UBC-Nepal Expedition: Phenotypical evidence for evolutionary adaptation in the control of cerebral blood flow and oxygen delivery at high altitude

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Running head: Cerebral blood flow in Sherpa at high-altitude

KEY POINTS SUMMARY

- Sherpa have lived in the Nepal Himalaya for 25-40 thousand years and display positive physiological adaptations to hypoxia
- Sherpa have previously been demonstrated to suffer less negative cerebral side effects of ascent to extreme altitude, yet little is known as to whether or not they display differential regulation of oxygen delivery to the brain compared to lowland natives
- We demonstrate that Sherpa have lower brain blood flow during ascent to and acclimatization at high-altitude compared to lowlanders and that this difference in flow is not attributable to factors such as mean arterial pressure, blood viscosity and pH
- The observed lower cerebral oxygen delivery in Sherpa likely represents a positive adaptation that may indicate a cerebral hypometabolic conservation of energy at altitude and/or decrease their risk of other cerebral consequences such as vasogenic edema.

ABSTRACT

Debilitating side effects of hypoxia manifest within the central nervous system; however, high-altitude natives of the Tibetan plateau, the Sherpa, experience negligible cerebral effects compared to lowland natives at extreme altitude. Phenotypical optimization of the oxygen cascade has been demonstrated in the systemic circulation of Tibetans and Sherpa, likely underscoring their adapted capacity to thrive at altitude. Yet, little is known as to how the cerebral circulation of Sherpa may be adapted. To examine potential differences in cerebral oxygen delivery in Sherpa compared to lowlanders we measured arterial blood gases and global cerebral blood flow (duplex ultrasound) during a nine-day ascent to 5050m. Although cerebral oxygen delivery was maintained during ascent in lowlanders, it was significantly reduced in the Sherpa at 3400m (-30.3±21.6%; $P<0.01$) and 4371m (-14.2±10.7%; $P=0.03$). Furthermore, linear mixed effects modeling indicated that independent of differences in mean arterial pressure, pH and blood viscosity, race accounts for an approximate 100mL · min^{-1} (~17-34%) lower CBF in Sherpa compared to lowlanders across ascent to altitude ($P=0.046$). To ascertain the role of chronic hypoxia independent of the ascent, Sherpa who had not recently descended were also examined at 5050m. In these Sherpa, cerebral oxygen delivery was also lower compared to lowlanders (~22% lower; $P<0.01$). We highlight new information about the influence of race and genetic adaptation in the regulation of cerebral oxygen delivery. The lower cerebral oxygen delivery in the Sherpa potentially represents a positive adaptation considering Sherpa endure less deleterious cerebral consequences than lowlanders at altitude.

Key words: Hypoxia; Cerebral Blood Flow; Sherpa; Adaptation; High-altitude

INTRODUCTION

The dramatically low atmospheric oxygen levels of the Tibetan Plateau represent one of the most hostile living conditions of modern human habitation. While this environment is debilitating for most humans, and may be fatal for those who ascend too high and too fast, it has been inhabited for millennia (30,000-40,000 years) by a lineage of Sherpa (Zhang & Li, 2002; Aldenderfer, 2011; Zhang *et al.*, 2018). The Sherpa, a highlander population of the Nepalese Khumbu region that shares a common genetic origin with Tibetans (Lu *et al.*, 2016; Zhang *et al.*, 2017), have clearly evolved under the selection pressures imposed by hypoxia and are consequently better suited for life at altitude than lowland natives (Lahiri & Milledge, 1965; Moore, 2017). Of critical importance in the setting of hypoxia is the oxygen sensitive brain, which due to a high metabolic demand and limited substrate storage is highly susceptible to metabolic deficiency and ensuing hypoxic damage [reviewed in: (Bailey *et al.*, 2009)]. Therefore, increases in cerebral blood flow (CBF) are integral to maintain oxygen delivery to the brain (CDO_2) in the face of hypoxemia and a reduced arterial oxygen content (CaO_2).

While several examples of phenotypical adaptations distinct from those in lowlanders have been observed in the oxygen cascade of Tibetans, such as a higher nitric oxide bioavailability (Beall *et al.*, 2001; Erzurum *et al.*, 2007), increased skeletal muscle capillary density (Beall, 2007), and improved muscle energetics in Sherpa (Horscroft *et al.*, 2017), little is known relative to potential adaptations in cerebral oxygen delivery/utilization [for review see: (Jansen & Basnyat, 2011; Gilbert-Kawai *et al.*, 2014)]. Tibetans may display a "high flow" phenotype to maintain oxygen delivery to various peripheral tissues in the presence of relatively normal arterial oxyhaemoglobin saturation (SaO_2) and haemoglobin concentrations [Hb] that are comparable to lowlanders at altitude (Beall, 2007). However, this high flow phenotype appears to be isolated to the arm, as femoral blood flow does not differ between lowlanders and Sherpa at rest (Ruggiero *et al.*, 2018; Tremblay *et al.*, 2018). Therefore, it is reasonable to expect the Sherpa brain may possess its own unique phenotype for the regulation of oxygen delivery. Preliminary evidence ostensibly indicates a high flow adaptation in the brain due to elevated internal carotid artery (ICA) velocity in Tibetan natives compared to lowlanders at 3658 m (Huang *et al.*, 1992). However, a recent cross-sectional study demonstrated lower CDO_2 in Tibetans than in Han Chinese at sea level and at 3658 m (Liu *et al.*, 2016). Given that velocity is a poor index of flow (and therefore CDO_2) in the setting of altered intra-cranial cerebral arterial diameter (Ainslie & Hoiland, 2014; Hoiland & Ainslie, 2016), which occurs at altitude (Wilson *et al.*, 2011; Imray *et al.*, 2014; Willie *et al.*, 2014*a*), previous theories related to a high-flow adaptation in the Sherpa brain (Huang *et al.*, 1992; Gilbert-Kawai *et al.*, 2014) need to be re-considered. Elucidation of hypoxia tolerant adaptation in humans may provide insight into potential therapeutic targets in chronic hypoxic diseases and further our basic understanding of human responses to low oxygen conditions.

It is well established that CDO_2 is maintained during ascent and at altitude in lowlanders [reviewed in: (Ainslie & Subudhi, 2014)]. However, volumetric CBF and CDO_2 have yet to be compared between lowlanders and Sherpa following a short duration at altitude (e.g., days). It is also unknown if CBF in lowlanders responds differentially to that of Sherpa during graded ascent to altitude. During ascent to and upon arrival at 5050 m, we hypothesized that CBF and CDO_2 would be lower in Sherpa compared to lowlanders. To examine these novel hypotheses, Sherpa and lowlanders were studied using a longitudinal experimental design during ascent to altitude. Further, to determine the influence of partial de-acclimatization on CBF regulation, Sherpa recently exposed to altitude for a short duration (those who ascended) and long duration (those residing at altitude) were compared to lowlanders using a cross-sectional design following arrival at 5050 m. Further mechanistic insight was also obtained within these groups via the concurrent assessment of established factors in the regulation of CBF [e.g. arterial blood gases (Kety & Schmidt, 1948), mean arterial pressure, MAP (Lucas *et al.*, 2010; Numan *et al.*, 2014) and blood viscosity (Hoiland *et al.*, 2016)].

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Clinical Research Ethics Board of the University of British Columbia (H16-01028) and the Nepal Health Research Council. All lowlander participants gave written informed consent in English prior to participating. All Sherpa participants read an in-depth study information form, spoke with a Nepalese physician and gave written informed consent in Nepalese prior to participating. This study conformed to the standards set by the Declaration of Helsinki, except for registration in a database.

Study participants

This study involved the recruitment of three distinct subject groups. First, the lowlander group comprised of 21 (1 female) healthy individuals (mean±standard deviation, Age: 29±6 years; body mass index (BMI): 23±2kg/m^2; height: 179±6 cm) who were recruited at the University of British Columbia's Okanagan campus and were part of the research team. All lowlander participants were free of cardiovascular, respiratory and neurological diseases and were non-smokers.

