CHEST™

Prevalence, Clinical Profile, Iron Status, and Subject-Specific Traits for Excessive Erythrocytosis in Andean Adults Living Permanently at 3,825 Meters Above Sea Level

Aldo De Ferrari, MD; J. Jaime Miranda, MD; Robert H. Gilman, MD, DTMH; Victor G. Dávila-Román, MD; Fabiola León-Velarde, DSc; Maria Rivera-Ch, DsC; Luis Huicho, MD; Antonio Bernabé-Ortiz, MD, MPH; Robert A. Wise, MD, FCCP; and William Checkley, MD, PhD; CRONICAS Cohort Study Group

BACKGROUND: Excessive erythrocytosis (EE) is a prevalent condition in populations living at high altitudes (> 2,500 m above sea level). Few large population-based studies have explored the association between EE and multiple subject-specific traits including oxygen saturation, iron status indicators, and pulmonary function.

METHODS: We enrolled a sex-stratified and age-stratified sample of 1,065 high-altitude residents aged ≥ 35 years from Puno, Peru (3,825 m above sea level) and conducted a standardized questionnaire and physical examination that included spirometry, pulse oximetry, and a blood sample for multiple clinical markers. Our primary objectives were to estimate the prevalence of EE, characterize the clinical profile and iron status indicators of subjects with EE, and describe subject-specific traits associated with EE.

RESULTS: Overall prevalence of EE was 4.5% (95% CI, 3.3%-6.0%). Oxygen saturation was significantly lower among EE than non-EE group subjects (85.3% vs 90.1%, $P < .001$) but no difference was found in iron status indicators between both groups ($P > .09$ for all values). In multivariable logistic regression, we found that age ≥ 65 years (OR = 2.45, 95% CI, 1.16-5.09), male sex (3.86, 1.78-9.08), having metabolic syndrome (2.66, 1.27-5.75) or being overweight (5.20, 1.95-16.77), pulse oximetry < 85% (14.90, 6.43-34.90), and % predicted FVC < 80% (13.62, 4.40-41.80) were strongly associated with EE. Attributable fractions for EE were greatest for being overweight (26.7%), followed by male sex (21.5%), pulse oximetry < 85% (16.4%), having metabolic syndrome (14.4%), and % predicted FVC < 80% (9.3%).

CONCLUSIONS: We found a lower prevalence of EE than in previous reports in the Peruvian Andes. Although the presence of hypoxemia and decreased vital capacity were strongly associated with excessive erythrocytosis, being overweight or having metabolic syndrome were associated with an important fraction of cases in our study population.

CHEST 2014; 146(5):1327-1336

Manuscript received February 5, 2014; revision accepted May 7, 2014; originally published Online First May 29, 2014.

ABBREVIATIONS: CMS = chronic mountain sickness; EE = excessive erythrocytosis; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; LDL = low-density lipoprotein; MMRC = modified Medical Research Council; pro-BNP = pro-brain natriuretic peptide; TIBC = total iron-binding capacity

AFFILIATIONS: From the Division of Pulmonary and Critical Care (Drs De Ferrari and Checkley and Prof Wise), School of Medicine, Johns Hopkins University, Baltimore, MD; CRONICAS Centre of Excellence for Chronic Diseases (Drs Miranda, Bernabé-Ortiz, and Checkley and Prof Gilman) and Departamento de Medicina, Escuela de Medicina (Dr Miranda and Prof Gilman), Universidad Peruana Cayetano Heredia, Lima, Peru; Program in Global Disease Epidemiology and Control (Prof Gilman and Dr Checkley), Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; Cardiovascular Imaging and Clinical Research Core Laboratory (Prof Dávila-Román), Cardiovascular Division, School of Medicine, Washington University in St. Louis, St. Louis, MO; and Departamento de Ciencias Biológicas y Fisiológicas (Prof León-Velarde and Drs Rivera-Ch

Chronic mountain sickness (CMS) is defined as a clinical syndrome characterized by excessive erythrocytosis (EE), severe hypoxemia, neurologic symptoms, sleep disorders, and, in some cases, pulmonary hypertension leading to cor pulmonale. This clinical picture resolves after descending to lower altitudes and reappears upon return to high altitude. In 2005, the Committee on Chronic and Subacute High Altitude Diseases established the current defining parameters for EE as a hemoglobin concentration ≥ 21 g/dL in men and ≥ 19 g/dL in women.[1] Such high levels of hemoglobin are associated with increased blood viscosity and, thus, with a higher risk of developing heart failure and thromboembolic disease. The committee also identified major risk factors for CMS including: being male or a postmenopausal woman, previous history of CMS, sleep apnea, hypopnea, being overweight, and an inability to respond to hypoxia.[1]

Approximately 140 million people worldwide live at altitudes > 2,500 m above sea level and are, therefore, at risk to develop CMS.[2] In South America alone, 35 million people live above this altitude, primarily in Bolivia, Peru, Colombia, and Ecuador.[1] In contrast to the low prevalence of CMS and EE reported among Tibetans living at high altitudes, a higher prevalence has been historically described in South America, ranging from 5% to 18% depending on the population under study.[3-5] The highest prevalence (34%) was found in Cerro de Pasco, Peru, at 4,300 m above sea level, in a group of miners aged ≥ 60 years.[6]

Although many studies have explored the relationship between high altitude, hemoglobin, and oxygen saturation, few have been undertaken in large, population-based studies. Moreover, the association between hemoglobin and iron status in normal subjects and EE participants is less well known.[7,8] We sought to characterize the prevalence, clinical profile, iron status, and subject-specific traits for EE in a population living at 3,825 m above sea level.

