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Hemoglobin Concentration of High-Altitude Tibetans and Bolivian Aymara

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ABSTRACT Elevated hemoglobin concentrations have been reported for high-altitude sojourners and Andean high-altitude natives since early in the 20th century. Thus, reports that have appeared since the 1970s describing relatively low hemoglobin concentration among Tibetan high-altitude natives were unexpected. These suggested a hypothesis of population differences in hematological response to high-altitude hypoxia. A case of quantitatively different responses to one environmental stress would offer an opportunity to study the broad evolutionary question of the origin of adaptations. However, many factors may confound population comparisons. The present study was designed to test the null hypothesis of no difference in mean hemoglobin concentration of Tibetan and Aymara native residents at 3,800–4,065 meters by using healthy samples that were screened for iron deficiency, abnormal hemoglobins, and thalassemias, recruited and assessed using the same techniques. The hypothesis was rejected, because Tibetan males had a significantly lower mean hemoglobin concentration of 15.6 gm/dl compared with 19.2 gm/dl for Aymara males, and Tibetan females had a mean hemoglobin concentration of 14.2 gm/dl compared with 17.8 gm/dl for Aymara females. The Tibetan hemoglobin distribution closely resembled that from a comparable, sea-level sample from the United States, whereas the Aymara distribution was shifted toward 3–4 gm/dl higher values. Genetic factors accounted for a very high proportion of the phenotypic variance in hemoglobin concentration in both samples (0.86 in the Tibetan sample and 0.87 in the Aymara sample). The presence of significant genetic variance means that there is the potential for natural selection and genetic adaptation of hemoglobin concentration in Tibetan and Aymara high-altitude populations. Am J Phys Anthropol 106:385–400, 1998.

Many studies of adaptation to high-altitude hypoxia have focused on the blood because of its role in oxygen transport. The French physician Viault reported in 1890 that European sojourners and Andean highlanders had an ". . .increase in the respiratory, oxygenating element. . ." of the blood (West, 1981, p. 334), and a 1913 publication reported that the total quantity of hemoglo-

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bin was increased in sojourners to Pike's Peak (Douglas et al., 1913). Subsequently, many studies of hemoglobin concentration confirmed those findings in sojourners and Andean high-altitude natives (for review, see Winslow and Monge, 1987). The inference was that this was the universal human adaptive hematological response to high-altitude hypoxia, because it was exhibited by Europeans with brief exposure to the stress and by Andean high-altitude natives with millennia of exposure. However, since the 1970s, numerous reports of relatively low hemoglobin concentration among Tibetan high-altitude natives suggested a hypothesis of Tibetan-Andean population differences in hematological response to high-altitude hypoxia. Generally, samples of Tibetan highlanders averaged 1.4 gm/dl less hemoglobin than their Andean counterparts at comparable (3,400–5,000 meters) altitude (Beall et al., 1990), and, in some cases, the hemoglobin concentration did not differ from sea level (Beall and Reichsman, 1984). These findings appeared to illustrate quantitatively different adaptations in two populations that have been exposed to the same stress and, presumably, to the same selective pressures for millennia. If a new model of two population-specific responses to life-long hypoxia replaces the old model of one universal response, then this raises important, broad, evolutionary questions about the origins and universality of human adaptations.

Although the existing body of data strongly suggested a hypothesis of Tibetan-Andean population differences in hematological response to high-altitude hypoxia, the possible confounding effect of widespread iron deficiency limiting hemoglobin synthesis had not been excluded firmly as an explanation for the lower Tibetan values. The present study was designed to test the null hypothesis of no difference in mean hemoglobin concentration of samples of Tibetan and Bolivian Aymara native residents at 3,800–4,065 meters who were recruited into the study, screened for iron-deficiency, and assayed by using the same procedures.

MATERIALS AND METHODS

Population and sample

The study was conducted in rural sites at 3,800–4,065 meters in the Tibet Autonomous Region (TAR) of China and in Bolivia. The Tibetan study site was Pen-Dri, two rural, agropastoral villages with a population of 773 ethnic Tibetans living at 3,800–4,065 meters in Lhasa Municipal District, TAR. The average barometric pressure was 479 Torr. Each household was contacted in May-June or October-November 1993 in order to invite household members and their biological relatives 9 years of age and older to participate in a study collecting interview, genealogical, anthropometric, and physiological data. Ninety-six percent of the households contributed one or more participants. Sixty-eight percent of those who were eligible by age participated and yielded a total sample of 428 people who ranged in age from 9 years to 82 years. Age was verified with reference to reported animal year of birth, which was then translated into the equivalent Western calendar year. Nearly all were residents of these two communities, which were traditionally inhabited only by Tibetans and where Tibetan is the everyday language. All were life-long, high-altitude, native residents at 3,600 meters or higher (with the exception of one low-altitude native who had lived in the village since 1950). Venipuncture blood samples were requested from volunteers 14 years of age and older.

The Bolivian study site was composed of four dispersed communities in Provincia Murillo, Departamento La Paz, Bolivia with a population of 1,175 ethnic Aymara living at 3900–4000 meters. The average barometric pressure was 478 Torr. Each household was contacted between May and August 1994 in order to invite household members and their biological relatives 14 years of age and older to participate in the study. Age was verified by birth certificates, by identity cards, and by reference to historical events for a few elderly people. Sixty-four percent provided a birth certificate or an identity card issued upon presentation of a birth certificate. Seventy-seven percent of all households participated. Fifty-seven percent of the residents who were eligible by age participated and yielded a total sample of 608 people 13–94 years of age. Seventy percent of the sample resided in the four communities, and the rest were relatives residing elsewhere (one at low altitude). All were Aymara (except for one Quechua) natives of this or nearby high-

altitude communities that were traditionally inhabited by Aymara and where Aymara is the everyday language. Venipuncture blood samples were requested from all study participants.

Methods

Following standard phlebotomy procedures, a venous blood sample was obtained in a 3-ml draw, EDTA-containing Vacutainer and then thoroughly mixed. Two 10-µl samples of whole blood were removed promptly, and each was mixed with Uni-Heme reagent in a Unopette microcollection system (Becton-Dickinson, Rutherford, NJ). The transmittances of the resulting cyanmethemoglobin solutions were read twice at 540 nm with a Milton Roy Mini 20 spectrophotometer (Milton Roy, Rochester, NY). Hemoglobin concentration was calculated from the average of the four transmittance values. The calculation used an equation relating the hemoglobin concentration of low, medium, and high Coulter hemoglobinometer controls/calibrator solutions (Coulter Diagnostics, Hialeah, FL) to their transmittance measured with a Beckman DU8 Spectrophotometer in our laboratory in Cleveland, Ohio, and confirmed with the Milton Roy Mini 20 at the time of data collection in the field. To ensure that the accuracy of hemoglobin measurement under field conditions did not differ significantly from those in a laboratory certified by the College of American Pathologists, hemoglobin concentrations were determined in the following manner: 1) The calibration of the field spectrophotometer was verified by comparison with a Beckman DU-8 spectrophotometer before and after visits to each field site. 2) On each day when measurements of hemoglobin were made in the field, the transmittances of the low, medium, and high Coulter hemoglobinometer controls/calibrator solutions were measured in association with the measurements of hemoglobin concentration in the study participants. Hemoglobin measurements were considered suitable for analysis when the field spectrophotometer readings of the low, medium, and high calibrating solutions yielded transmittance values within 2% of the known transmittance obtained for the three calibrating solutions and the cyanmethemoglobin standard solutions by a Beckman DU8 spectrophotometer in our laboratory in Cleveland. This avoided possible measurement noise due to daily or annual machine drift by using only data collected when the portable field instrument measured within the stated tolerance range defined on the basis of a reference laboratory instrument. On those days, the average difference between hemoglobin concentration estimated by the equation generated from the field instrument and that estimated by the equation generated from the reference laboratory instrument differed by 0.04 ± 0.06 (standard error of the mean) gm/dl, $n = 305$.

