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The influence of hemoconcentration on hypoxic pulmonary vasoconstriction in acute, prolonged and life-long hypoxemia.

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New and Noteworthy

Red blood cell concentration influences the pulmonary vasculature via direct frictional force and vasoactive signalling, but whether the magnitude of the response is modified with duration of exposure is not known. By assessing the pulmonary vascular response to haemodilution in acute normobaric and prolonged hypobaric hypoxia in lowlanders and life-long 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, haemodilution lowered pulmonary vascular resistance but a compensatory increase in cardiac output following haemodilution rendered PASP unchanged.

Abstract

Haemoconcentration can influence hypoxic pulmonary vasoconstriction (HPV) via increased frictional force and vasoactive signalling 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: (i) at sea level, in normoxia and isocapnic hypoxia with and without isovolumic haemodilution (n=10, aged 29±7 years); (ii) at altitude (6±2 days acclimatization at 5050 m), before and during hypervolumic haemodilution (n=11, aged 27±5 years) with room air and additional hypoxia (FiO_2 = 0.15), and; (iii) at altitude (4340 m) in Andean high-altitude natives with excessive erythrocytosis (EE; n=6, aged 39±17 years), before and during isovolumic haemodilution with room air and hyperoxia (end-tidal PO_2 = 100 mmHg). Results: At sea level, haemodilution 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 5050 m, haemodilution 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 4340 m, haemodilution 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 haematocrit level. In application, these findings emphasize the importance of accounting for haematocrit and duration of exposure when interpreting the pulmonary vascular responses to hypoxemia.

Introduction

Hypoxic pulmonary vasoconstriction (HPV) of the pulmonary artery is mediated by a range of mechanisms across the neuro-cardiopulmonary axis (for review see Swenson, 2013). 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 (PO_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 (7, 15) and attenuate (5, 10) 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 (46), 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 PO_2 (PAO_2) and mixed venous PvO_2, accounting for ~62% and ~38% respectively. A lower concentration of red cells will increase HPV by reducing PvO_2 at a constant arterial-venous O_2 difference. The vasodilatory action of deoxygenated haemaglobin will be modified by the concentration of red cells i.e. during polycythaemia, the greater concentration of red cells will lead to a larger signal, and *vice versa*. Therefore, the degree of HPV for a given PO_2 may vary across a range of haematocrit. Indeed, mild haemodilution has recently been demonstrated to exaggerate the pulmonary artery pressure response to acute poikilocapnic hypoxia (8). Haemoconcentration also occurs concomitantly to an increase in pulmonary artery pressure during acclimatisation to high altitude, but it's not known whether the increased concentration of red cells amplifies the HPV response through the action of deoxy-haemoglobin. During this period, the sensitivity of the pulmonary vasculature to changes in PO_2 is also modified, with pulmonary artery pressure remaining responsive to hypoxia after 6-10 days of incremental altitude exposure to 5300 m (20), but unresponsive to oxygen reversal after approximately three weeks' simulated ascent of Mt Everest in hypobaric hypoxia (11). With increasing time at altitude, pulmonary vascular remodelling may predominate the vasoactive effects of red blood cells and other mechanisms in the neuro-cardiopulmonary axis (28). Understanding the role of haematocrit 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) (18), often alongside elevated pulmonary artery pressures (30) *and RV enlargement (21)*. Current guidelines promote the consideration of EE in the diagnosis of high-altitude pulmonary hypertension (17), due to the potential for right heart failure in this population (25). However, only recently have mathematical models been developed to correct pulmonary vascular resistance (PVR) to haematocrit (26, 50). While 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 haemoglobin that is bound to oxygen is altered. Therefore, comprehensively understanding the interaction between haematocrit, 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 haematocrit and arterial PO_2 in lowlanders at sea level by performing an isovolumic haemodilution in normoxia and with acute isocapnic hypoxia. Second, we examined how changes in haematocrit and arterial PO_2 affect pulmonary haemodynamics in lowlanders at high altitude by performing a hypervolemic haemodilution (i.e. normalise haematocrit and blood volume to sea level values) following acclimatization to 5050 m. Finally, we assessed the pulmonary vascular responses to altered haematocrit and arterial PO_2 in Andeans chronically living at 4340 m with EE by performing an acute isovolumic haemodilution in normal room air and during oxygen supplementation. In line with these three aims, we hypothesised that haemodilution would (i) increase the pulmonary pressure response to acute isocapnic hypoxia (ii) augment pulmonary pressure at high altitude and increase the sensitivity of the pulmonary vasculature to acute hypoxia and (iii) 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 (#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 obtained from all volunteers.