Materials and Methods

Study Setting and Design

The study population was composed of adults aged ≥ 35 years living in Puno city (population 120,000) and surrounding nonmining rural communities at 3,825 m above sea level (Fig 1), located in the Puno Department in southwestern Peru. A large proportion of participants were of Aymaran ethnicity, the third largest ethnic group in Peru after mestizos (ie, mixed Amerindian and European ancestry) and Quechuas. Aymaran populations are mainly found in southern Peru and Bolivia. All participants provided verbal informed consent after our research team read the entire informed consent document to them and any questions were answered. Informed consents were verbal because sites included in this study are semiurban and rural with significant rates of illiteracy. All questionnaires were read aloud by trained field workers to study participants. Although most study participants were bilingual in Spanish and Aymara or Quechua, most of our team members were also bilingual and were able to perform the interview in Aymara or Quechua if necessary. The study was approved by the ethics committees of the Bloomberg School of Public Health (IRB2176), Johns Hopkins University (Baltimore, Maryland) and Universidad Peruana Cayetano Heredia (IRB55569) and Asociación Benéfica PRISMA (Lima, Peru).

Figure 1 – *Location of Puno city, Peru.*

and Huicho), Laboratorio de Adaptación a la Altura, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru.

FUNDING/SUPPORT: This work was supported in part by the Center for Global Health of Johns Hopkins University and by federal funds of the National Heart, Lung, And Blood Institute, National Institutes of Health, Department of Health and Human Services [under Contract No. HHSN268200900033C], and by the International Clinical Research Scholars and Fellows Program, Fogarty International Center and National Heart, Blood, and Lung Institute, National Institutes of Health [R24TW007988]. Dr Checkley was further supported by a Pathway to Independence Award [R00HL096955] from the National Heart, Lung and Blood Institute, National Institutes of Health.

CORRESPONDENCE TO: William Checkley, MD, PhD, Division of Pulmonary and Critical Care, School of Medicine, Johns Hopkins University, 1800 Orleans St, Ste 9121, Baltimore, MD 21205; e-mail: wcheckl1@jhmi.edu

DOI: 10.1378/chest.14-0298

We enrolled an age-stratified (35-44 years, 45-54 years, 55-64 years, ≥ 65 years), site-stratified (urban setting vs rural setting), and sex-stratified random sample of 1,066 participants as previously described.[9] Eligibility criteria were as follows: age ≥ 35 years, full-time resident of Puno, able to understand procedures and provide informed consent, not pregnant, without physical disability, and without active pulmonary TB. Only one participant was enrolled per household. Trained field workers conducted a medical history including cardiopulmonary risk factors in either Spanish, Aymara, or Quechua according to participant's primary language of preference, and measured BP, anthropometrics, heart rate, spirometry, and pulse oximetry. Certified phlebotomists collected fasting blood to measure hemoglobin, serum lipids, glucose, insulin, hemoglobin A1c, and high-sensitivity C-reactive protein (hs-CRP) levels. Hemoglobin was determined by an automated sodium lauryl sulfate method for the detection of methemoglobin. All tests were processed in a centralized testing facility.[9] We measured iron status indicators (n = 422), pro-brain natriuretic peptide (pro-BNP) (n = 519), and spirometry (n = 1,005) in subsets of participants that included most or all participants with EE.

Definitions

We defined study terms as follows:

- EE as a hemoglobin ≥ 21 g/dL in men and ≥ 19 g/dL in women[1]
- Rural dwelling as farming communities with low population density not included within an urban area
- Long-term residence at high-altitude as living in Puno ≥ 10 years
- Severe dyspnea as a modified Medical Research Council (MMRC) Dyspnea Scale score ≥ 3[10]
- History of cardiovascular disease as a self-reported history or current medication use for one or more of the following: arrhythmias, angina, myocardial infarction, heart failure, hyperlipidemia, or stroke
- Metabolic syndrome as having three or more of the following five conditions: (1) abdominal obesity (≥ 90 cm in men and ≥ 80 cm in women), (2) elevated serum triglycerides (≥ 150 mg/dL), (3) reduced levels of serum high-density lipoprotein (HDL) cholesterol (< 40 mg/dL), (4) hypertension (systolic BP ≥ 130 mm Hg or diastolic BP ≥ 85 mm Hg, use of antihypertensive medications, or a self-reported history of hypertension), and (5) insulin resistance (fasting glucose ≥ 100 mg/dL, use of antihyperglycemic agents, or self-reported history of diabetes)[11]
- Overweight as having a BMI ≥ 25 kg/m^2 and being obese as a BMI ≥ 30 kg/m^2
- Depression as a score of ≥ 21 in the 18-item version of the Spanish translation of the Center for Epidemiologic Study Depression (CES-D) Scale questionnaire[12]

First language was used as a proxy for ethnicity. We used reference equations for Mexican-Americans by Hankinson et al[13] to calculate % predicted values for prebronchodilator spirometry.

