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Title: UBC-Nepal Expedition: Haemoconcentration underlies the reductions in cerebral blood flow observed during acclimatization to high-altitude

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Running Title: Impact of haemoconcentration on cerebral blood flow at high-altitude

Abstract: At high-altitude, increases in haematocrit (Hct) are achieved through altitude-

induced diuresis and erythropoiesis, both of which result in increased arterial oxygen content (CaO2). Given the impact alterations in Hct have on CaO2, haemoconcentration has been hypothesized to partly mediate the attenuation of the initial elevation in cerebral blood flow (CBF) at high-altitude. To test this hypothesis, healthy males (n=13) ascended to 5050 m over nine days without the aid of prophylactic acclimatization medications. Following one-week of acclimatization at 5050 m, participants were haemodiluted by rapid saline infusion (2.10{plus minus}0.28 L) to return Hct towards pre-acclimatized levels. Arterial blood gases, Hct, global CBF (duplex ultrasound), and haemodynamic variables were measured following initial arrival to 5050 m, and after one-week of acclimatization at high-altitude, prior to and following the haemodilution protocol. Following one-week at 5050m, Hct increased from 42.5{plus minus}2.5 to 49.6{plus minus}2.5 % (P<0.001), and was subsequently reduced to 45.6{plus minus}2.3 % (P<0.001) following haemodilution. Global CBF decreased from 844{plus minus}160 to 619{plus minus}136 mL/min (P=0.033) following one-week of acclimatization and increased to 714{plus minus}204 mL/min (P=0.045) following haemodilution. Despite the significant changes in Hct, and thus CaO2, cerebral oxygen delivery was unchanged at all time points. Furthermore, these observations occurred in the absence of any changes in mean arterial blood pressure, cardiac output, arterial blood pH, or oxygen saturation pre- and post-haemodilution. These data highlight the influence of Hct in the regulation of CBF and are the first to demonstrate experimentally that haemoconcentration contributes to the reduction in CBF during acclimatization to altitude.

New Findings: 1. What is the central question of this study? To evaluate the degree to which increases in haematocrit alters cerebral blood flow and cerebral oxygen delivery during acclimatization to high-altitude 2. What is the main finding and its importance? Through haemodilution, we determined that, following one week of acclimatization, the primary mechanism contributing to the cerebral blood flow acclimatization response is generated by increases in haemoglobin and haematocrit, while the remaining contribution to the cerebral blood flow acclimatization response is likely attributable to ventilatory acclimatization

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New Findings

The central question of this study was to evaluate the degree to which increases in haematocrit alter cerebral blood flow and cerebral oxygen delivery during acclimatization to high-altitude. Through haemodilution, we determined that, following one-week of acclimatization, the primary mechanism contributing to the cerebral blood flow response during acclimatization is increases in haemoglobin and haematocrit, while the remaining contribution to the cerebral blood flow response during acclimatization is likely attributable to ventilatory acclimatization.

ABSTRACT

At high-altitude, increases in haematocrit (Hct) are achieved through altitude-induced diuresis and erythropoiesis, both of which result in increased arterial oxygen content (CaO_2). Given the impact alterations in Hct have on CaO_2, haemoconcentration has been hypothesized to partly mediate the attenuation of the initial elevation in cerebral blood flow (CBF) at high-altitude. To test this hypothesis, healthy males (n=13) ascended to 5050 m over nine days without the aid of prophylactic acclimatization medications. Following one-week of acclimatization at 5050 m, participants were haemodiluted by rapid saline infusion (2.10±0.28 L) to return Hct towards pre-acclimatized levels. Arterial blood gases, Hct, global CBF (duplex ultrasound), and haemodynamic variables were measured following initial arrival to 5050 m, and after one-week of acclimatization at high-altitude, prior to and following the haemodilution protocol. Following one-week at 5050m, Hct increased from 42.5±2.5 to 49.6±2.5 % ($P<0.001$), and was subsequently reduced to 45.6±2.3 % ($P<0.001$) following haemodilution. Global CBF decreased from 844±160 to 619±136 mL/min ($P=0.033$) following one-week of acclimatization and

increased to 714±204 mL/min (P=0.045) following haemodilution. Despite the significant changes in Hct, and thus CaO_2, cerebral oxygen delivery was unchanged at all time points. Furthermore, these observations occurred in the absence of any changes in mean arterial blood pressure, cardiac output, arterial blood pH, or oxygen saturation pre- and post-haemodilution. These data highlight the influence of Hct in the regulation of CBF and are the first to demonstrate experimentally that haemoconcentration contributes to the reduction in CBF during acclimatization to altitude.

INTRODUCTION

A reduction in the partial pressure of atmospheric oxygen results in hypoxemia, whereby necessary cerebrovascular responses are required to maintain adequate cerebral oxygen delivery (CDO_2). Upon ascent to high-altitude, cerebral blood flow (CBF) increases in proportion to the degree of hypoxemia to compensate for the reduced arterial oxygen content (CaO_2) (Severinghaus *et al.*, 1966; Ainslie & Subudhi, 2014). Within two- to three-days following initial arrival to high-altitude, CBF begins to decrease, returning to sea-level values typically between 7-14 days at a given altitude (Willie *et al.*, 2014), yet no mechanism(s) have been clearly ascribed to this pattern of CBF changes. It is well established that hypoxic CBF regulation is driven by changes in CaO_2 to maintain consistent CDO_2 during arterial hypoxemia at sea-level [reviewed in: (Hoiland *et al.*, 2016)]. This regulation of blood flow is similar to that of the systemic circulation during exercise (Roach *et al.*, 1999; Gonzalez-Alonso *et al.*, 2001), whereby flow is coupled to CaO_2, but not the partial pressure of arterial oxygen (PaO_2). Thus, the pattern of CBF changes observed at high-altitude are also likely regulated by changes in CaO_2 (Ainslie & Subudhi, 2014; Hoiland *et al.*,

2018). The primary factors influencing CaO_2 at high-altitude, and by association CBF, are changes in arterial oxygen saturation (SaO_2), hematocrit (Hct). This is in addition to the well documented influence of the partial pressure of carbon dioxide ($PaCO_2$) and pH on CBF. Alterations in cerebrovascular reactivity to CaO_2 and/or $PaCO_2$ may also underlie changes in CBF at altitude (Lucas *et al.*, 2011; Willie *et al.*, 2015) although their contribution to CBF changes during acclimatization is likely modest at best [reviewed in: (Hoiland *et al.*, 2018)].

