Tibetan highlanders. Hum. Biol. 69, 597–604.

Beall, C.M., Brittenham, G.M., Strohl, K.P., Blangero, J., Williams-Blangero, S., Goldstein, M.C., Decker, M.J., Vargas, E., Villena, M., Soria, R., Alarcon, A.M., Gonzales, C., 1998. Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. Am. J. Phys. Anthropol. 106, 385–400.

Beall, C.M., Laskowski, D., Strohl, K.P., Soria, R., Villena, M., Vargas, E., Alarcon, A.M., Gonzales, C., Erzurum, S.C., 2001. Pulmonary nitric oxide in mountain dwellers. Nature 414, 411–412.

Beall, C.M., Decker, M.J., Brittenham, G.M., Kushner, I., Gebremedhin, A., Strohl, K.P., 2002. An Ethiopian pattern of human adaptation to high-altitude hypoxia. Proc. Natl. Acad. Sci. U. S. A. 99, 17215–17218.

Becker, W.A., 1984. Manual of Quantitative Genetics, 4th edn. Academic Enterprises, Washington.

Bennett, A.F., 1987. Interindividual variability: an underutilized resource. In: Feder, M.E., Bennett, A.F., Burggren, W., Huey, R.B. (Eds.), New Directions in Ecological Physiology. Cambridge University Press, Cambridge, pp. 147–169.

Bennett, A.F., 2003. Experimental evolution and the Krogh principle: generating biological novelty for functional and genetic analyses. Physiol. Biochem. Zool. 76, 1–11.

Bland, J.M., Altman, D.G., 1986. Statistical methods of agreement between two methods of clinical measurement. The Lancet 1, 307–310.

Bothma, P.A., Joynt, G.M., Lipman, J., Hon, H., Mathala, B., Scribante, J., Kromberg, J., 1996. Accuracy of pulse oximetry in pigmented patients. S. Afr. Med. J. 86, 594–596.

Brutsaert, T.D., 2001. Genetic and environmental adaptation in high altitude natives. Conceptual, methodological, and statistical concerns. Adv. Exp. Med. Biol. 502, 133–151.

Brutsaert, T.D., Araoz, M., Soria, R., Spielvogel, H., Haas, J.D., 2000. Higher arterial oxygen saturation during submaximal exercise in Bolivian Aymara compared to European sojourners and Europeans born and raised at high altitude. Am. J. Phys. Anthropol. 113, 169–181.

Cavalli-Sforza, L.L., Menozzi, P., Piazza, A., 1994. The History and Geography of Human Genes (full version). Princeton University Press, New Jersey.

Clement, I.D., Pandit, J.J., Bascom, D.A., Dorrington, K.L., O'Connor, D.F., Robbins, P.A., 1995. An assessment of central-peripheral ventilatory chemoreflex interaction using acid and bicarbonate infusions in humans. J. Physiol. 485, 561–570.

Cherniack, N.S., 2004. Oxygen sensing: applications in humans. J. Appl. Physiol. 96, 352–358.

Cristosomo, I., Zayyad, A., Carley, D.W., Abubaker, J., Onal, E., Stepanski, E.J., Lopata, M., Basner, R.C., 1998. Chemo- and baroresponses differ in African-Americans and Caucasians in sleep. J. Appl. Physiol. 85, 1413–1420.

Curran, L.S., Zhuang, J., Sun, S.F., Moore, L.G., 1997. Ventilation and hypoxic ventilatory responsiveness in Chinese-Tibetan residents at 3,658 m. J. Appl. Physiol. 83, 2098–2104.

Dohm, M.R., 2002. Repeatability estimates do not always set an upper limit to heritability. Funct. Ecol. 16, 273–280.

D'Urzo, A.D., Jhirad, R., Jenne, H., Avendano, M.A., Rubenstein, I., D'Costa, M., Goldstein, M.A., 1990. Effect of caffeine on ventilatory responses to hypercapnia, hypoxia, and exercise in humans. J. Appl. Physiol. 68, 322–328.

Elegbeleye, O.O., Femi-Pearse, D., 1980. Relation between age and respiratory response to inhaled carbon dioxide in healthy Nigerians. Isr. J. Med. Sci. 16, 389–391.

Endler, J.A., 1986. Natural Selection in the Wild. Princeton University Press, New Jersey.