The second group of participants comprised of 12 high-altitude Sherpa (Age: 34±11, BMI: 24±4; height: 167±7 cm). These Sherpa were of Tibetan lineage. The Sherpa participants were recruited from local villages in the Solukhumbu Valley (Table 1) and descended to Kathmandu prior to experimental testing (see *Experimental Overview*). Four Sherpa were current smokers, with an average of 1.3±1.1 pack years. The Sherpa were free of cardiovascular, respiratory and neurological diseases. This cohort of Sherpa is referred to as the "Ascent Sherpa" throughout the manuscript. Notably, three of the Sherpa in this group had summited Mount Everest (8848 m) in the previous year, while the remaining Sherpa had reached maximum altitude of 4800 m to 7800 m (median: 5545 m) in the last year.

The third group of participants included 11 Sherpa of Tibetan lineage (Age: 23±7, BMI: 21±2; height: 170±6 cm) that were recruited from local villages in the Solukhumbu Valley (Table 1); however, unlike the Ascent Sherpa, these Sherpa were recruited while the research team was at the Ev-K2-CNR Pyramid laboratory. Therefore, these Sherpa did not descend to Kathmandu prior to testing. Five Sherpa were current smokers, with an average of 0.4±0.1 pack years. All Sherpa were free of cardiovascular, respiratory and neurological diseases. This cohort of Sherpa is referred to as the "Altitude Sherpa" throughout the manuscript. Notably, two of the Sherpa in this group had summited Mount Everest in the previous year, while the remaining Sherpa had reached a maximum altitude of 4200 m to 5545 m (median: 5300 m).

Experimental Overview

The logistics of the UBC-Nepal Expedition have been detailed extensively elsewhere (Willie *et al.*, 2018), therefore, only features of the expedition pertinent to the current study will be outlined. All lowlander participants spent 3-9 days in Kathmandu (1400 m) prior to flying to Lukla (2860 m) to begin the ascent to the EV-K2-CNR Pyramid Research laboratory (5050 m), while the Ascent Sherpa group descended to Kathmandu and remained there for 5-15 days (median: 7) prior to flying to Lukla with the lowlanders. Ascent to the Pyramid Laboratory took place over a slow and safe 9-day trekking protocol 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 5050 m.

In Kathmandu prior to the ascent and on the day following arrival to Namche, Pheriche, and the Pyramid laboratory, all participants underwent experimental measurement of arterial blood gases, venous blood viscosity, MAP, heart rate (HR), and CBF. These measurements are detailed below (see *Experimental Measures*).

Once the ascent study was completed, a second experimental protocol was conducted on the Altitude Sherpa group. The Altitude Sherpa group had not descended to low altitude (<2800 m) in the previous 6 months, were living at high-altitude prior to arrival at the Pyramid, ascended from 3400-4200 m to 5050 m in 1-2 days, and were tested 1-2 days following arrival. This timeline was utilized to match the time after arrival of testing to the lowlander and Ascent Sherpa groups (i.e. 1 day following arrival to 5050 m). They completed the same experimental protocol as the lowlanders and Ascent Sherpa (described below).

Experimental Measures

Following 10-minutes supine rest at each location during the ascent and after approximately 2 weeks at high-altitude when the Altitude Sherpa were tested, arterial blood samples were taken from the radial artery. A 23-G self-filling syringe (SafePico, Radiometer) was advanced into the radial artery under local anesthesia (lidocaine, 1.0%) and ultrasound guidance (Terason, uSmart 3300). Approximately 1mL of blood was withdrawn anaerobically and immediately assessed using an arterial blood gas analyzer for the partial pressure of arterial oxygen (PaO_2), the partial pressure of arterial carbon dioxide ($PaCO_2$), pH, bicarbonate (HCO_3^-), [Hb], and HCT (i-STAT 1, Abbott Point of Care). Further, following venipuncture of the median ante-cubital vein (21G needle, BD Vacutainer eclipse) 2mL of blood was collected into a lithium heparin vacutainer. From this venous sample, 1mL of whole blood was used to measure blood viscosity (Brookfield DVST Viscometer) at a shear rate of 225 s^{-1} with temperature controlled at 37.0°C.

Blood velocity and vessel diameter of the ICA and vertebral artery (VA) were measured using a 10MHz multi-frequency linear array duplex ultrasound (Terason T3200, Teratech, Burlington, MA) according to published technical recommendations (Thomas *et al.*, 2015). Arterial diameter was measured with B-mode imaging while pulse-wave mode was used to simultaneously measure peak envelope blood velocity. The ICA diameter and velocity were in most cases measured at least 1.5 cm distal to the common carotid bifurcation to eliminate recordings of turbulent and retrograde flow, while VA diameter and velocity were measured between C4-C5, C5-C6, or proximal to entry into the vertebral column. The location was determined on an individual basis in an attempt to select the most reproducible measures, with the same location repeated within participants and between testing dates. Images were recorded and stored as video files for offline analysis using automated edge detection software (Woodman *et al.*, 2001) following study completion. No less than a one-minute video was used for the assessment of ICA and VA flow. Volumetric blood flow was calculated using the following formula:

$$ICA\ or\ VA\ flow = (0.5 \cdot Peak\ envelope\ velocity) \cdot (\pi \cdot (0.5 \cdot diameter)^2)$$

The gCBF was estimated as twice the sum of the unilateral ICA and VA flow measurements. During the ascent, MAP was calculated as the average of three automated measurements on the brachial artery (Life Source, UA-767FAM). In the experimental trials following 2-weeks at the Pyramid, MAP was determined as the average of two manual sphygmomanometric measurements.

In an attempt to better understand the underlying physiology regulating gCBF in Sherpa and lowlanders we measured cerebral vascular reactivity to CO_2 using the hyperoxic rebreathe technique (Fan *et al.*, 2010). As we were unable to collect CO_2 reactivity data during our 2016 expedition, this particular data set was taken from 13 lowlanders and five Sherpa on our 2008 expedition to Nepal (Lucas *et al.*, 2011). The lowlanders from our 2008 expedition underwent a similar ascent protocol, and were tested following >2 weeks acclimatization at 5050 m (Lucas *et al.*, 2011). The Sherpa subjects from the 2008 expedition were tested under similar conditions as the Altitude Sherpa from the 2016 expedition in that they had not previously descended to low altitude for >6 months prior to testing. These data have not been previously published.

Calculations

In an attempt to account for the influence of changes in blood pressure on CBF upon ascent to high-altitude, we calculated cerebrovascular conductance (CVC) as gCBF divided by MAP (i.e. CVC = gCBF/MAP). Shear stress in the ICA and VA were calculated as the product of shear rate (s^{-1}) and venous blood viscosity (cP). Shear rate was determined as 4 times velocity divided by diameter (i.e. Shear rate = 4 * velocity / diameter). Cerebral oxygen delivery ($mL \cdot min^{-1}$) was calculated as the product of CaO_2 ($mL \cdot dL^{-1}$) and gCBF ($mL \cdot min^{-1}$) divided by 100 (i.e., $CDO_2 = CaO_2 \cdot gCBF / 100$).

Statistical Analyses

Data from the ascent were analyzed using a linear mixed effects model with a compound symmetry repeated measure co-variance structure. A linear mixed effects model was ran for each variable (e.g., those in figure 1 and table 2). The fixed effects were altitude and race (with altitude as a repeated effect) while subjects were included as a random effect. When significant main effects were detected, Bonferroni corrected post-hoc tests were used to make pairwise comparisons. Significance was assumed at $P<0.05$. There was no difference in the primary outcome variables between current and non-smokers in either Sherpa group.

As there were differences between lowlanders and Sherpa relative to several factors known to influence CBF (see Results), we also ran a linear mixed effects model for gCBF with MAP, pH, and viscosity as co-variates alongside altitude and race as fixed effects and subjects as a random effect. The selected variables

were chosen as they are considered important regulators of CBF in humans (Willie *et al.*, 2014*b*) and they improved the model fit, as determined by the -2 log likelihood indicating their acceptability in the model. When assessed for colinearity it was found that the covariates were only mildly colinear (all variable inflation factors < 1.2; note: >4 is considered moderately colinear). Residuals were normally distributed and equality of variance was confirmed by visually inspecting a residuals versus fitted scatterplot.

Differences between acclimatized lowlanders, the ascent Sherpa, and altitude Sherpa at 2-weeks were compared using a one-way ANOVA. When significant main effects were detected, Bonferroni corrected post-hoc tests were used to make pairwise comparisons. Sherpa versus lowlander cerebral vascular CO_2 reactivity were compared using a Mann-Whitney U test.