Early increases in Hct occur through high-altitude induced diuresis whereby elevated arterial pH stimulates bicarbonate to be excreted by the kidneys through the urine, reducing plasma volume. This response partially mitigates the initial reductions in CaO_2 (Pugh, 1964; Ryan *et al.*, 2014; Siebenmann *et al.*, 2017). Thereafter, following approximately one-week at altitude, Hct increases further through erythropoiesis (Siebenmann *et al.*, 2017). While PaO_2 and SaO_2 increase and $PaCO_2$ decreases as a result of ventilatory acclimatization [reviewed in: (Hoiland *et al.*, 2018], previous research has speculated that increased Hct provides a greater input into the changes in CaO_2 observed at altitude. Indeed, estimations indicate Hct may account for 60-70% of the increase in CaO_2 during acclimatization, and therefore possess a commensurate influence on the reduction in CBF observed across acclimatization (Hoiland *et al.*, 2018). Importantly, CaO_2 regulates hypoxic vasodilation via changes in PaO_2/SaO_2 as well as independent of alterations in PaO_2/SaO_2 such as when Hct is altered [reviewed in: (Hoiland *et al.*, 2016)]. However, this regulation of CBF by Hct appears unrelated to changes in blood viscosity [(Brown & Marshall, 1985; reviewed in: Hoiland *et al* 2016)]. This understanding of the fundamental importance of CaO_2 in the regulation of hypoxic cerebral vasodilation has been long standing (Brown *et al.*, 1985); however, the influence of Hct on CBF during acclimatization at altitude due to its relationship with CaO_2 has never been experimentally determined.

The aim of this study was to evaluate the degree to which Hct alters CBF and CDO_2 by acutely haemodiluting subjects to pre-acclimatized Hct concentrations observed upon initial arrival to 5050 m. We hypothesized that following approximately seven days of acclimatization at 5050 m above sea-level, CBF would be significantly decreased from initial arrival values concomitant with an increase in Hct. Further, we hypothesized that following haemodilution, due to the experimentally isolated reduction in Hct, CBF would concomitantly increase and thus be partially restored to near pre-acclimatized levels.

METHODS

Ethical approval

All participants gave written informed consent prior to participating. This study was approved by the University of British Columbia Clinical Research Ethics Board, the Nepal Health Research Council, and conformed to the standards set by the *Declaration of Helsinki* (except registry in a database) and the Canadian Government Tri-council Policy Statement (TCPS2) for integrity in research.

Participants

Thirteen healthy male participants (BMI: 23.2 ± 2.1 kg · m $^{-2}$, Age: 27 ± 5 years) were recruited to participate in this study. Participants were recruited at the University of British Columbia's Okanagan campus and were part of the research team. This project was part of a larger expedition to the EV-K2-CNR Italian Pyramid Research laboratory in the Khumbu Valley,

Nepal at 5050 m that took place September to November 2016 (Willie *et al.*, 2018). Although participants were recruited to other projects at the Pyramid Laboratory, all arrived to high-altitude at the same time and were tested >5 half-lives following any drug interventions. Further, all participants abstained from exercise, caffeine, and alcohol for 24 hours prior to testing and arrived having fasted for >4 hours. All participants were born and lived at or near sea-level and were free of cardiovascular, respiratory and neurological diseases and were non-smokers. One participant was taking Pentasa®, a medication used to treat inflammatory bowel disease, throughout the entire study.

Experimental Overview

The research team travelled to Nepal to begin the ascent to high-altitude. All participants spent 3-9 days in Kathmandu (1400 m) prior to flying to Lukla (2860 m) to begin the trek to the EV-K2-CNR Pyramid Research Laboratory (5050 m). Ascent to the Pyramid Laboratory took place over a slow and safe 9-day trek without the use of any acute mountain sickness prophylactics
(e.g., acetazolamide). Participants spent one night in Monjo (2800 m), three nights in Namche Bazaar (3400 m), one night in Deboche (3820 m), and then three nights in Pheriche (4371 m) followed by the final trekking day to the Pyramid Laboratory (5050 m). Due to the length of testing per participant, and hours available for testing at the remote laboratory, testing was limited to two participants per day. As such, participants underwent the haemodilution protocol across a small range of relative acclimatization with testing conducted on days 4-10 (avg. 7) at the Pyramid Laboratory (**Figure 1**). For more details on the ascent see (Willie *et al.*, 2018).

The day following initial arrival to the pyramid lab (i.e. day 1), subjects lay supine for 20 minutes of quiet rest, after which CBF, echocardiography and arterial blood samples were acquired. A minimum of 24 hours prior to the main experimental protocol participants' blood volumes were measured using the Carbon Monoxide Rebreathe technique (Schmidt & Prommer, 2005). This protocol is detailed in the following section (*Experimental Measures*). The following day, participants completed the experimental protocol prior to and following haemodilution. After 20 minutes of quiet supine rest participants were cannulated, following which, CBF, cardiac output (Q), and radial arterial blood measures were acquired. A hypervolemic haemodilution protocol was performed in an attempt to return Hct and plasma volume to pre-acclimatized values observed upon initial arrival at high-altitude (i.e., Hct ≈ 45 %) through rapid saline infusion (0.9 % NaCl). Prior to expedition testing, pilot work was conducted to determine the adequate infusion volume of saline required to elicit the desired decrease in Hct. A bolus infusion of saline equal to between 30-35 % of the participants total blood volume (avg 2.10 ± 0.28 L) was infused over 0.5-1 hr.