Biostatistical Methods

We used *t* tests to compare continuous variables between groups if normally distributed, Mann-Whitney *U* tests if nonnormally distributed, and χ^2 tests or Fisher exact tests for categorical variables, as appropriate. We used multivariable logistic regression to identify potential determinants of EE. Specifically, we regressed the presence or absence of EE as a function of age ≥ 65 years, being male (female is reference), having metabolic syndrome, oxygen saturation < 85%, categories of hs-CRP (1 mg/L-2.99 mg/L and ≥ 3 mg/L vs 0 mg/L-0.99 mg/L as reference), MMRC Dyspnea Scale score ≥ 3, and % predicted FVC < 80%. Because being overweight and having metabolic syndrome had a high tetrachoric correlation ($\tau = 0.62$) and may, thus, be collinear in regression analysis, we ran a second model in which we regressed the presence or absence of EE as a function of age ≥ 65 years, being male, being overweight, oxygen saturation < 85%, categories of hs-CRP, MMRC Dyspnea Scale score ≥ 3, and % predicted FVC < 80%. We also calculated population attributable fractions from multivariable logistic regression with all possible removal sequences and subsequent averaging.[14] We did not include living in an urban area as an explanatory variable in our final multivariable analyses because urban environment was likely collinear or in the causal pathway for other subject-specific traits. Although a large proportion of cases (75%) lived in an urban setting and site (urban vs rural) was an important determinant of EE in single variable analysis, it became nonsignificant after adjusting for subject-specific factors. We conducted statistical analyses in R (www.r-project.org).

Results

One out of 1,066 high-altitude dwellers did not have hemoglobin concentration available. Of the remaining 1065 participants, 518 (49%) were men, and 542 (51%) lived in rural areas. Mean age of study participants was 55.3 years (SD = 12.6), and mean hemoglobin concentration was 17.8 g/dL (SD = 1.7) in men and 15.9 g/dL (SD = 1.5) in women.

Clinical Profile by EE status

We contrast participant characteristics with and without EE in Table 1. Most participants with EE were men (65%), lived in an urban setting (75%), and had an average age of 61.7 years (SD = 12.3). Aymara was the most prevalent language spoken, with 47% of study participants reporting it as their first language vs 32% for Spanish and 21% for Quechua. Both men and women with EE had a higher BMI and waist circumference, had higher hs-CRP levels, and lower oxygen saturation than did counterparts without EE. Participants with EE had higher triglycerides and lower HDL than those without EE, but total cholesterol and low-density lipoprotein (LDL) levels were similar. The prevalence of metabolic syndrome was also higher in participants with vs without EE. Although there was no difference in FEV_1/FVC, participants with EE had a lower % predicted FEV_1 and % predicted FVC than those without EE (Table 1), suggesting that participants with EE were more likely to have a restrictive pattern vs participants without EE. Subgroup analysis of the 48 participants with EE showed obese participants with EE were more likely to live in an urban setting; had higher levels of cholesterol, LDL, HbA1c percentage, and insulin; and had a higher FEV_1/FVC than did those with EE who were not obese (Table 2); however, these differences were also similar among obese and nonobese participants without EE.

Prevalence of EE

The prevalence of EE in the study population was 4.5% (95% CI, 3.3%-6.0%). Participants with EE had mean hemoglobin concentrations of 22.0 g/dL in men and

TABLE 1] Laboratory and Clinical Parameters Among Participants With and Without EE

