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# Perinatal hypoxia increases susceptibility to high-altitude polycythemia and attendant pulmonary vascular dysfunction

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**Julian CG, Gonzales M, Rodriguez A, Bellido D, Salmon CS, Ladenburger A, Reardon L, Vargas E, Moore LG.** Perinatal hypoxia increases susceptibility to high-altitude polycythemia and attendant pulmonary vascular dysfunction. *Am J Physiol Heart Circ Physiol* 309: H565–H573, 2015. First published June 19, 2015; doi:10.1152/ajpheart.00296.2015.—Perinatal exposures exert a profound influence on physiological function, including developmental processes vital for efficient pulmonary gas transfer throughout the lifespan. We extend the concept of developmental programming to chronic mountain sickness (CMS), a debilitating syndrome marked by polycythemia, ventilatory impairment, and pulmonary hypertension that affects ~10% of male high-altitude residents. We hypothesized that adverse perinatal oxygenation caused abnormalities of ventilatory and/or pulmonary vascular function that increased susceptibility to CMS in adulthood. Subjects were 67 male high-altitude (3,600–4,100 m) residents aged 18–25 yr with excessive erythrocytosis (EE, Hb concentration  $\geq 18.3$  g/dl), a preclinical form of CMS, and 66 controls identified from a community-based survey ( $n = 981$ ). EE subjects not only had higher Hb concentrations and erythrocyte counts, but also lower alveolar ventilation, impaired pulmonary diffusion capacity, higher systolic pulmonary artery pressure, lower pulmonary artery acceleration time, and more frequent right ventricular hypertrophy, than controls. Compared with controls, EE subjects were more often born to mothers experiencing hypertensive complications of pregnancy and hypoxia during the perinatal period, with each increasing the risk of developing EE (odds ratio = 5.25,  $P = 0.05$  and odds ratio = 6.44,  $P = 0.04$ , respectively) after other factors known to influence EE status were taken into account. Adverse perinatal oxygenation is associated with increased susceptibility to EE accompanied by modest abnormalities of the pulmonary circulation that are independent of increased blood viscosity. The association between perinatal hypoxia and EE may be due to disrupted alveolarization and microvascular development, leading to impaired gas exchange and/or pulmonary hypertension.

polycythemia; altitude; excessive erythrocytosis; developmental programming; hypoxia

### NEW & NOTEWORTHY

*We describe the first systematic evaluation of the relationship between perinatal hypoxia and excessive erythrocytosis in later life. Our findings suggest that adverse perinatal oxygenation*

*raises the risk of excessive erythrocytosis accompanied by a modest elevation of systolic pulmonary artery pressure that is independent of increased blood viscosity.*

DEVELOPMENTAL PROGRAMMING centers on the concept that environmental exposures during perinatal life influence physiological function and disease susceptibility throughout the lifespan by altering the developmental trajectory of numerous organ systems. In humans, the lung and its pulmonary circulation may be particularly vulnerable to perinatal exposures, given that developmental processes vital for efficient pulmonary gas transfer are incomplete at birth (49). Perinatal hypoxia, for instance, impairs alveolarization (34, 39) and promotes cardiopulmonary remodeling and alters vasoreactivity of the pulmonary arteries in the neonatal period (10, 21). The effects of perinatal hypoxia persist into adulthood, influencing ventilatory sensitivity (6, 36) and enhancing pulmonary vascular response to hypoxia (58). In this study we extend the concept of developmental programming to chronic mountain sickness (CMS), a condition that is induced by environmental exposure to hypoxia.

CMS is a debilitating and progressive syndrome marked by pronounced polycythemia, persistent arterial hypoxia, and ventilatory impairment without underlying cardiopulmonary disease (32, 60). A clinical diagnosis of CMS requires a Hb concentration ( $[Hb] \geq 19$  g/dl for women or  $\geq 21$  g/dl for men that is accompanied by three or more of the following symptoms: breathlessness and/or palpitations, sleep disturbance, cyanosis, dilatation of veins, paresthesia, headache, or tinnitus (32). These cutoff values for Hb originated from data obtained in the central Andes of Peru at an altitude of 4,340 m (reviewed in Ref. 32). A large proportion of high-altitude residents develop CMS, with 8–15.6% of men being affected (43, 61) and 8.8% of postmenopausal women presenting with hematocrit levels  $>56\%$  (33). Extensive complications of the pulmonary circulation, including elevated pulmonary artery pressure ( $P_{PA}$ ), pulmonary hypertension, and right ventricular (RV) hypertrophy (RVH), frequently complicate CMS (13, 37, 38, 53, 54) and, together with other severe symptoms that accompany the condition, increase the risk of major disability and mortality from RV failure and other causes. Consequently, CMS is an important public health issue for highland regions worldwide, most prominently in South America, but also in Asia and the Rocky Mountain region of North America.

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Progress toward understanding the etiology of CMS has proven challenging, in part, because the condition is most frequently diagnosed in older adults in settings where the lack of medical records or diagnostic testing limits the ability to identify pathophysiological changes that precede established disease and confounds the ability to distinguish the physiological consequences of CMS from those of normal aging (31, 44). Such progress is facilitated by the recognition of a preclinical condition, “excessive erythrocytosis” (EE), which is defined by  $[Hb] \geq 18.3$  g/dl or 2 SDs above the mean  $[Hb]$  of 17.3 g/dl for men in La Paz, Bolivia; such individuals experienced a progressive decline in ventilatory function, worsening hypoxia, rising hematocrit, and increasing severity of CMS symptoms over a 4-yr period (60).