Experimental Measures

A radial artery catheter (20-gauge; Arrow, Markham, ON, Canada) was inserted into the left radial artery under local anesthesia (Lidocaine, 1.0%) and ultrasound guidance using sterile technique. The arterial catheter was attached to an inline waste-less sampling system (Edwards Lifesciences, VAMP, CA, USA) and a pressure transducer that was placed at the height of the right atrium (TruWave transducer). Following cannulation, subjects rested quietly for 20-minutes, after which an arterial blood sample was taken, and analyzed (ABL90 FLEX, Radiometer, Copenhagen, Denmark).

Internal carotid artery (ICA) and vertebral artery (VA) image acquisition was obtained using a 10 MHz multi-frequency linear array probe attached to a high-resolution ultrasound machine (15L4, Terason t3200, Burlington, MA, USA). Arterial diameter was measured with B-mode imaging while pulse-wave mode simultaneously measured blood velocity within the vessel. Image location was selected on an individual basis in order to ensure clear, highly reproducible measures with VA imaging occurring between cervical vertebrate 4 and 5, 5 and 6 or proximal to entry into the vertebral column and ICA imaging taken ≥1.5 cm distal to the common carotid bifurcation. The sum of both ICA and VA flow multiplied by two was used to estimate global cerebral blood flow (gCBF). Cerebrovascular reactivity to CaO_2 was also assessed using the concurrent ultrasound blood flow values and arterial blood samples. CaO_2 was calculated from the arterial blood sample utilizing the arterial SpO_2, PaO_2, and [Hb] ($CaO_2 = (1.34 \times [Hb] \times SaO_2) + (0.003 \times PaO_2)$).

Upon initial arrival (day 1), blood pressure was assessed using an automated blood pressure cuff (HEM775CAN, Omron Healthcare). During the haemodilution protocol, blood pressure was assessed continuously by means of intra-radial pressure through the radial arterial catheter and was corrected to manual brachial blood pressure measurements (i.e. auscultation). A portable ultrasound (Vivid Q, GE Healthcare, Piscataway, NJ, USA) was used for the echocardiography assessment of cardiac output (Q). Participants were rolled into the left lateral decubitus position whereby a trained cardiac sonographer acquired parasternal and apical images. Stroke volume (SV) was calculated from the diameter of the left ventricular outflow tract diameter and the velocity-time integral from a five-chamber view (Lang *et al.*, 2015). Reliability values for a range of echocardiographic variables from our group have previously been reported

(Stembridge *et al.*, 2014). Heart rate was obtained from the R-R intervals recorded from a three lead ECG and multiplied by SV to calculate Q.

Assessment of total blood volume

Total blood volume was determined using the previously validated carbon monoxide rebreathing method, as described in detail elsewhere (Schmidt & Prommer, 2005). Using a custom glass spirometer (Blood Tec) and a 5-liter reservoir bag of 100 % O_2, participants were instructed to maximally inhale a specific volume of carbon monoxide (0.8 mL · kg^{-1}) which was inserted into the apparatus mid inspiration. Participants then held their breath at maximal inspiration for 10 seconds before rebreathing on the apparatus for two-minutes. A venous blood sample was obtained at baseline and seven minutes following onset of the carbon monoxide rebreathing to determine total haemoglobin [Hb] and carboxyhaemoglobin (ABL 90; Radiometer). Expired carbon monoxide was measured using a portable carbon monoxide analyzer (Dräger Pac 3500; Draeger Safety) at baseline and at four- and seven-minutes post rebreathe onset. Total red cell volume assessed by this measure was used to accurately calculate total blood volume pre- and post- haemodilution in conjunction with Hct %. Blood volume post haemodilution was calculated using the initial blood volume, and Hct % recorded from arterial blood samples pre- and post- haemodilution (i.e. $Hct_{Initial}$ x blood $volume_{Initial}$ = Hct_{Final} x blood $volume_{Final}$).

Data and Statistical Analyses

Statistical analyses were performed using IBM SPSS 24. All data is reported as mean ± standard deviation. Linear mixed effects models, with repeated measures compound symmetry covariance structures, were used to determine significant changes in CBF, arterial blood gases, and cardiovascular variables from initial arrival and pre- vs post-haemodilution (fixed factor: time, random factor: subjects). Estimated CBF was calculated using the slope of the response between gCBF and Hct, pre- to post-haemodilution, multiplied by the change in Hct required to elicit a full reversal of haemoconcentration to initial arrival levels in individual participants. The "estimated" CBF was compared to initial arrival using a paired t-test. A linear mixed effects model analysis with repeated measures (compound symmetry covariance structure) was used to determine cerebrovascular reactivity to changes in CaO_2 during the acclimatization process (day 1 to pre hemo; condition 1) and during haemodilution (pre hemo to post hemo; condition 2). The dependent variable was gCBF, while CaO_2 and condition were fixed effects. Subjects were included as a random effect and MAP and $PaCO_2$ were added as co-variates, which improved the model fit (-2 Log Likelihood). This was repeated for ICA and VA flow as well. Relative changes in ICA and VA flow and reactivity were compared using a t-test following acclimatization and haemodilution to examine regional CBF differences. Finally, linear regression analysis was performed between both ΔgCBF and gCBF reactivity versus days spent at altitude to delineate the effect of testing day. Significance was set at $P<0.05$ for all statistical analyses.