Characteristics	EE	No.	No EE	No.	*P* Value
Demographics					
Male, %	64.6	48	47.9	1,017	.04
Age, mean (SD), y	61.7 (12.3)	48	55.0 (12.5)	1,017	<.001
Urban, %	75	48	47.9	1,017	<.001
Daily smoker, %	4.2	48	1.0	1,017	.19
>20-y exposure to biomass fuel smoke, %	41.7	48	57.8	1,010	.04
Clinical parameters, mean (SD)					
BMI in men, kg/m^2	29.0 (4.5)	31	25.6 (3.4)	487	<.001
BMI in women, kg/m^2	30.9 (5.4)	17	26.9 (4.7)	530	.001
Waist circumference in men, cm	100.4 (11.5)	31	90.3 (9.4)	487	<.001
Waist circumference in women, cm	97.4 (15.2)	17	86.4 (12.4)	530	<.001
Systolic BP, mm Hg	117.4 (14.5)	48	114.0 (17.3)	1,017	.19
Diastolic BP, mm Hg	75.5 (10.5)	48	73.5 (10.6)	1,017	.21
Metabolic syndrome, %	60.0	45	35.8	1,014	.002
Pulse oximetry, %	85.3 (4.0)	44	90.0 (3.2)	923	<.001
MMRC Dyspnea Scale score	1.5 (1.2)	48	1.3 (0.8)	1,017	.06
Severe dyspnea, %	14.6	48	8.8	1,017	.24
Depression, %	33.3	48	25.9	1,005	.33
Serum parameters, mean (SD)					
Hemoglobin in men, g/dL	22.0 (1.0)	31	17.5 (1.4)	487	<.001
Hemoglobin in women, g/dL	20.1 (1.2)	17	15.8 (1.3)	530	<.001
hs-CRP, mg/L	6.5 (21.0)	48	2.7 (6.9)	1,017	.001
Fasting glucose, mg/dL	95.7 (15.5)	48	93.2 (27.9)	1,017	.55
Insulin, micro-International Units/mL	10.8 (7.4)	48	7.7 (8.4)	1,017	.01
Hemoglobin A1c, %	6.2 (0.5)	48	5.9 (1.0)	1,017	.06
Total cholesterol, mg/dL	200.1 (50.9)	48	194.6 (41.1)	1,017	.37
HDL, mg/dL	37.7 (8.9)	48	43.1 (11.3)	1,017	.001
Triglycerides, mg/dL	185.5 (78.8)	48	148.1 (92.6)	1,017	.006
LDL, mg/dL	125.4 (40.9)	48	121.9 (35.9)	1,017	.51
N-terminal pro-BNP, pg/mL	98.4 (179.1)	41	76.7 (256.1)	478	.59
Iron status indicators, mean (SD)					
Serum ferritin, ng/mL	156.0 (132.2)	41	167.1 (145.0)	381	.64
Total serum iron, μg/dL	124.4 (72.4)	41	116.4 (55.1)	381	.39
Transferrin saturation, %	33.4 (20.4)	41	32.6 (14.6)	381	.75
Transferrin, mg/dL	297.3 (120.4)	41	281.3 (70.4)	381	.20
UIBC, μg/dL	281.8 (146.1)	41	252.6 (103.8)	381	.09
TIBC, μg/dL	397.5 (127.5)	41	368.2 (97.9)	381	.09
Pulmonary function, mean (SD)					
Prebronchodilator FEV_1, L	2.35 (0.93)	48	2.77 (0.82)	957	.006
% predicted prebronchodilator FEV_1	92.9 (20.8)	48	107.0 (18.2)	957	<.001
Prebronchodilator FVC, L	3.16 (1.19)	48	3.67 (1.05)	957	.009
% predicted prebronchodilator FVC	95.8 (19.1)	48	110.8 (17.0)	957	<.001
Prebronchodilator FEV_1/FVC, %	74.1 (7.7)	48	75.6 (6.6)	957	.23

EE = excessive erythrocytosis; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; LDL = low-density lipoprotein; MMRC = modified Medical Research Council; pro-BNP = pro-brain natriuretic peptide; TIBC = total iron-binding capacity; UIBC = unsaturated iron-binding capacity.

TABLE 2] Comparison Between Obese and Nonobese Participants With EE

Characteristics	Obese	No.	Nonobese	No.	*P* Value
Demographics					
Men, %	55.6	18	74.1	27	.33
Age, mean (SD), y	59.1 (10.3)	18	64.4 (13.6)	27	.14
Urban, %	94.4	18	59.3	27	.01
Daily smoker, %	5.6	18	3.8	27	1
Clinical parameters, mean (SD)					
Systolic BP, mm Hg	120.7 (15.3)	18	115.3 (13.8)	27	.23
Diastolic BP, mm Hg	79.2 (7.3)	18	73.1 (11.7)	27	.04
Pulse oximetry, %	84.8 (3.3)	18	85.7 (4.3)	27	.42
MMRC Dyspnea Scale score	1.8 (1.5)	18	1.0 (1.4)	27	.37
Depression, %	38.8	18	48.1	27	.76
Serum parameters, mean (SD)					
Hemoglobin in men, g/dL	21.9 (0.9)	18	21.9 (0.9)	27	.91
Hemoglobin in women, g/dL	20.1 (0.8)	18	20.2 (1.7)	27	.80
hs-CRP, mg/L	1.02 (1.1)	18	0.74 (1.2)	27	.43
Fasting glucose, mg/dL	98.2 (12.6)	18	95.2 (17.4)	27	.51
Insulin, micro-International Units/mL	15.5 (8.1)	18	7.3 (4.8)	27	$<$.001
Hemoglobin A1c, %	6.4 (0.6)	18	6.1 (0.4)	27	.05
Total cholesterol, mg/dL	219.3 (50.8)	18	188.3 (50.3)	27	.05
HDL, mg/dL	36.6 (6.5)	18	38.3 (9.9)	27	.48
Triglycerides, mg/dL	206.9 (67.5)	18	175.7 (86.8)	27	.18
LDL, mg/dL	141.3 (44.4)	18	114.8 (37.3)	27	.04
N-terminal pro-BNP, pg/mL	59.9 (68.2)	18	135.2 (239.2)	21	.18
Iron status indicators, mean (SD)					
Serum ferritin, ng/mL	165.5 (183)	18	158.5 (71.8)	21	.88
Total serum iron, μg/dL	110.6 (59.8)	18	135.4 (83.8)	21	.29
Transferrin saturation, %	30.3 (15.5)	18	37.3 (24)	21	.28
Transferrin, mg/dL	282.4 (63.3)	18	287.7 (138.9)	21	.88
UIBC, μg/dL	271.4 (69.4)	18	264.1 (169.7)	21	.86
TIBC, μg/dL	366.8 (69.7)	18	395.5 (133.6)	21	.40
Pulmonary function, mean (SD)					
Prebronchodilator FEV_1, L	2.42 (0.85)	16	2.32 (0.99)	27	.73
% predicted prebronchodilator FEV_1	99.8 (15.6)	16	88.9 (22.6)	27	.07
Prebronchodilator FVC, L	3.13 (1.12)	16	3.18 (1.24)	27	.91
% predicted prebronchodilator FVC	99.6 (16.1)	16	93.5 (20.7)	27	.29
Prebronchodilator FEV_1/FVC, %	77.1 (4.2)	16	72.3 (8.7)	27	.02

See Table 1 legend for expansion of abbreviations.