Experimental Design

Study 1- Acute Hypoxia

A total of ten male participants (aged 29±7, height 176±4 cm, weight 72±2 kg) participated in this laboratory study, all of whom were sea level residents who were normotensive, non-smokers with no previous history of cardiovascular, respiratory or haematological conditions and were taking no prescription medications. The participants visited the laboratory once, and first underwent radial artery and internal jugular vein catheterisation. Pre-haemodilution cardiovascular assessments were taken during normoxia and acute isocapnic hypoxia ($P_{ET}O_2$ = 40 ± 2 mmHg). Dynamic end-tidal forcing (12) was used to closely match arterial PO_2 to that experienced in Study 2 described below, and carbon dioxide fixed throughout to avoid the additional influence on the pulmonary vasculature (3). Next, isovolumic haemodilution was 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 haematocrit of ~10% (e.g. 45 to 35% haematocrit). 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 (52) and experimental design (14) 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 hours prior to testing. Following a cautious ascent profile over 10 days, participants were enrolled into the study following 6 ± 2 days of acclimatization to 5050 m. Eleven male sea level residents (aged 27±5, height 177±5 cm, weight 75±8 kg) participated in Study 2. The participants arrived at the laboratory in the fasted state (>4 hrs) and first underwent arterial and peripheral venous catheterisation. Pre-haemodilution measurements were made whilst breathing room air and during poikilocapnic hypoxia (FiO_2 = 0.15). All measurements were repeated following successful

hypervolemic haemodilution via rapid infusion of saline, a procedure our group has successfully performed previously at high altitude (41). Similar to Study 1, the intervention aimed to reduce haematocrit by ~10%. In contrast to Study 1, hyper- rather than isovolemic haemodilution was chosen. This was to normalise total blood volume and cardiac filling to sea level values (41, 44, 45), as a lower blood volume would have lowered stroke volume and, in turn, pulmonary artery pressure.

Study 3- Life-long High-Altitude Residence

A total of ten male Andean highlanders were recruited from the town of Cerro de Pasco, Peru (4340 m) (49). 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 years, height 162±7 cm, body mass 69±12 kg) and haematological data in five participants. Following medical screening that included blood pressure, haematocrit 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 PaO_2 of 100 mmHg). Hyperoxia was chosen as it allowed a greater magnitude of change in oxygen saturation compared to what was safe and realistic 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 haematocrit of ~10% (48).

Experimental Measures

Haematological and haemodynamic measures

Arterial (20G, Arrow, Markham, Ontario, Canada) and central venous catheterisation (13G, 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, haemoglobin concentrations and haematocrit were determined via co-oximetry (ABL90 Flex, Radiometer, Copenhagen, Denmark) and used to calculate arterial oxygen content (CaO_2). Absolute blood and plasma volumes were determined using the modified carbon monoxide rebreathing technique (35). Blood viscosity was determined from venous samples using a cone and plate viscometer (Model DV2T, Brookfield Amtek, USA) at 37°C and a shear rate of 225 s^{-1}.