RESULTS

Arterial blood content changes

Arterial blood metrics are presented in **Table 1**. Following one-week of acclimatization at 5050 m, [Hb] and Hct were elevated and then subsequently decreased following haemodilution. However, [Hb] ($P<0.01$) and, Hct ($P<0.001$), were not reduced to their initial arrival levels and remained slightly elevated following haemodilution. Both PaO_2 and SaO_2 increased following acclimatization (SaO_2, $P<0.01$; PaO_2, $P<0.001$) and remained unchanged following haemodilution (SaO_2, $P=0.78$; PaO_2, $P=0.79$). Though $PaCO_2$ significantly decreased following acclimatization and following the haemodilution protocol, pH was only altered (increased) following acclimatization, but remaining unchanged with haemodilution ($P=0.39$).

Cardiovascular variables

Resting cardiovascular data are presented in **Table 2**. Following acclimatization mean arterial pressure (MAP) was elevated ($P=0.03$), but did not increase further with haemodilution ($P=0.32$). Following haemodilution MAP was higher compared to initial arrival ($P<0.01$). Heart rate did not decrease following acclimatization ($P=0.29$), but was reduced following haemodilution compared to initial arrival ($P=0.03$). Notably, heart rate did not change pre- to post-haemodilution ($P=0.15$). Stroke volume decreased following acclimatization ($P<0.01$) and then increased following hemodilution ($P=0.02$). Following haemodilution, SV was not *statistically* different compared to initial arrival ($P=0.051$). These changes ultimately led to a reduction in Q following acclimatization ($P<0.01$), whereby Q remained below initial arrival

values following haemodilution (P=0.01). However, Q was not altered following haemodilution (P=0.62).

CBF Results

Cerebrovascular results are presented in **Figure 2**. Following approximately one-week at 5050 m gCBF decreased by 20.9 ± 23.6% from 843.8 ± 160.2 to 619.6 ± 135.9 mL · min^{-1} (P<0.001). This decrease was mediated by reductions in blood flow through both the ICA and VA (-14.2 ± 23.2%; P<0.01 and -22.3 ± 33.2%; P<0.01, respectively) (**Figure 2**). Following the haemodilution trial, gCBF was elevated by 15.3 ± 18.8% to 714.1 ± 203.5 mL · min^{-1} (P=0.045) due to an increase in ICA blood flow (+15.7 ± 19.7%; P=0.03) while blood flow in the VA only increased marginally and non-significantly (+15.5 ± 28.4%; P=0.305). Post haemodilution, gCBF remained significantly lower compared to initial arrival at high-altitude (P=0.033). At each time point, gCBF was regulated to the extent that CDO_2 was unaltered across acclimatization (126.2 ± 25.5 vs. 114.4 ± 22.7 mL · min^{-1}) and hemodilution (121.6 ± 30.2 mL · min^{-1}; main effect: P=0.302). No regional differences were observed between the relative changes in ICA and VA flow following acclimatization (-14.20 ± 23.15 vs -22.34 ± 33.16 %; P=0.10) or following haemodilution (15.72 ± 19.67 vs 15.52 ± 28.36 %; P=0.98). Linear regression analysis indicated there was no relationship between ΔgCBF and day of testing (R^2=0.191; P=0.135).

Blood volume

Blood volume data prior to and following haemodilution are presented in **Table 3**. Pre haemodilution, individual blood volumes ranged from 5.47 to 7.75 L with a mean of 6.18 ± 0.83

L. Of this, red cell volume accounted for 3.07 ± 0.49 L while plasma volume accounted for 3.11 ± 0.40 L. Assuming blood volume and hematocrit were relatively unchanged within 24 hrs following the carbon monoxide rebreathe assessment, blood volume post haemodilution was calculated based on the pre-haemodilution blood data. From these calculations, the mean increase in plasma volume and thus total blood volume observed following saline infusion was 0.54 ± 0.14 L. Notably, hydration status, as assessed by arterial blood osmolarity was not different pre- to post-haemodilution (288.14 ± 2.21 vs 289.00 vs 2.11 mmol · Kg^{-1}) and falls within euhydration ranges (Armstrong et al., 2010).

Mathematical estimation of CBF with a fully normalized Hct

Despite the large saline infusion utilized (2.10 ± 0.28 L), the haemodilution protocol failed to fully revert Hct to initial arrival values (**Table 1**) and estimations based on total Hb mass and Hct concentration indicate on average 540 mL of saline remained within the vasculature at the time of measurement. Thus, an estimation of the CBF that would occur at a fully reversed haemoconcentration was utilized. The group mean regression for ΔgCBF (mL · min^{-1}) per ΔHct (%) was 22.4 mL · min^{-1} · Hct^{-1} with individual values ranging from -17.96 to 82.33 mL · min^{-1} · Hct^{-1}. Utilizing these reactivity slopes on an individual subject basis, we calculated that CBF would have increased to 790.5 mL · min^{-1} had Hct been reduced by an additional 3.1% and fully restored to the initial arrival value of ~42.5 %. Cumulatively, addition of this theoretical change in Hct leads to an overall 165.46 ml · min^{-1} increase in gCBF from pre- to post-hemodilution. This is inclusive of the experimentally elicited increase and additional estimated change (**Figure 2**). While accounting for the change in CBF with full normalization of Hct statistically eliminates

the difference between initial arrival gCBF and that of the theoretical post-hemo gCBF (843.8 ± 160.2 vs. 790.5 ± 266.8 mL · min^{-1}; P=0.62), this increase in gCBF is not sufficient to explain the entire CBF response during acclimatization, which was a 224.13 ml · min^{-1} decrease. Indeed, these changes in gCBF are significantly different (224.1±189.2 vs. 165.5±212.1 mL · min^{-1}; P=0.02).