The remaining sample was allowed to separate, and the plasma and cells were pipetted into separate microtubules and frozen in liquid nitrogen for transport to Cleveland. Iron status was evaluated by radioimmunoassay of plasma ferritin (RAMCO, Houston, TX) and assay of zinc erythrocyte protoporphyrin (Aviv model 206D, ZP hematofluoremeter, Aviv Biomedical, Lakewood, NJ). Hemoglobin A_2 was measured by minicolumn chromatography (Isolab Quik-SEP A2 columns, Isolab, Akron, OH), and hemoglobin electrophoresis was performed by using cellulose acetate (Helena Labs, Beaumont, TX). The percent oxygen saturation (SaO_2) of arterial hemoglobin was measured by using a Criticare 501 + finger pulse oximeter (Criticare Systems, Inc., Waukeshau, WI), as described elsewhere (Beall et al., 1997; Beall and Goldstein, 1990). Anthropometry was performed following standard protocols. Body mass index (BMI) was calculated as weight in kg divided by the square of height in meters. All measurements were conducted with awake, quietly resting study participants during a single visit to a field laboratory established in a central location in the study community.

Socioeconomic and lifestyle data were obtained by a questionnaire that was administered in Tibetan, Spanish, or Aymara. It elicited information on occupation, work history, education, smoking, alcohol consumption, and coca chewing. Occupation was categorized as full-time farmer or other. Work history was categorized according to whether or not an individual was currently working in a nearby urban area (La Paz in Bolivia or Lhasa in the TAR), had previously worked in that urban area, or had spent his entire life

in the rural sector. Student status was assessed in terms of whether or not the individual was currently enrolled in school. Educational experience was measured in terms of the number of years of school attendance and was categorized as no years versus any years. Cigarette smoking behavior was assessed by a question asking whether the individual used tobacco products, including cigarettes, cigars, pipes, or snuff. Answers were categorized as presence or absence of cigarette smoking (irrespective of the frequency). Alcohol use was assessed by a question asking whether the individual drank alcoholic beverages, including traditional, locally brewed beverages and commercially produced ones: Answers were categorized as no use or some use (irrespective of the frequency). Coca chewing was assessed in Bolivia by a question asking whether the individual chewed coca leaves: Answers were categorized as no use or some use (irrespective of the frequency).

TABLE 1. Exclusion criteria applied to total sample with available hemoglobin concentration to identify a subsample that was not iron deficient, had normal electrophoretic pattern, was not pregnant, had not recently delivered an infant, was healthy, and had technically acceptable hemoglobin concentration determination

Criteria	Tibetan sample (n)	Bolivian Aymara sample (n)
Total with hemoglobin concentration determinations	349	607
Iron deficiency	9	19
No iron status determination	61	20
Abnormal electrophoretic pattern	6	0
Pregnant and not excluded by the above criteria	5	14
Delivered an infant in past year and not excluded by the above	15	37
Self-reported symptoms consistent with illness influencing hemoglobin concentration and not excluded by the above	29	19
Healthy, not iron deficient, normal electrophoresis, not pregnant, no recent delivery subsample	219	492
Technically acceptable subsample	136	174

Analysis

The total sample consisted of 349 Tibetans 14–90 years of age and 607 Bolivian Aymara 13–94 years of age. To control for several potential sources of confounding, the analysis is based on subsamples of healthy (by self report) individuals who were not iron deficient and who had a normal hemoglobin electrophoretic pattern in cellulose acetate and less than 3.5% hemoglobin A_2 (to exclude abnormal hemoglobin variants and thalassemias, respectively). The absence of iron deficiency was defined as having normal values on two laboratory tests of iron status. Those were plasma ferritin level greater than 12 ng/ml and zinc erythrocyte protoporphyrin level less than or equal to 70 µg/dl red blood cells (Expert Scientific Working Group, 1985; Looker et al., 1995). In addition, women who were pregnant or who had given birth in the past year were excluded. Individuals reporting symptoms consistent with illnesses, such as tuberculosis or frequent nose bleeds, that might influence hemoglobin concentration were also excluded from analysis. Table 1 describes the number of individuals in the various exclusion categories, resulting in a potential sample for analysis of 219 Tibetans and 492 Aymara. Technically acceptable measurements were available for 136 Tibetans and 174 Aymara and formed the basis of this report.

Tables 2 and 3 demonstrate that the samples of healthy people who were not iron deficient and the subsamples for hemoglobin concentration analysis did not differ in physical, socioeconomic, and lifestyle characteristics among the Tibetans or the Aymara. The tables demonstrate the representativeness of the hemoglobin concentration subsamples that were 39% and 29% of the healthy Tibetan and Aymara samples of people who were not iron deficient, respectively. The average age, BMI, plasma ferritin, plasma zinc erythrocyte protoporphyrin, and SaO_2 of the healthy people who were not iron deficient and the hemoglobin concentration subsamples did not differ. The healthy, not iron-deficient samples and the hemoglobin concentration subsamples also resembled one another closely in socioeconomic characteristics, such as the percent of full-time farmers and the percent of individuals who were living or working outside the rural community, currently enrolled in school, and uneducated. They had similar lifestyle characteristics, such as the percent of people who

TABLE 2. Biological, socioeconomic, and lifestyle characteristics of the Tibetan sample[1]

Characteristic	Tibetan males		Tibetan females	
	Healthy, not iron-deficient sample	Subsample for hemoglobin analyses	Healthy, not iron-deficient sample	Subsample for hemoglobin analyses
Number	110	75	109	61
Biological characteristics (mean ± SEM)				
Age (years)	32 ± 1.5	34 ± 1.8	36 ± 1.7	35 ± 2.3
BMI (kg/m^2)	18.3 ± 0.18	18.4 ± 0.20	18.6 ± 0.22	18.5 ± 0.30
Plasma ferritin (ng/ml)	106.3 ± 10.57	111.6 ± 12.39	42.2 ± 5.40	40.8 ± 3.47
Plasma zinc erythrocyte protoporphyrin (µg/dl red blood cells)	23.9 ± 1.18	25.2 ± 1.53	31.4 ± 1.86	29.6 ± 2.71
SaO_2 (%)	89 ± 0.3	89 ± 0.3	89 ± 0.3	90 ± 0.4
Socioeconomic and lifestyle characteristics (% of total)				
Full-time farmers (%)	86.4	90.7	90.8	91.8
Presently working in urban area or did so in the past (%)	8.2	9.3	4.6	3.3
Presently enrolled as a student	5.5	0	4.6	4.9
Had no formal education (%)	40.0	48.0	66.1	70.5
Does not smoke cigarettes (%)	26.2	27.8	100	100
Does not drink alcoholic beverages (%)	39.1	38.7	57.8	54.1

[1] BMI, body mass index; SaO_2, percent of oxygen saturation of arterial hemoglobin.

