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RESEARCH ARTICLE

Integrative Cardiovascular Physiology and Pathophysiology

The influence of hemoconcentration on hypoxic pulmonary vasoconstriction in acute, prolonged, and lifelong hypoxemia

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Abstract

Hemoconcentration can influence hypoxic pulmonary vasoconstriction (HPV) via increased frictional force and vasoactive signaling from erythrocytes, but whether the balance of these mechanism is modified by the duration of hypoxia remains to be determined. We performed three sequential studies: *1*) at sea level, in normoxia and isocapnic hypoxia with and without isovolumic hemodilution (n = 10, aged 29 ± 7 yr); *2*) at altitude (6 ± 2 days acclimatization at 5,050 m), before and during hypervolumic hemodilution (n = 11, aged 27 ± 5 yr) with room air and additional hypoxia [fraction of inspired oxygen ($F_{I_{O_2}}$)= 0.15]; and *3*) at altitude (4,340 m) in Andean high-altitude natives with excessive erythrocytosis (EE; n = 6, aged 39 ± 17 yr), before and during isovolumic hemodilution with room air and hyperoxia (end-tidal P_{O_2} = 100 mmHg). At sea level, hemodilution mildly increased pulmonary artery systolic pressure (PASP; + 1.6 ± 1.5 mmHg, P = 0.01) and pulmonary vascular resistance (PVR; + 0.7 ± 0.8 wu, P = 0.04). In contrast, after acclimation to 5,050 m, hemodilution did not significantly alter PASP (22.7 ± 5.2 vs. 24.5 ± 5.2 mmHg, P = 0.14) or PVR (2.2 ± 0.9 vs. 2.3 ± 1.2 wu, P = 0.77), although both remained sensitive to additional acute hypoxia. In Andeans with EE at 4,340 m, hemodilution lowered PVR in room air (2.9 ± 0.9 vs. 2.3 ± 0.8 wu, P = 0.03), but PASP remained unchanged (31.3 ± 6.7 vs. 30.9 ± 6.9 mmHg, P = 0.80) due to an increase in cardiac output. Collectively, our series of studies reveal that HPV is modified by the duration of exposure and the prevailing hematocrit level. In application, these findings emphasize the importance of accounting for hematocrit and duration of exposure when interpreting the pulmonary vascular responses to hypoxemia.

NEW & NOTEWORTHY Red blood cell concentration influences the pulmonary vasculature via direct frictional force and vasoactive signaling, but whether the magnitude of the response is modified with duration of exposure is not known. By assessing the pulmonary vascular response to hemodilution in acute normobaric and prolonged hypobaric hypoxia in lowlanders and lifelong hypobaric hypoxemia in Andean natives, we demonstrated that a reduction in red cell concentration augments the vasoconstrictive effects of hypoxia in lowlanders. In high-altitude natives, hemodilution lowered pulmonary vascular resistance, but a compensatory increase in cardiac output following hemodilution rendered PASP unchanged.

hemodilution; hypoxia; hypoxic pulmonary vasoconstriction; pulmonary pressure; viscosity

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INTRODUCTION

Hypoxic pulmonary vasoconstriction (HPV) of the pulmonary artery is mediated by a range of mechanisms across the neurocardiopulmonary axis (for review, see Ref. 1). Pulmonary vascular smooth muscle cells are intrinsically sensitive to hypoxia, causing the pulmonary arterioles and veins to constrict in response to a decrease in partial pressure of oxygen (P_{O_2}) (2). Extrinsic factors such as vascular endothelium, neurohormonal, and erythrocyte-dependent mechanisms can also alter the balance of vasoactive forces during hypoxic exposure. Erythrocytes can both augment (3, 4) and attenuate (5, 6) the HPV response, through nitric oxide (NO) scavenging and release, depending on whether they are in the oxygenated or deoxygenated state. The balance between these regulatory processes has been suggested to vary depending on the duration of hypoxic exposure (1), but different time domains are often explored in separate studies with differing methodologies making comparisons problematic.

The signal for HPV is predominately derived from alveolar P_{O_2} (Pa_{O_2}) and mixed venous Pv_{O_2}, accounting for ~62% and ~38%, respectively. A lower concentration of red cells will increase HPV by reducing Pv_{O_2} at a constant arterial-venous O_2 difference. The vasodilatory action of deoxygenated hemoglobin will be modified by the concentration of red cells; i.e., during polycythemia, the greater concentration of red cells will lead to a larger signal, and vice versa. Therefore, the degree of HPV for a given P_{O_2} may vary across a range of hematocrit. Indeed, mild hemodilution has recently been demonstrated to exaggerate the pulmonary artery pressure response to acute poikilocapnic hypoxia (7). Hemoconcentration also occurs concomitantly to an increase in pulmonary artery pressure during acclimatization to high altitude, but it is not known whether the increased concentration of red cells amplifies the HPV response through the action of deoxyhemoglobin. During this period, the sensitivity of the pulmonary vasculature to changes in P_{O_2} is also modified, with pulmonary artery pressure remaining responsive to hypoxia after 6–10 days of incremental altitude exposure to 5,300 m (8) but unresponsive to oxygen reversal after approximately 3 wk of simulated ascent of Mt. Everest in hypobaric hypoxia (9). With increasing time at altitude, pulmonary vascular remodeling may predominate the vasoactive effects of red blood cells and other mechanisms in the neurocardiopulmonary axis (10). Understanding the role of hematocrit on HPV is especially pertinent to high-altitude residents of the Andean mountains, where up to one-third of the population experiences excessive erythrocytosis (EE) (11), often alongside elevated pulmonary artery pressures (12) and right ventricular (RV) enlargement (13). Current guidelines promote the consideration of EE in the diagnosis of high-altitude pulmonary hypertension (14) due to the potential for right heart failure in this population (15). However, only recently have mathematical models been developed to correct pulmonary vascular resistance (PVR) to hematocrit (16, 17). Although such models attempt to account for the mechanical effect of blood viscosity, they overlook the vasoactive processes that occur when erythrocyte concentration is reduced or the proportion of hemoglobin that is bound to oxygen is altered. Therefore, comprehensively understanding the interaction between hematocrit, pulmonary vasoconstriction, and duration of exposure will aid the diagnosis and treatment of lifelong high-altitude residents.