Cardiovascular Assessments

Pulmonary vascular haemodynamic and cardiac function were determined via echocardiography following the American Society of Echocardiography recommendations (33), as previously performed by our group at high altitude (42, 43). 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, USA) for subsequent offline analysis (Echopac, GE Healthcare, Piscataway, NJ, USA). 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 3-lead electrocardiograph. PVR was estimated by calculating mean pulmonary artery pressure from PASP (4), then subtracting left atrial pressure derived from early mitral inflow velocity and early tissue Doppler velocity of the septal and lateral mitral annulus(27) 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 (47).

HPV is largely determined by alveolar (PAO_2; ~38%) and venous PO_2 (PvO_2; 62%), and the interventions we employed will alter the degree of HPV in line with these relative contributions. For example, haemodilution will reduce arterial oxygen content, such that maintained a-vO_2 differences will result in a lower PvO_2. Changes in inspired oxygen will reduce both PaO_2 and PvO_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 PO_2 so that our data can be interpreted relative to the stimulus applied. PAO_2 was estimated from the alveolar gas equation, using $PaCO_2$ from our blood gas data and a fixed respiratory quotient of 0.82. PvO_2 was estimated by assuming a fixed arterial-venous oxygen difference of 5ml under resting conditions and subtracting that from CaO_2 measured from arterial blood gas data. The proportional contribution towards HPV was then applied using the following equation from Marshall and Marshall (23):

- Stimulus $PO_2 = PvO_2^{0.375} \times PAO_2^{0.626}$

Statistical Analysis

All statistical analyses were performed in Graphpad Prism (Version 7, San Diego, US). Distribution normality was confirmed with the Shapiro-Wilk test. Two-way repeated measures analysis of variance (factors: haemodilution 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 PO_2 was calculate 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 mean ± SD. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Study 1- Acute Hypoxia

Haemodilution was effective in reducing both haematocrit (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 the hypoxia pre-haemodilution and normoxia post-haemodilution, and was lowest in hypoxia following haemodilution (Table 1). Haemodilution did not alter mean arterial pressure, but it was elevated in response to hypoxia.

As expected, acute hypoxia elevated PASP in both pre- and post-haemodilution conditions (main effect p<0.001). Haemodilution 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 to pre-haemodilution hypoxia (+4.5±2.4 mmHg, P<0.001, Figure 1). The changes in PASP in normoxia occurred independent of changes in cardiac output, with no significant main effect for haemodilution (P=0.237). Whilst there was a significant main effect for the change in oxygen saturation (P=0.037, Figure 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 pre-haemodilution (P=0.002) and post haemodilution (P=0.030) states, and increased in normoxia pre-post haemodilution (P=0.015).

Study 2- Prolonged Exposure to High Altitude

At high altitude, haemodilution decreased haematocrit from 49.2±2.9 to 43.2±3.2 (P<0.001), so that it was comparable to sea level baseline in Study 1 (43.5%). Concomitant with the decrease in haematocrit was a reduction in blood viscosity (4.5±0.6 vs. 3.7±0.4 cP, P<0.001) and CaO_2 (Table 1). Acute poikilocapnic hypoxia decreased arterial PO_2 and CaO_2 to similar degrees pre- and post-haemodilution (Table 1). Despite the hypervolemic nature of the haemodilution, mean arterial pressure was not altered by the intervention (main effect P=0.097).

PASP was not significantly altered (P=0.14) following haemodilution (Figure 2) whilst breathing room air, but it was increased pre-post haemodiltuion during the acute poikilocapnic hypoxia condition (P<0.001) suggesting haemodilution may only increase PASP under acute hypoxic stress and not in the acclimatised state. Consistent with Study 1, acute poikilocapnic hypoxia increased PASP pre-haemodilution (P=0.004) and this response remained post-haemodilution (P=0.002) (Figure 2). Cardiac output and PVR were unchanged by haemodilution in room air or acute poikilocapnic hypoxia (main effects P=0.395 and P=0.116, respectively).