CBF reactivity to CaO_2

Reactivity values from the linear mixed effects modelling are reported as mean ± SD (**Figure 3**). Following one-week of acclimatization and haemodilution gCBF reactivity was unchanged (-58.7 ± 51.2 vs -64.5 ± 56.2 [Δ(mL · min^{-1} · mL^{-1} · dL^{-1})] P=0.72). Additionally there was no change in ICA reactivity to CaO_2 (-18.1 ± 18.7 vs -22.9 ± 20.2 [Δ(mL · min^{-1} · mL^{-1} · dL^{-1})]; P=0.43) or VA reactivity (-10.3 ± 12.6 vs -8.0 ± 14.1 [Δ(mL · min^{-1} · mL^{-1} · dL^{-1})]; P=0.56) between acclimatization and haemodilution. Additionally, there were no differences detected in relative ICA and VA reactivity to CaO_2 (9.87 ± 6.49 and 9.11 ± 5.43 [Δ(% · mL^{-1} · dL^{-1}), respectively)] following haemodilution (P=0.97). No regional differences between relative ICA and VA reactivity to CaO_2 were observed following acclimatization (-1.80 ± 22.14 vs -9.18 ± 29.43 [Δ(% · mL^{-1} · dL^{-1})]; P=0.07) or haemodilution (-1.08 ± 29.58 vs 9.05 ± 15.33 [Δ(% · mL^{-1} · dL^{-1})]; P=0.23). Linear regression analysis indicated there was no relationship between gCBF reactivity and day of testing (R^2=0.209; P=0.116).

DISCUSSION

This study aimed to mechanistically examine the degree to which haemoconcentration at high-altitude contributes to changes in CBF during acclimatization. The main findings of this study were that; 1) Similar to previous studies, elevations in CaO_2 through increases in Hct, as well as PaO_2, occurred following approximately one-week at high-altitude, resulting in a reduction in CBF but maintained CDO_2 (Severinghaus *et al.*, 1966; Huang *et al.*, 1987; Jensen *et al.*, 1990; Baumgartner *et al.*, 1994; Lucas *et al.*, 2011; Willie *et al.*, 2014; Subudhi *et al.*, 2014); 2) Following haemodilution, an absolute reduction in Hct by ~4 % resulted in a significant increase in gCBF; however, this response was blunted compared to that during acclimatization where CBF did not return to initial arrival values. Further, changes in CBF following haemodilution and mathematical extension of this response indicate that haemoconcentration is responsible for ~74% of the decrease in CBF observed during acclimatization to high-altitude. This finding is further supported when the tight coupling of CBF to CaO_2 is considered (Brown *et al.*, 1985). Indeed, CBF both during acclimatization and following haemodilution appears to be tightly dictated by changes in CaO_2. These findings imply that though ventilatory acclimatization and elevations in haematocrit occur concomitantly during acclimatization, the haemoconcentration response contributes to the CBF response during acclimatization to a greater degree at high-altitude.

Cerebral Blood Flow

Increased CBF in response to a reduction in CaO_2 has been observed in previous studies, which demonstrates the brain's ability to maintain CDO_2 during hypoxemia (Ainslie *et al.*, 2014).

This relationship is further evidenced by the reduction in CBF that occurs concomitant to increased CaO_2 with acclimatization to high-altitude [reviewed in: (Ainslie & Subudhi, 2014)]. Therefore, it appears that CBF is regulated to maintain convective oxygen delivery to the brain. However, the relative contribution that haemoconcentration has on the reduction in CBF observed across acclimatization had not yet been investigated.

Notably, due to the rapid diffusion of saline out of the intravascular space (Greenfield *et al.*, 1989), Hct was not fully reversed to values observed upon initial arrival to high-altitude. However, as Hct has a linear inverse relationship with CBF through its relationship with CaO_2 (Brown & Marshall, 1985; Ainslie *et al.*, 2014), the fully reversed response can be calculated by extrapolating this linear inverse relationship (**Figure 2**). Following mathematical correction to estimate the influence of a full reversal of haemoconcentration (see "*Mathematical estimation of CBF with a fully normalized Hct*"), gCBF did not completely return to initial arrival values despite the lack of statistical difference between pre-acclimatization and the theoretical post-haemodilution CBF. The increase in gCBF following haemodilution (and mathematical extension of this increase) explained 74% of the reduction in gCBF observed following acclimatization. This suggests that the majority of the reduction in gCBF following one-week of acclimatization is mediated through increases in [Hb] and Hct, and their subsequent influence on CaO_2. This is in agreement with recent estimations made by our group (Hoiland *et al.*, 2018).

Cerebrovascular hypoxic reactivity did not differ between acclimatization and haemodilution, highlighting previous findings demonstrating that CBF is primarily governed by changes in CaO_2 and suggests that the difference in the CBF responses between acclimatization and haemodilution are not due to alterations in hypoxic reactivity. Therefore, it stands to reason that the difference between initial arrival CBF and following haemodilution (and correction for

full restoration of Hct) is primarily a result of ventilatory acclimatization whereby SaO_2, PaO_2, and pH are also elevated from initial arrival at high-altitude. Indeed, SaO_2 and PaO_2 were 5.0% and 6.1 mmHg higher following acclimatization, respectively, while pH was increased by 0.03 units (**Table 1**). Given SaO_2, PaO_2, and pH were unaltered during haemodilution, their influence on cerebral vasomotor tone would have been unaltered and likely explains the difference between initial arrival and post-haemodilution CBF.

Differences in regional CBF have been noted at high-altitude and in hypoxia with an apparent preference of flow to the posterior regions of the brain which houses the primary centers for regulating physiological function upon arrival to high-altitude (Subudhi *et al.*, 2014; Feddersen *et al.*, 2015) and during exposure to normobaric hypoxia (Willie *et al.*, 2012). Of note, we did not observe any significant regional differences between relative changes in flow following acclimatization or haemodilution. Further, no differences were observed in the hypoxic reactivity between the ICA and VA. This finding agrees with previous work from our group conducted at the same altitude in subjects using acetazolamide (Willie *et al.*, 2014). Differences between these findings and previous research showing regional flow disparities may be related to the methodology of data collection, primarily the blood flow measurement (transcranial Doppler ultrasound vs duplex ultrasound) and the severity and mode of exposure to the hypoxic stimulus (Hoiland *et al.*, 2018; Willie *et al.*, 2018) whereby a more severe step change or exposure may necessitate regional blood flow prioritization to the posterior circulation as a form of survival response. Further, an important consideration is that while these regional differences appear to be more prevalent at highly localized levels (Binks *et al.*, 2008; Lawley *et al.*, 2017), bulk flow measures at the VA and ICA may fail to detect these differences in some studies given that flow measures at these sites (VA & ICA) represent the summation of multiple discrete brain regions.