We therefore sought to determine the role of erythrocyte-dependent modulation of hypoxic pulmonary vasoconstriction in humans by performing three sequential studies. First, we explored the influence of hematocrit and arterial P_{O_2} in lowlanders at sea level by performing an isovolumic hemodilution in normoxia and with acute isocapnic hypoxia. Second, we examined how changes in hematocrit and arterial P_{O_2} affect pulmonary hemodynamics in lowlanders at high altitude by performing a hypervolemic hemodilution (i.e., normalize hematocrit and blood volume to sea level values) following acclimatization to 5,050 m. Finally, we assessed the pulmonary vascular responses to altered hematocrit and arterial P_{O_2} in Andeans chronically living at 4,340 m with EE by performing an acute isovolumic hemodilution in normal room air and during oxygen supplementation. In line with these three aims, we hypothesized that hemodilution would *1*) increase the pulmonary pressure response to acute isocapnic hypoxia, *2*) augment pulmonary pressure at high altitude and increase the sensitivity of the pulmonary vasculature to acute hypoxia, and *3*) lower pulmonary pressure in Andean high-altitude natives mediated by reduced viscosity.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was granted by the Clinical Research Ethics Board at the University of British Columbia (H16-01297 and HS17-02687 for *studies 1* and *2*, respectively) and the Universidad Peruana Cayetano Heredia, Lima, Peru (No. 101686 for *study 3*) and conformed to the Declaration of Helsinki except for registration in a database. All experimental procedures were explained in writing and verbally in the participants' native language, and written informed consent was obtained from all volunteers.

Experimental Design

Study 1: acute hypoxia.

A total of 10 male participants (age, 29 ± 7 yr; height, 176 ± 4 cm; and weight, 72 ± 2 kg) participated in this laboratory study, all of whom were sea-level residents who were normotensive, nonsmokers with no previous history of cardiovascular, respiratory, or hematological conditions and were taking no prescription medications. The participants visited the laboratory once and first underwent radial artery and internal jugular vein catheterization. Prehemodilution cardiovascular assessments were taken during normoxia and acute isocapnic hypoxia [end-tidal pressure of oxygen ($P_{ET_{O_2}}$) = 40 ± 2 mmHg]. Dynamic end-tidal forcing (18) was used to closely match arterial P_{O_2} to that experienced in *study 2* described below, and carbon dioxide was fixed throughout to avoid the additional influence on the pulmonary vasculature (19). Isovolumic hemodilution was next performed via the removal of whole blood from the internal jugular vein and replaced with infusion of volume-matched human serum

albumin to achieve an absolute drop in hematocrit of ~10% (e.g., 45%–35% hematocrit). Following which, cardiovascular measurements were repeated in normoxia and acute hypoxia.

Study 2: prolonged exposure.

This study was conducted as part of the University of British Columbia Nepal 2016 expedition. Precise details of the adopted ascent profile (20) and experimental design (21) are detailed elsewhere. Although the expedition consisted of numerous studies, care was taken to avoid overlap with drug interventions, and all participants avoided exercise and caffeine >24 h before testing. Following a cautious ascent profile over 10 days, participants were enrolled into the study following 6 ± 2 days of acclimatization to 5,050 m. Eleven male, sea-level residents (age 27 ± 5 yr, height 177 ± 5 cm, weight 75 ± 8 kg) participated in *study 2*. The participants arrived at the laboratory in the fasted state (>4 h) and first underwent arterial and peripheral venous catheterization. Prehemodilution measurements were made while breathing room air and during poikilocapnic hypoxia [fraction of inspired oxygen ($F_{I_{O_2}}$) = 0.15]. All measurements were repeated following successful hypervolemic hemodilution via rapid infusion of saline, a procedure our group has successfully performed previously at high altitude (22). Similar to *study 1*, the intervention aimed to reduce hematocrit by ~10%. In contrast to *study 1*, hypervolemic rather than isovolemic hemodilution was chosen. This was to normalize total blood volume and cardiac filling to sea-level values (22–24), as a lower blood volume would have lowered stroke volume and, in turn, pulmonary artery pressure.

Study 3: lifelong high-altitude residence.

A total of 10 male Andean highlanders were recruited from the town of Cerro de Pasco, Peru (4,340 m) (25). Of these, two did not complete the experiments due to clotting of the arterial line, one did not tolerate the catheterization procedure and withdrew, and one had unacceptable echocardiographic windows. Analysis of blood samples was not possible in one individual due to equipment failure. Therefore, cardiopulmonary data are reported on six Andeans (aged 39 ± 17 yr, height 162 ± 7 cm, body mass 69 ± 12 kg) and hematological data in five participants. Following medical screening that included blood pressure, hematocrit, and full medical and altitude history, the participants reported to the laboratory on one occasion. Instrumentation began with radial artery catheterization for blood gas sampling and antecubital vein cannulation for volume infusion. Cardiovascular assessments were completed in (hypoxic) room air and simulated isocapnic normoxia (end-tidal forcing with hyperoxia to achieve a Pa_{O_2} of 100 mmHg). Hyperoxia was chosen as it allowed a greater magnitude of change in oxygen saturation compared with what was safe and realistic in individuals with a starting saturation of ~75%–80%. These measurements were taken before and after blood volume removal via the arterial catheter and replacement with human serum albumin to achieve an absolute drop in hematocrit of ~10% (26).

Experimental Measures

Hematological and hemodynamic measures.