Perinatal hypoxia influences the maturation of organ systems involved in  $O_2$  transport, as evidenced by an increased incidence of restricted fetal growth, Doppler indices indicative of intrauterine hypoxia, and impaired postnatal arterial oxygenation ( $SA_{O_2}$ ) (11, 28, 50). We hypothesized that perinatal hypoxia increased susceptibility to CMS and attendant abnormalities of the pulmonary circulation by impairing maturation of the lung at the level of the airways and the pulmonary vasculature. This hypothesis was supported by a pilot study (45) in which we identified lower birth weights and a higher incidence of perinatal hypoxia in a convenience sample of young men with EE ( $n = 12$ ). Here we conducted a large-scale community-based survey ( $n = 981$ ) to identify an unbiased sample of young highland men with clearly delineated EE and appropriate controls to evaluate 1) whether modest polycythemia was associated with impaired ventilatory function or abnormalities of the pulmonary circulation and 2) whether perinatal hypoxia increased susceptibility to EE. Understanding the mechanisms by which perinatal hypoxia influences structure and function of the lung, pulmonary circulation, and ventilatory control mechanisms has important clinical applications for identifying the environmental triggers for adult-onset cardiopulmonary disease. Moreover, interventions such as postnatal  $O_2$  supplementation and other measures that could improve the health of large numbers of the 140 million high-altitude residents worldwide are readily available (46).

## METHODS

**Study subjects and protocol.** Using a community-based survey, we screened 1,149 individuals at four postsecondary schools in the La Paz-El Alto metropolitan area in Bolivia to establish a representative sample of young men with EE and healthy controls of similar age, ancestry, and socioeconomic status living across a narrow high-altitude range (3,600–4,100 m) (29). Of the 1,149 individuals screened, 981 were eligible for study. Women were excluded, since polycythemia is rare in women prior to menopause (33). Additional exclusion criteria for the survey were age  $<18$  or  $>25$  yr, a history or evidence of cardiac or pulmonary disease on clinical examination (e.g., essential hypertension, type 1 or type 2 diabetes mellitus, or chronic bronchitis), frequent smoking ( $>4$  cigarettes per week), or residence at  $<3,600$  m. Survey participants completed a brief questionnaire to document age, altitude of birth, residential and medical history, and CMS symptoms of dyspnea, exercise intolerance, headache, tinnitus, cyanosis, and sleep disturbance. Initial clinical examinations, health history, self-reported symptoms, and  $SA_{O_2}$  measurements were collected prior to our knowledge or the subjects’ knowledge of EE status to reduce the potential for recall bias. Hematocrit

and  $[Hb]$  were measured in triplicate using the microcentrifuge and cyanmethemoglobin method, respectively, at the time of the survey.

Survey participants were then classified as control or EE, defined as  $[Hb] \geq 18.3$  g/dl (60), and subsamples were selected at random within these two categories for participation in the complete study protocol. Of the 150 individuals selected, 133 highland men, 67 with EE and 66 healthy controls, agreed to participate. All studies were performed at the Bolivian Institute of High Altitude Biology (3,600 m) in La Paz. On the first visit, peripheral blood samples were collected from an antecubital vein by routine venipuncture for a repeat measurement of  $[Hb]$  and hematocrit and a complete blood count and to test for iron deficiency anemia using established serum ferritin and transferrin saturation cutoff values of  $<10$  ng/ml and  $<15\%$ , respectively, for highland Bolivian men (59). An in-depth interview was also conducted on the first visit to obtain a more detailed residential and health history and information regarding exposures to substances known to influence pulmonary function. Respiratory testing and cardiopulmonary evaluations were performed on subsequent visits. Medical record reviews and an in-depth interview with the subject’s mother were then conducted to obtain information regarding pregnancy, delivery, and perinatal characteristics. The Colorado Multiple Institutional Review Board and Colegio Medico, its Bolivian counterpart, approved all survey and study procedures.

**Systolic  $P_{PA}$  and RVH.** Transthoracic echocardiography (GE Vivid I Ultrasound 2007) was used to estimate systolic  $P_{PA}$  ( $sP_{PA}$ ) and to evaluate right heart structure and function. Subjects rested in the supine or lateral recumbent position for the duration of the examination. Studies began with anatomic surveys and dimensional measurements to screen for structural heart abnormalities. Tricuspid regurgitation was measured with the use of continuous-wave Doppler and averaged from three complete waveforms of good quality. Maximal tricuspid regurgitation jet flow velocity ( $TR_{max}$ ) was used to estimate  $sP_{PA}$  using a modified Bernoulli equation as previously reported (24, 41, 63). Estimation of  $sP_{PA}$  by Doppler transthoracic echocardiography has been validated by right heart catheterization at sea level and at high altitude (4, 8). Tricuspid regurgitation was absent in three subjects (1 control and 2 EE); therefore,  $sP_{PA}$  could not be calculated for these individuals. Pulsed-wave Doppler was used to evaluate pulmonary artery acceleration time (PAAT) from the onset of pulmonary flow to the peak flow velocity (23). Pulmonary vascular resistance (PVR) was calculated from the ratio of  $TR_{max}$  to the RV outflow tract time-velocity integral (TVI-RVOT) using the following formula:  $PVR = (TR_{max}/TVI-RVOT \times 10) + 0.16$  (3).

For electrocardiographic evaluations, the following were considered to be indicative of RVH: 1) peaked P waves with increased voltage in leads II and III and aVF and right precordial leads, 2) rightward axis QRS deviation, 3) a rS pattern in the right precordial leads and complexes of RS or rS type in the left precordial leads, or 4) T wave inversion over the right precordial leads. A single cardiologist-investigator (C.S.S.) performed all transthoracic echocardiography examinations to protect against interoperator effects.