Further, differences in reactivity that have been noted at high-altitude are thus likely not attributable to changes in Hct based on our results.

Following acclimatization, PaO_2 and SaO_2 increased through well documented mechanisms of ventilatory acclimatization [reviewed in; (Hoiland *et al.*, 2018)]. Both were unaltered following haemodilution, isolating any effects of reversing ventilatory acclimatization induced blood gas changes from our CBF changes attributed to acclimatization/Hct. Thus, the persisting influence of SaO_2 and PaO_2 may represent the remaining stimulus for lower CBF post haemodilution compared to initial arrival. Further, while pH was unaltered following haemodilution, both pre- and post-haemodilution pH were higher than initial arrival. This may also be driving a reduction in CBF and indicate our results may in fact be underestimating (albeit modestly) the influence of haemoconcentration on CBF at altitude.

Methodological considerations

Effect of viscosity on CBF

Alterations in Hct occurring both chronically during acclimatization (increases) and acutely during haemodilution (decreases) will lead to concomitant changes in whole blood viscosity, which may subsequently affect flow through the vasculature. However, experimental study has to date refuted this [(Brown & Marshall, 1985) reviewed in (Hoiland *et al.*, 2016)]. Indeed, a reduction in blood viscosity through plasma exchange in patients with paraproteinemia, whereby CaO_2 and $PaCO_2$ were constant, does not alter CBF (Brown & Marshall, 1985). While at

odds with basic physical principles (i.e., Poiseuille's Law), it is important to consider that the cerebral vasculature is a complex network of compliant vessels conveying a non-newtonian fluid. Thus, the conditions do not reflect those in which Poiseuille's Law was defined. Further, alterations in blood viscosity can influence vascular paracrine signalling. To speculate, reductions in blood viscosity would reduce the direct resistive effects of blood flow through the vessel, however, the shear stress stimuli induced by viscosity would also be reduced, potentially limiting dilation through reduced stimulation of shear dependent pathways (Melkumyants, Balashov, & Khayutin, 1989). This would act to increase vascular resistance. Thus, it is likely that a balance exists between these two stimuli, possibly explaining why viscosity has been previously shown to have a negligible effect on CBF (Brown & Marshall, 1985). Indeed, that haemodilution leads to greater increases in CBF than blood flow to other vascular beds (Crystal & Salem, 2002; Van Bommel et al., 2002) indicate the increases in CBF observed during haemodilution reflect active vascular regulation.

Effect of $PaCO_2$ and pH

As expected, $PaCO_2$ was significantly reduced following one-week of acclimatization at high-altitude due to ventilatory acclimatization (Rahn & Otis, 1949). However, we also observed a 2.6 mmHg reduction in $PaCO_2$ following haemodilution, though both PaO_2 and SaO_2 were unchanged. This decrease in $PaCO_2$ in the presence of unchanged PaO_2 and SaO_2 has been reported in a previous saline infusion study (Prisk *et al.*, 2010) and is likely due to the dilutional acidosis effect of the saline solution (Muir *et al.*, 1975). Notably, at least at sea level, $PaCO_2$ has

been shown to effect ICA and VA flow by ~6-8 % per 1 mmHg change in $PaCO_2$ (Willie *et al.*, 2012; Hoiland *et al.*, 2015). These observations indicate that, upon correction for this small alteration in $PaCO_2$, the CBF response would be greater following haemodilution, suggesting that the contribution of Hct on the CBF response would be more substantial. However, as $PaCO_2$ primarily alters CBF through changes in arterial pH - which notably was not different pre to post haemodilution - the difference in $PaCO_2$ pre-to post-haemodilution likely had a negligible effect.

Blood volume expansion

Due to the logistical constraints and remote nature of the high-altitude expedition, a hypervolemic hemodilution protocol using saline infusion was utilized as opposed to a normovolemic haemodilution protocol in which blood volume would be maintained. However, this may be most appropriate as the initial increases in Hct observed at altitude are due to a reduced plasma volume (Siebenmann et al., 2015), thus our intervention manipulated Hct in the same manner as the environmental stress of high-altitude hypoxia. Though the volume of fluid utilized in this protocol was relatively large in relation to the overall blood volume of participants, the crystalloid properties of the fluid infused and the time at rest between measures resulted in a large portion of the infused saline leaving the vasculature (Greenfield *et al.*, 1989). This is primarily evidenced by the relatively marginal reduction in hematocrit compared to volume infused and blood volume data which indicates only 540 mL of saline remained within the vasculature at the time of CBF measurement. Further, if increases in blood volume resulted in alterations in CBF, this would be expected to coincide with alterations in cardiac parameters, (i.e.

increased Q). Of note, neither of these variables were significantly elevated by hemodilution suggesting there was no direct impact of volume expansion on CBF changes in this study.

CONCLUSIONS

This study was the first to experimentally investigate the degree to which CBF acclimatization is driven by haemoconcentration. Through haemodilution, we were able to determine that, following one-week of acclimatization, the primary mechanism contributing to the CBF response during acclimatization response is generated by diuresis and erythropoiesis-mediated increases in [Hb] and Hct, while the remaining contribution to the CBF response during acclimatization response is likely attributable to ventilatory acclimatization.