RESULTS

Arterial and Venous Blood Variables

All arterial and venous blood data are summarized in Table 2. Notably, CaO_2, SaO_2, and the PaO_2 did not differ between lowlanders and Sherpa at any high-altitude location. Although a reduction in the $PaCO_2$ was observed in both groups, $PaCO_2$ was higher in Sherpa at 4371 m and 5050 m (vs. lowlanders $P<0.01$). Accordingly, there was a main effect for lower pH in Sherpa across all altitudes ($P<0.01$). At 1400 m Sherpa had an elevated haemoglobin concentration ([Hb]) relative to lowlanders ($P<0.01$), but following an increase in lowlander [Hb] at 3400 m ($P<0.01$), there was no difference present between groups throughout the remainder of the ascent. Both haematocrit (HCT) and venous whole blood viscosity followed the same pattern as [Hb], with Sherpa HCT and viscosity elevated compared to lowlanders at 1400 m, but not different during the remainder of ascent.

Cerebrovascular and Cardiorespiratory Variables

In lowlanders, global CBF (gCBF) was unchanged from Kathmandu (1400 m) values at 3400 m and 4371 m (+0.4±15.5% & -0.1±21.5%, respectively), but increased by 23.3±26.1% at 5050 m (main effect of altitude, $P<0.01$) (Figure 1). Flow through the ICA and VA followed a similar pattern – both were unaltered at 3400 m or 4371 m, but were elevated by 18.4±26.2% and 24.6±31.9% at 5050 m, respectively (main effect of altitude, $P<0.01$). In the Sherpa, gCBF appeared 21.3±24.0% lower at 3400 m compared to Kathmandu, returned to Kathmandu values by 4371 m (-4.3±8.8%), and was elevated by 13.5±22.3% at 5050 m (main effect of altitude, $P<0.01$). Regional flow through the ICA and VA appeared to follow

differential patterns in the Sherpa, with ICA flow reduced by 22.8±21.6% at 3400 m compared to Kathmandu, returned to Kathmandu values by 4371 m (-5.8±11.1%), and then elevated by 10.0±22.8% at 5050 m. However, VA flow was unaltered at 3400 m (-5.9±44.2%), albeit this response showed substantial inter-individual variability (see statistical outputs in Figure 1). At 4371 m, VA flow in the Sherpa was slightly elevated by 10.6±51.2%, with a further significant elevation at 5050 m where VA flow was 20.7±31.8% greater than in Kathmandu (main effect of altitude, $P<0.01$). There was a significant main effect of race, with ICA flow, VA flow, and gCBF greater in lowlanders compared to Sherpa across all altitudes (Figure 1). Changes in blood velocity and diameter of the ICA and VA are summarized in Table 3. Anterior (i.e. ICA flow) versus posterior (i.e. VA flow) distributions of CBF in lowlanders and Sherpa are presented in Figure 2. Sherpa had a lower posterior distribution of CBF than lowlanders across all altitudes during ascent (main effect of race, $P=0.018$).

There was a significant interaction effect (i.e. altitude * race) for ICA delivery of oxygen (DO_2; $P=0.008$) but not VA DO_2 ($P=0.443$; Table 3). Collectively, global CDO_2 was maintained in the lowlanders during ascent (Figure 1D). In contrast, Sherpa CDO_2 was reduced at 3400 m (-30.3±21.6%; $P<0.01$), 4371 m (-14.2±10.7%; $P=0.03$), but was not statistically different from Kathmandu at 5050 m (-12.0±18.0%; $P=0.28$). Compared to lowlanders, Sherpa CDO_2 was lower at 3400 m ($P<0.01$), 4371 m ($P=0.03$), and 5050 m ($P<0.01$).

Lowlander mean arterial pressure (MAP) across ascent in lowlanders and Sherpa is summarized in Table 3. The combination of changes in gCBF and MAP lead to a main effect of both altitude ($P<0.001$) and race ($P<0.001$) on CVC (interaction, $P=0.166$). Lowlander (7.91±1.42 vs. 6.97±1.32 mL · min^{-1} · $mmHg^{-1}$) and Sherpa (5.80±1.32 vs. 4.02±1.15 mL · min^{-1} · $mmHg^{-1}$) CVC were reduced at 3400 m compared to Kathmandu (main effect of altitude, $P=0.001$). Lowlander and Sherpa CVC were not different from Kathmandu at 4371 m (7.04±1.58 & 5.22±1.23 mL · min^{-1} · $mmHg^{-1}$, respectively) or 5050 m (7.99±1.74 & 6.41±1.64 mL · min^{-1} · $mmHg^{-1}$, respectively).

Covariate Analysis: Linear mixed effects modeling was utilized to determine the significance of race on CBF at altitude by controlling for important regulators of CBF as covariates. Including MAP, pH, and viscosity led to the best model fit (-2 log likelihood). As outlined in Figure 3, irrespective of including covariates into the analysis, race possesses a significant influence on gCBF at altitude (see section *Cerebrovascular and Cardiorespiratory Variables* for results of initial linear mixed model). Indeed, race had a similar effect on gCBF in both models given the similar parameter effects with no covariates (-118.93±47.34; $P=0.014$) or with the inclusion of MAP, pH and viscosity as covariates (-99.67±48.93; $P=0.046$). Further illustrating the significant effect of race as a factor in CBF control at altitude, upon

controlling for the aforementioned covariates, there was a significant interaction between altitude and race (P=0.012), with post-hoc tests revealing that gCBF was lower in Sherpa compared to lowlanders at 1400m (P=0.046), 3400 m (P<0.001), 4371 m (P=0.022) and 5050 m (P=0.001) (Figure 3). Note: the gCBF data plotted in Figure 1C and Figure 3A are the same, except Figure 1C is the mean± standard deviation of the raw data whereas Figure 3A is the mean± standard error of the model estimates. The data is reproduced in Figure 3 for the purpose of easy comparison with the covariate model.

Lowlander Comparison to Both the Ascent and Altitude Sherpa

Comparison of lowlanders to both the Ascent Sherpa and Altitude Sherpa at 5050 m revealed their gCBF were similarly 24% (P<0.01) and 27% (P<0.01) lower, respectively (Figure 4). While differences in ICA flow between groups approached statistical significance (main effect, P=0.06), the difference in gCBF between lowlanders and both Sherpa groups is largely attributable to a 40% (Ascent Sherpa, P<0.01) and 44% (Altitude Sherpa, P<0.01) lower VA flow. This is consistent with the lower posterior CBF distribution in both Sherpa groups compared to lowlanders at 5050 m (P<0.05 for both; Figure 2). These differences in CBF - in the face of similar CaO_2 values (Table 4) - were reflected in a 21% (P=0.02) and 22% (P<0.01) lower CDO_2 in the Ascent Sherpa and Altitude Sherpa, respectively. Cerebral vascular CO_2 reactivity was not different between Sherpa and lowlanders (5.4±2.1 vs. 5.0±1.1 % · $mmHg^{-1}$; P=0.77) at 5050 m (Figure 5).

DISCUSSION

The present study examined the novel hypothesis that CBF and CDO_2 would be lower in Sherpa compared to lowlanders during ascent to high altitude. To address this question, unique comparisons of CBF, CDO_2 and related physiological parameters were made between lowlanders and partially de-acclimatized Sherpa during graded ascent to 5050 m. Further comparisons were made between lowlanders, the Sherpa that had ascended (Ascent Sherpa), and Sherpa that were residing at altitude prior to travel to the Pyramid Laboratory (Altitude Sherpa). The primary findings are: 1) gCBF during ascent to high altitude is significantly lower (~17-34%) in the Sherpa with an approximate 100 to 120 mL · min^{-1} of the difference in gCBF between lowlanders and Sherpa attributable to factors associated with race; 2) consequently, upon ascent to high-altitude, CDO_2 is higher in lowlanders compared to Sherpa, and higher than both the Ascent and Altitude Sherpa at 5050 m; 3) CBF distribution to the posterior circulation was lower across all altitudes during ascent in Sherpa compared to lowlanders, and lower in both Sherpa groups compared to lowlanders

at 5050 m. Collectively, these data indicate that there is a unique role of race in governing differential CBF regulation between lowlanders and Sherpa, irrespective of partial de-acclimatization, implicating long-term (i.e., generational) adaptations in the regulation of CDO_2.