**Pulmonary function tests.** Forced vital capacity (FVC), forced expiratory volume at 1 s ( $FEV_1$ ), forced inspiratory flow, forced inspiratory vital capacity, and peak expiratory flow were determined by spirometry (model 03001, Collins), and flow-volume curves measured for forced expiratory flow at 75% of FVC ( $FEF_{75\%}$ ) by whole body plethysmography (Autobox U6200, Sensor Medics). Pulmonary function testing was performed according to international standards (42), and values are reported as the best of three efforts meeting repeatability criteria established by the American Thoracic Society and the European Respiratory Society (42). The percent predicted values for spirometry were calculated using published reference equations for Mexican Americans (19). Pulmonary diffusion capacity ( $DL_{CO}$ ) was measured by the single-breath method using  $CO$  (1). Alveolar volume ( $VA$ ) was calculated by measuring tracer gas (i.e., helium) dilution during the breath-hold phase of the single-breath  $DL_{CO}$  test (1).  $DL_{CO}$  was corrected for  $[Hb]$  and altitude (1) and

expressed as a raw value and as a function of VA ( $DL_{CO}/VA$ ). Subjects performed practice efforts for all ventilatory procedures, with the exception of the single-breath  $DL_{CO}$  test, before formal examinations were conducted to minimize the impact of learning effects.

**Medical records review and maternal interview.** Perinatal data were obtained by structured interview of the subject's mother and a review of the medical records from the hospital where the subject was born concerning 1) maternal health and reproductive history, 2) pregnancy or delivery complications, 3) fetal complications, and 4) newborn characteristics and complications. Prematurity was defined as a gestational age at delivery <37 wk from the last menstrual period or as noted in the medical records. Perinatal hypoxia was defined as any one of the following: clinical diagnosis of fetal or newborn distress, birth asphyxia, bronchopneumonia, nuchal cord, severe cyanosis, meconium aspiration, resuscitation, neonatal O<sub>2</sub> treatment, or acute neonatal respiratory infection as noted in the medical records or as obtained from the maternal interview. Hypertensive disorders of pregnancy were defined as two or more blood pressures  $\geq 140/90$  mmHg  $\geq 6$  h apart in a woman who was normotensive when non-pregnant and classified as preeclampsia if accompanied by proteinuria ( $>0.3$  g·l protein<sup>-1</sup>·24 h<sup>-1</sup>) or gestational hypertension in the absence of proteinuria as noted in the medical records or as obtained from the maternal interview. For analysis, gestational hypertension and preeclampsia were grouped into a single category ["hypertensive pregnancy" (HTN<sub>PREG</sub>)], since both conditions can impair fetal growth (2).

Of the 133 subjects included for study, we obtained maternal and newborn information for 91 individuals (52 EE subjects and 39 controls). Recovery of the remaining 42 records was prevented by maternal death ( $n = 2$ ) or the inability to contact the mother due to rural residence or no response to the request for interview ( $n = 40$ ). The number of missing perinatal records was not unexpected, given that 43% of women deliver outside the health-care system in Bolivia (56). To maximize the availability of labor and delivery records, we selected schools in and around La Paz, where the proportion of hospital deliveries is higher (65%) than the national average and likely even greater in the educated segment of the population represented by our school-based samples. Age, weight, [Hb], hematocrit, SaO<sub>2</sub>, rural birth, and altitude of birth were equivalent between EE subjects and controls with and without maternal and newborn information; therefore, it is likely that the subjects with records were representative of the larger study population.

**Data interpretation and analysis.** Differences between EE subjects and controls with respect to demographic variables and physiological and perinatal characteristics were determined using ANOVA or  $\chi^2$  tests as appropriate. Data are reported as means  $\pm$  SE or the proportion [odds ratio (OR)] and 95% confidence interval (CI). Logistic regression was used to determine variables that were significantly associated with EE status. Significant variables were introduced into multiple logistic regression models simultaneously to determine the relative contribution of pregnancy or perinatal or other physiological characteristics that were significantly associated with EE status. Age was also included as an independent variable, given its known association with CMS. Statistical analyses were performed using SPSS 19.0. Two-sided  $P < 0.05$  was taken as evidence of significant association, and trends were considered when  $0.05 < P < 0.10$ .

## RESULTS

Individuals included in our community-based survey ( $n = 981$ ) averaged  $21.1 \pm 2.0$  yr of age, and nearly all (95%) were born at  $\geq 2,500$  m. Mean [Hb] and hematocrit values for these individuals were  $16.8 \pm 1.8$  g/dl and  $52.1 \pm 3.1\%$ , respectively. Of those surveyed, 10.4% ( $n = 102$ ) had EE, with a mean [Hb] of  $18.8 \pm 0.5$  g/dl and hematocrit of  $57.5 \pm 2.2\%$  compared with  $16.6 \pm 1.0$  g/dl and  $51.5 \pm 2.7\%$  for controls.

Characteristics of the 133 research subjects (67 EE subjects and 66 controls) selected at random from the community-based survey for physiological study are shown in Table 1. Age, height, weight, arterial blood pressure, and the proportion of individuals born at high altitude were equivalent between EE subjects and controls (97% and 94%, respectively; Table 1). Self-reported ancestry was also similar between groups (controls: 33% Andean, 63% Mestizo, and 4% European; EE subjects: 26% Andean, 71% Mestizo, and 3% European). Known exposure to synthetic fiber, paint, gas, or chemicals was minimal (<10%) and similar in EE subjects and controls. Smoking, alcohol, or coca use was also equivalent in EE subjects and controls (34 and 35%, 55 and 57%, and 12 and 15%, respectively). EE subjects more frequently reported experiencing headaches, cyanosis, dyspnea, or palpitations while resting or with exercise, numbness and tingling of the upper or lower extremities, and dizziness than controls (Fig. 1). Sleep disturbances, including insomnia, excess sleepiness, or interrupted sleep, and mental or physical fatigue also tended to be more common in EE subjects (Fig. 1). By definition, polycythemia was more common in EE subjects than controls; namely, [Hb], hematocrit, and erythrocyte and reticulocyte counts were elevated (Table 1, Fig. 2, A–C).