Fagan, K.A., Weil, J.V., 2001. Potential genetic contributions to control of the pulmonary circulation and ventilation at high altitude. High Alt. Med. Biol. 2, 165–171.

Fahlman, A., Fisher, J.A., Vesely, A., Sasano, H., Terblanche, J., Jackson, S., Myburg, K., 2002. A simple breathing circuit to maintain isocapnia

during measurements of the hypoxic ventilatory response. Respir. Physiol. Neurobiol. 133, 259–270.
Falconer, D.S., Mackay, T.F., 1996. Introduction to Quantitative Genetics, 4th edn. Longman Harlow, London.
Garland Jr., T., 2001. Phylogenetic comparison and artificial selection. Adv. Exp. Med. Biol. 502, 107–132.
Garland, T.J., Dickerman, A.W., Janis, C.M., Jones, J.A., 1993. Phylogenetic analysis of covariance by computer simulation. Syst. Biol. 42, 265–292.
Gene, M., Moreno, P., Borrego, N., Pique, E., Xifro, A., Fuentes, M., Bert, F., Corella, A., Perez-Perez, A., Turbon, D., Corbella, J., Huguet, E., 2000. Population study of Aymara Amerindians for the PCR-DNA polymorphisms HUMTH01, HUMVWA31A, D3S1358, D8S1179, D18S51, D19S253, YNZ22 and HLA-DQ alpha. Int. J. Leg. Med. 113, 126–128.
Gozal, D., Ben-Ari, J.H., Harper, R.M., Keens, T.G., 1995. Ventilatory responses to repeated short hypercapnic challenges. J. Appl. Physiol. 78, 1374–1381.
Hackett, P.H., Reeves, J.T., Reeves, C.D., Grover, R.F., Rennie, D., 1980. Control of breathing in Sherpas at low and high altitude. J. Appl. Physiol. 49, 374–379.
Han, F., Strohl, K.P., 2000. Inheritance of ventilatory behaviour in rodent models. Respir. Physiol. 121, 247–256.
Harrison, G.A., Kucheman, C.F., Moore, M.A.S., Boyce, A.J., 1969. The effects of altitudinal variation in Ethiopian populations. Philos. Trans. R. Soc. Lond. 256, 147–182.
Harvey, P.H., Pagel, M.D., 1993. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford.
Hochachka, P.W., Monge, C., 2000. Evolution of human hypoxia tolerance physiology. Adv. Exp. Med. Biol. 475, 25–43.
Hochachka, P.W., Somero, G., 2002. Biochemical Adaptations: Mechanism and Process in Physiological Evolution. Oxford University Press, Oxford.
Hochachka, P.W., Clark, C.M., Holden, J.E., Stanley, C., Ugurbil, K., Menon, R.S., 1996. P-31 magnetic resonance spectroscopy of the Sherpa heart: a phosphocreatine adenosine triphosphate signature of metabolic defense against hypobaric hypoxia. Proc. Natl. Acad. Sci. U. S. A. 93, 1215–1220.
Hochachka, P.W., Gunga, H.C., Kirsch, K., 1998. Our ancestral physiological phenotype: an adaptation for hypoxia tolerance and for endurance performance? Proc. Natl. Acad. Sci. U. S. A. 95, 1915–1920.
Hochachka, P.W., Rupert, J.L., Monge, C., 1999. Adaptation and conservation of physiological systems in the evolution of human hypoxia tolerance. Comp. Biochem. Physiol., A 124, 1–17.
Jackson, D.A., Somers, K.M., 1989. Are probabilities estimates from the permutation model of Mantel's test stable? Can. J. Zool. 67, 766–769.
Johnston, I.D., Bland, J.M., Anderson, H.R., 1987. Ethnic variation in respiratory morbidity and lung function in childhood. Thorax. 42, 542–548.
Joseph, C.L.M., Ownby, D.R., Peterson, E.L., Johnson, C.C., 2000. Racial differences in physiological parameters related to asthma among middle-class children. Chest 117, 1336–1344.
Kawakami, Y., Irie, T., Kishi, F., Asanuma, Y., Shida, A., Yoshikawa, T., Kamishima, K., Hasegawa, H., Murao, M., 1981. Familial aggregation of abnormal ventilatory control and pulmonary function in chronic obstructive pulmonary disease. Eur. J. Respir. Dis. 62, 56–64.
Khoo, M.C.K., 2001. Respiratory instability and variability: why is respiration such a mess? In: Poon, C.S., Kazemi, H. (Eds.), Frontiers in Modeling and Control of Breathing. Kluwer/Plenum, New-York. Adv. Exp. Med. Biol. 499, 495–500.
Kobayashi, S., Nishimura, M., Yamamoto, M., Akiyama, Y., Kishi, F., Kawakami, Y., 1993. Dyspnea sensation and chemical control of breathing in adult twins. Am. Rev. Respir. Dis. 147, 1192–1198.
Krebs, C.J., 1999. Ecological Methodology, 2nd ed. Benjamin/Cummings, Menlo Park.