Cerebral blood flow at altitude: Influence of race

Our study corroborates recent research demonstrating that Tibetans' possess lower CBF than lowlanders at altitude (Liu *et al.*, 2016) (Figures 1; 3 & 4). Although not quantified in this latter study (Liu *et al.*, 2016), we extend these findings using a longitudinal design to show that these differences are present despite a similar blood gas profile during ascent between groups. Notably, however, pH was lower, and $PaCO_2$ was higher in the Sherpa (typically these two factors would increase gCBF in Sherpa relative to lowlanders – see *Potential Mechanism(s) of Reduced CDO_2*). When lowlander CBF was compared to Sherpa that had not recently descended to low altitude, lowlander CBF remained higher; therefore, irrespective of recent time at altitude, Sherpa possess lower CBF than lowlanders. Yet, the recent study by Lui *et al.*, reported similar CBF at sea level between Tibetans and Han-Chinese (Liu *et al.*, 2016). Our group has also recently demonstrated that gCBF is lower in Sherpa children compared to lowlander children which, given the consistently lower CBF across the lifespan, suggests developmental differences do not account for our observation of lower CBF in Sherpa at high altitude compared to lowlanders (Flück *et al.*, 2017). Further research, using a longitudinal study design where Tibetans/Sherpa born at sea-level ascend to altitude is necessary to explicitly determine the influence of Tibetan genetic adaptation on CBF regulation independent of previous hypoxic exposure. Overall, the lower gCBF in Sherpa during ascent, and in both Sherpa groups at 5050 m, led to a lower CDO_2 in Sherpa compared to lowlanders at every high-altitude time point.

Previous studies have indicated a greater preservation of VA flow (i.e. posterior oxygen delivery) relative to ICA flow (i.e. anterior oxygen delivery) in both high-altitude natives (Liu *et al.*, 2016) and lowlanders rapidly ascending to 5260 m (Subudhi *et al.*, 2014). In other words, reactivity of the posterior circulation to altitude appears greater than the anterior circulation, with posterior oxygen delivery not changing from normoxic values. These findings are further corroborated by the present study (Figure 1) where posterior CBF distribution did not decrease during ascent in lowlanders or Sherpa. However, the Ascent and Altitude Sherpa demonstrated a lower percentage of gCBF distributed to the posterior circulation across all altitudes compared to lowlanders (Figure 2). At 5050 m lower posterior CBF distribution appeared to represent the majority of the lower gCBF in both Sherpa groups compared to lowlanders. This lower percentage

distribution to the posterior circulation in Sherpa adults is consistent with data in Sherpa children (Flück *et al.*, 2017).

Potential Mechanism(s) of Reduced CDO_2

Several factors that are implicated in the integrative regulation of CBF should theoretically be cause for a *higher* – rather than the observed *lower* - CBF in Sherpa compared to lowlanders in the present study. For example, higher $PaCO_2$ and MAP alongside a lower pH in Sherpa compared to lowlanders (Table 2) should theoretically lead to a higher CBF in Sherpa (Kety & Schmidt, 1948; Lucas *et al.*, 2010). Despite these differences, and that CaO_2, [Hb], and blood viscosity were similar throughout ascent, CBF is paradoxically lower in Sherpa compared to lowlanders. This suggests that simply assessing absolute changes in CBF alone may lead to a misrepresentation of differences in CBF regulation between lowlanders and Sherpa. When the contributions of MAP, pH, and viscosity were accounted for statistically as covariates, CBF became *significantly* lower in the Sherpa compared to the lowlanders at each altitude. Therefore, independent of physiological regulators of CBF, race is an important mechanism that underscores the lower CBF observed in Sherpa compared to lowlanders.

Race being an independent predictor of gCBF at altitude (~100 mL · min^{-1}; Figure 3; ~15% of resting lowlander CBF) further affirms that the difference in CBF between groups cannot be explained by our measured variables. When considering cerebrovascular responsiveness to the aforementioned inputs (e.g. CaO_2, $PaCO_2$, MAP, etc.,) there remains no apparent explanation of reduced CBF and CDO_2 in Sherpa during ascent. For example, cerebrovascular CO_2 reactivity is not different between lowlander and Sherpa at altitude during ascent at 4371 m (Jansen *et al.*, 1999) and following acclimatization to 5050 m (Figure 5). Further, we have demonstrated previously that the cerebrovascular response to driven changes in MAP (i.e. cerebral autoregulation) does not differ between Sherpa and partially acclimatized lowlanders (Smirl *et al.*, 2014), although this has not been consistently demonstrated during infusion of the vasoactive drug phenylephrine (Jansen *et al.*, 2014). However, infusion of a vasoactive drug likely invalidates transcranial Doppler ultrasound as an index of CBF as used in the aforementioned study by Jansen and colleagues (Hoiland & Ainslie, 2016). Endothelial function, as indexed in the brachial artery, is greater in Sherpa at 5050m following ascent in the same cohort of individuals tested in this present study (Tremblay *et al.*, 2018). When compared between acclimatized (~2 weeks) lowlanders and Sherpa, endothelial function appears similar (Lewis *et al.*, 2014). If differences in endothelial function were to manifest similarly in the cerebral circulation this would be cause for higher CBF. Therefore, potential differences in endothelial, function is another factor that cannot explain the reduced CDO_2 observed in Sherpa during ascent.

While cerebral metabolism does not differ between Sherpa and lowlanders at sea level (Hochachka *et al.*, 1996), it has not been compared between groups following acclimatization or prolonged stay at altitude and may represent the most tenable mechanism for reduced CDO_2. In keeping CDO_2 did not differ between groups at 1400 m where metabolism is apparently not different after a similar duration of de-acclimatization (Hochachka *et al.*, 1996); however, upon hypoxic exposure (3400 m and above) CDO_2 was lower in Sherpa, which, if coupled to cerebral metabolic demand (as it is at sea-level) (Ainslie *et al.*, 2014), is perhaps related to some form of hypoxia induced hypometabolism. This may represent a genetic/hypoxic interaction, whereby adaptive phenotypes only present following exposure to hypoxia (i.e., genotype-phenotype interaction). Such a hypometabolic adaptation is key for the purposes of oxygen conservation in various vertebrates such as the crucian carp and some fresh water turtles that possess an ability to reduce cerebral metabolism among a number of other adaptive physiological processes to tolerate hypoxic and anoxic environments (Nilsson & Lutz, 2004). A similar hypometabolic response is also observed in elite apnea divers who display a reduction in cerebral metabolism during long duration apneas (3-7 min) (Bain *et al.*, 2016). Further, greater mitochondrial efficiency has recently been demonstrated in skeletal muscle of Sherpa compared to lowlanders (Horscroft *et al.*, 2017), which if such a phenomenon is present in cerebral tissue may be related to the differential flow regulation observed in the present study. However, if the difference in CDO_2 is unrelated to metabolic differences between lowlander and Sherpa (i.e., no difference in cerebral metabolism), one may expect a role of elevated angiogenesis and capillary density (Xu & Lamanna, 2006) in Sherpa that allows for a greater extraction of oxygen. If the case, this would necessitate less bulk flow to maintain metabolic homeostasis as is seen in skeletal muscle of varying capillary density (Gayeski *et al.*, 1988), however, one would expect this to be reflected by a higher (not lower as we observed) cerebrovascular conductance in the Sherpa group. Although these mechanism(s) remain to be established, it would seem reasonable to suggest that the observed reductions in CDO_2 in the Sherpa are adaptive rather than maladaptive (see *Significance and Implications*).