Study 3- Life-long high-altitude residence

Haemodilution reduced haematocrit 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). While these represent substantial reductions, Andean natives still remained polycythaemic even compared to lowlanders at high altitude in Study 2. Mean arterial pressure was not altered by haemodilution or acute hyperoxia (Table 2). PASP remained unchanged following haemodilution (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 haemodilution (Figure 3). For example, in room air, haemodilution increased cardiac output (P=0.027) but decreased PVR (P=0.019). A similar effect of haemodilution was observed under hyperoxia.

Influence of Stimulus PO_2 on pulmonary haemodynamics

In lowlanders, haemodilution increased the slope of the PASP response to an increased stimulus PO_2 at both sea level and high altitude (Figure 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 haemodilution. While the slope of the PVR response was unchanged, as discussed above, PVR for a given stimulus PO_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 haemodilution when they stimulus PO_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: (i) haemodilution augments the pulmonary pressure response to acute isocapnic hypoxia; (ii) in lowlanders at high altitude, haemodiltion had no effect on pulmonary pressure but the pulmonary vasculature was more responsive to changes in the stimulus PO_2; and (iii) pulmonary pressure remained unchanged in Andeans following acute haemodilution because of an increase in cardiac output but reciprocal reduction in PVR. Collectively, our data indicate that the influence of arterial PO_2 and haematocrit on the pulmonary pressure response to hypoxia is modified by the duration of hypoxic exposure and starting haematocrit.

Haemodilution and acute hypoxia

As hypothesised, the reduction in haematocrit increased the magnitude of pulmonary pressure response to isocapnic hypoxia i.e. there was a greater increase in pulmonary artery pressure for a given change in stimulus PO_2 following haemodilution. A similar response has recently been reported whereby greater increases in pulmonary pressure and cardiac output were observed during graded poikilocapnic hypoxia (8). We confirm and extend these findings by controlling for changes in end-tidal PCO_2, as the ventilation-induced hypocapnia in the study of Duke et al. (2016) would likely have elicited vasodilatation that would have effectively masked the haemodilution-induced elevation in pulmonary pressure (3). The increased sensitivity to hypoxia following haemodilution may be mediated by the vasodilatory action of red blood cells. The lower concentration of red cells would attenuate the generation of S-nitrosothiol (10), NO (5) and ATP (40) by deoxyhaemoglobin, and low haemoglobin levels can increase free radical signalling (9, 29). Together, these mechanisms would shift the vasoactive balance towards constriction. In normoxia, haemodilution also induced a mild increase in PASP and PVR, suggesting the erythrocyte-associated vasodilatory signalling mechanisms outlined above also influence pulmonary vascular tone under baseline normoxic conditions. Interestingly, haemodilution did not alter the slope of the PVR response to hypoxia for a given stimulus PO_2 (i.e. alveolar and venous PO_2). Given that PVR increased, but was not directly related to the change in stimulus PO_2, reduced vasodilatory action from the lower concentration of red blood cells could be responsible for the drop in PVR for a given PO_2.

Pulmonary vascular resistance at altitude

In the landmark study by Groves, Reeves, Sutton, Wagner, Cymerman, Malconian, Rock, Young and Houston (11), 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 (20) by data showing no alteration to the slope of relationship between pulmonary artery pressure and oxygen saturation during a shorter period of acclimatisation (14 days) up to 5300 m. This is despite the haemoconcentration that occurs during acclimatisation, a process that serves to normalise arterial oxygen content and has the potential to augment erythrocyte-dependent mechanisms of pulmonary vascular control. In line with previous observations (20), 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 PO_2. Further work is required to explore the time-dependency of the responsiveness to acute changes in PO_2, as it appears this is lost somewhere between 2-4 weeks of high-altitude exposure. Despite the known effects of viscosity, haemodilution did not alter PVR while breathing room air at 5050 m. The absence of change in lowlanders may be due to poor signal-to-noise ratio in our experiment, as models for the PVR-haematocrit predict only a ~0.3 mmHg/L/min change in PVR with a haematocrit shift from 49% to 43% lying on the flat portion of the exponential relationship (50). One may have also expected to observe an increase in cardiac output following hypervolemic haemodilution, as the normalisation of cardiac output with acclimatisation has traditionally thought to be due to the restoration of oxygen content via haemoconcentration. However, haemoconcentration occurs progressively over the first seven days at high altitude via plasma volume constriction (34), but cardiac output remains elevated throughout this period (24). Therefore, cardiac output and oxygen content (i.e. delivery) may not be the regulated physiological variable, with blood pressure control a more likely candidate (38).