Arterial (20G, Arrow, Markham, ON, Canada) and central venous catheterization (13-G, Cook Medical, Bloomington, IN) were performed with local lidocaine (1%) and under sterile conditions with ultrasound guidance. The arterial catheters were connected to an inline waste-less sampling system containing a pressure transducer located at the height of the right atrium (VAMP System, Edwards Lifesciences) for the monitoring and assessment of systemic blood pressure. Arterial blood gases, hemoglobin concentrations, and hematocrit were determined via cooximetry (ABL90 Flex, Radiometer, Copenhagen, Denmark) and used to calculate arterial oxygen content (Ca_{O_2}). Absolute blood and plasma volumes were determined using the modified carbon monoxide rebreathing technique (27). Blood viscosity was determined from venous samples using a cone and plate viscometer (Model DV2T, Brookfield Amtek) at 37°C and a shear rate of 225 s^{-1}.

Cardiovascular assessments.

Pulmonary vascular hemodynamic and cardiac function were determined via echocardiography following the American Society of Echocardiography recommendations (28), as previously performed by our group at high altitude (29, 30). Participants were supine and tilted into the left-lateral position where images were acquired in the parasternal and apical imaging windows. Five successive cardiac cycles were recorded on a portable ultrasound (Vivid q, GE Healthcare, Piscataway, NJ) for subsequent offline analysis (Echopac, GE Healthcare, Piscataway, NJ). Pulmonary artery systolic pressure (PASP) was estimated as the maximum systolic pressure gradient across the tricuspid valve. The modified Bernoulli equation ($4V^2$) was applied to the peak systolic regurgitation jet velocity measured via continuous wave Doppler, and right atrial pressure was estimated from the collapsibility of the inferior vena cava. Cardiac output was determined from stroke volume obtained from the velocity-time integral of the left ventricular outflow tract in the five-chamber view and heart rate acquired from a three-lead electrocardiograph. PVR was estimated by calculating mean pulmonary artery pressure from PASP (31), then subtracting left atrial pressure derived from early mitral inflow velocity and early tissue-Doppler velocity of the septal and lateral mitral annulus (32) and dividing by cardiac output. This approach was chosen as our volume interventions may have caused changes in left atrial pressure, rather than assuming left atrial pressure to be zero or applying a set value obtained from the literature (33).

HPV is largely determined by alveolar P_{O_2} (Pa_{O_2}; ~38%) and venous P_{O_2} (Pv_{O_2}; 62%), and the interventions we employed will alter the degree of HPV in line with these relative contributions. For example, hemodilution will reduce arterial oxygen content, such that maintained arterial-venous oxygen differences will result in a lower Pv_{O_2}. Changes in inspired oxygen will reduce both Pa_{O_2} and Pv_{O_2} due to decreased diffusion in the lung and, therefore, provide a stronger stimulus for HPV. To compare the effects of both interventions across our three protocols, we calculated the estimated stimulus P_{O_2} so that our data can be interpreted relative to the stimulus applied. Pa_{O_2} was estimated from the alveolar gas equation, using Pa_{CO_2} from our blood gas data and a fixed respiratory quotient of 0.82. Pv_{O_2} was estimated by assuming a fixed arterial-venous oxygen difference of 5 mL under resting conditions and subtracting that from Ca_{O_2}

measured from arterial blood gas data. The proportional contribution toward HPV was then applied using the following equation from Marshall and Marshall (34): Stimulus P_{O_2} = $Pv_{O_2}^{0.375} \times Pa_{O_2}^{0.626}$.

Statistical Analysis

All statistical analyzes were performed in Graphpad Prism (version 7, San Diego, CA). Distribution normality was confirmed with the Shapiro–Wilk test. Two-way repeated-measures analysis of variance (factors: hemodilution and oxygen saturation) was conducted for dependent data in each study separately, and when a significant main effect was detected, post hoc comparisons were performed with Bonferroni correction to account for multiple comparisons and adjusted P values reported. Where appropriate, effect sizes (Cohen's d) are reported to help indicate the magnitude of change. The slope of the response for a given change in stimulus P_{O_2} was calculated using the rise over run method and tested for differences using a paired-samples t test. Significance was established at $P < 0.05$, and data are presented as means ± SD. The data that support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

Study 1: Acute Hypoxia

Hemodilution was effective in reducing both hematocrit (43.5 ± 2.6% vs. 35.0 ± 1.6%, $P < 0.001$) and blood viscosity [3.5 ± 0.3 vs. 2.8 ± 0.3 centipoise (cP), $P < 0.001$]. By design, there was only a small difference in oxygen content between hypoxia prehemodilution and normoxia posthemodilution, with oxygen content lowest in hypoxia following hemodilution (Table 1). Hemodilution did not alter mean arterial pressure, but it was elevated in response to hypoxia.

As expected, acute hypoxia elevated PASP in both pre- and posthemodilution conditions (main effect $P < 0.001$). Hemodilution resulted in a small increase in PASP in normoxia (+1.6 ± 1.5 mmHg, $P = 0.008$) and a relatively larger increase during hypoxia compared with prehemodilution hypoxia (+4.5 ± 2.4 mmHg, $P < 0.001$, Fig. 1). The changes in PASP in normoxia occurred independent of changes in cardiac output, with no significant main effect for hemodilution ($P = 0.237$). Although there was a significant main effect for the change in oxygen saturation ($P = 0.037$, Fig. 1), post hoc analysis revealed no significant changes in cardiac output. Therefore, observed changes in PVR are likely attributable to changes in pulmonary vasculature tone. Indeed, PVR was increased in response to acute hypoxia in both prehemodilution ($P = 0.002$) and posthemodilution ($P = 0.030$) states and increased in normoxia pre- and posthemodilution ($P = 0.015$).