Lahiri, S., 2000. Historical perspectives of cellular oxygen sensing and responses to hypoxia. J. Appl. Physiol. 88, 1467–1473.
Lahiri, S., Kao, F.F., Velasquez, T., Martinez, C., Pezzia, W., 1970. Respiration of man during exercise at high altitude: highlander vs. lowlander. Respir. Physiol. 8, 361–375.
Lahiri, S., Di Giulo, C., Roy, A., 2002. Lessons from chronic intermittent and sustained hypoxia at high altitudes. Respir. Physiol. Neurobiol. 130, 223–233.
Lane, A.B., Soodyall, H., Arndt, S., Ratshikhopha, M.E., Jonker, E., Freeman, C., Young, L., Morar, B., Toffie, L., 2002. Genetic substructure in South African Bantu-Speakers: evidence from autosomal DNA and Y-chromosome studies. Am. J. Phys. Anthropol. 119, 175–185.
Manly, B.F.J., 1986. Randomization and regression methods for testing for associations with geographical, environmental and biological distances between populations. Res. Popul. Ecol. 28, 201–281.
Marais, E., Chown, S.L., 2003. Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach *Perisphaeria* sp. J. Exp. Biol. 206, 4565–4574.
Masuyama, S., Kimura, H., Sugita, T., Kuriyama, T., Tatsumi, K., Kunitomo, F., Okita, S., Tojima, H., Yuguchi, Y., Watanabe, S., Honda, Y., 1986. Control of ventilation in extreme altitude climbers. J. Appl. Physiol. 61, 500–506.
McNab, B.K., 2003. Sample sizes and the estimation of physiological parameters in the field. Funct. Ecol. 17, 82–86.
Mitchell, G.S., Baker, T.L., Nanda, S.A., Fuller, D.D., Zabka, A.G., Hodgeman, B.A., Bavis, R.W., Mack, K.J., Olson Jr., E.B., 2001. Intermittent hypoxia and respiratory plasticity. J. Appl. Physiol. 90, 2466–2475.
Møller, A.P., 2001. Heritability of arrival date in a migratory bird. Proc. R. Soc. Lond., B 268, 203–206.
Monge, C.M., León-Velarde, F., Arregui, A., 2001. Chronic mountain sickness in Andeans. In: Schoene, T.H.R. (Ed.), High Altitude: An Exploration of Human Adaptation. Marcel Dekker Inc., New York, pp. 815–838.
Moore, L.G., 2000. Comparative human ventilatory adaptation to high altitude. Respir. Physiol. 121, 257–276.
Moore, L.G., 2003. Fetal growth restriction and maternal oxygen transport during high altitude pregnancy. High Alt. Med. Biol. 4, 141–156.
Moore, L.G., Zamudio, S., Zhuang, J., Sun, S., Droma, T., 2001. Oxygen transport in Tibetan women during pregnancy at 3,658 M. Am. J. Phys. Anthropol. 114, 42–53.
Muza, S.R., Rock, P.B., Fulco, C.S., Zamudio, S., Braun, B., Cymerman, A., Butterfield, G.E., Moore, L.G., 2001. Women at altitude: ventilatory acclimatization at 4,300 m. J. Appl. Physiol. 91, 1791–1799.
Niermayer, S., Zamudio, S., Moore, L.G., 2001. The people. In: Schoene, T.H.R. (Ed.), High Altitude: An Exploration of Human Adaptation. Marcel Dekker Inc., New York, pp. 43–100.
Nishimura, M., Yamamoto, M., Yoshioka, A., Akiyama, Y., Kishi, F., Kawakami, Y., 1991. Longitudinal analyses of respiratory chemosensitivity in normal subjects. Am. Rev. Respir. Dis. 143, 1278–1281.
Piersma, T., Drent, J., 2003. Phenotypic flexibility and the evolution of organismal design. Trends Ecol. Evol. 18, 228–233.
Powell, F.L., 2003. Functional genomics and the comparative physiology of hypoxia. Ann. Rev. Physiol. 65, 203–230.
Powell, F.L., Milsom, W.K., Mitchell, G.S., 1998. Time domains of the hypoxic ventilatory response. Respir. Physiol. 112, 123–134.
Prabhakar, N.R., 2001. Oxygen sensing during intermittent hypoxia: cellular and molecular mechanisms. J. Appl. Physiol. 90, 1986–1994.
Rebuck, A.S., Campbell, E.J.M., 1974. A clinical method for assessing the ventilatory response to hypoxia. Am. Rev. Respir. Dis. 109, 345–350.
Reeves, J.T., McCullough, R.E., Moore, L.G., Cymerman, A., Weil, J.V., 1993. Sea-level PCO_2 relates to ventilatory acclimitization. J. Appl. Physiol. 75, 1117–1122.
Sahn, S.A., Zwillich, C.W., Dick, N., McCullough, R.E., Lakshminarayan, S., Weil, J.V., 1977. Variability of ventilatory responses to hypoxia and hypercapnia. J. Appl. Physiol. 43, 1019–1025.