TABLE 3. Biological, socioeconomic, and lifestyle characteristics of the Aymara sample[1]

Characteristic	Aymara males		Aymara females	
	Healthy, not iron-deficient sample	Subsample for hemoglobin analyses	Healthy, not iron-deficient sample	Subsample for hemoglobin analyses
Number	283	91	209	83
Biological characteristics (mean ± SEM)				
Age (years)	36 ± 1.0	37 ± 1.9	36 ± 1.2	38 ± 2.0
BMI (kg/m^2)	22.2 ± 0.19	22.3 ± 0.35	23.1 ± 0.29	23.4 ± 0.43
Plasma ferritin (ng/ml)	58.5 ± 2.25	61.8 ± 4.33	40.3 ± 2.60	47.0 ± 4.06
Plasma zinc erythrocyte protoporphyrin (µg/dl red blood cells)	38.0 ± 0.98	36.8 ± 2.07	51.8 ± 1.70	53.0 ± 2.29
SaO_2 (%)	92 ± 0.2	92 ± 0.4	91 ± 0.3	90 ± 0.4
Socioeconomic and lifestyle characteristics (% of total)				
Full-time farmers (%)	27.2	36.3	85.2	84.3
Presently working in urban area or did so in the past (%)	52.3	53.8	82.8	86.7
Presently enrolled as a student (%)	17.0	22.0	9.6	10.8
Had no formal education (%)	7.4	8.8	21.5	26.5
Does not smoke cigarettes (%)	37.8	37.4	57.9	66.3
Does not drink alcoholic beverages (%)	0	0	0	0
Does not chew coca leaves (%)	39.2	38.5	46.4	54.2

[1] BMI, body mass index; SaO_2, percent of oxygen saturation of arterial hemoglobin.

did not smoke cigarettes, drink alcohol, or chew coca leaves. Thus, the hemoglobin concentration subsample accurately reflected the sample of healthy people in both sites.

The repeatability of height, weight, and SaO_2 was determined by calculating the mean and standard deviation of the difference between measurements of the same individual on different days (Bland and Altman, 1986). The repeatability of height was +1 ± 2 mm (n = 22) in the Tibetan sample and +9 ± 22 mm (n = 22) in the Aymara sample. The repeatability of weight was +0.1 ± 2 pounds (n = 22) in the Tibetan sample and −1.3 ± 5 pounds (n = 22) in the Aymara sample. The repeatability of SaO_2 was +1.3 ± 2.7% (n = 22) in the Tibetan sample and +0.3 ± 2.9% (n = 22) in the Aymara sample. Study participants were not asked for a second venous blood sample to determine repeatability of hemoglobin concentration.

The normality of the distribution of the variables involved in the statistical analyses

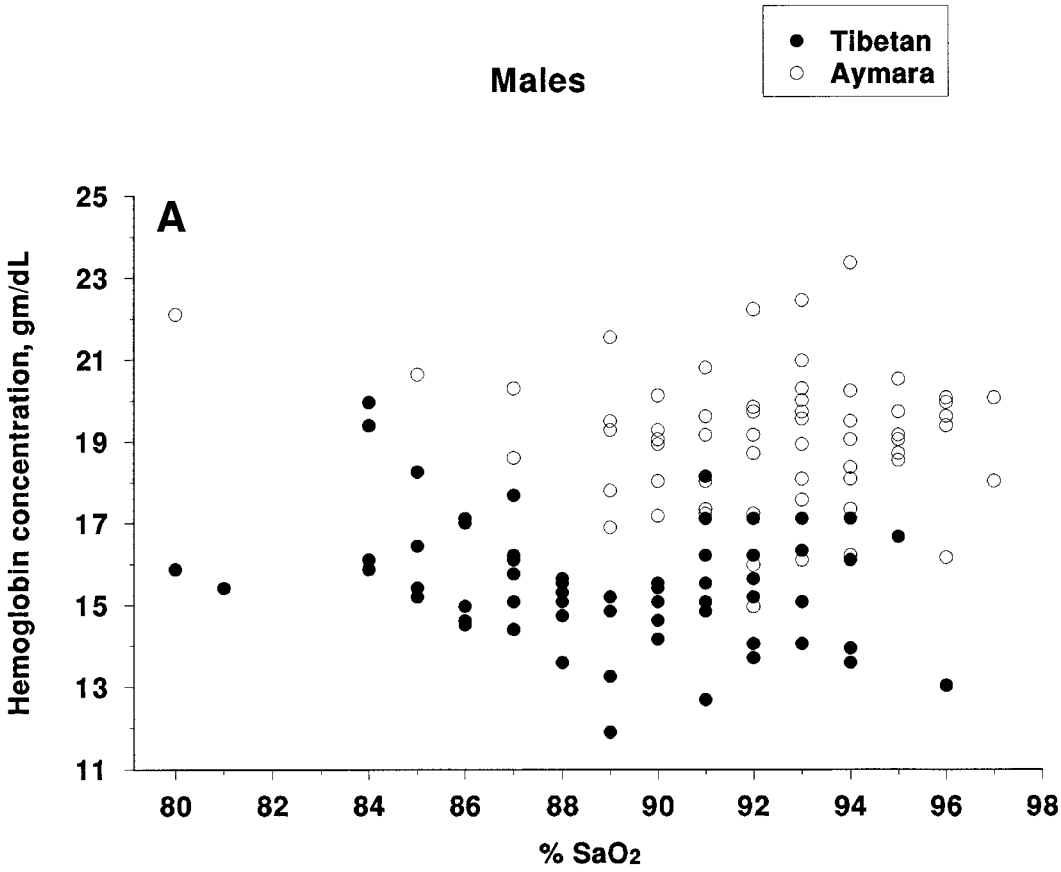

Fig. 1. Scatter plot of Tibetan and Bolivian Aymara hemoglobin concentration with age. **A:** Males. **B:** Females.

(age, BMI, SaO_2, and hemoglobin concentration) was evaluated by the inspection of probability plots displaying the observed cumulative proportions of each variable plotted on the y-axis against the expected cumulative proportions under the assumption of a normal distribution of that variable plotted on the x-axis. The data points clustered about the straight line y = x, and no values deviated markedly from that line. Therefore, it was concluded that they were distributed normally in the males and females from both study sites.