Maternal, pregnancy, and newborn characteristics are shown in Table 2. Maternal age and parity at the time the research subject was born were similar between EE subjects and controls, and an equivalent proportion of EE subjects and controls were delivered in a hospital (67% and 55%, respectively,  $P = 0.27$ ). None of the women reported smoking during pregnancy.

Thirty percent of EE mothers developed HTN<sub>PREG</sub>, a rate threefold higher than that of control mothers (Table 2). Similarly, 33% of EE subjects experienced hypoxia-related complications during the perinatal period compared with 8% of controls, with birth asphyxia and fetal or newborn distress being the largest contributors to this difference (Table 2). Birth weight, corrected or uncorrected for gestational age, was similar between EE subjects and controls (Table 2). Premature delivery occurred twice as often in EE subjects as in controls,

Table 1. *Subject characteristics*

Variable	Control ( $n = 66$ )	EE ( $n = 67$ )	<i>P</i> Value
<i>General characteristics</i>			
Age, yr	$21.0 \pm 0.3$	$21.5 \pm 0.3$	0.19
Height, m	$1.68 \pm 0.78$	$1.67 \pm 0.50$	0.43
Weight, kg	$23.6 \pm 0.31$	$22.3 \pm 0.36$	<0.01
Heart rate, beats/min	$58.9 \pm 9.9$	$63.3 \pm 10.1$	0.01
Systemic blood pressure, mmHg			
Diastolic	$70.6 \pm 1.3$	$70.4 \pm 1.1$	0.47
Systolic	$101.3 \pm 1.4$	$102.7 \pm 1.1$	0.34
Hematocrit, %	$51.2 \pm 0.3$	$56.9 \pm 0.4$	<0.001
<i>Ventilatory function</i>			
FVC, %predicted	$118.5 \pm 2.1$	$114.5 \pm 2.2$	0.20
FEV <sub>1</sub> , %predicted	$122.6 \pm 1.9$	$117.1 \pm 2.1$	0.06
FEV <sub>1</sub> /FVC, %predicted	$88.5 \pm 0.8$	$86.6 \pm 0.8$	0.08
FEF <sub>75%</sub> , %predicted	$129.1 \pm 3.9$	$119.3 \pm 5.6$	0.05
PEF, %predicted	$123.0 \pm 2.1$	$119.8 \pm 3.1$	0.39
FIVC, %predicted	$112.2 \pm 2.2$	$107.3 \pm 2.3$	0.13

Values are means  $\pm$  SE;  $n$ , number of subjects. EE, excessive erythrocytosis; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s; FEF<sub>75%</sub>, forced expiratory flow at 75% of FVC; PEF, peak expiratory flow; FIVC, forced inspiratory vital capacity.



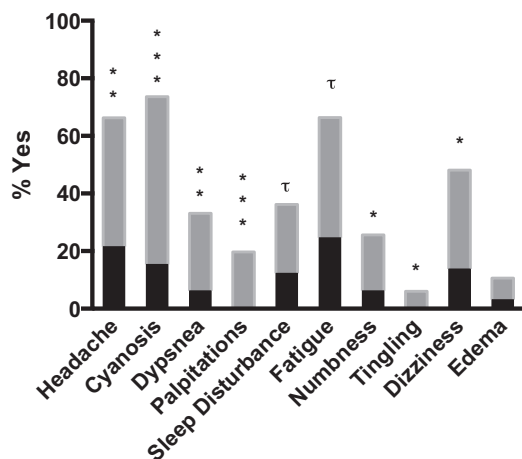


Fig. 1. Self-reported symptoms prior to our knowledge or the subjects' knowledge of excessive erythrocytosis (EE) status. Headaches, cyanosis, dyspnea, or palpitations while resting or with exercise, numbness and tingling of the upper or lower extremities, and dizziness were reported more frequently by EE subjects (gray bars) than controls (black bars). Sleep disturbances, including insomnia, excess sleepiness, or interrupted sleep, and mental or physical fatigue also tended to be more common in EE subjects. Significant differences between controls and EE subjects for each symptom are indicated as follows: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; trends are indicated by  $\tau$ .

although this difference was not statistically significant (30.6% and 16.6%, respectively,  $P = 0.14$ ).

Pulmonary function parameters were similar between EE subjects and controls, with only  $FEV_{75\%}$ ,  $FEV_1/FVC$ , and  $FEV_1$  tending to be lower in EE subjects (Table 1).  $SaO_2$  and  $V_A$  were mildly reduced in EE subjects (Fig. 2, *D* and *E*).  $DL_{CO}$  was reduced in EE subjects compared with controls whether expressed as percent predicted (Fig. 2*F*) or absolute value (data not shown). However, EE subjects had a higher  $DL_{CO}$  relative to their  $V_A$  ( $DL_{CO}/V_A$ ) than controls (7.8 vs. 7.0,  $P = 0.001$ ), indicating that their apparent diffusion limitation compared with controls was likely due to reduced  $V_A$ .

PAAT was lower, pulmonary artery trunk diameter was larger, and  $sP_{PA}$  was slightly elevated in EE subjects compared with controls (Fig. 2, *G–I*), but PVR was similar in controls and EE subjects ( $1.4 \pm 0.0$  and  $1.4 \pm 0.02$ , respectively,  $P = 0.43$ ). As expected, PVR was inversely proportional to PAAT and positively associated with  $sP_{PA}$  in the entire subject population and the EE subjects and controls when considered separately (Fig. 3). A higher proportion of EE subjects had  $sP_{PA} \geq 40$  mmHg (52% vs. 37%,  $P = 0.02$ ) and showed electrocardiographic evidence of RVH (72% vs. 53%  $P < 0.05$ ). RV wall thickness also tended to be greater in EE subjects than controls ( $0.54 \pm 0.01$  vs.  $0.57 \pm 0.01$  cm,  $P = 0.09$ ). All other chamber, valvular, and wall dimensions were within normal limits and did not differ by EE status.