Methodological Considerations

This study demonstrated that in lowlanders ascending to altitude, CBF does not increase until approximately 5050 m (Figure 1). This CBF pattern is distinctly different from that demonstrated in individuals performing a similar ascent profile with concurrent acetazolamide prescription for acute mountain sickness prophylaxis (Willie *et al.*, 2014*a*). However, given the maintenance of CDO_2 despite unaltered CBF, and the disconnect between CBF at altitude and acute mountain sickness severity (Ainslie & Subudhi, 2014) there appears no

460 likely maladaptive consequences of the present CBF response to altitude. This is also likely the case for
461 Sherpa, who despite reduced CDO_2 present with no acute mountain sickness symptoms.
462
463 In any high-altitude study, especially those comparing lowland native to high altitude native populations, it
464 is important to consider: i) the level of acclimatization of the lowland group; and ii) the comparability of
465 this acclimatization to that of the high altitude native population (i.e. Sherpa in the present study). In an
466 attempt to match the extent of acclimatization between lowlanders and the Ascent Sherpa, the Ascent
467 Sherpa descended to Kathmandu for 5-15 days prior to ascent with the lowlanders. As each group was
468 tested 1 day following arrival at each altitude, neither group was likely acclimatized to a large extent at the
469 time of each measurement. While a matching of acclimatization between groups cannot be explicitly
470 confirmed, assessment of the hematological factors regulating CBF at altitude can provide inferential
471 evidence for acceptable matching of acclimatization within the context of the present experimental design.
472 In other words, there was close matching of [Hb] and CaO_2 from 3400 m onwards. Other studies have
473 observed that following >2 years of de-acclimatization Tibetans possess a lower [Hb] compared to
474 lowlanders at low altitude (Liu *et al.*, 2016); however, in the present study Sherpa [Hb] was elevated above
475 lowlanders following 5-15 days of de-acclimatization in Kathmandu (Table 2). In lowlanders returning
476 from altitude, [Hb] has been shown to return to sea-level values within one to two weeks (Ryan *et al.*, 2014;
477 Siebenmann *et al.*, 2015) although this did not appear to be the case for the Sherpa population we tested.
478 There was no correlation between the variability in duration (days) of de-acclimatization with [Hb] in
479 Kathmandu ($r<0.01$; $P=0.87$). Whether a greater extent of chronic hypoxia in the Sherpa prolongs the
480 presence of elevated [Hb] (or Hb mass) is currently unknown. Nevertheless, upon reaching 3400 m, there
481 were no recorded hematological differences between groups. This indicates that differences in CBF and
482 CDO_2 throughout ascent cannot be attributed to [Hb], as it was similar between groups.
483
484 In an attempt to provide greater insight into the regulatory mechanisms underlying the differences in CBF
485 regulation between Sherpa and lowlanders observed herein, we utilized unpublished data from a prior 2008
486 expedition to Nepal. While these data indicated there is no difference in cerebrovascular CO_2 reactivity
487 between Sherpa and Lowlanders, it is important to consider the small sample size of the Sherpa group from
488 2008 ($n=5$). Nevertheless, a post hoc power calculation using the effect size derived from our CO_2 reactivity
489 data ($d=0.27$), while assuming a power of 0.8, indicates >200 subjects would be required in each group
490 (lowlander and Sherpa) to observe a statistical difference in CO_2 reactivity. While this indicates the lack of
491 difference our data demonstrates is relatively robust, it is still important to note that the small sample size
492 limits the certainty to which this comparison truly representative.
493

Typically, Sherpa are of smaller weight and stature than that of western counterparts (as in the present study), which may be cause to scale CBF to body size. Indeed, there appears to be a relationship between stature and brain weight, albeit quite modest and with a large standard error of the estimate (Heymsfield *et al.*, 2009). If we apply the allometric equation derived by Heymsfield *et al.*, 2009, we obtain an estimated brain mass of 1344±25 g for lowlanders, 1298±28 g for Ascent Sherpa, and 1309±23 g for Altitude Sherpa. Both the Ascent Sherpa (-3.5%) and Altitude Sherpa (-2.7%) estimates of brain mass are significantly lower than that of lowlanders (1-way ANOVA, $P<0.01$ for both). Under the assumption that these differences in brain mass are correct, it still remains unlikely that a 2-4% lower brain mass can explain the 24 and 27% lower CBF observed in our Ascent and Altitude Sherpa groups, respectively. Therefore, if there is indeed a lower cerebral metabolism in Sherpa at altitude, as speculated, this is likely primarily due to a signaled down-regulation of metabolic processes, versus that of a size principle (i.e., lower brain mass = lower cerebral metabolism).

Significance and Implications

Since their involvement in the attempted conquests of Mt. Everest in the early 1920's, high altitude native Sherpa have been recognized for their exceptional performance at altitude and tolerance to hypoxia. In contrast to lowland native mountaineers who display deleterious consequences of ascent above 8000 m such as cortical atrophy and/or hyper-intensities (Garrido *et al.*, 1993; Paola *et al.*, 2008), Sherpa are unaffected in this regard (Garrido *et al.*, 1996), indicating the presence of functional adaptations that confer protection to the brain. Therefore, it stands to reason that the lower CBF observed herein represents a positive adaptation. While lower CBF and CDO_2 may indicate a hypometabolic adaptation in Sherpa, as speculated above, the potential implications of lower CBF extend beyond that of metabolic homeostasis. For example, lower CBF may predispose Sherpa to a lower risk of vasogenic edema and/or intracranial hypertension (Lawley *et al.*, 2015; Sagoo *et al.*, 2016) than lowlanders (Schoonman *et al.*, 2008). This may be of particular importance at extreme altitudes (e.g. ≥8000 m) where lowlander CBF is reportedly 200% greater than at sea-level (Wilson *et al.*, 2011).

CONCLUSION

Sherpa have lived at high altitude for >25,000 years and possess specific physiological adaptations to the low atmospheric oxygen. In comparison to lowland natives, Sherpa perform exceptionally well at altitude from the perspective of physical performance, display a negligible incidence of altitude illness, and have

529 greater reproductive success. Many forms of altitude illness reside in the central nervous system and likely
530 involve the cerebral circulation. Detecting the advantageous hypoxia tolerant adaptation possessed by the
531 Sherpa may illuminate potential therapeutic targets in hypoxemic disorders, and further our basic
532 understanding of human responses and survival in low oxygen conditions.
533
534
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536
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542

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550

551 **AUTHOR CONTRIBUTIONS**

552 RLH, CAH, HHC, JCT, CKW, JD, DBM, CG, MS, SN & PNA were involved in data collection. RLH,
553 CAH, LMB & PNA were involved in data analyses and interpretation. RLH, CAH & PNA drafted the
554 manuscript. All authors critically reviewed the manuscript. RLH, CAH, CKW & PNA conceived the study
555 design. All authors approved the final version of this manuscript.

556

557 **CONFLICT OF INTEREST**

558 The authors declare no conflicts, financial or otherwise.

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710

711 **TABLES**

712

713 **Table 1. Age and time at altitude for the Sherpa participants.**

Ascent Sherpa						Altitude Sherpa				
Subject Number	**Age**	**Childhood Residence**	**Altitude (m)**	**Total Years at altitude**		**Subject Number**	**Age**	**Childhood Residence**	**Altitude (m)**	**Total Years at altitude**
101	22	Thame	3800	16.5		113	43	Pangbouche	3985	43
102	31	Thamo	3440	31		114	19	Khunde	3800	14
103	30	Namche	3400	12		115	23	Darjeeling	2040	6
104	25	Thamo	3440	25		116	26	Pheriche	4371	26
105	38	Salleri	2300	24		117	18	Thame	3800	18
106	39	Khumjung	3790	39		118	20	Thame	3800	16
107	44	Khumjung	3790	44		120	18	Thame	3800	18
108	26	Thamo	3440	21		121	20	Thame	3800	20
109	44	Pangbouche	3985	41		122	23	Thamo	3440	23
110	22	Thamo	3440	20		123	24	Thamo	3440	25
111	59	Khunde	3840	59		124	20	Thamo	3440	10
112	31	Pheriche	4371	16						