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AUTHOR CONTRIBUTIONS

C.A.H., P.N.A., & R.L.H. conceived the study design. C.A.H., P.N.A., J.C.T., H.H.C., A.P., M.S., A.W., E.D., M.G.R., M.T., C.G., A.S., D.B.M., & R.L.H. were involved in data collection. C.A.H., P.N.A., J.C.T., M.S., A.L.D., & R.L.H. were involved in data analyses and interpretation. C.A.H., P.N.A., & R.L.H. drafted the manuscript. All authors critically reviewed and approved the final version of this manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

TABLES

Table 1. Arterial blood gases at high altitude.

	Arrival at 5050m	Pre-Haemodilution	Post-Haemodilution
CaO_2 (mL · dL)	15.4 ± 1.6	18.6 ± 1.3*	17.2 ± 1.0*†
Hct (%)	42.5 ± 2.5	49.6 ± 2.5*	45.6 ± 2.3*†
[Hb] (g · dL)	14.3 ± 0.8	16.2 ± 0.8*	14.9 ± 0.8*†

$PaCO_2$ (mmHg)	29.6 ± 1.8	23.6 ± 2.1*	21.0 ± 1.5*†
pH	7.47 ± 0.02	7.50 ± 0.02*	7.49 ± 0.02*
PaO_2 (mmHg)	40.6 ± 4.7	46.7 ± 4.2*	47.0 ± 3.4*
SaO_2 (%)	78.3 ± 5.6	83.3 ± 4.1*	83.7 ± 3.5*

595 * significant difference from arrival at 5050m

596 † significant difference between pre- and post-haemodilution

Table 2. Cardiovascular parameters upon arrival to high-altitude and pre- and post-haemodilution

	Arrival at 5050m	Pre-Haemodilution	Post-Haemodilution
MAP (mmHg)	99±12	108±10*	111±9*
HR (bpm)	65±18	58±11	53±12*
SV (mL)	69±13	54±11*	63±8.8†
Q ($L \cdot min^{-1}$)	4.45±1.52	3.09±0.65*	3.28±0.65

* significant difference from arrival at 5050m

† significant difference between pre- and post-haemodilution

Table 3. Individual blood volume data pre- and post-haemodilution

	Pre-Haemodilution				Post-Haemodilution			
Subject	Hct (%)	Hct Volume (L)	Plasma Volume (L)	Blood Volume (L)	Hct (%)	Hct Volume (L)	Plasma Volume (L)	Blood Volume (L)
1	47.4	2.661	2.953	5.613	43.4	2.661	3.470	6.131
2	53.2	2.911	2.561	5.472	48.9	2.911	2.989	5.900
3	50.2	2.569	2.549	5.118	46.6	2.569	2.923	5.492
4	50.8	3.555	3.443	6.997	46.1	3.555	4.110	7.665
5	49	3.799	3.954	7.753	45.4	3.799	4.550	8.349
6	49	2.787	2.900	5.687	45.5	2.787	3.325	6.111
7	49.8	2.927	2.951	5.878	45.2	2.927	3.522	6.449
8	50.6	3.607	3.521	7.128	45.4	3.607	4.288	7.895
9	50.8	3.474	3.365	6.839	45.6	3.474	4.094	7.569
10	50.1	2.896	2.884	5.780	46.8	2.896	3.271	6.167
11	52.9	3.695	3.290	6.985	49.3	3.695	3.747	7.442
12	43.3	2.411	3.157	5.568	40.5	2.411	3.572	5.983
13	47	2.604	2.936	5.540	43.6	2.604	3.619	6.223
MEAN	49.5	3.069	3.113	6.181	45.6	3.069	3.652	6.721
SD	2.6	0.486	0.396	0.834	2.3	0.486	0.492	0.924

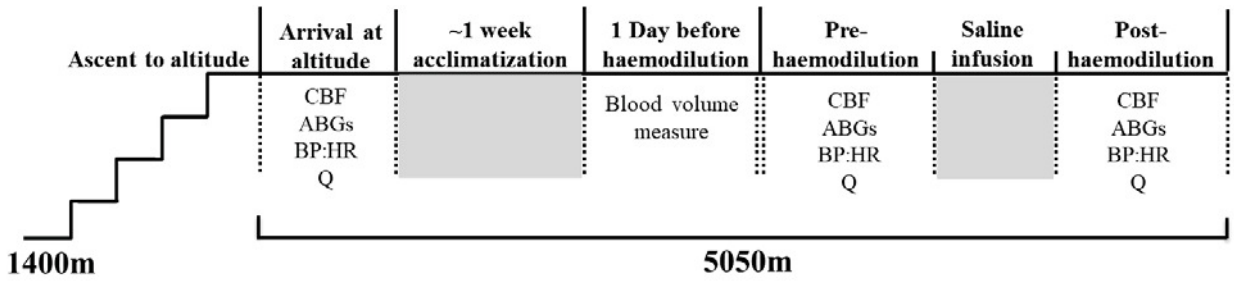
Individual blood volume data pre- and post-haemodilution. Plasma volume and total blood volume are derived from the Hct % and total Hct volume measured using a CO re-breath test performed one-day prior to the haemodilution protocol.