Because  $HTN_{PREG}$ , perinatal hypoxia,  $DL_{CO}$ ,  $sP_{PA}$ ,  $SaO_2$ , body mass index (BMI), and  $V_A$  were significantly associated with EE status [ $HTN_{PREG}$ :  $\exp(\beta) = 5.48$ ,  $P = 0.01$ ; perinatal hypoxia:  $\exp(\beta) = 5.99$ ,  $P = 0.01$ ;  $DL_{CO}$ :  $\beta = -0.039$ ,  $P = 0.005$ ;  $sP_{PA}$ :  $\beta = 0.357$ ,  $P = 0.10$ ;  $SaO_2$ :  $\beta = 0.86$ ,  $P = 0.06$ ; BMI:  $\beta = 1.22$ ,  $P = 0.02$ ;  $V_A$ :  $\beta = 0.73$ ,  $P = 0.05$ ] when examined individually by logistic regression in subjects with birth-related information, we performed multiple logistic regression analyses to determine the independent contribution of each pregnancy-related factor to EE. Age was also included as

an independent variable, because the incidence of CMS is known to increase with age. As shown in Table 3,  $HTN_{PREG}$  and perinatal hypoxia significantly raised the risk of developing EE [OR = 5.25 (CI = 1.00, 27.42),  $P = 0.03$  and OR = 6.44 (CI = 1.07, 38.94),  $P = 0.02$ , respectively] after the influences of  $DL_{CO}$ ,  $sP_{PA}$ , age, BMI,  $SaO_2$ , and  $V_A$  were taken into account.

Finally, to evaluate whether the modest pulmonary vascular differences between EE subjects and controls were influenced by blood viscosity, we used Pearson's correlations to determine whether  $sP_{PA}$  or PVR was independent of  $SaO_2$  or [Hb], since such an association would likely be due to increased blood viscosity. Neither PVR nor  $sP_{PA}$  was associated with  $SaO_2$  or [Hb] when the EE subjects and controls were considered separately or when both groups were combined (data not shown), suggesting that the modest pulmonary circulation abnormalities between EE subjects and controls were not likely due to increased blood viscosity.

## DISCUSSION

This is the first study to systematically examine the influence of perinatal hypoxia on an increase in the susceptibility to EE and its attendant pulmonary circulatory abnormalities. Our findings are novel in three respects. 1) Our observation that the modest pulmonary circulation abnormalities accompanying EE are independent of blood viscosity suggests that polycythemia itself is not a primary determinant of increased  $sP_{PA}$  or PVR in this context. In support of this interpretation,  $sP_{PA}$  was higher, PAAT was lower, and there was more frequent evidence of RVH in EE subjects than controls, while there was no correlation between  $sP_{PA}$  or PVR and [Hb] or  $SaO_2$  during wakefulness. 2) We demonstrate that adverse perinatal oxygenation and  $HTN_{PREG}$  are more common in EE subjects than controls and are significantly associated with EE status, even after other characteristics of EE (e.g., impaired  $DL_{CO}$  and elevated  $sP_{PA}$ ) are taken into account. Specifically,  $HTN_{PREG}$  and perinatal hypoxia increased the probability of developing EE 5.3- and 6.4-fold, respectively. 3) Our large-scale community-based survey highlights the high prevalence (10.4%) of EE in early adulthood and, therefore, the public health importance of screening for elevated Hb in young individuals and for hypoxia-related perinatal complications at high altitude. In sum, our findings suggest an association between hypoxic exposures in early life and the development of CMS during adulthood.

Conducting the study in La Paz, Bolivia was advantageous, given that >1.5 million persons live in the La Paz-El Alto metropolitan area at an altitude that is sufficient to induce polycythemia (60) and increase the incidence of hypoxia-related pregnancy complications (18, 28, 30). With respect to the latter, 13% of infants of Andean ancestry and 33% of infants of European ancestry are affected by hypoxia-induced fetal growth restriction at 3,600 m in Bolivia (28).  $SaO_2$  is also reduced from birth through the first few months of life in infants of Andean and European ancestry at 3,600 m (51). The incidence of hypertensive pregnancy complications and other hypoxia-related perinatal complications, including fetal distress and newborn respiratory distress, are also more frequent at high altitude than at sea level (30). For these reasons, conducting our study in Bolivia enabled us to identify a sufficient number of individuals who experienced hypoxia

