TITLE

Sustained vasomotor control of skin microcirculation in Sherpas versus altitude-naïve Lowlanders – experimental evidence from Xtreme Everest 2

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NEW FINDINGS

What is the central question of this study?

Do Sherpa highlanders, when exposed to graded hypobaric hypoxia, exhibit enhanced vasomotor and neurovascular control to maintain microcirculatory flux, and thus tissue oxygenation, when compared to altitude-naïve Lowlanders?

What is the main finding and its importance?

Sherpas, when exposed to hypobaric hypoxia at high altitude, demonstrated superior preservation of their peripheral microcirculatory perfusion, a greater oxygen-unloading rate and sustained microvascular reactivity with enhanced vasomotion, when compared to altitude-naïve Lowlanders. These differences have not previously been reported and may improve our understanding of the multifactorial responses to sustained environmental hypoxia.

ABSTRACT (250)

Enhanced oxygen delivery consequent to an increased microvascular perfusion, has been postulated to play a key role in the physiological adaptation of Tibetan highlanders to the hypobaric hypoxia encountered at high altitude. We tested the hypothesis that Sherpas, when exposed to graded hypobaric hypoxia, demonstrate enhanced vasomotor and neurovascular control to maintain microcirculatory flux, and thus tissue oxygenation when compared to altitude-naïve Lowlanders. Eighty three Lowlanders (39M/44F, 38.8(13.1)y mean±SD) and 61 Sherpas (28M/33F, 27.9(6.9)y) were studied on ascent to Everest Base Camp over 11 days. Skin blood flux and tissue oxygen saturation were measured simultaneously using combined laser Doppler fluximetry and white light spectroscopy at baseline, 3500m and 5300m. In both cohorts, ascent resulted in a decline in the sympathetically mediated microvascular constrictor response ($p<0.001$), which was more marked in Lowlanders than Sherpas ($p<0.001$). The microvascular dilator response evaluated by post occlusive reactive hyperaemia was significantly greater in Sherpas than Lowlanders at all sites ($p<0.002$). Spectral analysis of the blood flux signals revealed enhanced myogenic (vasomotion) activity in Sherpas, which was unaffected by ascent to 5300m. Whilst skin tissue oxygenation (StO_2) was lower in Sherpas than Lowlanders, oxygen-unloading rate was faster, and deoxyHb levels higher, at all altitudes. Together, these data suggest that Sherpas, when exposed to hypobaric hypoxia, demonstrated superior preservation of peripheral microcirculatory perfusion compared to altitude-naïve Lowlanders. The physiological differences in local microvasculature vasomotor and neurovascular control may play a key role in Sherpa adaptation to high altitude hypobaric hypoxia by sustaining local perfusion and tissue oxygenation.

INTRODUCTION

Sherpa highlanders are the direct descendants of nomadic Tibetans who have successfully resided at altitudes of over 4000m for the last 500 generations (Aldenderfer, 2003). Their extraordinary tolerance to hypobaric hypoxia is likely to have resulted from the process of natural selection leading to genotypic and downstream phenotypic adaptations enabling the population to cope with the rigors of life at high altitude. Interestingly, previous data demonstrates Sherpas do not exhibit increased arterial oxygen content (CaO_2) when compared to Lowlanders on exposure to similar altitudes (Beall et al. 1998; Samaja et al. 1979; Wu et al. 2004). Consequently, attention has since moved away from the traditional focus of global haemodynamics towards other potential phenotypes to explain their improved performance at altitude. One such area increasingly considered to be vital for the development of hypoxia tolerance, is at the distal end of the oxygen cascade - the microcirculation (Martin et al. 2010, Martin et al. 2013). Anatomically, the microcirculation consists of a network of blood vessels whose primary role is regulation of convective blood flow to match micro-regional oxygen demand (Levick, 2009). In a recent study using incident dark field imaging, Sherpa highlanders demonstrated significantly greater sublingual microcirculatory blood flow and capillary density when compared to Lowlanders at high altitude (Gilbert-Kawai et al. 2017). The authors postulated that this increase could provide both a greater flow per unit tissue volume, and flow per unit time, both of which would enhance oxygen delivery. No studies to date however, have explored this directly in a single microvascular bed, and therefore the relationship between microvascular perfusion and tissue oxygenation remains

unknown, as do the regulatory control mechanisms behind these phenotypical differences.