714

715

716

Table 2. Arterial and venous blood data

		Kathmandu (1400m)	Namache (3400m)	Pheriche (4371m)	Pyramid (5050m)
CaO_2		*Race: P=0.244; Altitude: P<0.001; Interaction: P=0.005*			
($ml \cdot dl^{-1}$)	Lowlander	17.8±1.1†	17.3±1.0	16.7±0.9*	15.6±1.4*
	Ascent Sherpa	19.3±1.1	17.3±1.4*	16.8±1.0*	15.7±1.4*
SaO_2		*Race: P=0.152; Altitude: P<0.001; Interaction: P=0.809*			
(%)	Lowlander	95.4±1.2	87.4±2.7*	84.5±3.2*	79.0±4.9*
	Ascent Sherpa	94.5±1.9	86.7±3.2*	82.5±4.5*	77.4±4.5*
PaO_2		*Race: P=0.523; Altitude: P<0.001; Interaction: P=0.693*			
(mmHg)	Lowlander	77.0±6.5	51.8±4.1*	47.6±3.7*	41.2±4.4*
	Ascent Sherpa	74.8±7.6	52.2±4.6*	46.7±4.5*	40.6±4.3*
$PaCO_2$		*Race: P=0.050; Altitude: P<0.001; Interaction: P=0.011*			
(mmHg)	Lowlander	40.3±2.6	34.5±1.4*	32.2±1.6*†	29.9±1.9*†
	Ascent Sherpa	39.8±1.6	35.4±2.3*	34.3±2.9*	32.1±2.7*
pH		*Race: P<0.001; Altitude: P<0.001; Interaction: P=0.918*			
	Lowlander	7.42±0.02	7.44±0.02*	7.43±0.02	7.46±0.02*
	Ascent Sherpa	7.40±0.01	7.41±0.02*	7.40±0.02	7.44±0.02*
HCO_3^-		*Race: P=0.111; Altitude: P<0.001; Interaction: P=0.002*			
($mEq \cdot L^{-1}$)	Lowlander	26.33±1.42*†	23.57±1.21*†	21.55±1.34*	21.37±1.57*
	Ascent Sherpa	24.55±1.24*	22.32±1.63*	21.47±1.83*	21.66±2.01*
BEecf		*Race: P=0.034; Altitude: P<0.001; Interaction: P=0.029*			
($mEq \cdot L^{-1}$)	**Lowlander**	1.95±1.56*†	-0.62±1.40*†	-2.81±1.60*	-2.39±1.93*
	Ascent Sherpa	-0.11±1.45*	-2.18±1.83*	-3.27±2.15*	-2.67±2.17*
[Hb]		*Race: P=0.017; Altitude: P=0.012; Interaction: P=0.001*			
($g \cdot dl^{-1}$)	Lowlander	13.56±0.80†	14.46±0.70*	14.44±0.52*	14.46±0.72*
	Ascent Sherpa	14.83±0.92	14.50±0.75	14.85±0.89	14.81±0.65
HCT		*Race: P=0.020; Altitude: P=0.010; Interaction: P=0.001*			
(%)	Lowlander	39.90±2.39†	42.52±2.04*	42.48±1.54*	42.78±2.24*
	Ascent Sherpa	43.64±2.69	42.64±2.20	43.64±2.62	43.66±2.17
Venous viscosity		*Race: P=0.240; Altitude: P<0.001; Interaction: P=0.004*			
(cP)	Lowlander	3.78±0.48†	4.41±0.30*	4.53±0.43*	4.78±0.36*
	Ascent Sherpa	4.32±0.40	4.46±0.67	4.63±0.58	4.62±0.29

Bolded **Lowlander** or **Sherpa** indicates a main effect of race, with the bolded group possessing a higher value

* Significant difference from Kathmandu, P<0.05

† Significant difference between Sherpa and Lowlander within an altitude, P<0.05

CaO_2, arterial oxygen content; SaO_2, arterial oxyhaemoglobin saturation; PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide; HCO_3^-, bicarbonate ion; BEecf, base excess extracellular fluid; [Hb], haemoglobin concentration; HCT, haematocrit.

Table 3. Influence of ascent to altitude on cerebrovascular parameters.

		Kathmandu (1400m)	Namache (3400m)	Pheriche (4371m)	Pyramid (5050m)
ICA velocity		*Race: P=0.105; Altitude: P<0.001; Interaction: P=0.021*			
($cm \cdot s^{-1}$)	**Lowlander**	39.3±8.2	40.9±9.7	40.8±10.3	44.4±10.2*
	Ascent Sherpa	37.2±5.8	32.1±5.6†	37.8±4.5	42.2±7.1
ICA diameter		*Race: P=0.315; Altitude: P<0.001; Interaction: P=0.270*			
(mm)	Lowlander	5.05±0.51	4.89±0.45*	4.85±0.47*	5.08±0.53
	Ascent Sherpa	4.97±0.60	4.63±0.66*	4.74±0.40*	4.81±0.54
ICA flow		*Race: P=0.014; Altitude: P<0.001; Interaction: P=0.061*			
($mL \cdot min^{-1}$)	**Lowlander**	236.2±49.3	229.0±50.5*	226.7±63.9	267.0±59.8*
	Ascent Sherpa	215.2±32.4	165.8±50.3*	199.4±22.9	229.3±41.7*
ICA DO_2		*Race: P=0.073; Altitude: P<0.001; Interaction: P=0.008*			
($mL \cdot min^{-1}$)	Lowlander	42.1±0.2	39.4±8.0	36.0±12.9*	41.2±8.1
	Ascent Sherpa	41.8±6.6	28.6±9.5*†	34.4±2.3	35.5±6.6
VA velocity		*Race: P=0.035; Altitude: P=0.001; Interaction: P=0.996*			
($cm \cdot s^{-1}$)	**Lowlander**	24.3±5.4	26.1±6.1	25.4±7.7	29.6±7.3*
	Ascent Sherpa	19.5±5.8	21.7±10.0	21.2±5.1	25.2±7.0*
VA diameter		*Race: P<0.001; Altitude: P=0.001; Interaction: P=0.033*			
(mm)	**Lowlander**	4.03±0.42	3.9±0.40	4.04±0.52	4.19±0.43
	Ascent Sherpa	3.56±0.52†	3.16±0.57*†	3.36±0.57†	3.39±0.57†
VA flow		*Race: P=0.001; Altitude: P<0.001; Interaction: P=0.357*			
($mL \cdot min^{-1}$)	**Lowlander**	94.3±30.0	96.8±30.1	98.3±34.7	122.3±35.0*
	Ascent Sherpa	60.3±25.4	54.8±34.4	64.3±32.9	72.4±36.9
VA DO_2		*Race: P=0.003; Altitude: P=0.091; Interaction: P=0.443*			
($mL \cdot min^{-1}$)	**Lowlander**	16.7±5.3	16.7±5.0	16.4±5.8	19.0±5.7
	Ascent Sherpa	12.2±4.7	9.9±6.3	11.5±5.6	12.6±6.2
MAP		*Race: P=0.017; Altitude: P<0.001; Interaction: P=0.002*			
(mmHg)	Lowlander	84.5±6.3	93.4±9.1*	93.9±8.6*	99.9±10.2*
	Ascent Sherpa	95.2±9.2†	101.5±10.7†	100.7±9.6†	96.5±5.3

Bolded **Lowlander** or **Sherpa** indicates a main effect of race, with the bolded group possessing a higher value

* Significant difference from Kathmandu, P<0.05

† Significant difference between Sherpa and Lowlander within an altitude, P<0.05

DO_2, delivery of oxygen; ICA, internal carotid artery; MAP, mean arterial pressure; VA, vertebral artery

Table 4. Comparison between lowlanders, the ascent Sherpa, and the altitude Sherpa following arrival at 5050m.

	Lowlander	Ascent Sherpa	Altitude Sherpa	ANOVA
CaO_2 (ml · dl^{-1})	15.66±1.35	15.68±1.35	16.95±2.36	0.102
SaO_2 (%)	78.95±4.94	77.43±6.32	86.73±3.42*†	**<0.001**
PaO_2 (mmHg)	41.20±4.44	40.58±4.25	41.65±6.23	0.876
$PaCO_2$ (mmHg)	29.93±1.58	32.06±2.71*	31.88±2.44	**0.020**
pH	7.46±0.02	7.44±0.02*	7.41±0.02*†	**<0.001**
HCO_3^- (mEq · L^{-1})	21.37±1.57	21.66±2.01	20.26±1.56	0.123
BEecf (mEq · L^{-1})	-2.39±1.93	-2.67±2.17	-4.35±1.69*	**0.030**
[Hb] (g · dl^{-1})	14.46±0.72	14.81±0.65	15.94±1.03*†	**<0.001**
HCT (%)	42.78±2.24	43.66±2.17	47.05±3.58*†	**<0.001**
MAP (mmHg)	99.86±10.22	96.55±5.30	88.87±7.69*	**0.006**
CVC (ml · min^{-1}· $mmHg^{-1}$)	7.99±1.74	6.41±1.64	6.54±1.99	**0.047**

*denotes a significant difference from lowlanders; †, denotes a significant difference from the ascent Sherpa group. CaO_2, arterial oxygen content; SaO_2, arterial oxyhaemoglobin saturation; PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide; HCO_3^-, bicarbonate ion; BEecf, base excess extracellular fluid; [Hb], haemoglobin concentration; HCT, haematocrit; MAP, mean arterial pressure; CVC, cerebral vascular conductance; $\dot{V}E$, minute ventilation.