Haemodilution 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 haemodilution. These findings are consistent with early invasive studies in chronic mountain sickness patients directly measuring PVR following a comparable 10% reduction in haematocrit (22). The greater influence of haemodilution on PVR is due to the higher starting haematocrit being on the steep portion of the PVR-haematocrit curve (50). However, in contrast to our data and predictive models, when a group of chronic mountain sickness patients were gradually haemodiluted across a four day period (39), 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 signalling via S-nitrosothiol (10), NO (5) and ATP (40), as well as amplifying free radical signalling (9, 29). 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 acclimatised to high altitude (20). Therefore, consistent with the blunted ventilatory response in high altitude natives (36), the pulmonary vasculature of Andeans with EE appears to be unresponsive to acute changes in arterial PO_2.

Influence of Stimulus PO_2

The degree of HPV is largely driven by a combination of mixed venous PO_2 (~37%) and alveolar PO_2 (~63%) (23). By estimating the "stimulus PO_2" from our data, we are able to observe that PASP is greater in lowlanders following haemodilution for a given stimulus PO_2. The greater HPV response for the same stimulus PO_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 haematocrit (e.g. ~68%), with a 10% reduction being a smaller relative stimulus compared to the change in lowlanders. Even at 58% haematocrit, 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 PO_2. Future studies, preferably with long-term relocation to lower altitudes as has been employed previously (37), should look to quantify the pulmonary vascular response across a wider range of stimulus PO_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 (51). However, the benefits are short lived, as red cell production will eventually restore blood volume to pre-removal levels. This approach has been associated iron deficiency resulting in further elevation of PASP (39). Herein, we demonstrate that despite a haemodilution 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. Anaemia and iron deficiency are also receiving increasing attention for their role in idiopathic and heritable forms of pulmonary arterial hypertension (32), given the relationship with exercise capacity, symptoms and survival (31). We report that, even in healthy individuals, changes in haematocrit can alter pulmonary vascular haemodynamics at rest and the response to acute changes in PaO_2, highlighting the importance of haematology in pulmonary vascular regulation in health and disease.

Limitations

There are several limitations to our study that require acknowledgement. We used indirect measures of pulmonary vascular haemodynamics. However, these measurements have been shown to correlate well with invasive methodologies (6, 53), 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 haemodilution, and the complex nature of high altitude field work (1), we were unable to recruit a larger sample. Our sample sizes are, however, comparable to previous work in the field (13, 20, 39), and we have reported individual data in our figures to be as transparent with as possible. The average age of our Andean high altitude native group was also greater by 10 years, and pulmonary vascular tone is known to increase with age (16, 19). However, when the change in PVR to altered PO_2 was plotted as a function of age during both pre- and post-haemodilution states, there was no significant relationship.

Conclusion

In summary, our series of studies demonstrate that the influence of haemodilution is modified by the duration of hypoxic exposure in lowlanders, exerting a more profound effect under acute vs. prolonged hypoxia. Rapid haemodilution in life-long high-altitude natives results in an acute drop in pulmonary vascular resistance due to the higher starting haematocrit, but any benefits in unloading the right ventricle are largely negated by an acute increase in cardiac output. Collectively, our data suggest haemodilution to dampen the vasodilatory action of deoxygenated red blood cells, and highlight the need to consider haematology and stimulus PO_2 when investigating pulmonary vascular pathophysiology.