Study 2: Prolonged Exposure to High Altitude

At high altitude, hemodilution decreased hematocrit from 49.2 ± 2.9 to 43.2 ± 3.2 ($P < 0.001$), so that it was comparable with sea-level baseline in *study 1* (43.5%). Concomitant with the decrease in hematocrit was a reduction in blood viscosity (4.5 ± 0.6 vs. 3.7 ± 0.4 cP, $P < 0.001$) and Ca_{O_2} (Table 1). Acute poikilocapnic hypoxia decreased arterial P_{O_2} and Ca_{O_2} to similar degrees pre- and posthemodilution (Table 1). Despite the hypervolemic nature of the hemodilution, mean arterial pressure was not altered by the intervention (main effect $P = 0.097$).

PASP was not significantly altered ($P = 0.14$) following hemodilution (Fig. 2) while breathing room air, but it was increased pre- and posthemodiltuion during the acute poikilocapnic hypoxia condition ($P < 0.001$) suggesting hemodilution may only increase PASP under acute hypoxic stress and not in the acclimatized state. Consistent with *study 1*, acute poikilocapnic hypoxia increased PASP prehemodilution ($P = 0.004$) and this response remained posthemodilution ($P = 0.002$; Fig. 2). Cardiac output and PVR were unchanged by hemodilution in room air or acute poikilocapnic hypoxia (main effects $P = 0.395$ and $P = 0.116$, respectively).

Table 1. *Hematological and hemodynamic effects of hemodilution in acute and prolonged hypoxia*

	Prehemodilution		Posthemodilution		ANOVA *P* Values		
	Normoxia	Hypoxia	Normoxia	Hypoxia	Hemodilution	O_2 saturation	Interaction
Study 1: acute hypoxia							
Hematocrit, %	43.5 ± 2.6	44.5 ± 2.4*	35.0 ± 1.6*#	35.7 ± 1.4*†	<0.001	<0.001	0.09
Arterial oxygen saturation, %	98 ± 0	75 ± 2*#	98 ± 1	74 ± 4*#	0.6	<0.001	0.6
Arterial P_{O_2}, mmHg	94 ± 4	40 ± 2*#	96 ± 7	40 ± 2*#	0.26	<0.001	0.3
Arterial P_{CO_2}, mmHg	42 ± 2	42 ± 2	41 ± 2	42 ± 1#	0.87	0.02	0.02
Arterial oxygen content, mL/dL	19.3 ± 1.1	15.7 ± 0.7*#	15.1 ± 1*#	11.9 ± 0.8*†	<0.001	<0.001	0.002
Mean arterial pressure, mmHg	99 ± 6	107 ± 14*#	98 ± 5	107 ± 8*#	0.18	0.01	0.07
Heart rate, beats/min	61 ± 12	74 ± 13	61 ± 10	75 ± 11*#	0.885	<0.001	0.839
Stroke volume, mL	61.5 ± 10.6	60.5 ± 17.6	63.1 ± 14.7	66.7 ± 16.6	0.095	0.738	0.365
Study 2: prolonged exposure							
Hematocrit, %	49.2 ± 2.9	49.6 ± 2.7*#	43.2 ± 3.2*	45.7 ± 2.4*†	<0.001	<0.001	0.002
Arterial oxygen saturation, %	85 ± 3	70 ± 7*#	86 ± 3	73 ± 7*#	0.397	<0.001	0.98
Arterial P_{O_2}, mmHg	49 ± 3	35 ± 4*#	52 ± 4*	37 ± 4*#†	<0.001	0.001	0.149
Arterial P_{CO_2}, mmHg	25 ± 2	23 ± 3	23 ± 4	20 ± 1*#†	0.001	<0.001	0.244
Arterial oxygen content, mL/dL	18.8 ± 1.4	15.7 ± 1.9*#	16.7 ± 1.3*	15.0 ± 1.6*#	<0.001	<0.001	0.003
Mean arterial pressure, mmHg	107 ± 11	96 ± 10*	111 ± 8	100 ± 13*#	0.097	0.005	0.802
Heart rate, beats/min	59 ± 13	65 ± 16*#	56 ± 15	65 ± 15*#	0.576	0.002	0.145
Stroke volume, mL	62.5 ± 13.6	63.9 ± 13.5	70.3 ± 14.2	63.7 ± 10.9	0.137	0.316	0.2

Values are means ± SD. $P < 0.05$ vs. *normoxia prehemodilution, vs. #normoxia posthemodilution, and vs. †hyperoxia prehemodilution.

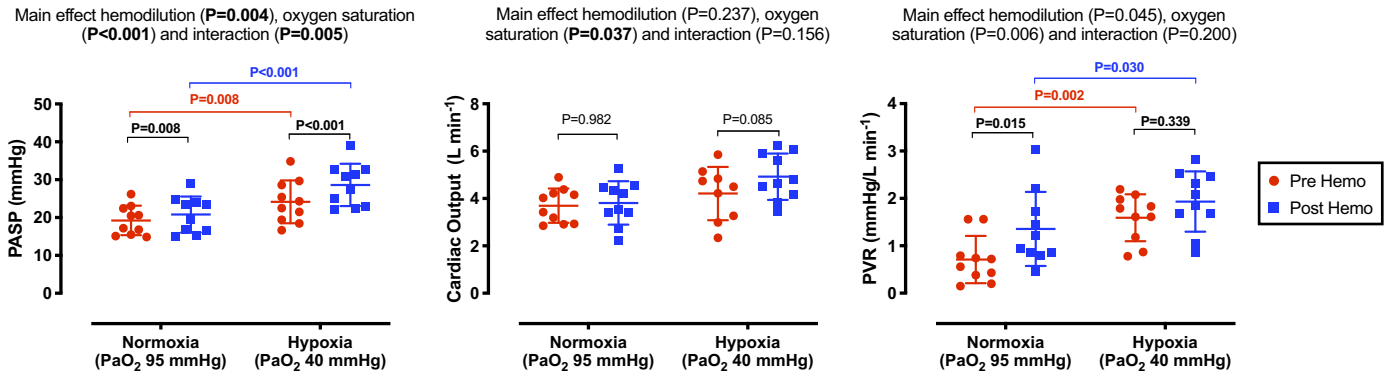

Figure 1. Pulmonary vascular response to acute hypoxia before and after hemodilution at sea level. Pulmonary artery systolic pressure (PASP) and pulmonary vascular resistance (PVR) were increased in response to both hemodilution and acute hypoxia. Significant *P* values in bold. Pa_{O_2}, arterial partial pressure of oxygen.