761 **FIGURES**
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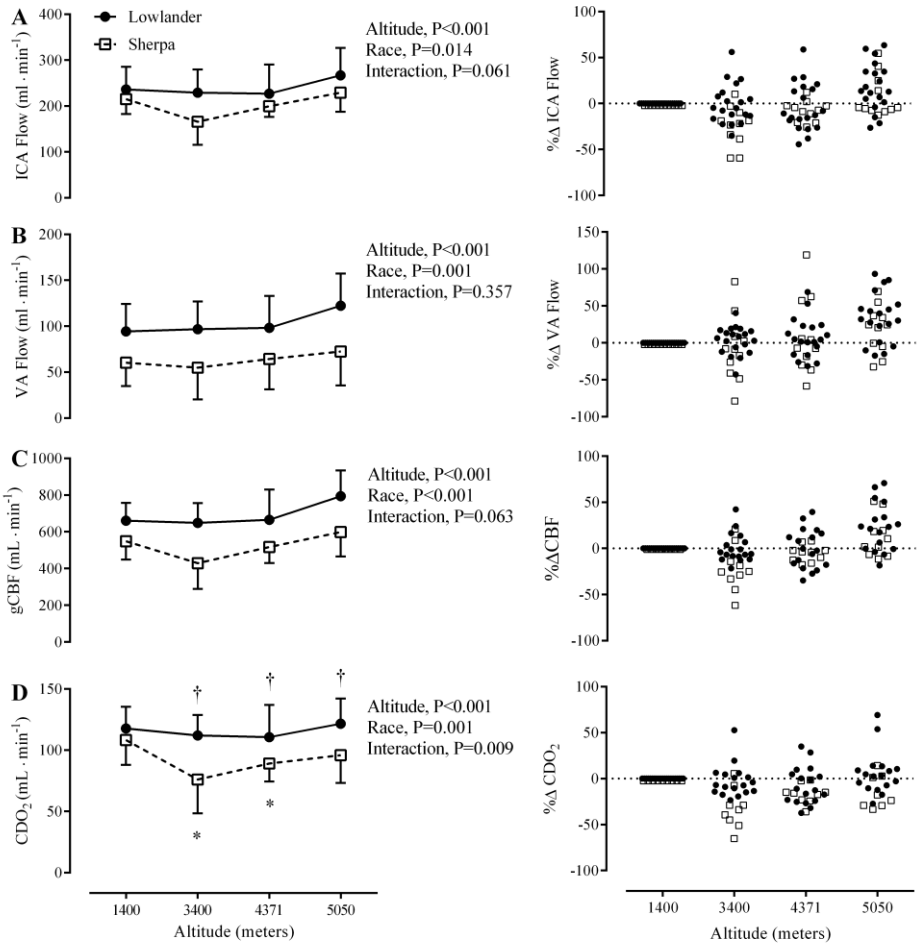

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764 **Figure 1. Regional cerebral blood flow and cerebral oxygen delivery upon ascent to 5050 m in**
765 **lowlanders and Sherpa.** For all panels lowlanders are denoted by the filled circle symbol (•), and Sherpa
766 by the open square symbol (▫). **A**. Internal carotid artery (ICA) flow in lowlander and Sherpa. ICA flow
767 was reduced across groups at 3400 m (marginal means, P=0.01) and increased across groups at 5050 m
768 (marginal means, P=0.02). **B**. Vertebral artery (VA) flow in lowlander and Sherpa. VA flow was increased
769 across groups at 5050 m (marginal means, P<0.01). **C.** Global cerebral blood flow (gCBF) in lowlander
770 and Sherpa. gCBF was reduced across groups at 3400 m (marginal means, P=0.038) and increased across
771 groups at 5050 m (marginal means, P<0.01). **D.** Cerebral oxygen delivery (CDO_2) in lowlander and Sherpa.
772 CDO_2 was lower in Sherpa compared to lowlanders at 3400 m (P<0.01), 4371 m (P=0.03), and 5050 m
773 (P<0.01). While lowlander CDO_2 was maintained across ascent, Sherpa CDO_2 decreased from Kathmandu
774 values at 3400 m (P<0.01) and 4731 m (P=0.04). In all panels, the % change from Kathmandu values for
775 lowlanders and Sherpa throughout ascent are presented in the right figures.* Denotes a significant change
776 from Kathmandu values (P<0.05)
777 † Denotes a significant difference between lowlanders and Sherpa at a given altitude (P<0.05)
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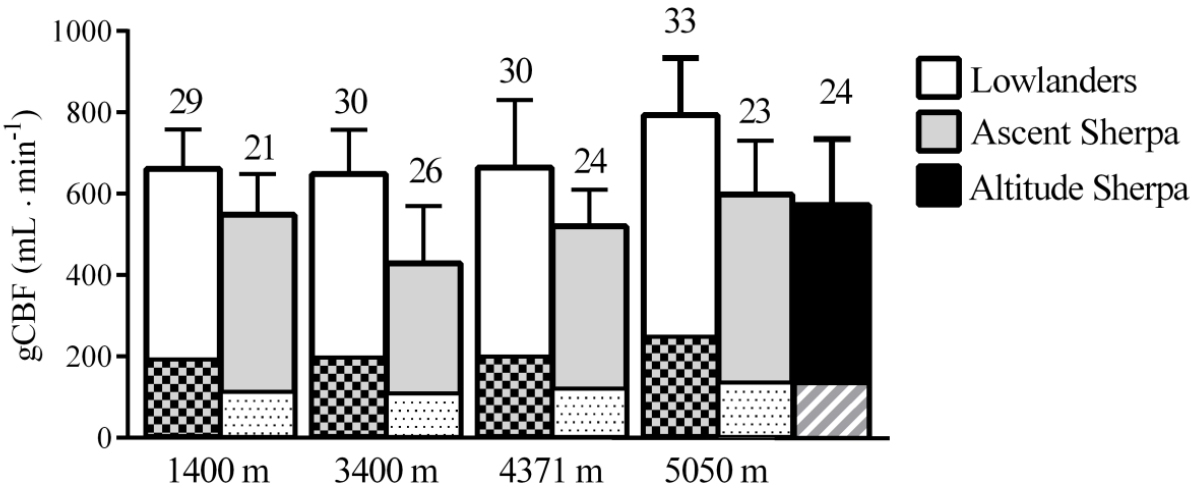
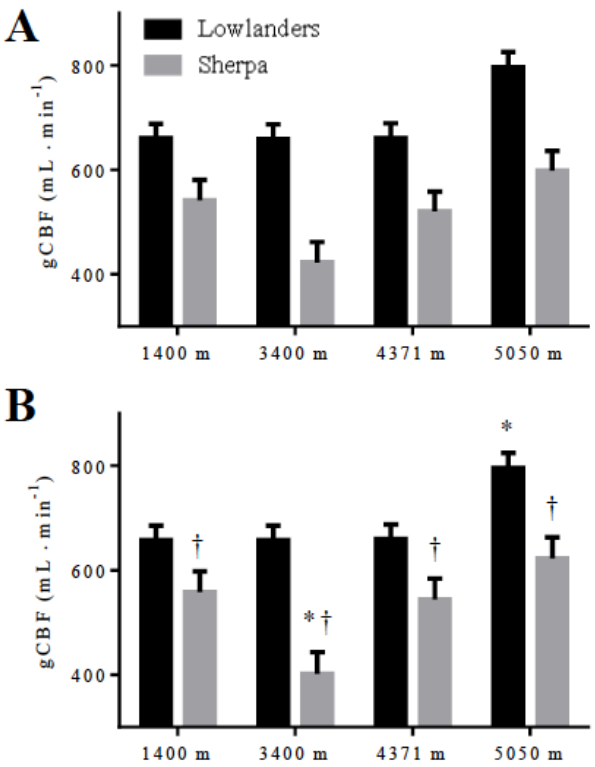


Figure 2. Cerebral blood flow distribution in lowlanders and Sherpa upon ascent and following acclimatization. The presented data are global cerebral blood flow (gCBF) with open bars for the lowlanders, grey bars for the ascent sherpa and a black bar for the altitude sherpa. Overlayed on the gCBF data, a patterned bar, is an estimate of bilaterial VA flow (2 · unilateral VA flow), with the estimated %contribution of VA flow to gCBF denoted above each time point. For the ascent protocol a 2-way ANOVA was used for statistical comparisons (Factors: Race and Altitude). Their was a main effect of Race ($P=0.018$) with the %CBF distribution to the posterior circulation less in Sherpa across altitudes. Following 2-weeks at 5050 m, the three groups (right side of figure) were compared using a 1-way ANOVA. Both the ascent Sherpa ($P<0.01$) and altitude Sherpa ($P<0.01$) had lower VA distribution than lowlanders following arrival at 5050 m.