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Contributions

MS had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data collection, data analysis, including and especially any adverse effects. MS, RLH and PNA contributed substantially to the study design, data analysis and interpretation, and the writing of the manuscript. AMW, CAH, JD, TGD, AD, MMT, CG, JA, LLS,

JPM, DMB and DBM contributed substantially to data collection, data analysis and interpretation, and the writing of the manuscript. All authors approved the final version of this manuscript.

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Figure Legends

Figure 1- Pulmonary vascular response to acute hypoxia before and after haemodilution at sea level. Pulmonary artery systolic pressure (PASP) and pulmonary vascular resistance (PVR) were increased in response to both haemodilution and acute hypoxia. PaO_2 = arterial partial pressure of oxygen; CaO_2 arterial oxygen content. Significant p values in **bold**.

Figure 2- Pulmonary vascular response to haemodilution following acclimatisation to 5050 m in room air and acute hypoxia. Pulmonary artery systolic pressure (PASP) was unaltered by haemodilution, but remained responsive to acute hypoxia. FiO_2 =. Fraction of inspired oxygen. Significant p values in **bold**.

Figure 3- Pulmonary vascular response to haemodilution in six 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 haemodilution, but pulmonary vascular resistance was reduced following haemodilution despite an increase in cardiac output. PaO_2 = arterial partial pressure of oxygen. Significant p values in **bold**.

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 haemodilution, 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 haemodilution. The slope of the response for a given change in stimulus PO_2 was calculate using the rise over run method, and tested for differences using a paired-samples t test. PaO_2 = arterial partial pressure of oxygen. Significant p values in **bold.**

627 Table 1- Haematological and haemodynamic effects of haemodilution in acute and prolonged hypoxia.
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	Pre-Haemodilution		Post-Haemodilution		ANOVA P Value		
	Normoxia	Hypoxia	Normoxia	Hypoxia	*Haemodilution*	*O2 Saturation*	*Interaction*
Study 1- Acute Hypoxia							
Haematocrit (%)	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 PO2 (mmHg)	94 ± 4	40 ± 2*#	96 ±7	40 ± 2*#	0.26	<0.001	0.3
Arterial PCO2 (mmHg)	42 ± 2	42 ± 2	41 ± 2	42 ± 1#	0.87	0.02	0.02
Arterial Oxygen Content (ml dl-1)	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 (bpm)	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							
Haematocrit (%)	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 PO2 (mmHg)	49 ± 3	35 ± 4*#	52 ± 4*	37 ± 4*#†	<0.001	0.001	0.149
Arterial PCO2 (mmHg)	25 ± 2	23 ± 3	23 ± 4	20 ± 1*#†	0.001	<0.001	0.244
Arterial Oxygen Content (ml dl-1)	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 (bpm)	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

629 *= vs. normoxia pre-haemodilution, #= vs. normoxia post haemodilution and †= vs. hypoxia pre-haemodilution.
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634 Table 2- Haematological and haemodynamic effects of haemodilution in Andean natives during room air and hyperoxia
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	Pre-Haemodilution		Post-Haemodilution		ANOVA P Value		
	Normoxia	Hyperoxia	Normoxia	Hyperoxia	*Haemodilution*	*O2 Saturation*	*Interaction*
Study 3- Life-long High Altitude Residence							
Haematocrit (%)	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 PO2 (mmHg)	44 ± 4	101 ± 7*#	41 ± 3	98 ± 5*#	0.255	<0.001	0.098
Arterial PCO2 (mmHg)	36 ± 2	36 ± 3	36 ± 2	36 ± 2	0.503	<0.001	0.265
Arterial Oxygen Content (ml dl-1)	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 (bpm)	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

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637 *= vs. normoxia pre-haemodilution, #= vs. normoxia post haemodilution and †= vs. hyperoxia pre-haemodilution.
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