Means and standard errors of the mean are reported. The t-tests address the hypothesis of population differences, and correlation-regression analyses describe the strength of association between hemoglobin concentration and other physical characteristics. Maximum likelihood variance decomposition methods that are available in the computer program PAP (Hasstedt, 1989), as modified by Blangero (1993), provide information regarding the relative importance of covariates, additive genetic, shared household environment, and random environmental effects on phenotypic variation in hemoglobin concentration. This approach uses maximum likelihood estimation techniques to partition the variance in a trait into components that are attributable to additive genetic factors and environmental factors while allowing for the systematic influence of known covariate effects. The heritability, h^2, is determined as the proportion of additive genetic variance relative to the total phenotypic variance. The Tibetan sample was composed of eight pedigrees ranging in

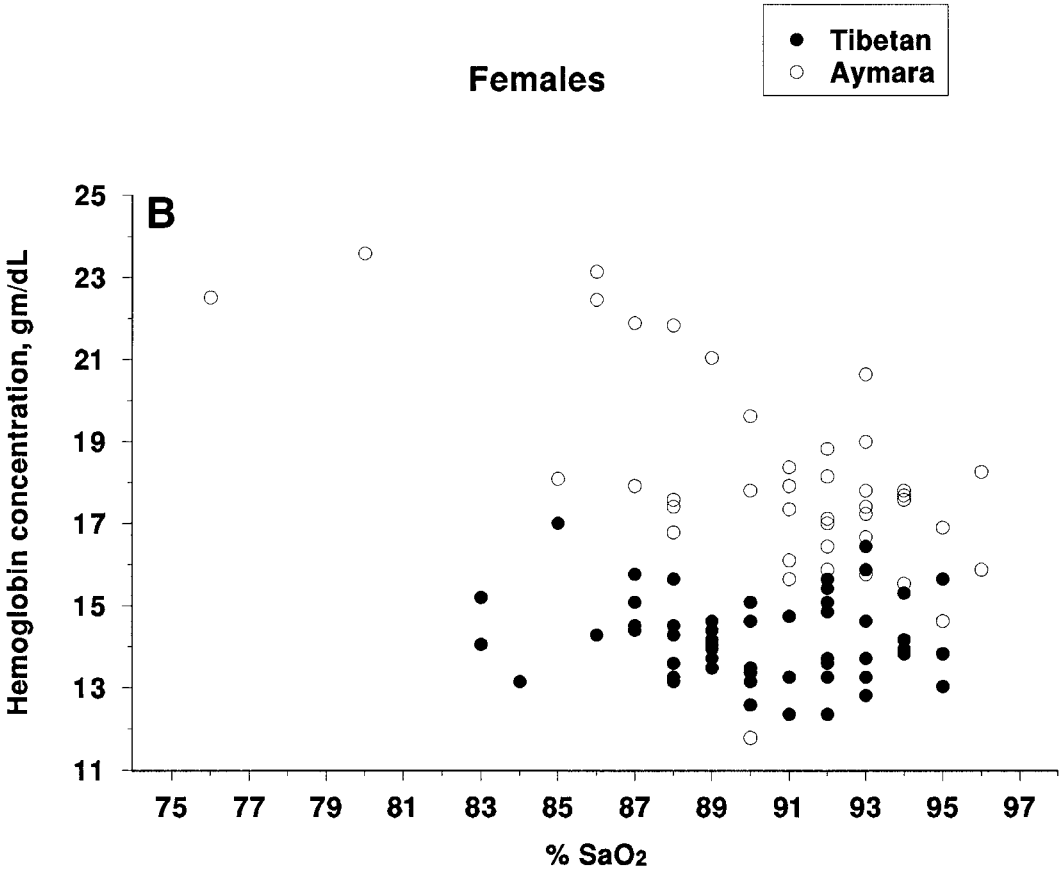

Fig. 1.

size from 2 to 67 people and 13 unrelated individuals who were retained in the analysis to improve parameter estimation. The Bolivian sample consisted of 13 pedigrees with between 2 and 90 individuals and an additional 18 independent individuals who were included in the analysis. Pedigrees were constructed by using all available information on relationships between individuals obtained from the genealogies provided by the study participants. Individuals were defined as belonging to a pedigree if they were biologically related to anyone else in the pedigree.

RESULTS

The Tibetan male sample had a mean hemoglobin concentration of 15.6 ± 0.17 gm/dl, with a range of 11.9–20.0 (n = 75). The Aymara male sample had a 3.5 gm/dl higher mean of 19.1 ± 0.18 gm/dl, with a range of 15.0–25.1 (n = 91; t = −13.8; $P < 0.05$). The Tibetan female sample had a mean hemoglobin concentration of 14.2 ± 0.14 gm/dl, with a range of 12.4–17.6 (n = 61). The Aymara female sample had a 3.6 gm/dl higher mean of 17.8 ± 0.23 gm/dl, with a range of 11.8–23.6 (n = 83; t = −13.0; $P < 0.05$) for Aymara. By study design, the difference was not explained by differences in the prevalence of iron deficiency.

Additional analyses evaluated the potential influence of biological factors contributing to the lower Tibetan hemoglobin concentration. Figure 1 presents scatter plots of hemoglobin concentration with age and illustrates that Tibetans had lower hemoglobin concentration at all ages. Both male samples

TABLE 4. Bivariate correlations of hemoglobin concentration with age, BMI, and SaO_2 in the Tibetan and Bolivian Aymara samples[1]

Group	Age	BMI	SaO_2
Tibetan males	r = 0.06, n = 75	r = 0.07, n = 75	r = −0.26,* n = 68
Tibetan females	r = −0.08, n = 61	r = 0.02, n = 60	r = −0.08, n = 58
Aymara males	r = 0.15, n = 91	r = 0.24,* n = 90	r = −0.14, n = 62
Aymara females	r = 0.29,* n = 83	r = 0.39,* n = 78	r = 0.63,* n = 46

[1] BMI, body mass index; SaO_2, percent of oxygen saturation of arterial hemoglobin.
* $P < 0.05$.

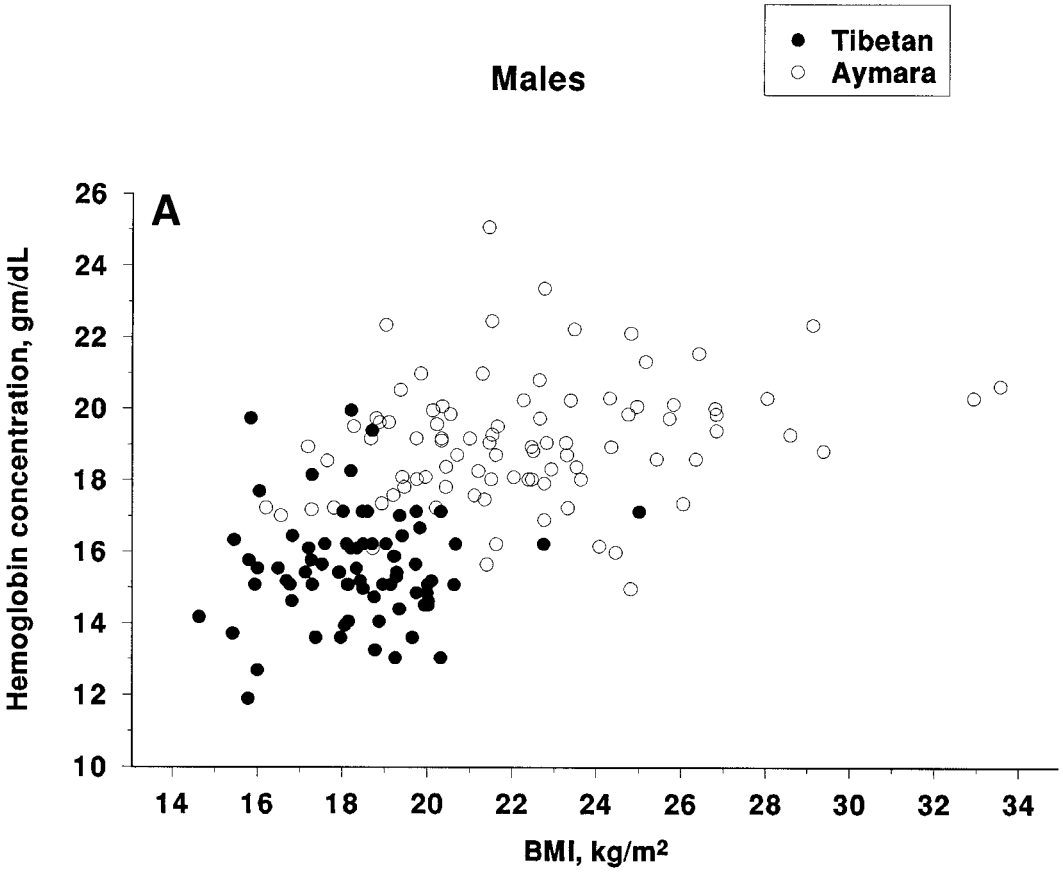

Fig. 2. Scatter plot of Tibetan and Bolivian Aymara hemoglobin concentration with body mass index (BMI). **A:** Males. **B:** Females.