Samaja, M., 1997. Blood gas transport at high altitude. Respiration 64, 422–428.

Sarkar, S., Banerjee, P.K., Selvamurthy, W., 2003. High altitude hypoxia: an intricate interplay of oxygen responsive macroevents and micromolecules. Mol. Cell. Biochem. 253, 287–305.

Sato, F., Nishimura, M., Igarashi, T., Yamamoto, M., Miyamoto, K., Kawakami, Y., 1996. Effects of exercise and CO_2 inhalation on intersubject variability in ventilatory and heart rate responses to progressive hypoxia. Eur. Respir. J. 9, 960–967.

Scoggin, C.H., Doekel, R.D., Kryger, M.H., Zwillich, C.W., Weil, J.H., 1978. Familial aspects of decreased hypoxic drive in endurance athletes. J. Appl. Physiol. 44, 464–468.

Schoene, R.B., 1982. Control of ventilation in climbers to extreme altitude. J. Appl. Physiol. 53, 886–890.

Semenza, G.L., Koury, S.T., Nejfelt, M.K., Gearhart, J.D., 1991. Cell-type-specific and hypoxia-inducible expression of the human erythropoietin gene in transgenic mice. Proc. Natl. Acad. Sci. U. S. A. 88, 8725–8729.

Shea, S.A., 1996. Behavioural and arousal-related influences on breathing in humans. Exp. Physiol. 81, 1–26.

Sørensen, S.C., Severinghaus, J.W., 1968. Irreversible respiratory insensitivity to acute hypoxia in man born at high altitude. J. Appl. Physiol. 25, 217–220.

Soutiere, S.E., Tankersley, C.G., 2001. Challenges implicit to gene discovery during research in the control of ventilation during hypoxia. High Alt. Med. Biol. 2, 191–200.

Spengler, C.M., Shea, S.A., 2001. Explained and unexplained variability of CO_2-sensitivity in humans. In: Poon, C.S., Kazemi, H., (Eds.), Frontiers in Modeling and Control of Breathing. Kluwer/Plenum, New-York. Adv. Exp. Med. Biol. 499, 483–488.

Spicer, J.I., Gaston, K.J., 1999. Physiological Diversity and its Ecological Implications. Blackwell, Oxford.

Strohl, K.P., Thomas, A.J., St Jean, P., Schlenker, E.H., Koletsky, R.J., Schork, N.J., 1997. Ventilation and metabolism among rat strains. J. Appl. Physiol. 82, 317–323.

Tankersley, C.G., 2000. A genomic model for differential hypoxic ventilatory responses. Adv. Exp. Med. Biol. 475, 75–85.