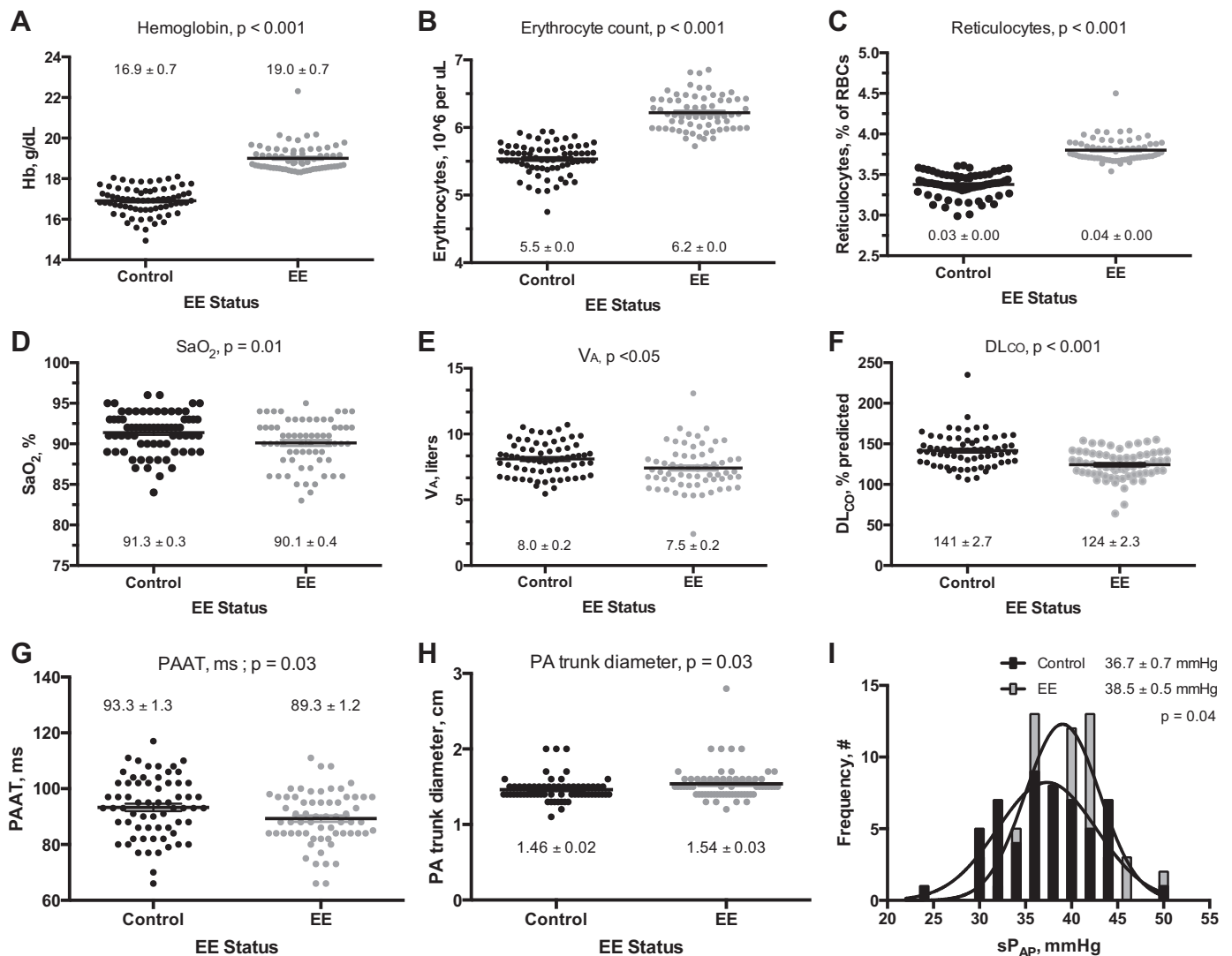


Fig. 2. Hematologic and physiological characteristics of young male highlanders with EE ( $n = 67$ ) compared with healthy high-altitude controls (Control,  $n = 66$ ). Black (control) and gray (EE) dots represent individual subject values; horizontal black lines within each cluster indicate the corresponding mean  $\pm$  SE. As expected, Hb levels (A) and erythrocyte (B) and reticulocyte (C) counts were elevated in EE subjects. Compared with controls, EE subjects also had reduced arterial oxygenation (SaO<sub>2</sub>, D), lower alveolar volume (VA, E), lower pulmonary diffusion capacity corrected for altitude and [Hb] (DL<sub>CO</sub>, F), reduced pulmonary artery acceleration time (PAAT, G), increased pulmonary artery (PA) trunk diameter (H), and elevated pulmonary artery systolic pressure (sP<sub>PA</sub>, I).

during perinatal life and to assess the relationship of these pregnancy-related complications to EE. Another prominent strength of our study was its use of a large-scale, community-based survey to minimize the selection bias introduced by convenience sampling methods.

Our observation that perinatal hypoxia increases the occurrence of EE and modest abnormalities in the pulmonary circulation is consistent with experimental animal and human studies demonstrating that O<sub>2</sub> deficit in early life adversely affects the function and structure of the pulmonary circulation. For instance, lambs gestated at 3,500–3,800 m have greater basal PVR, higher sP<sub>PA</sub>, and enhanced postnatal pulmonary vascular contractile response to hypoxia and pharmacological agonists (10, 20, 21). Highland-gestated lambs retain a higher baseline sP<sub>PA</sub> and PVR under normoxic, low-altitude conditions, indicating that pulmonary circulation abnormalities induced by O<sub>2</sub> deficit in early life persist in the absence of sustained hypoxia (10). In murine models, perinatal exposure to 10% O<sub>2</sub> reduces

pulmonary artery compliance, increases the hypoxic pulmonary vasoconstrictor response, decreases PAAT, and increases small pulmonary artery medial thickness (26, 57). Postnatal normalization of sP<sub>PA</sub> is also impaired in humans at high altitude, as indicated by a delayed or absent drop in sP<sub>PA</sub> (16). Basal P<sub>PA</sub> is higher in offspring of preeclamptic women than offspring of normotensive women at high altitude, regardless of birth weight (24), supporting our observation that hypertensive pregnancy increases susceptibility to EE with modest sP<sub>PA</sub> elevation.

Perinatal hypoxia is also an important determinant of the surface area available for pulmonary gas exchange (39, 40, 47). Specifically, hypoxia impairs septation of peripheral air spaces, a process that is required for the formation of the pulmonary alveoli and extends well into postnatal life (49). In rats, hypoxic (13% O<sub>2</sub>) exposure during the time frame that encompasses alveolarization (gestation to 14 days after birth) irreversibly alters septation and, thereby, increases alveolar size