20.1 g/dL in women. In contrast, mean hemoglobin concentrations among high-altitude residents without EE were 17.5 g/dL in men and 15.8 g/dL in women. We found a higher prevalence of EE with older age (Table 3), particularly among those aged ≥ 65 years (7.8% vs 3.4%, $P = .004$). The prevalence of EE was also higher among male participants (6.0% vs 3.1%, $P = .03$) and among those living in an urban setting (6.9% vs 2.2%, $P = .001$).

Subject-Specific Traits Associated With EE

We identified several subject-specific traits for EE in multivariable logistic regression (Table 4). Traditional subject-specific traits included older age (≥ 65 years

TABLE 3] Prevalence of EE by Age and Sex

Age Group, y	Total		Men		Women		*P* Value
	No.	% EE	No.	% EE	No.	% EE	
35-44	260	2.0	128	4.0	132	0	.03
45-54	266	4.1	121	6.6	145	2.1	.12
55-64	271	4.1	130	6.2	141	2.1	.13
65+	268	7.8	139	7.2	129	8.5	.82
Total	1,065	4.5	518	6.0	547	3.1	.03

See Table 1 legend for expansion of abbreviation.

vs younger), male sex (vs female), hypoxemia (pulse oximetry < 85% vs higher), and being overweight (adjusted OR = 5.20; 95% CI, 1.95-16.77; *P* = .002). Having metabolic syndrome, a higher hs-CRP level (vs 0 mg/L-0.99 mg/L) and % predicted FVC < 80% (vs higher) were also all strongly associated with EE. Although the ORs for EE were highest for pulse oximetry < 85% and % predicted FVC < 80%, the average attributable factor was highest for being overweight (average attributable fraction of 27%). Living in an urban setting was associated with an increased odds of EE (unadjusted OR = 3.28; 95% CI, 1.73-6.64); however, this association was no longer significant when adjusted for subject-specific factors (*P* = .24). Specifically, some subject-specific factors were strongly associated with living in an urban setting including: being overweight (OR = 3.79; 95% CI, 2.89-4.99), abdominal obesity (3.39, 2.59-4.46), low HDL (1.67, 1.3-2.16), high triglycerides (2.31, 1.79-3.02), insulin resistance (2.05, 1.47-2.87), having metabolic syndrome (2.4, 1.8-3.7), and having an elevated hs-CRP (3.25, 2.5-4.22).

Iron Status Indicators in EE

We did not find differences in iron status indicators between participants with and without EE (Table 1) including transferrin (297.3 mg/dL vs 281.3 mg/dL, *P* = .20) and total iron-binding capacity (TIBC) (397.5 μg/dL vs 368.2 μg/dL, *P* = .09).

Discussion

In this large, population-based study of 1,065 high-altitude residents living at 3,825 m above sea level, we found an overall EE prevalence of 4.5%. EE was more common among participants who were male, older, had metabolic syndrome or were overweight, had lower oxygen saturation and a decreased forced vital capacity, and a large proportion were urban dwellers. In contrast, iron status indicators were similar between individuals with and without EE. Although the presence of hypoxemia and decreased FVC were strongly associated with EE, having metabolic syndrome or being overweight were associated with an important fraction of cases in our study population.

TABLE 4] Subject-Specific Traits Associated With EE

Traits	Single Variable		Multivariable		Attributable Fraction
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	
Age ≥ 65 y (younger is reference)	2.42 (1.33-4.35)	.003	2.45 (1.16-5.09)	.02	9.4
Being male (female is reference)	1.98 (1.58-7.65)	.03	3.86 (1.78-9.08)	.001	21.5
Having metabolic syndrome	3.42 (1.87-6.47)	< .001	2.66 (1.27-5.75)	.011	14.4
hs-CRP (0 mg/L-0.99 mg/L is reference), mg/L					
1-2.99	3.85 (1.81-8.92)	.001	3.52 (1.47-9.09)	.01	14.6
≥ 3	5.49 (2.46-13.08)	< .001	2.92 (1.07-8.11)	.04	7.3
Pulse oximetry < 85% (higher is reference)	12.97 (6.57-25.29)	< .001	14.44 (6.32-33.23)	< .001	16.4
% predicted FVC < 80% (higher is reference)	8.43 (3.80-17.68)	< .001	12.09 (4.12-34.64)	< .001	9.3
MMRC Dyspnea Scale score ≥ 3 (lower is reference)	1.77 (0.71-3.82)	.18	2.18 (0.66-6.27)	.17	2.5

Subject-specific traits associated with EE and attributable fraction from multivariable logistic regression with all possible removal sequences and subsequent averaging. See Table 1 legend for expansion of abbreviations.