and Tibetan females had stable hemoglobin concentration across the reported age span, whereas Aymara females exhibited a small, significant, positive correlation between hemoglobin concentration and age (Table 4). Figure 2 presents scatter plots of hemoglobin concentration with BMI and illustrates that Tibetans had lower hemoglobin concentrations in the BMI ranges where there was overlap. Aymara had significantly higher hemoglobin concentration at higher BMI, whereas Tibetans did not. Figure 3 shows that Tibetans had lower hemoglobin concentration at all SaO_2. Tibetan males had a small, significant, negative correlation, and Aymara females had a moderate, significant, negative correlation of hemoglobin concentration with SaO_2. The three Aymara

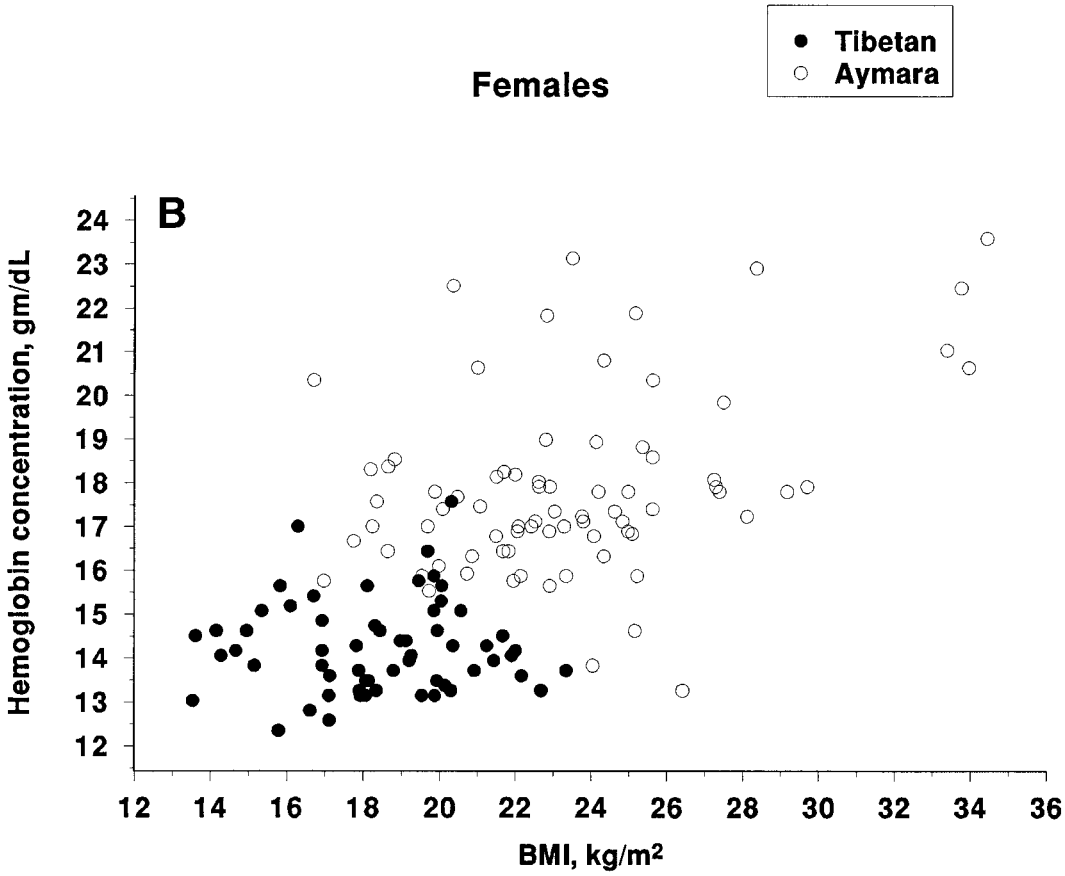

Fig. 2.

women with low SaO_2 did not account for this finding (a correlation analysis excluding them yielded an $r = -0.51$; $P < 0.05$).

The heritability (h^2) of hemoglobin concentration in the Tibetan and Aymara samples was 0.86 ± 0.14 and 0.87 ± 0.12, respectively, considering sex, sex-specific age, and age^2, smoking, and BMI effects as covariates. These results indicated that between 86% and 87% of the phenotypic variance in hemoglobin concentration in each population sample was attributable to additive genetic effects. There was no evidence for the significant effects of shared household environment in either sample.

DISCUSSION

These results confirm the body of evidence that Tibetan highlanders have lower hemoglobin concentration than their Andean counterparts at the same altitude. They strengthen the inference of population differences by indicating that the difference is not an artifact of iron deficiency. Iron-deficiency anemia in the Tibetan sample is extremely unlikely to account for the differences between these two samples, because iron-deficient individuals were eliminated from both samples based on values of two laboratory tests of iron status. The absence of iron deficiency was defined as having ferritin above a standard cut-off value of 12 ng/ml and zinc erythrocyte propoporphyrin below a standard cut-off value of 70 µg/dl red blood cells. Because chronic inflammation can cause elevation of both indicators, there was an acknowledged potential for inclusion of false positives—chronically ill individuals