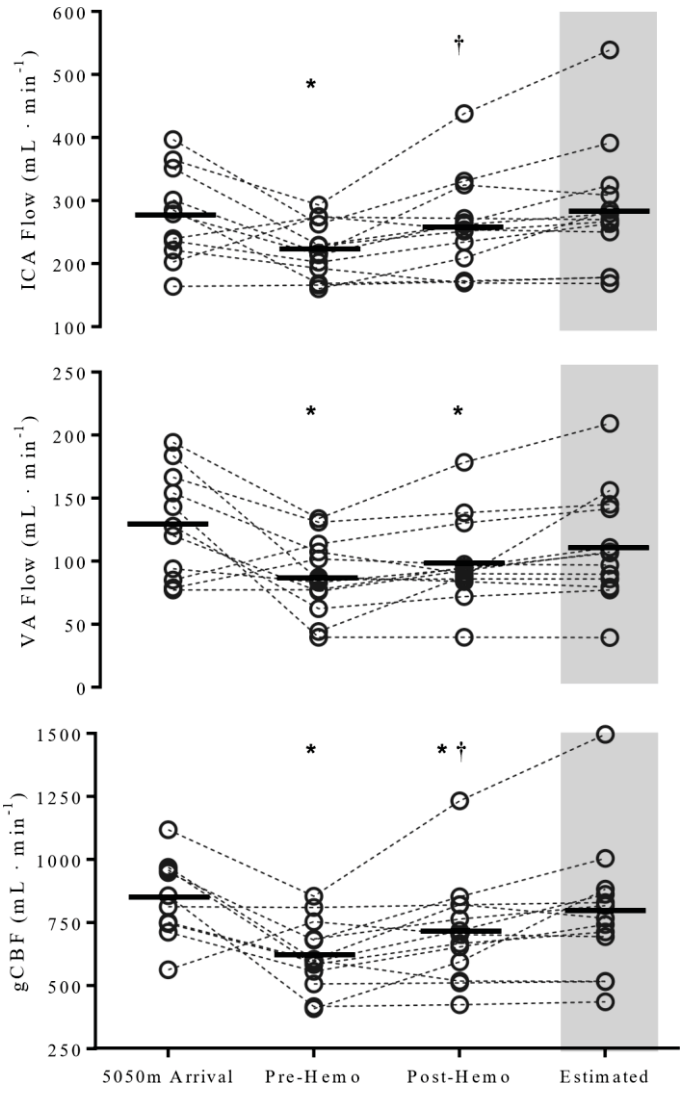
FIGURES

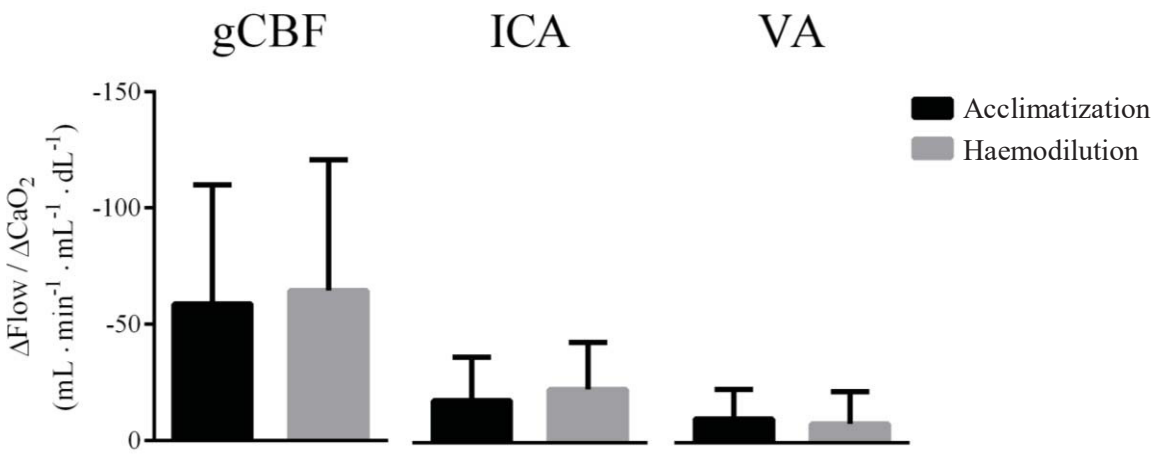
Figure 1. Experimental overview of the study protocol. Participants ascended to 5050 m over a 9-day period involving rapid ascent by plane to 2860 m, and ambulatory ascent onward to 5050 m. Cerebral blood flow (CBF), arterial blood gases (ABGs), blood pressure (BP), cardiac output (Q) and heart rate (HR) were recorded at all three time points.

Figure 2. Cerebral blood flow during acclimatization and following haemodilution. At the arrival time point, sample sizes were reduced for the internal carotid artery (ICA; n=11), vertebral artery (VA; n=11), and global cerebral blood flow (gCBF; n=10). Data was successfully collected in all participants (n=13) following one-week of acclimatization at 5050 m (pre-hemo) and following hypervolemic haemodilution (post-hemo). "Estimated" data (denoted by the grey background) are based on theoretical calculations for a fully normalized Hct (see "*Theoretical calculation of CBF with a fully normalized Hct*"). Blood flow was calculated to assume a complete reversal of haematocrit to initial arrival values (i.e. greater extent of haemodilution). Open circles represent individual data points with dotted lines tracking within subject changes. Black horizontal lines represent the average flow at each time point. * signifies a significant difference from arrival at 5050m. † signifies a significant difference pre- to post- haemodilution. Significance is set a
$P<0.05$

Figure 3. Cerebrovascular reactivity to acclimatization and haemodilution. Reactivity to changes in CaO_2 was not different across acclimatization (Initial arrival to pre-hemo) compared to during haemodilution (pre-hemo to post-hemo). gCBF, global cerebral blood flow; ICA, internal carotid artery; VA, vertebral artery. No differences in reactivity were observed between the ICA and VA at any time point. Data are presented as mean ± SD.


Ascent to altitude
Arrival at altitude
~1 week acclimatization
1 Day before haemodilution
Pre-haemodilution
Saline infusion
Post-haemodilution
CBF
ABGs
BP:HR
Q
Blood volume measure
CBF
ABGs
BP:HR
Q
CBF
ABGs
BP:HR
Q
1400m
5050m


ICA Flow (mL · min-1)
600
500
400
300
200
100
*
†
VA Flow (mL · min-1)
250
200
150
100
50
0
*
*
gCBF (mL · min-1)
1500
1250
1000
750
500
250
*
* †
5050m Arrival
Pre-Hemo
Post-Hemo
Estimated


gCBF
ICA
VA
-150
-100
-50
0
ΔFlow / ΔCaO2
(mL . min-1 . mL-1 . dL-1)
Acclimatization
Haemodilution