Tankersley, C.G., 2003. Genetic aspects of breathing: on interactions between hypercapnia and hypoxia. Respir. Physiol. Neurobiol. 135, 167–178.

Tankersley, C.G., Fitzgerald, R.S., Levitt, R.C., Mitzner, W.A., Ewart, S.L., Kleeberger, S.R., 1997. Genetic control of differential baseline breathing pattern. J. Appl. Physiol. 82, 874–881.

Tarbichi, A.G.S., Rowley, J.A., Shkoukani, M.A., Mahadevan, K., Badr, M.S., 2003. Lack of gender difference in ventilatory chemoresponsiveness and post-hypoxic ventilatory decline. Respir. Physiol. Neurobiol. 137, 41–50.

Terblanche, J.S., Fahlman, A., Myburgh, K.H., Jackson, S., 2004. Measurement reliability of highly variable physiological responses to experimentally-manipulated gas fractions. Physiol. Meas. 25, 1189–1197.

Thomas, D.A., Swaminathan, S., Beardsmore, C.S., McArdle, E.K., MacFadyen, U.M., Goodenough, P.C., Carpenter, R., Simpson, H., 1993. Comparison of peripheral chemoreceptor responses in monozygotic and dizygotic twin infants. Am. Rev. Respir. Dis. 148, 1605–1609.

van de Wal, B.W., Erasmus, L.D., Hechter, R., 1970. Stem and standing heights in Bantu and White South Africans: their significance in relation to pulmonary function values. S. Afr. Med. J. 45, 568–570.

Van Dongen, S., 1998. How repeatable is the estimation of developmental stability by fluctuating asymmetry? Proc. R. Soc. Lond., B 265, 1423–1427.

Vargas, M., Leon-Velarde, F., Monge-C, C., Palacios, J.A., Robbins, P.A., 1998. Similar hypoxic ventilatory responses in sea-level natives and high-altitude Andean natives living at sea level. J. Appl. Physiol. 84, 1024–1029.

Vizek, M., Pickett, C.K., Weil, J.V., 1987. Interindividual variation in hypoxic ventilatory response: potential role of carotid body. J. Appl. Physiol. 63, 1884–1889.

Waddle, D.M., 1994. Matrix correlation tests support a single origin for modern humans. Nature 368, 452–454.

Weil, J.V., 2003. Variation in human ventilatory control—genetic influence of the hypoxic ventilatory response. Respir. Physiol. Neurobiol. 135, 239–246.

Weil, J.V., Stevens, T., Pickett, C.K., Tatsumi, K., Dickinson, M.G., Jacoby, C.R., Rodman, D.M., 1998. Strain-associated differences in hypoxic chemosensitivity of the carotid body in rats. Am. J. Physiol. 274, L767–L774.

Westerterp, K.R., Robach, P., Wouters, L., Richalet, J.P., 1996. Water balance and acute mountain sickness before and after arrival at high altitude of 4,350 m. J. Appl. Physiol. 80, 1968–1972.

Winer, B.J., Brown, D.R., Michels, K.M., 1991. Statistical Principles in Experimental Design. McGraw-Hill, New York, pp. 497–582.

Winslow, R.M., Monge, C.M., 1997. Hypoxia, Polycythemia, and Chronic Mountain Sickness. Johns Hopkins University Press, Baltimore.

Wood, S., Norboo, T., Lilly, M., Yoneda, K., Eldridge, M., 2003. Cardiopulmonary function in high altitude residents of Ladakh. High Alt. Med. Biol. 4, 445–454.

Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, New Jersey.

Zhivotovsky, L.A., Rosenberg, N.A., Feldman, M.W., 2003. Features of evolution and expansion of modern humans, inferred from genomewide microsatellite markers. Am. J. Hum. Genet. 72, 1171–1186.

Zhang, S., Robbins, P.A., 2000. Methodological and physiological variability within the ventilatory response to hypoxia in humans. J. Appl. Physiol. 88, 1924–1932.

Zhuang, J., Droma, T., Sun, S., Janes, C., McCullough, R.E., McCullough, R.G., Cymerman, A., Huang, S.Y., Reeves, J.T., Moore, L.G., 1993. Hypoxic ventilatory responsiveness in Tibetan compared with Han residents of 3658 m. J. Appl. Physiol. 74, 303–311.