Maintenance of an adequate tissue perfusion and oxygenation is dependent on the neural, humoral and local vaso-mechanisms that determine vascular tone and flow patterns within the microvascular bed (Intaglietta et al. 1990). These mechanisms have been explored using time series analyses of the rhythmic oscillatory fluctuations attributed to local vasomotion and flowmotion control (Stefanovska et al. 1999). Recent studies of skin blood flux and oxygenation signals recorded simultaneously using combined laser Doppler fluximetry and white light reflectance spectroscopy have shown these signals to oscillate over broad, generally similar frequency ranges (Kuliga et al. 2014, Bernjak et al. 2012). They further suggest that local flowmotion may influence oxygen delivery and extraction (Kuliga et al. 2017; Thorn et al. 2011; Thorn et al. 2016). Real-time collection of robust measures of microvascular blood flux and oxygenation parameters may thus provide novel information that enhances our understanding of the ways in which the peripheral vasculature of Sherpa highlanders adapts to sustained exposure to hypobaric hypoxia and of how the microcirculation and microvascular perfusion are influenced by hypoxia.

It is unclear whether exposure to hypobaric hypoxia alters microvascular reactivity and whether the adaptive mechanisms in Sherpas differ from those in altitude-naïve Lowlanders on ascent to altitude. We tested the hypothesis that Sherpas, when exposed to hypobaric hypoxia at high altitude, demonstrate enhanced vasomotor and neurovascular control to maintain microcirculatory flux and tissue oxygenation, when compared to altitude-naïve Lowlanders. In order to explore this we studied two

cutaneous microvascular beds: that of the forearm, which is a well characterised bed under both endothelium dependent and neurovascular control, and that of the finger pulp, where superficial microvascular perfusion is largely determined by abundantly present arteriovenous anastomoses under sympathetically mediated constrictor tone (Braverman, 2005).

MATERIALS AND METHODS

Ethical Approval

The study was undertaken as part of the Xtreme Everest 2 research expedition (XE2) (Gilbert-Kawai et al. 2015). Approval of the study design, risk management plan and protocol were obtained from both the University College London Research Ethics Committee (Ref: 3750/002) and the Nepal Health Research Council (NHRC) (1334). The study was performed to the standards set by the Declaration of Helsinki, except for registration in a database.

Study Participants

Eligible participants were adults (aged 18 years or above) of either Lowlander or Sherpa origin who were declared fit to travel to altitude following medical screening (Gilbert-Kawai et al. 2015). A Lowlander was defined as an individual whose known descendants were not one of the following high altitude populations; Tibetan, Andean or Ethiopian. A Sherpa was defined as a person who is of direct Sherpa ancestry (for at least two generations) - both parents and grandparents originating in the Solukhumbu region of Nepal. Local translators were available at all times and all participants provided written informed consent for participation in the studies.

Setting and ascent profile

Baseline measurements were performed in London for Lowlanders (35m) and in Kathmandu (1300m) for Sherpas. Repeat measurements were then collected at Namche Bazaar (NB) (3500m) and Everest Base Camp (EBC) (5300m). At the beginning of the trek, all Lowlanders flew to Lukla (2800m) after spending one night in Kathmandu, and all Sherpas flew from Katmandhu to Lukla. All participants followed an identical ascent profile over the eleven-day trek to EBC. Subjects were tested in the morning on day two after arrival at each high altitude laboratory.

Physiological measurements

The measurements taken were: peripheral oxygen saturations (SpO_2) (Nonin Onyx 9500, Nonin Medical Inc, Minnesota, USA), heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) (Omron M3H, Moron Healthcare, Japan) and respiratory rate (RR).They were recorded after ten minutes seated at rest. From whole blood samples, we measured haemoglobin concentration (Hb) (Hemocue AB, Hemocue, Sweden) and haematocrit (Hct) values (Sigma 1-14 microcentrifuge, Sigma, Germany).

Tissue blood flux and oxygenation

For the duration of the LDF study, the participants lay still in the supine position with their non-dominant arm resting at the level of their right atrium. Participants were required to rest for ten minutes before any measurements were taken. Probes were placed on the forearm approximately 10cm proximal to wrist (combined LDF™/OXY™/temperature probe (CP1T-1000), Moor, Axminster, UK), and pulp of the index finger (combined LDF™/temperature probe (VP1T), Moor, Axminster, UK).

Probe position was recorded with a photograph and permanent marker pen to ensure use of the same anatomical site in subsequent measurements.