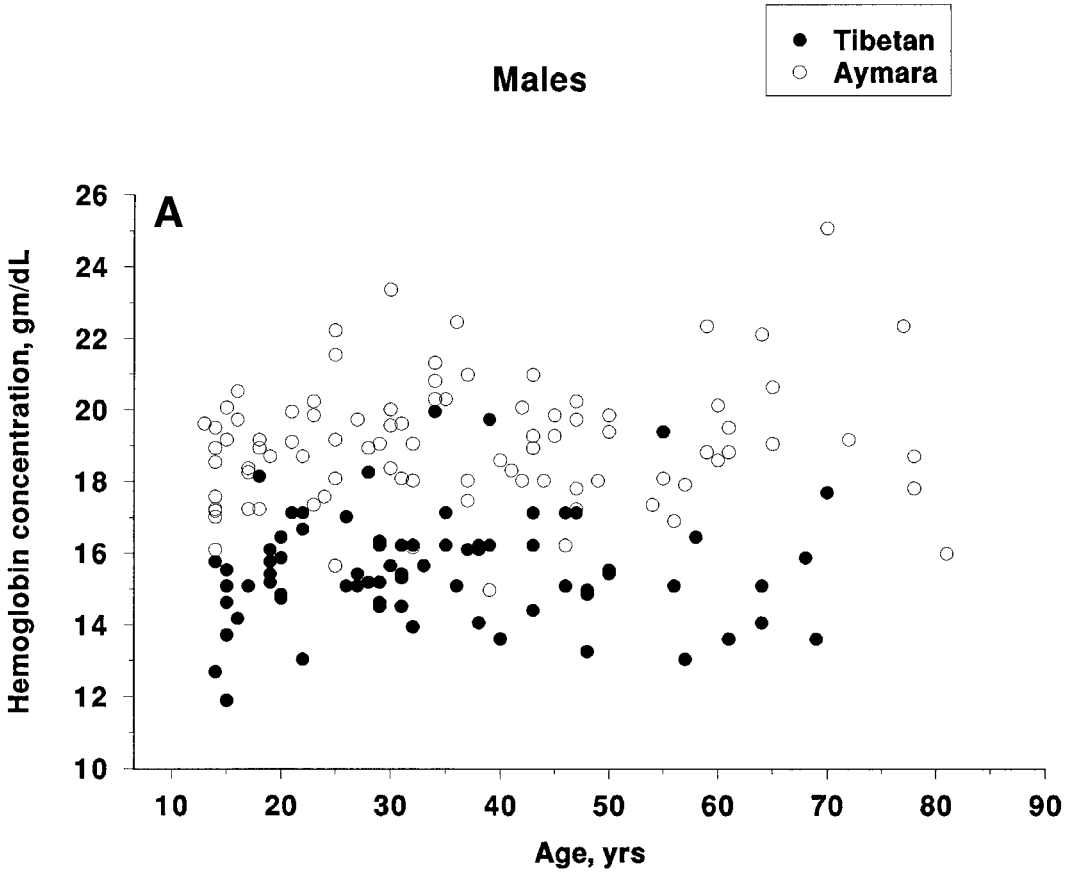

Fig. 3. Scatter plot of Tibetan and Bolivian Aymara hemoglobin concentration with percent oxygen saturation (SaO_2). **A:** Males. **B:** Females.

without iron reserves with ferritin that was nevertheless within the normal range due to their illness. The high cut-off for zinc erythrocyte protoporphyrin countered this by potentially eliminating some individuals with high zinc erythrocyte protoporphyrin values due to infection alone. Epidemiological or laboratory data on chronic inflammation were not available for these two samples. Health was evaluated by self report, and those reporting chronic illness were not included in the sample for analysis. Thus, it is unlikely that widespread, chronic inflammation accounts for the lower Tibetan hemoglobin concentration. Folate deficiency was extremely unlikely to account for the lower Tibetan hemoglobin concentration, because the Tibetan staple food was whole grain barley, a rich source of folate. Overall, it is unlikely that hemoglobin synthesis was limited by nutritional constraints in the Tibetan sample.

It is also improbable that air pollution from cigarette smoking (see, e.g., Ramirez et al., 1991) or burning biomass fuels (wood and dung) for domestic cooking and heating (see, e.g., Norboo et al., 1991) accounts for the higher Aymara hemoglobin concentration. Both practices could exacerbate hypoxia by producing carbon monoxide, which binds to hemoglobin to form carboxyhemoglobin and thereby reduces the amount of oxyhemoglobin and may stimulate a compensatory increase in erythropoiesis and hemoglobin concentration. However, the Aymara sample had substantially lower risk of these forms of pollution. Smoking was much lighter among Aymara than Tibetan males.

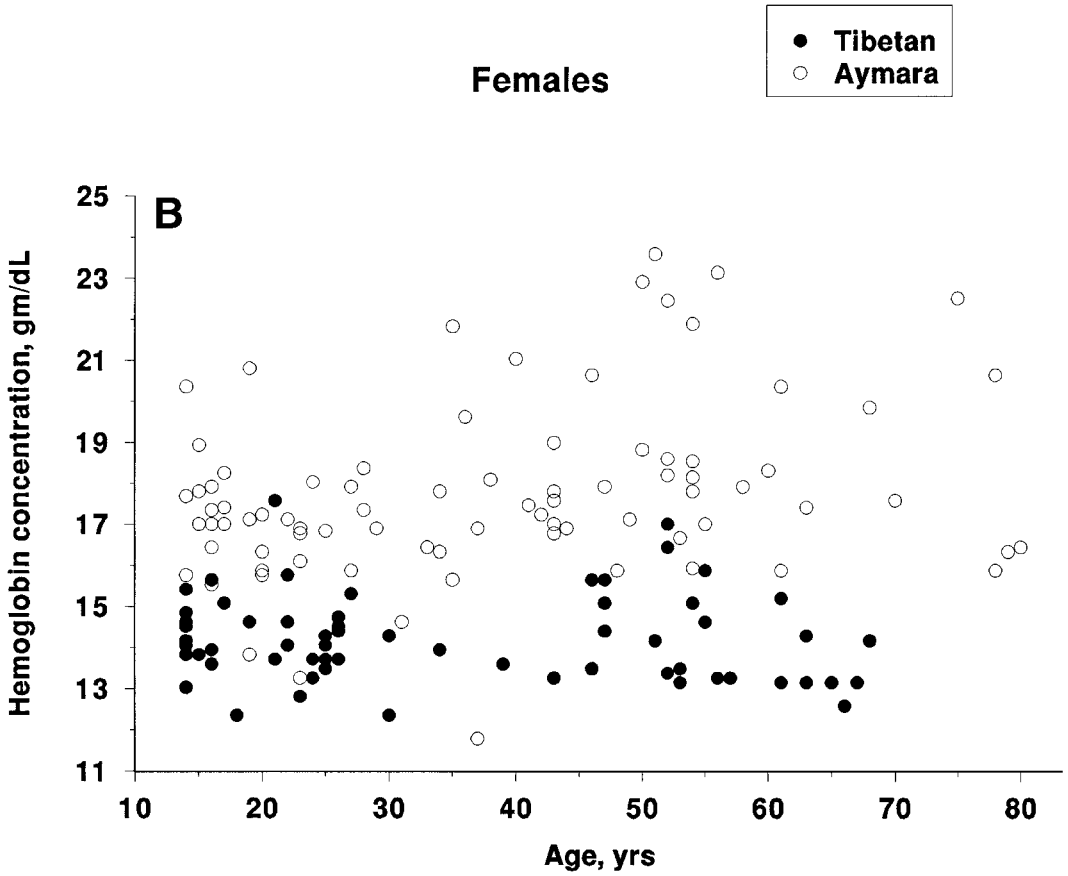

Fig. 3.

Most Aymara males who reported cigarette smoking claimed only occasional smoking at parties. Just 6% of the Aymara males smoked daily compared with 69% of Tibetan males. Similarly, just 1% of the Aymara females reported smoking cigarettes daily, whereas the rest of the female smokers reported only occasional smoking at parties. Exposure to indoor air pollution from biomass fuels was evaluated by using answers to interview questions about the location of the kitchen (attached to the house or in a separate structure) and the type of fuel used (wood, dung, or propane). Only 20% of Aymara males and 17% of Aymara females reported having kitchens in the house, and only 6% of Aymara males and 10% of Aymara females reported using wood fuel only (another 69% of males and 71% of females reported using both wood and propane gas). In contrast, the Tibetans all had kitchens attached to the house and burned both wood and dung. [Alternatives were not available in the villages. After one of the investigators (C.M.B.) walked through them to confirm that there were no detached kitchens and talked with several informants to ascertain that the reported kitchen and fuel-use pattern was uniform, those questions were dropped from the questionnaire shortly after the beginning of the study.] The lighter smoking of Aymara males and the lower proportion of people exposed to daily biomass fuel burning in the house both argue against the possibility that indoor air pollution accounts for their higher hemoglobin concentration.