Study 3: Lifelong High-Altitude Residence

Hemodilution reduced hematocrit from 68.2 ± 5.4% to 58.4 ± 4.9% ($P < 0.001$) and lowered viscosity from 8.1 ± 1.4 to 5.4 ± 0.7 cP ($P = 0.002$). Although these represent substantial reductions, Andean natives still remained polycythaemic even compared with lowlanders at high altitude in *study 2*. Mean arterial pressure was not altered by hemodilution or acute hyperoxia (Table 2). PASP remained unchanged following hemodilution ($P = 0.201$) and during hyperoxia ($P = 0.504$). The consistent PASP during both interventions are likely underpinned by an increase in cardiac output and a decrease in PVR in response to hemodilution (Fig. 3). For example, in room air, hemodilution increased cardiac output ($P = 0.027$) but decreased PVR ($P = 0.019$). A similar effect of hemodilution was observed under hyperoxia.

Influence of Stimulus P_{O_2} on Pulmonary Hemodynamics

In lowlanders, hemodilution increased the slope of the PASP response to an increased stimulus P_{O_2} at both sea level and high altitude (Fig. 4), but the slope of the PVR response remained unchanged suggesting that the increase in pressure is largely related to the higher cardiac output needed to sustain oxygen delivery following hemodilution. Although the slope of the PVR response was unchanged, as discussed above, PVR for a given stimulus P_{O_2} was elevated by hemodilution at sea level (main effect $P = 0.045$) but not at high altitude. In contrast, neither the slope of the PVR nor PASP response was altered by hemodilution when the stimulus P_{O_2} was decreased during hyperoxia in Andeans. Moreover, PVR was reduced (main effect $P = 0.029$) by hemodilution in Andean natives, highlighting differences between the temporal domains of hypoxic exposure.

DISCUSSION

In relation to our three hypotheses, the primary novel findings of this series of studies are *1*) hemodilution augments the pulmonary pressure response to acute isocapnic hypoxia; *2*) in lowlanders at high altitude, hemodiltion had no effect on pulmonary pressure but the pulmonary vasculature was more responsive to changes in the stimulus P_{O_2}; and *3*) pulmonary pressure remained unchanged in Andeans following acute hemodilution because of an increase in cardiac output but reciprocal reduction in PVR. Collectively, our data indicate that the influence of arterial P_{O_2} and hematocrit on the pulmonary pressure response to hypoxia is modified by the duration of hypoxic exposure and starting hematocrit.

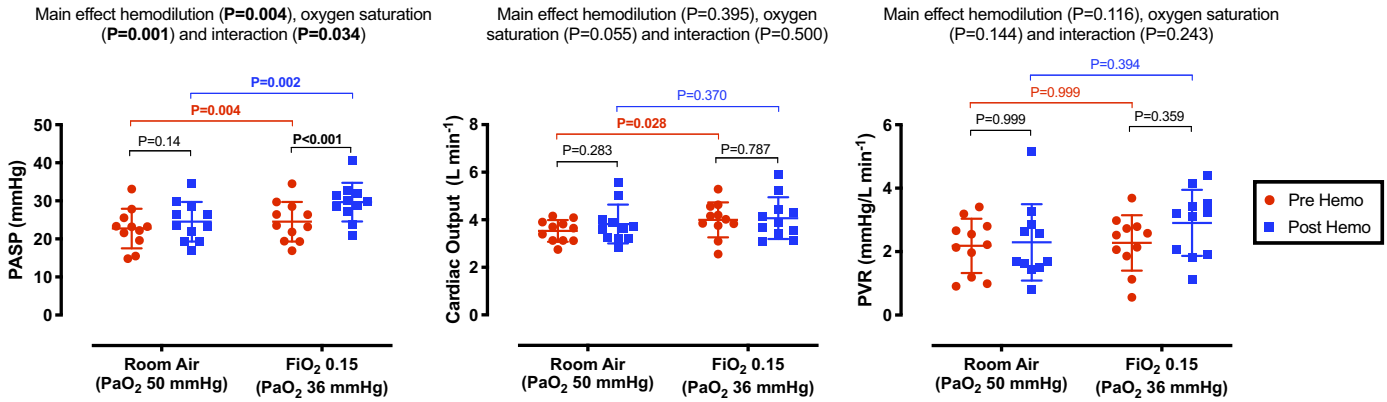

Figure 2. Pulmonary vascular response to hemodilution following acclimatization to 5,050 m in room air and acute hypoxia. Pulmonary artery systolic pressure (PASP) was unaltered by hemodilution but remained responsive to acute hypoxia. Significant *P* values in bold. FI_{O_2}, fraction of inspired oxygen; Pa_{O_2}, arterial partial pressure of oxygen.