Previous studies in Andean populations residing at comparable altitudes have reported similar or moderately increased mean hemoglobin values[5,15] with a highly variable prevalence of EE. Monge et al[16] evaluated 2,875 healthy Peruvian miners living at 4,300 m above sea level and found EE prevalence that varied from 7% to 34%. Another study found a prevalence of 9% in a high-altitude Andean female population.[3] Our results contrast with the relatively high prevalence of EE historically reported in South America and are similar to values found in Han immigrants residing in the Qinghai-Tibet plateau (5.6%), although not as low as Tibetan natives (1.2%).[17] We also found a higher prevalence of EE with age, a well-described phenomenon,[6] especially among those aged ≥ 65 years.

Differences in the prevalence of EE reported in South America could be explained by the altitude at which previous studies were conducted and differences in genetic admixture. For example, the study conducted by Monge et al[16] was in Cerro de Pasco, Peru, which is located at 4,330 m above sea level (ie, 500 m higher than Puno) and the majority of the population is of Quechuan ethnicity, whereas the study conducted by Beall et al[5] was in Provincia Murillo near La Paz, Bolivia, which is located at 3,900 to 4,000 m above sea level (ie, 100-200 m higher than Puno) in an ethnic Aymara population. Studies have identified candidate genes that help explain differences in prevalence of EE reported in South America, Tibet, and Africa,[18-20] but none of these studies have addressed potential genetic differences among Quechuas and Aymaras. Moreover, Gayà-Vidal et al[21] propose that Aymaras have been genetically more isolated than Quechuas which could have helped Aymaras preserve genes that favor high-altitude adaptation. Therefore, it may not be surprising that higher altitude and genetic admixture may help explain differences in the prevalence of EE between our study and previous work.

Iron deficiency did not appear to be an important factor associated with EE in our study population. Because proteosomal degradation of hypoxia-inducible factor is iron dependent[22] and the hypoxia-inducible factor pathway is associated with the development of EE, we would have expected to find differences in iron status indicators between participants with and without EE. Moreover, a previous study conducted in Cerro de Pasco, Peru found that hypoxic pulmonary hypertension may be attenuated by iron supplementation and exacerbated by iron depletion.[8] However, we did not find differences in iron status by EE status. One alternative explanation could be that those without EE did not have sufficient iron stores to make more hemoglobin. Iron deficiency limits erythropoietic activity and can consequently counterbalance the development of EE. We found it surprising, however, that despite the increased bone marrow requirement, these individuals did not appear to develop iron deficiency. Acute and prolonged exposure to high altitude in lowlanders may induce a marked suppression of hepcidin allowing increased iron absorption and release of iron from stores despite inflammatory activation.[7,23] In highlanders exposed to chronic hypoxia, iron regulation may, thus, reach equilibrium at a different level of hemoglobin, but proving this would require additional biomarkers including erythropoietin, hepcidin, and soluble transferrin receptor in EE group and in a representative subgroup of non-EE to better understand the underlying mechanism.

We identified strong associations between EE and several subject-specific traits in our multivariable model, some of which are well recognized such as hypoxemia, older age, being overweight, and being male.[4,6,24,25] Our study also identified potentially new risk factors for EE, including having metabolic syndrome and a decreased vital capacity. The former is consistent with previous studies that have reported both altered lipid profiles and glucose metabolism in high-altitude dwellers.[26,27] These studies found increased levels of total and LDL cholesterol, increased triglycerides, low levels of HDL cholesterol, glucose intolerance, and both increased BMI and waist circumference in highlanders, all of which are related to metabolic syndrome. However, the relationship between these variables and EE has been less well described in the literature. The results of our study show an increased prevalence of metabolic syndrome among participants with EE vs participants without EE. Moreover, being overweight had the largest attributable fractions for EE, even larger than hypoxemia and having a low FVC, which emphasizes its importance as a potential contributing factor leading to the development of EE because it was highly prevalent in our study population. Although we cannot determine the mechanism by which obesity and metabolic syndrome could lead to EE in this study, we think that it might be related to a combination of their detrimental effects on respiratory physiology[28] and other metabolic disturbances leading to an exaggerated production of erythrocytes.

Our data also suggest that inflammation was independently associated with EE. A study conducted on 25 high-altitude dwellers and 12 lowland control subjects found a higher elevation in oxidative-nitrosative stress among patients with EE vs those without EE, which could

contribute to systemic vascular dysfunction.[29] Moreover, EE has been associated with endothelial dysfunction in animal models[30] and in human observational studies.[31,32] In our study, hs-CRP was elevated among participants with EE vs participants without EE with an average value ≥ 3 mg/L in the EE group, which denotes a high cardiovascular risk.[33] The association between EE and increased cardiovascular risk has been previously described. Rimoldi et al[31] showed that EE leads to systemic vascular dysfunction as evidenced by impaired flow-mediated dilation, increased vascular stiffness, and increased carotid intima-media thickness. Additionally, several studies have shown elevations in inflammatory markers (eg, IL-6, CRP) associated with increased cardiovascular risk in both animal[30] and human studies.[32] Further damage to blood vessels could be secondary to increased blood viscosity, a hallmark of EE, which is a strong predictor of cardiovascular disease and an important pathophysiological factor in the development of atherothrombosis as shown by Jeong et al.[34] In severe cases, EE may lead to pulmonary vascular dysfunction, pulmonary hypertension, and finally cor pulmonale.[35]