The substantially lower (nearly 20%) Tibetan than Aymara hemoglobin concentration in the present study was unlikely to reflect systematic differences in recruitment

TABLE 5. Summary of analyses of covariance of site differences in hemoglobin concentration using age, BMI, and SaO_2 as covariates[1]

Source	SS	df	MS	F ratio	Adjusted mean hemoglobin concentration (adjusted for age, BMI, SaO_2) Tibetan	Aymara
Male						
Model	428.44	4	107.11	47.83*	15.6	19.0
Error	279.92	125	2.24			
Total	708.36	129				
Female						
Model	417.00	4	104.25	47.03*	14.3	17.8
Error	210.57	95	2.22			
Total	627.57	99				

[1] BMI, body mass index; SaO_2, percent of oxygen saturation of arterial hemoglobin; df, Degrees of freedom.
* $P < 0.05$.

or measurement technique, because both were controlled by study design and implementation. The process of subject recruitment was the same in both sites, and similar proportions of households and individuals participated. Ethnicity was established on the basis of birth in a traditional Tibetan or Aymara rural area and use of the native language. Although more Aymara than Tibetan participants had been exposed to urban life, and more Aymara lived outside the study community, this did not explain the sample differences. There was an insignificant trend toward lower hemoglobin concentration in the Aymara with urban exposure (defined as past or current work or residence in La Paz), and there was an insignificant trend toward higher hemoglobin concentration in the few Tibetans with urban exposure (Lhasa). The analytic procedures were the same in the two sites. Only data obtained on days when the field spectrophotometer provided transmittance readings of the control/calibrating solutions within 2% of the expected values were used. Thus, a systematic or time-dependent assay bias was avoided.

The population differences were not due to the higher SaO_2 and BMI of the Aymara. This was evaluated by analyses of covariance, which are summarized in Table 5. The estimated mean hemoglobin concentration, adjusted for age, BMI, and SaO_2, differed from the observed sample means by only 0.1 gm/dl.

The findings of the present study were broadly consistent with previous reports of similar samples. The Aymara male sample mean hemoglobin concentration was higher than that reported for other rural Aymara samples and resembled that for urban samples near the same altitude (for review, see Beall et al., 1990). The Aymara female mean was higher (by 1.5–2.0 gm/dl) than previously reported at 3,600–4,000 meters (see, e.g., Moreno-Black et al., 1984; Beall et al., 1990). The Tibetan male mean hemoglobin concentration was 1–2.5 gm/dl lower than that previously reported at 3,600–4,000 meters altitude in rural Nepal and urban TAR (see, e.g., Adams and Shrestha, 1974; Adams and Strang, 1975; Groves et al., 1993; Sun et al., 1990; Zhuang et al., 1993).

The Tibetan mean and distribution of hemoglobin concentration closely resembled those of a United States sea-level sample drawn from the third National Health and Nutrition Examination Survey, 1988–1994 (NHANES III). The NHANES III was designed to represent the civilian, noninstitutionalized population of the United States aged 2 months and older (U.S. Department of Health and Human Services, 1996a–c). A sea-level sample comparable to the present samples was drawn from the published NHANES III data set by selecting "whites" 20–49 years of age residing in the United States but outside of the "West" (to exclude high-altitude residents of the mountain states) whose self-reported health was good, very good, or excellent and who were not iron deficient, as defined by the same two criteria employed in the present study. The United States sea-level male mean hemoglobin concentration was 15.3 ± 0.02 gm/dl, with a range of 10.4–18.7 (n = 1703), and the United States sea-level female mean hemoglobin concentration was 13.4 ± 0.02 gm/dl, with a range of 5.2–16.9 (n = 1797). These means were only 0.3 gm/dl and 0.8 gm/dl lower than Tibetan males and females, respectively, whereas they were 3.8 gm/dl and 3.4 gm/dl lower than Aymara. Figure 4 illustrates the similar cumulative frequency distributions of hemoglobin concentration for the United States and Tibetan samples in contrast to the Aymara curve,

which is shifted to the right by 3–4 gm/dl. The Tibetans in the present study lived at altitudes of 3,800–4,065 meters without significant erythrocytosis. This was also reported for a rural Tibetan sample at 3,250–3,560 meters (Beall and Reichsman, 1984). However, another Tibetan sample at the extremely high altitude of 4,850–5,450 meters had mean male and female hemoglobin concentrations of 18.2 gm/dl and 16.7 gm/dl, respectively (Beall et al., 1987). That finding indicated that Tibetans can respond to high-altitude hypoxia with erythrocytosis; however, the hypoxic stimulus must be stronger than that encountered at the 3,800–4,065 meters of the present study. In contrast, Aymara had a marked erythrocytosis at these altitudes. These findings confirm earlier ones and strengthen the hypothesis that there are population genetic differences in the size of the hematological response to high-altitude hypoxia.

Identifying population differences in genetic factors that determine quantitative traits, such as hemoglobin concentration, requires several steps. A necessary first step is determining that genetic factors influence hemoglobin concentration in one or both samples. The present study estimated the proportion of phenotypic variance in hemoglobin concentration that was attributable to genetic factors (i.e., the heritability of hemoglobin concentration) in each population. Genetic factors accounted for 86% of the total variation in hemoglobin concentration in the Tibetan and 87% of the total variation in the Aymara sample. These h^2 values are higher than the 66% for another high-altitude Tibetan sample (Beall et al., 1994), 59% for a sea-level Australian twin sample (Whitfield and Martin, 1985), and 48% for a sea-level sample of Canadian nuclear families (Perusse et al., 1987). An h^2 of only 22% was reported for a sample that included two Chilean ethnic groups at three altitudes (Chakraborty et al., 1983). That finding was weakened by including different populations in different environments in a single calculation—h^2 values, by definition, are specific to a single population in a single environment. Overall, the present and previous studies indicate that genetic factors account for a large proportion of phenotypic variance in hemoglobin concentration in a wide range of altitudes and samples. The presence of significant genetic variance is a necessary prerequisite for natural selection. The high h^2 values in the present study mean that both populations have a high potential for response to natural selection.

To determine whether the genetic factors that influence hemoglobin concentration in the Tibetan and Aymara populations are different requires additional information on the specific loci and alleles involved. This information is not presently available. However, the design and analysis of the present study suggest that the genetic factors are different. The present study sought to compare two populations in a single environment with respect to relevance for hemoglobin concentration by controlling for the major known environmental influences. The combination of the existence of variation in hemoglobin concentration, the common finding that 86–87% of the variation in hemoglobin concentration is attributable to genetic factors, and the large differences in population means suggest that different alleles influence the hematological response to high-altitude hypoxia in the two populations. These data do not identify the specific genetic factors underlying intrapopulation or interpopulation variation in response or provide insight into the mechanism. Possibilities include factors that attenuate or enhance hemoglobin concentration itself and pleiotropic or epistatic effects of alleles for other traits.