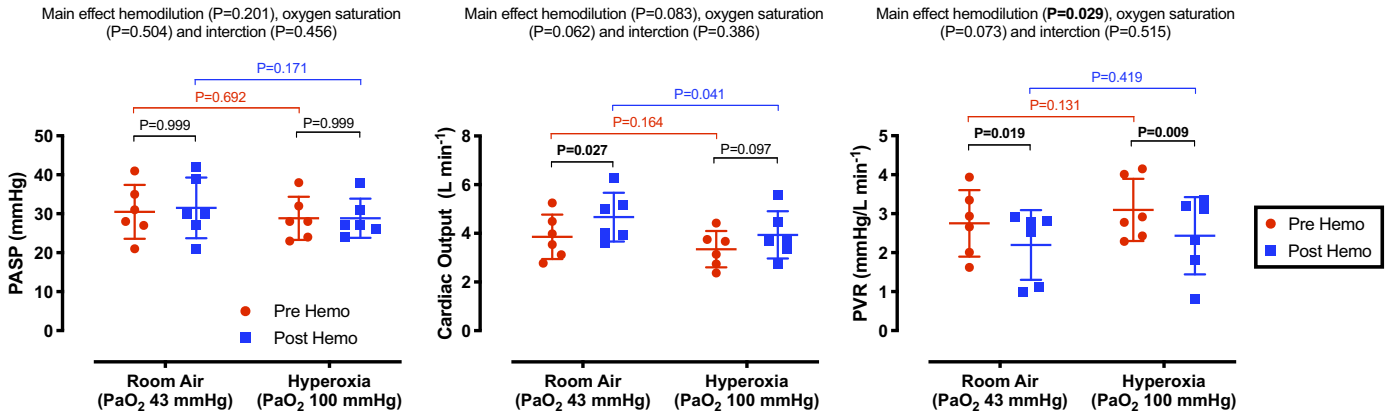

Figure 3. Pulmonary vascular response to hemodilution in 6 Andeans with excessive erythrocytosis in room air and after restoration of oxygen saturation to sea level values. Pulmonary artery systolic pressure (PASP) was unresponsive to both acute hypoxia and hemodilution, but pulmonary vascular resistance was reduced following hemodilution despite an increase in cardiac output. Significant *P* values in bold. Pa_{O_2}, arterial partial pressure of oxygen.

Hemodilution and Acute Hypoxia

As hypothesized, the reduction in hematocrit increased the magnitude of pulmonary pressure response to isocapnic hypoxia; that is, there was a greater increase in pulmonary artery pressure for a given change in stimulus P_{O_2} following hemodilution. A similar response has recently been reported whereby greater increases in pulmonary pressure and cardiac output were observed during graded poikilocapnic hypoxia (7). We confirm and extend these findings by controlling for changes in end-tidal P_{CO_2}, as the ventilation-induced hypocapnia in the study of Duke et al. (7) would likely have elicited vasodilatation that would have effectively masked the hemodilution-induced elevation in pulmonary pressure (19). The increased sensitivity to hypoxia following hemodilution may be mediated by the vasodilatory action of red blood cells. The lower concentration of red cells would attenuate the generation of *S*-nitrosothiol (6), NO (5), and ATP (35) by deoxyhemoglobin, and low hemoglobin levels can increase free radical signaling (36, 37). Together, these mechanisms would shift the vasoactive balance toward constriction. In normoxia, hemodilution also induced a mild increase in PASP and PVR, suggesting the erythrocyte-associated vasodilatory signaling mechanisms outlined above also influence pulmonary vascular tone under baseline normoxic conditions. Interestingly, hemodilution did not alter the slope of the PVR response to hypoxia for a given stimulus P_{O_2} (i.e., alveolar and venous P_{O_2}). Given that PVR increased but was not directly related to the change in stimulus P_{O_2}, reduced vasodilatory action from the lower concentration of red blood cells could be responsible for the drop in PVR for a given P_{O_2}.

Pulmonary Vascular Resistance at Altitude

In the landmark study by Groves et al. (9), the pulmonary vasculature was shown to be unresponsive to acute restoration of arterial oxygen saturation during simulated ascent to the summit of Mt. Everest over 40 days. Recently, this premise has been somewhat challenged (8) by data showing no alteration to the slope of relationship between pulmonary artery pressure and oxygen saturation during a shorter period of acclimatization (14 days) up to 5,300 m. This is despite the hemoconcentration that occurs during acclimatization, a process that serves to normalize arterial oxygen content and has the potential to augment erythrocyte-dependent mechanisms of pulmonary vascular control. In line with previous observations (8), we report an increase in pulmonary pressure in response to an additional hypoxic stimulus at high altitude but no change in absolute PVR or the slope of the response to a change in stimulus P_{O_2}. Further work is required to explore the time dependency of the responsiveness to acute changes in P_{O_2}, as it appears this is lost somewhere between 2 and 4 wk of high-altitude exposure. Despite the known effects of viscosity, hemodilution did not alter

Table 2. *Hematological and hemodynamic effects of hemodilution in Andean natives during room air and hyperoxia*

	Prehemodilution		Posthemodilution		ANOVA *P* Values		
	Normoxia	Hyperoxia	Normoxia	Hyperoxia	Hemodilution	O_2 saturation	Interaction
Study 3: lifelong high-altitude residence							
Hematocrit, %	67.7 ± 5.9	69.0 ± 5.7#	57.1 ± 4.1*†	56.2 ± 4.5*#†	<0.001	0.673	0.148
Arterial oxygen saturation, %	78 ± 5	96 ± 1*#	76 ± 5	96 ± 1*#	0.098	<0.001	0.092
Arterial P_{O_2}, mmHg	44 ± 4	101 ± 7*#	41 ± 3	98 ± 5*#	0.255	<0.001	0.098
Arterial P_{CO_2}, mmHg	36 ± 2	36 ± 3	36 ± 2	36 ± 2	0.503	<0.001	0.265
Arterial oxygen content, mL/dL	24.3 ± 1.8	30.5 ± 2.4*#	19.8 ± 1.8*	25.0 ± 2.0#†	0.003	<0.001	0.491
Mean arterial pressure, mmHg	106 ± 18	103 ± 17	96 ± 22	95 ± 21	0.400	0.179	0.214
Heart rate, beats/min	62 ± 9	61 ± 10	68 ± 11	62 ± 19	0.180	0.058	0.955
Stroke volume, mL	60.9 ± 7.4	60.8 ± 10.5	67.8 ± 14.1	65.5 ± 11.3	0.271	0.694	0.305

Values are means ± SD. $P < 0.05$ vs. *normoxia prehemodilution, vs. #normoxia posthemodilution, and vs. †hyperoxia prehemodilution.