No covariates

Race, P<0.001; Altitude, P<0.001; Interaction, P=0.063

gCBF = 660.89 - 118.93 (Sherpa) – 2.28 (Namche) - 0.38 (Pheriche) + 135.89 (Pyramid) – 116.78 (Namche*Sherpa) - 20.93 (Pheriche*Sherpa) - 79.04 (Pyramid*Sherpa)

Parameter	Estimate	Std. Error	P-value
Sherpa	-118.93	47.34	0.014
Namche	-2.28	27.96	0.94
Pheriche	-0.38	27.96	0.99
Pyramid	135.89	28.53	<0.001
Namche*Sherpa	-116.78	47.50	0.016
Pheriche*Sherpa	-20.93	47.03	0.66
Pyramid*Sherpa	-79.04	47.37	0.01

Covariates: MAP, pH, Viscosity

Race, P<0.001; Altitude, P<0.001; Interaction, P=0.01

gCBF = 657.87 - 99.67 (Sherpa) – 0.20 (Namche) + 1.73 (Pheriche) + 138.01 (Pyramid) – 156.46 (Namche*Sherpa) – 15.56 (Pheriche*Sherpa) – 73.41 (Pyramid*Sherpa)

Parameter	Estimate	Std. Error	P-value
Sherpa	-99.67	48.93	0.046
Namche	-0.20	28.13	0.99
Pheriche	1.73	28.13	0.95
Pyramid	138.01	28.69	<0.001
Namche*Sherpa	-156.46	50.03	0.003
Pheriche*Sherpa	-15.56	49.53	0.75
Pyramid*Sherpa	-73.41	49.10	0.14

Figure 3. Covariate analysis of cerebral blood flow regulation in sherpa and lowlanders. This figure depicts the estimated marginal means (±standard error) and model output for the linear mixed effects model analysis of gCBF without (Panel A) and with (Panel B) adjustment for important factors in the control of gCBF at altitude. Following inclusion of covariates into the statistical model, there is an interaction between altitude and race, with post-hoc analysis (Bonferroni corrected) indicating the gCBF was lower in Sherpa compared to lowlanders at all time points. Each table includes the overall model equation and the individual coefficients for each of the fixed effects (altitude and race). Subjects were included as a random effect. gCBF, global cerebral blood flow; MAP, mean arterial pressure.

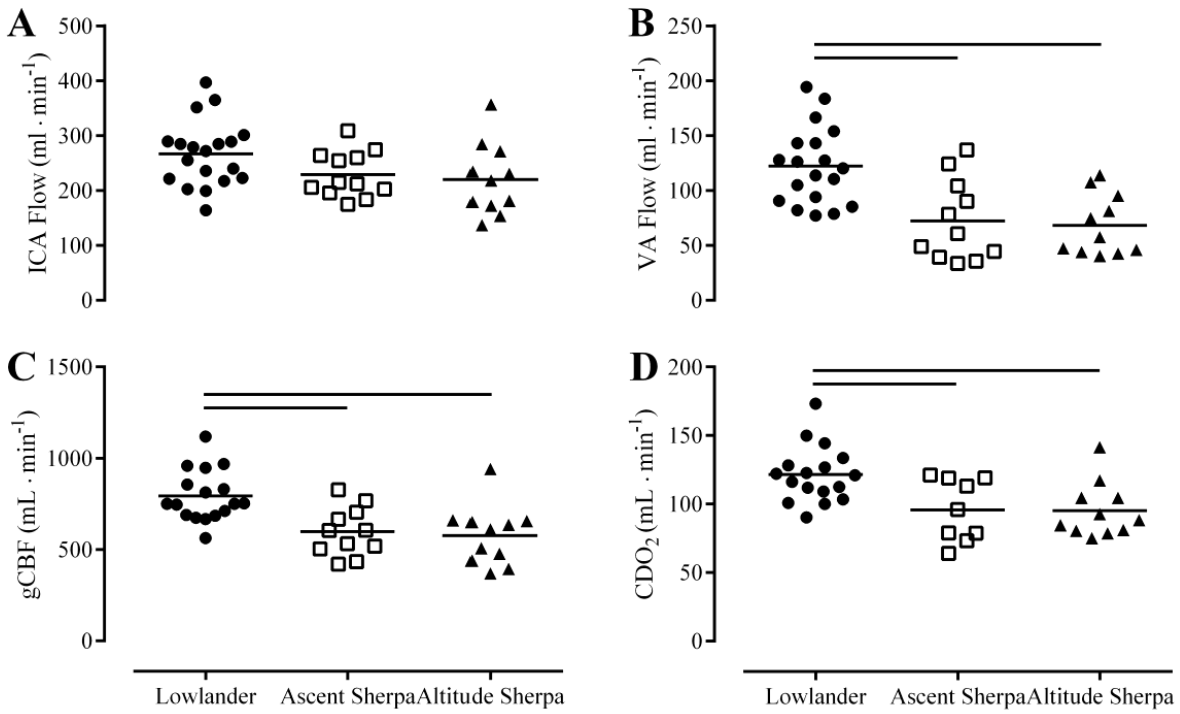


Figure 4. Cerebral blood flow and oxygen delivery in acclimatized lowlanders, Sherpa following ascent, and Sherpa recruited at altitude. In all panels lowlanders are denoted by the filled circle symbol, Sherpa that took part in the ascent with the open square symbol, and Sherpa who were recruited at altitude by the filled triangle. ICA, internal carotid artery; VA, vertebral artery; gCBF, global cerebral blood flow; CDO_2, cerebral oxygen delivery. Horizontal bars indicate a significant difference from lowlanders.

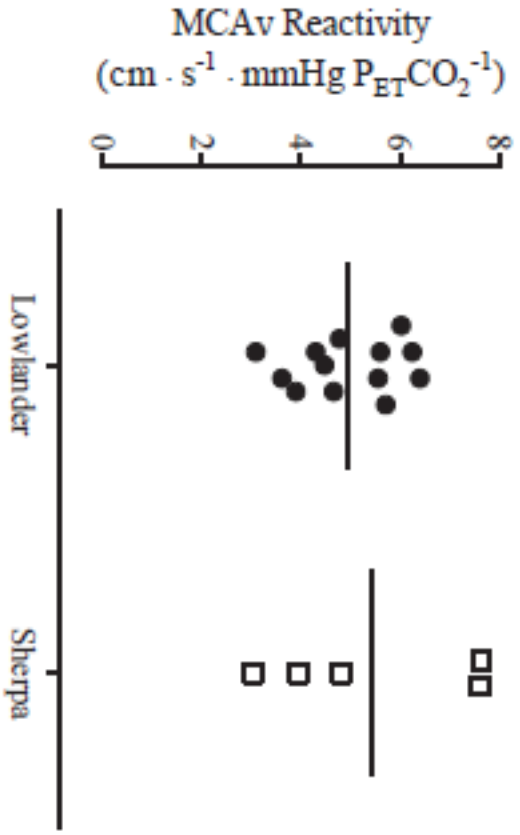


Figure 5. Lowlander and Sherpa cerebrovascular CO_2 reactivity. The presented cerebrovascular reactivity data are the relative reactivity (i.e. %change) values for lowlanders and Sherpa collected at 5050 m in 2008. The hyperoxic rebreathe technique was used for these tests [Previously described: (Fan *et al.*, 2010)]. There is no difference in CO_2 reactivity between sherpa and acclimatized lowlanders as determined by the non-parametric Mann Whitney U Test (P=0.77).