The Pen-Dri Tibetan and Ventilla Aymara samples described in this report differ in other oxygen-transport traits that are relevant to high-altitude adaptation. For example, the Tibetans had 2–3% lower SaO_2 than the Aymara, indicating that the same ambient hypobaric hypoxia results in greater Tibetan arterial hypoxia (Beall et al., 1998). At the same time, Tibetans had 1.5 times higher resting ventilation and roughly double the hypoxic ventilatory response (increase in ventilation upon exposure to further experimental hypoxia) of the Aymara (Beall et al., 1997). These findings, together with the lower Tibetan hemoglobin concentration, suggest that different combinations of quantitative traits have been selected for in these

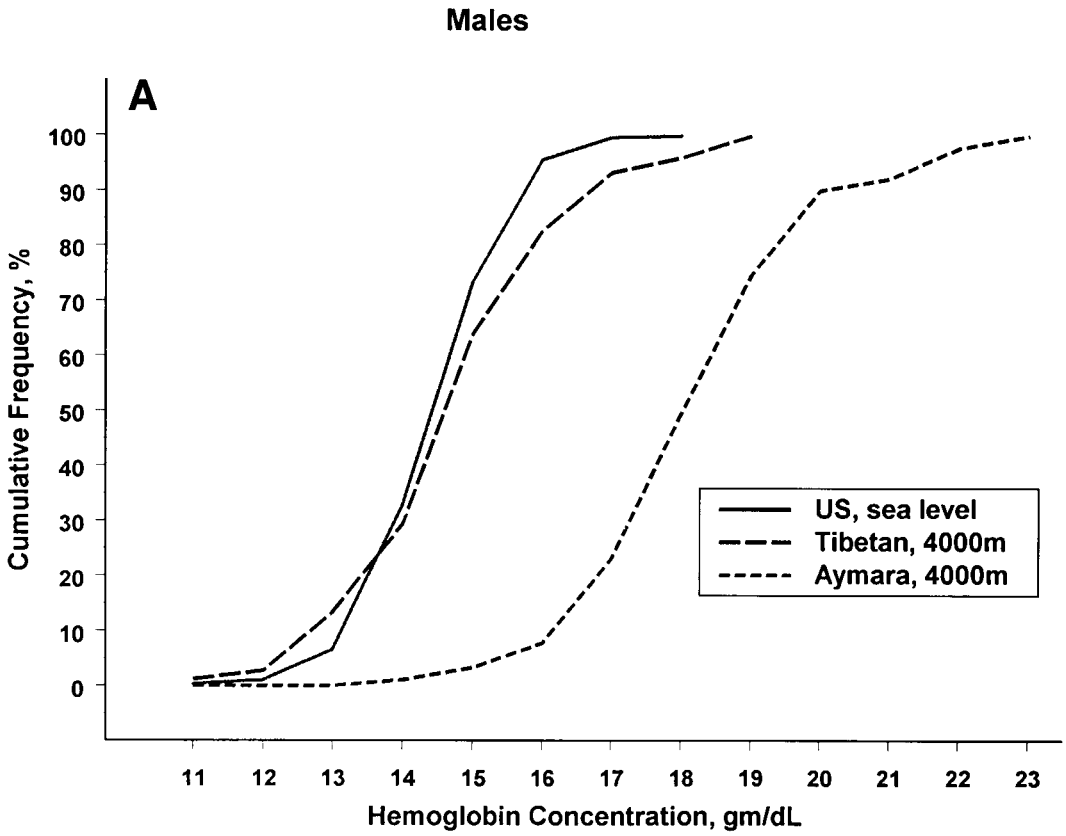

Fig. 4. Cumulative frequency distribution of United States, Tibetan, and Aymara hemoglobin concentrations. **A:** Males. **B:** Females.

two populations with millennia of exposure to the same stress of high-altitude hypoxia. They indicate that human biology is capable of more than one successful response to an environmental stress, and they pose broad questions about the origin of adaptations and the processes whereby populations come to differ in their adaptations. The Tibetan and Andean models of high-altitude adaptation provide a rich natural laboratory for addressing these questions.

CONCLUSIONS

A sample of 136 male and female high-altitude Tibetans had mean hemoglobin concentrations that were 3.5–3.6 gm/dl lower than those from a sample of 174 male and female Aymara from Bolivia. The mechanism that produces the mean differences is not known; however, major environmental influences, such as iron deficiency, were controlled for in the present study. The h^2 values of 86–87% demonstrated that genetic factors accounted for a very high proportion of phenotypic variance, and they revealed a high potential for the action of natural selection on hemoglobin concentration in both populations.

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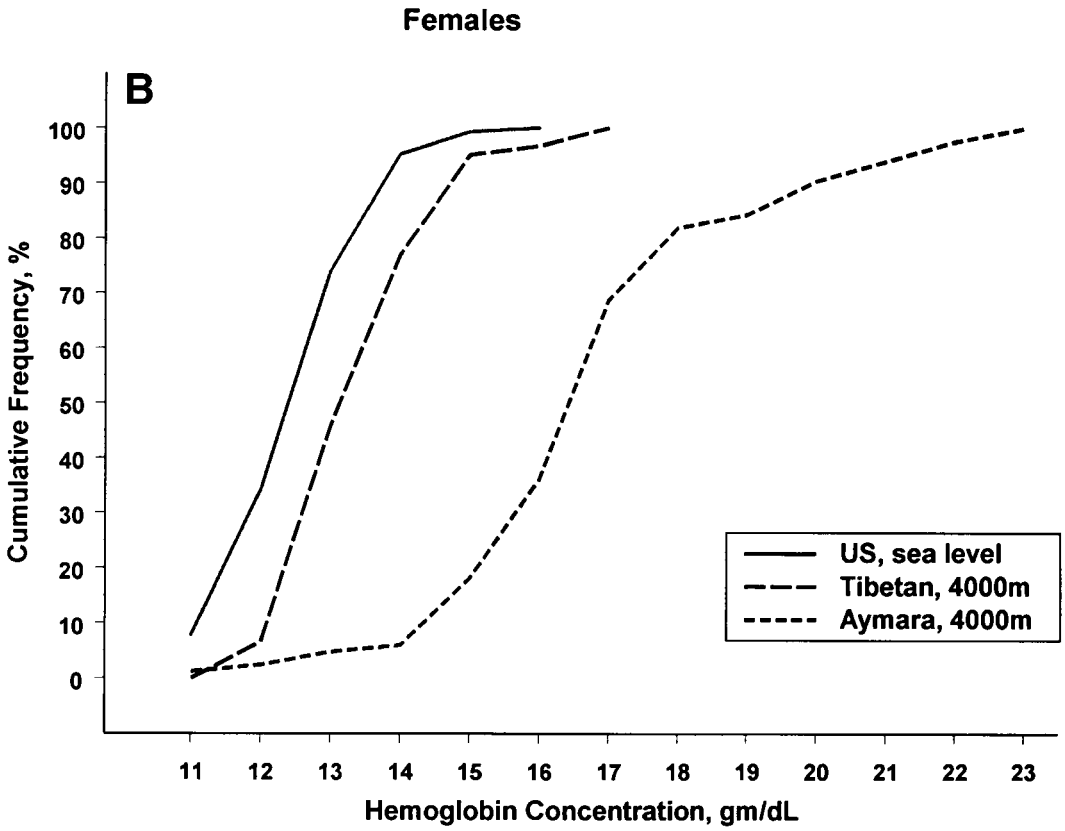

Fig. 4.

some of the Bolivian anthropometry and interviews. Ms. Linda Bowman and Ms. Judy Miniun performed laboratory analyses in Cleveland.

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