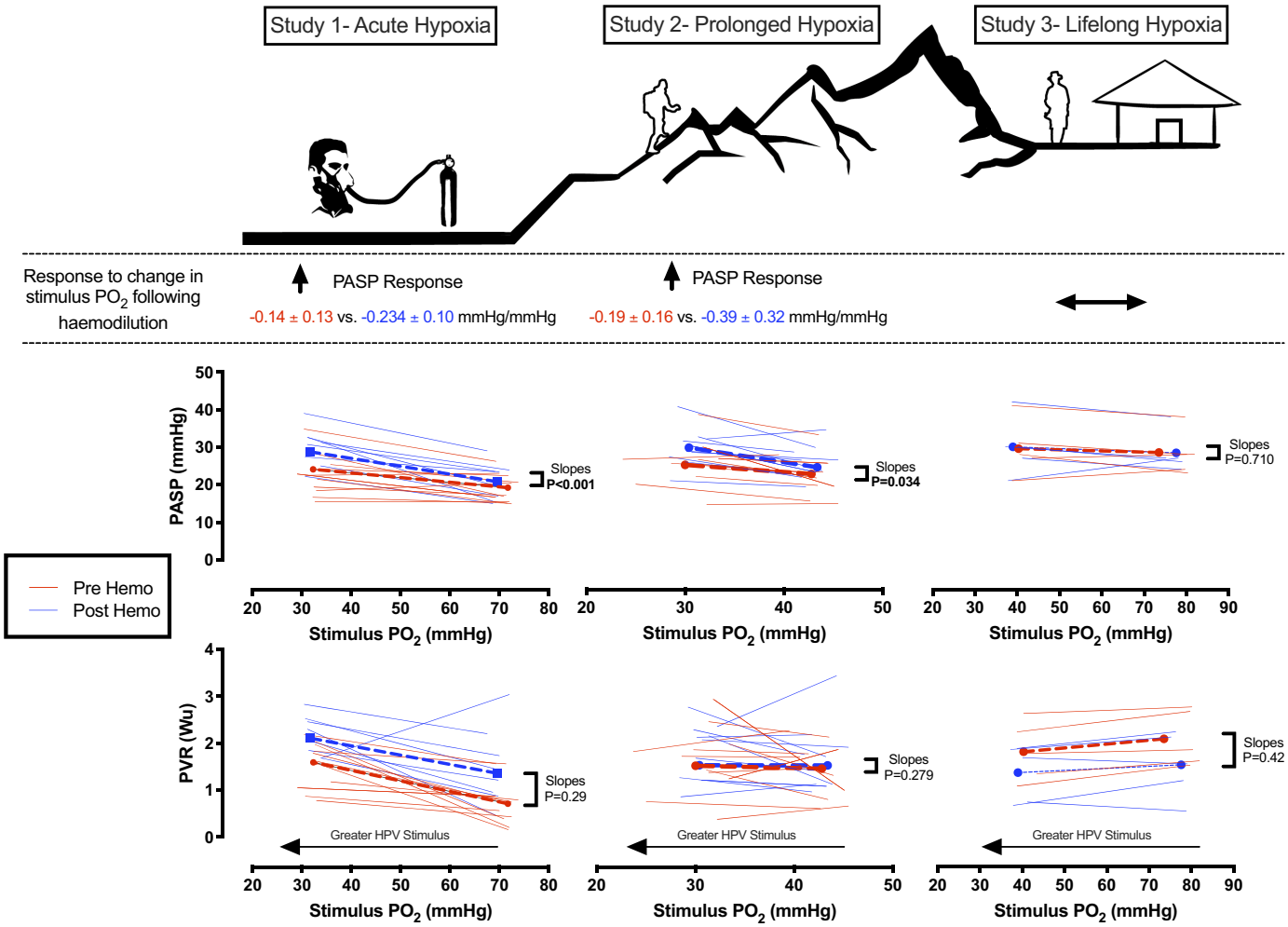

Figure 4. Pulmonary vascular changes for a given stimulus Po_2 across all three time domains of hypoxic exposure. The slope of the PASP response was greater in lowlanders following hemodilution but PVR remained unchanged. The slope of the PASP and PVR response to a change in stimulus Po_2 via hyperoxia was not altered by hemodilution. The slope of the response for a given change in stimulus Po_2 was calculated using the rise over run method and tested for differences using a paired-samples *t* test. Significant *P* values in bold. HPV, hypoxic pulmonary vasoconstriction; PASP, pulmonary artery systolic pressure; PVR, pulmonary vascular resistance.

PVR while breathing room air at 5,050 m. The absence of change in lowlanders may be due to poor signal-to-noise ratio in our experiment, as models for the PVR-hematocrit predict only a ~0.3 mmHg/L/min change in PVR with a hematocrit shift from 49% to 43% lying on the flat portion of the exponential relationship (17). One may have also expected to observe an increase in cardiac output following hypervolemic hemodilution, as the normalization of cardiac output with acclimatization has traditionally thought to be due to the restoration of oxygen content via hemoconcentration. However, hemoconcentration occurs progressively over the first 7 days at high altitude via plasma volume constriction (38), but cardiac output remains elevated throughout this period (39). Therefore, cardiac output and oxygen content (i.e., delivery) may not be the regulated physiological variable, with blood pressure control a more likely candidate (40).