Table 2. Maternal and newborn characteristics

	Control (n = 39)	EE (n = 52)	P Value
<i>Maternal characteristics</i>			
Age at time of delivery, yr	27.5 ± 1.0	28.3 ± 1.1*	0.60
Parity, no. of live births	4.8 ± 0.4	4.0 ± 0.3*	0.11
HTN <sub>PREG</sub> , %yes	10.3 (1, 20)	30.8 (18, 43)	< 0.05
Other complications, %yes	5.1 (0, 12)	15.4 (6, 25)	0.12
Placenta previa	2.6 (2, 8)	0 (0, 0)	0.24
Premature labor	2.6 (2, 8)	1.9 (0, 6)	0.84
Anemia	0 (0, 0)	3.8 (1, 9)	0.22
Hemorrhage	0 (0, 0)	1.9 (0, 6)	0.38
Premature rupture of membranes	0 (0, 0)	1.9 (0, 6)	0.38
<i>Delivery and newborn characteristics</i>			
Birth wt, g	3,170 ± 12†	3,002 ± 12†	NS
Perinatal hypoxia-related complications, %yes	7.7 (0, 16)	32.7 (20, 45)	<0.01
Birth asphyxia, fetal or newborn distress	5.1 (0, 12)	21.2 (10, 32)	<0.05
Prolonged need for O <sub>2</sub> treatment	2.6 (0, 8)	1.9 (0, 6)	0.84
Bronchopneumonia	0 (0, 0)	3.8 (0, 9)	0.22
Nuchal cord	0 (0, 0)	1.9 (0, 6)	0.38
Severe cyanosis	0 (0, 0)	1.9 (0, 6)	0.38
Meconium aspiration	0 (0, 0)	1.9 (0, 6)	0.38
Acute respiratory infection	0 (0, 0)	1.9 (0, 6)	0.38

Values are means ± SE or percentage and 95% confidence interval; n, number of subjects. HTN<sub>PREG</sub>, hypertension in pregnancy. \*n = 49; †n = 24.

(9, 39). Even brief hypoxic bouts are sufficient to compromise the development of key structures involved in pulmonary gas exchange; 9 h of maternal hypoxia on the last day of pregnancy combined with 1–2 h of hypoxia after birth delays septation, slows the postnatal increase in lung volume, and accelerates alveolar wall thinning in rats (40). Our observation that V<sub>A</sub> was lower in EE subjects than controls is in line with the hypothesis that a reduced V<sub>A</sub> resulting from perinatal hypoxia raises the risk of developing EE. Moreover, smaller V<sub>A</sub>, rather than a pronounced diffusion limitation or reduced pulmonary capillary blood volume, is likely responsible for the lower DL<sub>CO</sub> evident in EE subjects than controls, since values normalized after correction for V<sub>A</sub> (15, 17). However, DL<sub>CO</sub> values are well above 100% predicted values in EE subjects and controls. Hypoxic exposure in later life has been shown to accelerate alveolar formation in animal models (9, 12, 22, 25). Therefore, it is possible that the high DL<sub>CO</sub> values we observed in all subjects are the result of rapid lung development during postnatal life and that this adaptive process may be slightly impaired in individuals who go on to develop EE. We speculate

Table 3. Hypertensive pregnancy and perinatal hypoxia are associated with EE status

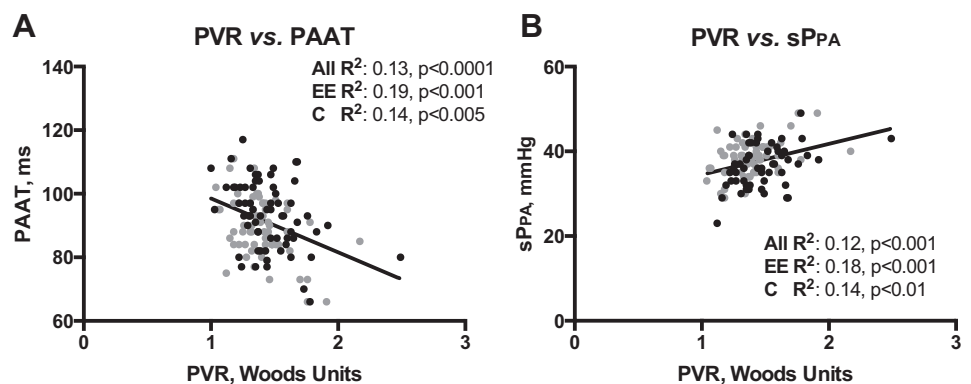
Dependent variable: EE	Exp(β)	95% CI for Exp(β)		P Value
		Lower	Upper	
<i>A. Covariate</i>				
Hypertensive pregnancy, yes	5.25	1.00	27.42	0.049
sP <sub>PA</sub> , mmHg	1.11	0.99	1.24	0.068
DL <sub>CO</sub> , %predicted	-1.02	1.00	1.04	0.092
SaO <sub>2</sub> , %	-0.87	0.71	1.06	0.167
V <sub>A</sub> , liters	-0.67	0.45	0.99	0.045
Age, yr	0.98	0.76	1.25	0.841
BMI, kg/m <sup>2</sup>	1.21	0.99	1.46	0.056
<i>B. Covariate</i>				
Perinatal hypoxia, yes	6.44	1.07	38.94	0.042
sP <sub>PA</sub> , mmHg	1.14	1.02	1.28	0.017
DL <sub>CO</sub> , %predicted	-1.02	1.00	1.04	0.124
SaO <sub>2</sub> , %	-0.93	0.75	1.15	0.469
V <sub>A</sub> , liters	-0.70	0.47	1.05	0.088
Age, yr	0.92	0.71	1.18	0.490

DL<sub>CO</sub>, pulmonary diffusion capacity of CO corrected for altitude and Hb; sP<sub>PA</sub>, estimated systolic pulmonary artery pressure; SaO<sub>2</sub>, arterial O<sub>2</sub> saturation; V<sub>A</sub>, alveolar volume; BMI, body mass index; Exp(β), odds ratio for variable.

that subtle, but persistent, impairment of pulmonary gas exchange efficiency due to reduced V<sub>A</sub> contributes to the etiology of EE and that such effects are mediated, in part, by adverse perinatal oxygenation.