The clinical parameters with the highest odds of having EE were hypoxemia (pulse oximetry saturation < 85%) and a low vital capacity (% predicted FVC < 80%). Hypoxemia is the driving force in the development of EE and our findings agree with previous work.[4] While we cannot discuss causation due to the nature of our cross-sectional study, low oxygen saturation in those with EE could also be a consequence of elevated hemoglobin concentration and blood viscosity rather than a cause. We also found decreased lung volumes (both FVC and FEV_1) in participants with vs participants without EE, suggesting that decreased vital capacity is one likely mechanism that drives the hypoventilation seen in patients with EE.[36,37] Because the FEV_1/FVC ratio was similar in both groups, the results also suggest that, in comparison with healthy highlanders, a restrictive pattern predominates among individuals with EE. Although smoking might play an important role in the development of excessive erythrocytosis, only 1% of our study participants reported daily smoking and there was no difference in the prevalence of daily smoking by EE status.

Our study has some potential shortcomings. First, some subject-specific traits were obtained through questionnaires which are subject to reporting biases. We tried to overcome these shortcomings by using validated questionnaires and training our field workers. Second, it is impossible to determine the causality of associations found in cross-sectional studies. A major strength of our work was that it captured a large sample of people living at high altitudes and studied it very extensively, evaluating important clinical and serological parameters, pulmonary function tests, and EE risk factors.

In summary, our results show a lower prevalence of EE than previous Andean reports. Iron status indicators were similar in both groups, suggesting that EE is not related to alterations in iron metabolism. We also found alterations in lipid and glucose metabolism as well as in BMI and waist circumference among subjects with EE, which, together with an elevated hs-CRP level, is compatible with a high prevalence of metabolic syndrome and increased risk of cardiovascular disease. Although presence of hypoxemia and decreased FVC were strongly associated with EE, having metabolic syndrome or being overweight were associated with a large fraction of cases in our study population. Finally, our study confirms some of the traditional risk factors for EE historically described in the literature and adds having metabolic syndrome and % predicted FVC < 80% as new potential ones.

Acknowledgments

Author contributions: W. C., A. B.-O., R. H. G., and J. J. M. conceived the original study design and were responsible for study conduct. F. L.-V., M. R.-C., and L. H. provided expert guidance on high altitude medicine; V. G. D.-R. and R. A. W. provided expert guidance on cardiovascular and pulmonary medicine, respectively; A. D. F. and W. C. led the analysis and writing of the manuscript; all authors contributed equally to the analysis, interpretation of results, and writing of the manuscript; and W. C. had ultimate oversight over study conduct, analysis plan, and writing of the manuscript.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