Hemodilution Lowers PVR in Andeans

In contrast to our observations in lowlanders at sea level and high altitude, we observed no change in PASP and a reduction in PVR in individuals with EE following hemodilution. These findings are consistent with early invasive studies in chronic mountain sickness patients directly measuring PVR following a comparable 10% reduction in hematocrit (41). The greater influence of hemodilution on PVR is due to the higher starting hematocrit being on the steep portion of the PVR-hematocrit curve (17). However, in contrast to our data and predictive models, when a group of chronic mountain sickness patients were gradually hemodiluted across a 4-day period (42), pulmonary artery pressure and cardiac output both increased, suggesting there was no change in PVR. This incongruency may be related to differences in the temporal response of the pulmonary vasculature, where the effects of a gradual reduction in blood viscosity on PVR are offset by the vasoconstrictive effects of lowering red blood cell concentration. As in lowlanders in *study 1*, fewer red blood cells per unit volume of blood would lead to decreased vasodilatory signaling via *S*-nitrosothiol (6), NO (5), and ATP (35), as well as amplifying free radical signaling (36, 37). Therefore, the pulmonary vasculature may retain responsiveness to

changes in red blood cells in Andeans native to high altitude. However, PVR was unresponsive to acute hyperoxia in our Andean population, which is in direct contrast to lowlanders acclimatized to high altitude (8). Therefore, consistent with the blunted ventilatory response in high-altitude natives (43), the pulmonary vasculature of Andeans with EE appears to be unresponsive to acute changes in arterial P_{O_2}.

Influence of Stimulus P_{O_2}

The degree of HPV is largely driven by a combination of mixed venous P_{O_2} (~37%) and alveolar P_{O_2} (~63%) (34). By estimating the "stimulus P_{O_2}" from our data, we are able to observe that PASP is greater in lowlanders following hemodilution for a given stimulus P_{O_2}. The greater HPV response for the same stimulus P_{O_2} suggests an alternative vasoactive pathway is acting on the pulmonary vasculature in lowlanders. From our experiments, we speculate that this is erythrocyte-mediated vasodilation. This, however, was not the case in Andeans. The absence of this response in Andeans could be due to the higher starting hematocrit (e.g., ~68%), with a 10% reduction being a smaller relative stimulus compared with the change in lowlanders. Even at 58% hematocrit, the vasoactive effects of erythrocytes may be maximally effective, and a more substantial reduction needed to alter the PASP response for a given change in stimulus P_{O_2}. Future studies, preferably with long-term relocation to lower altitudes as has been employed previously (44), should look to quantify the pulmonary vascular response across a wider range of stimulus P_{O_2}.

Translational Perspective

Venesection (i.e., the removal of blood) has historically been used to treat the clinical manifestations of EE and reduce the cardiovascular burden of high viscosity in those diagnosed with chronic mountain sickness (45). However, the benefits are short lived, as red cell production will eventually restore blood volume to preremoval levels. This approach has been associated with iron deficiency resulting in further elevation of PASP (42). Herein, we demonstrate that despite a hemodilution resulting in a reduced PVR, PASP remained elevated due to a compensatory increase in cardiac output, adding further doubt to the effectiveness of this therapeutic approach. Anemia and iron deficiency are also receiving increasing attention for their role in idiopathic and heritable forms of pulmonary arterial hypertension (46), given the relationship with exercise capacity, symptoms, and survival (47). We report that, even in healthy individuals, changes in hematocrit can alter pulmonary vascular hemodynamics at rest and the response to acute changes in $P_{a_{O_2}}$, highlighting the importance of hematology in pulmonary vascular regulation in health and disease.

Limitations

There are several limitations to our study that require acknowledgment. We used indirect measures of pulmonary vascular hemodynamics. However, these measurements have been shown to correlate well with invasive methodologies (48, 49), and invasive techniques were not practicable in such remote mountainous locations. Each of our three studies had a relatively small sample size, especially our study of high-altitude natives. Given the invasiveness associated with hemodilution and the complex nature of high-altitude field work (50), we were unable to recruit a larger sample. Our sample sizes are, however, comparable with previous work in the field (8, 42, 51), and we have reported individual data in our figures to be as transparent as possible. The average age of our Andean high-altitude native group was also greater by 10 yr, and pulmonary vascular tone is known to increase with age (52, 53). However, when the change in PVR to altered P_{O_2} was plotted as a function of age during both pre- and posthemodilution states, there was no significant relationship.

Conclusions

In summary, our series of studies demonstrate that the influence of hemodilution is modified by the duration of hypoxic exposure in lowlanders, exerting a more profound effect under acute versus prolonged hypoxia. Rapid hemodilution in lifelong high-altitude natives results in an acute drop in pulmonary vascular resistance due to the higher starting hematocrit, but any benefits in unloading the right ventricle are largely negated by an acute increase in cardiac output. Collectively, our data suggest hemodilution to dampen the vasodilatory action of deoxygenated red blood cells and highlight the need to consider hematology and stimulus P_{O_2} when investigating pulmonary vascular pathophysiology.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

M.S., R.L.H., A.M.W., C.A.H., T.G.D., M.M.T., C.G., J.A., L.L.S., J.P.M., and P.N.A. conceived and designed research; M.S., R.L.H., A.M.W., C.A.H., J.D., T.G.D., A.D., M.M.T., C.G., J.A., L.L.S., J.P.M., D.M.B., D.B.M., and P.N.A. performed experiments; M.S., R.L.H., A.M.W., C.A.H., J.D., T.G.D., A.D., M.M.T., C.G., J.A., L.L.S., J.P.M., D.M.B., D.B.M., and P.N.A. analyzed data; M.S., R.L.H., A.M.W., C.A.H., J.D., T.G.D., A.D., M.M.T., C.G., J.A., L.L.S., J.P.M., D.M.B., D.B.M., and P.N.A. interpreted results of experiments; M.S. and T.G.D. prepared figures; M.S. drafted manuscript; M.S., R.L.H., A.M.W., C.A.H., J.D., T.G.D., A.D., M.M.T., C.G., J.A., L.L.S., J.P.M., D.M.B., D.B.M., and P.N.A. edited and revised manuscript; M.S., R.L.H., A.M.W., C.A.H., J.D., T.G.D., A.D., M.M.T., C.G., J.A., L.L.S., J.P.M., D.M.B., D.B.M., and P.N.A. approved final version of manuscript.

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