Counter to our expectations, neither birth weight nor the frequency of preterm delivery differed between EE subjects and controls. Previous studies indicate that prematurity increases the risk of diseases characterized by airway obstruction (62), pulmonary circulation abnormalities (5), and lower DL<sub>CO</sub> and impaired exercise capacity in later life (14, 35). We therefore anticipated that prematurity would be associated with impaired ventilatory function, elevated sP<sub>PA</sub>, and higher [Hb]. While the absolute frequency of preterm delivery was higher in the EE group, several of these cases were due to early induction of labor or cesarean section prompted by complications associated with HTN<sub>PREG</sub> (n = 4) or hemorrhage (n = 1). The lack of a statistically significant relationship between the incidence of prematurity and impaired pulmonary function may be explained, in part, by a normalization of lung function during childhood and early adulthood, as has been observed in cases of prematurity without bronchopulmonary dysplasia (48).

Fig. 3. Correlations between characteristics of the pulmonary circulation [pulmonary vascular resistance (PVR), PAAT, and sP<sub>PA</sub>]. Black (control) and gray (EE) dots represent individual subject values. Pearson's correlation coefficients and significance values are shown for all subjects, EE subjects, and controls. As expected, PVR is inversely related to PAAT (A) and positively associated with sP<sub>PA</sub> (B).





Perinatal hypoxia also delays peripheral chemoreceptor maturation, an effect that induces a persistent blunting of the hypoxic ventilatory response and is more pronounced in men than women (6, 27, 36, 52). Therefore, it is possible that the relationship of perinatal hypoxia to EE we observed is due, in part, to altered ventilatory control. In support of this possibility, young men with EE have sleep-disordered breathing patterns compared with controls (29). As speculated elsewhere (51), enhanced neonatal oxygenation in Tibetans compared with Andeans may be a factor in their greater hypoxic ventilatory sensitivity and protection from high-altitude polycythemia (7).

The conduct of these studies faced some limitations due to ethical constraints and logistical aspects of the health-care system in Bolivia. For ethical reasons,  $sP_{PA}$  was estimated using well-established transthoracic echocardiographic methods, rather than directly measured by right heart catheterization (24, 37, 41, 63). Using this strategy, we identified an elevation of  $sP_{PA}$  in EE subjects compared with controls that was statistically significant but likely to be of limited clinical relevance when considered in isolation. In this regard, it is important to emphasize that our aim was to characterize EE, rather than established CMS, which, according to the Qinghai diagnostic criteria, requires specific characteristics including  $[Hb] \geq 21.0$  g/dl and the presence of severe hypoxemia (32); such characteristics were purposefully absent in the present study population, given the early (preclinical) manifestations of the disease. Therefore, while the  $sP_{PA}$  difference we observed was small, we consider that higher  $sP_{PA}$  in conjunction with reduced PAAT increased pulmonary artery trunk diameter and that a tendency toward greater RV wall thickness in EE may reflect an early pathophysiological stage of CMS that could become clinically important with advancing age. Prospective studies are required to determine the clinical progression of pulmonary circulatory abnormalities in EE. An additional consideration for the interpretation of our findings relates to the criteria used for electrocardiographic evidence of RVH. Specifically, because right axis deviation and precordial T wave inversion are common in healthy adolescents and young adults prior to 20 yr of age, the high frequency of RVH we report may indicate the persistence of a juvenile electrocardiographic pattern, rather than RVH (55). However, because we have no reason to believe that the retention of juvenile electrocardiographic patterns would be more common in EE subjects than controls, our findings suggest that RVH is more frequent in EE.

The perinatal data available for these subjects were limited by the types of medical records available in a developing country such as Bolivia. We maximized the recovery of perinatal-related information by surveying segments of the population expected to have a higher proportion of hospital deliveries. Similarly, the inclusion of young adults improved the likelihood of recovering perinatal-related information, given that their births were more recent and the availability of health care and medical records in Bolivia have improved over time. One potential explanation for the higher proportion of EE subjects than controls with perinatal information may be that mothers of EE subjects were more inclined to participate, because they had a complicated pregnancy and/or they were motivated by the fact that their child had EE or was otherwise symptomatic. However, given the similarity in age, weight,

$[Hb]$ , hematocrit,  $SaO_2$ , residence in a rural area, and altitude of birth between EE subjects and controls with and without maternal and newborn information, we considered it likely that the EE subjects and controls with records were representative of individuals without records.

Collectively, our findings demonstrate for the first time that adverse oxygenation during perinatal life is associated with EE and attendant abnormalities of the pulmonary circulation during young adult life at high altitude. We propose that the relationship of perinatal hypoxia to polycythemia may be driven, in part, by impaired efficiency of gas exchange secondary to disrupted alveolarization, pulmonary microvascular development, and/or maturation of chemosensory pathways. Our findings highlight the importance of screening for EE in young adults and for hypoxia-related perinatal complications at high altitude. Further study using experimental animal models to manipulate the degree and magnitude of perinatal hypoxia accompanied by prospective human studies will be useful to clarify the role of  $O_2$  deficit in the onset of hypoxia-related polycythemia and to distinguish whether genetic predispositions to vascular disorders of pregnancy also predispose male offspring to vascular dysfunction and/or polycythemia.

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

#### AUTHOR CONTRIBUTIONS

C.G.J., E.V., and L.G.M. developed the concept and designed the research; C.G.J., M.G., A.R., D.B., C.S.S., A.L., L.R., and E.V. performed the experiments; C.G.J. analyzed the data; C.G.J., E.V., and L.G.M. interpreted the results of the experiments; C.G.J. prepared the figures; C.G.J. drafted the manuscript; C.G.J., M.G., C.S.S., A.L., L.R., E.V., and L.G.M. edited and revised the manuscript; C.G.J., M.G., A.R., D.B., C.S.S., A.L., L.R., E.V., and L.G.M. approved the final version of the manuscript.

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