References

1. León-Velarde F, Maggiorini M, Reeves JT, et al. Consensus statement on chronic and subacute high altitude diseases. *High Alt Med Biol*. 2005;6(2):147-157.
2. Niermeyer S, Zamudio S, Moore LG. The people. In: Hornbein TF, Schoene RB, eds. *High Altitude: An Exploration of Human Adaptation*. New York, NY: Marcel Dekker Inc.; 2001:43-100.
3. León-Velarde F, Ramos MA, Hernández JA, et al. The role of menopause in the development of chronic mountain sickness. *Am J Physiol*. 1997;272(1 pt 2): R90-R94.
4. Monge-C C, Arregui A, León-Velarde F. Pathophysiology and epidemiology of chronic mountain sickness. *Int J Sports Med*. 1992;13(suppl 1):S79-S81.
5. Beall CM, Brittenham GM, Strohl KP, et al. Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. *Am J Phys Anthropol*. 1998;106(3):385-400.
6. León-Velarde F, Arregui A, Monge C, Ruiz y Ruiz H. Aging at high altitudes and the risk of chronic mountain sickness. *J Wilderness Med*. 1993;4(2): 183-188.
7. Talbot NP, Lakhal S, Smith TG, et al. Regulation of hepcidin expression at high altitude. *Blood*. 2012;119(3):857-860.
8. Smith TG, Talbot NP, Privat C, et al. Effects of iron supplementation and depletion on hypoxic pulmonary hypertension: two randomized controlled trials. *JAMA*. 2009;302(13):1444-1450.
9. Miranda JJ, Bernabe-Ortiz A, Smeeth L, Gilman RH, Checkley W; CRONICAS Cohort Study Group. Addressing geographical variation in the progression of non-communicable diseases in Peru: the CRONICAS cohort study protocol. *BMJ Open*. 2012;2(1):e000610.
10. Stenton C. The MRC breathlessness scale. *Occup Med (Lond)*. 2008;58(3):226-227.
11. Alberti KG, Eckel RH, Grundy SM, et al; International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120(16): 1640-1645.
12. Lewinsohn PM, Seeley JR, Roberts RE, Allen NB. Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. *Psychol Aging*. 1997;12(2): 277-287.
13. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general US population. *Am J Respir Crit Care Med*. 1999;159(1):179-187.
14. Rückinger S, von Kries R, Toschke AM. An illustration of and programs estimating attributable fractions in large scale surveys considering multiple risk factors. *BMC Med Res Methodol*. 2009;9:7.
15. Vásquez R, Villena M. Normal hematological values for healthy persons living at 4000 meters in Bolivia. *High Alt Med Biol*. 2001;2(3):361-367.
16. Monge C, Léon-Velarde F, Arregui A. Increasing prevalence of excessive erythrocytosis with age among healthy high-altitude miners. *N Engl J Med*. 1989;321(18):1271.
17. Wu TY, Li W, Li Y, et al. Epidemiology of chronic mountain sickness: ten years study in Qinghai-Tibet. In: Ohno H, Kobayashi T, Masuyama S, Nakashima M, eds. *Progress in Mountain Medicine and High Altitude Physiology*. Matsumoto, Japan: Dogura & Co, Ltd; 1998:120-125.
18. Xing G, Qualls C, Huicho L, et al. Adaptation and mal-adaptation to ambient hypoxia; Andean, Ethiopian and Himalayan patterns. *PLoS ONE*. 2008;3(6):e2342.
19. Beall CM, Cavalleri GL, Deng L, et al. Natural selection on EPAS1 (HIF2alpha) associated with low hemoglobin concentration in Tibetan highlanders. *Proc Natl Acad Sci U S A*. 2010;107(25): 11459-11464.
20. Bigham AW, Mao X, Mei R, et al. Identifying positive selection candidate loci for high-altitude adaptation in Andean populations. *Hum Genomics*. 2009;4(2):79-90.
21. Gayà-Vidal M, Moral P, Saenz-Ruales N, et al. mtDNA and Y-chromosome diversity in Aymaras and Quechuas from Bolivia: different stories and special genetic traits of the Andean Altiplano populations. *Am J Phys Anthropol*. 2011;145(2): 215-230.
22. Jaakkola P, Mole DR, Tian YM, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science*. 2001;292(5516):468-472.
23. Piperno A, Galimberti S, Mariani R, et al; HIGHCARE Investigators. Modulation of hepcidin production during hypoxia-induced erythropoiesis in humans in vivo: data from the HIGHCARE project. *Blood*. 2011;117(10): 2953-2959.
24. Sime F, Monge C, Whittembury J. Age as a cause of chronic mountain sickness (Monge's disease). *Int J Biometeorol*. 1975;19(2):93-98.
25. León-Velarde F, Vargas M, Huicho L, Arregui A, Acosta R. Chronic mountain sickness and chronic lower respiratory tract disorders. *Chest*. 1994;106(1):151-155.
26. Málaga G, Zevallos-Palacios C, Lazo Mde L, Huayanay C. High frequency of dyslipidemia and impaired fasting glycemia in a high altitude Peruvian population [in Spanish]. *Rev Peru Med Exp Salud Publica*. 2010;27(4):557-561.
27. Gonzales GF, Tapia V. Association of high altitude-induced hypoxemia to lipid profile and glycemia in men and women living at 4,100m in the Peruvian Central Andes [in Spanish]. *Endocrinol Nutr*. 2013;60(2):79-86.
28. Parameswaran K, Todd DC, Soth M. Altered respiratory physiology in obesity. *Can Respir J*. 2006;13(4):203-210.
29. Bailey DM, Rimoldi SF, Rexhaj E, et al. Oxidative-nitrosative stress and systemic vascular function in highlanders with and without exaggerated hypoxemia. *Chest*. 2013;143(2):444-451.
30. Ogunshola OO, Djonov V, Staudt R, Vogel J, Gassmann M. Chronic excessive erythrocytosis induces endothelial activation and damage in mouse brain. *Am J Physiol Regul Integr Comp Physiol*. 2006;290(3):R678-R684.
31. Rimoldi SF, Rexhaj E, Pratali L, et al. Systemic vascular dysfunction in patients with chronic mountain sickness. *Chest*. 2012;141(1):139-146.
32. Hartmann G, Tschöp M, Fischer R, et al. High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. *Cytokine*. 2000;12(3):246-252.
33. Grundy SM, Hansen B, Smith SC Jr, Cleeman JI, Kahn RA; American Heart Association; National Heart, Lung, and Blood Institute; American Diabetes Association. Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. *Circulation*. 2004;109(4):551-556.
34. Jeong SK, Cho YI, Duey M, Rosenson RS. Cardiovascular risks of anemia correction with erythrocyte stimulating agents:

should blood viscosity be monitored for risk assessment? *Cardiovasc Drugs Ther*. 2010;24(2):151-160.

35. Penaloza D, Arias-Stella J. The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. *Circulation*. 2007;115(9):1132-1146.
36. Reeves JT, Weil JV. Chronic mountain sickness. A view from the crow's nest. *Adv Exp Med Biol*. 2001;502:419-437.
37. Sun SF, Huang SY, Zhang JG, et al. Decreased ventilation and hypoxic ventilatory responsiveness are not reversed by naloxone in Lhasa residents with chronic mountain sickness. *Am Rev Respir Dis*. 1990;142(6 pt 1):1294-1300.