Skin microvascular blood flux, tissue oxygen saturation (StO_2), and temperature were measured simultaneously using combined laser Doppler fluximetry (LDF) and white light reflectance spectroscopy. LDF is used widely to evaluate microvascular function under physiological and pathophysiological conditions (Holowatz et al. 2008; IJzerman et al. 2003; Yamamoto et al. 2009; Cracowski et al. 2016). When combined with dynamic reactivity tests, such as post-occlusive reactive hyperaemia (PORH) or deep inspiratory breath-hold (IBH), LDF provides a measure of microvascular perfusion capacity and mechanisms underlying vaso-control (Cracowski et al. 2016; Cracowski et al. 2006). StO_2 represents a dynamic balance between oxygen delivery (DO_2) by the microvascular bed and oxygen consumption (VO_2) in the tissue (Liu et al. 2011; Kuliga et al. 2017). Resting blood flux, StO_2 and skin temperature signals were recorded continuously for 10 min prior to dynamic perturbation of blood flux by; i) three 6 second duration deep IBH separated by 60 seconds to elicit a rapid and transient sympathetically-mediated vasoconstriction that can be detected in the cutaneous microvasculature of the finger tip pulp (Allen et al. 2002; Rauh et al. 2003; Feger et al. 2005), and ii) inflation of an automated blood pressure cuff (VMS-PRES, Moor, Axminster, UK) placed around the upper arm to a supra-systolic pressure of 250mmHg for 3 minutes to elicit a PORH response measured at the forearm. We have previously shown that the inter-individual coefficients of variation (CV) for resting blood flux and StO_2 measured using the combined probe in healthy individuals at ambient room temperature are 0.15 and 0.09, respectively (Kuliga et al. 2014).

Data Analysis

Signals were recorded using a 40 Hz sampling rate. Blood flux was recorded in arbitrary perfusion units (PU), and StO_2 in percentage (%) - derived from measures of oxyHb, deoxyHb and totalHb (expressed in arbitrary units; AU); ([totalHb] = [oxyHb] + [deoxyHb]); StO_2 = ([oxyHb]/[totalHb]) x 100%. Analysis was performed using the manufacturer's own validated software (moorVMS-PC software, Moor, Axminster, UK).

Blood flux values were determined in the forearm and finger; i) Resting flux (RF) - rest in the final five minutes prior to the dynamic tests; ii) Maximum flux (MF) - the peak value after the release of the three minute arterial occlusion cuff; and iii) IBH - over the last three seconds of each breath hold. Skin temperature was monitored continuously at both skin sites throughout the study.

The relative change during the reactive hyperaemic response to arterial occlusion at the forearm (RH) was calculated as (RH = (MF - RF) / RF) x 100)%. The fall in blood flux in response to the IBH measured at the finger, was calculated as the difference between the minimal blood flux and blood flux measured immediately prior to each IBH, at the finger. The vasoconstrictor response for each participant was presented as the mean IBH response expressed as a percentage of RF. Cutaneous vascular conductance (CVC) at the forearm, was calculated at rest as the ratio of RF to MAP and at peak RH as the ratio of MF to MAP.

Mean resting values for the oxygenation signals recorded at the forearm (oxyHb, deoxyHb, totalHb and StO_2), were calculated over the five minutes prior to arterial occlusion. Oxygen removal (consumption) by the tissue was estimated as the rate of oxygen desaturation from the decrease in the oxyHb signal during the first 60 seconds of arterial occlusion (Boas et al. 2011; Thorn et al. 2016).

Spectral density was estimated by Welch's method of Fourier transform with Hanning window size of 200s and 50% overlap between windows using the manufacturer's software. The power contribution was evaluated within frequency range (0.0095-1.6 Hz), divided into frequency intervals corresponding to the frequency bands described previously as attributable to endothelial (I, 0.0095 – 0.02 Hz), neurogenic (II, 0.02 – 0.06 Hz) and myogenic (III, 0.06 – 0.15Hz) activity (Bollinger et al. 1993; Kvernmo et al. 1999; Söderström et al. 2003; Stewart et al. 2007). Total spectral power was estimated as the sum of absolute power across the frequency range (0.0095-1.6 Hz) and expressed in U^2/Hz. Power spectral density contribution (PSD contribution) was calculated relative to total spectral power, and is expressed as a percentage.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows Version 23.0 (IBM, USA). Data were first tested for Gaussian distribution using the Kolmogorov-Smirnov test and visual inspection of histograms. As the data did not show a normal distribution, all data are presented as median (interquartile range). Two-way ANOVA was conducted to examine the effect of cohort (Sherpa/Lowlander) and site (baseline, NB, EBC), on key output variables including RF, StO_2, and microvascular dilator and constrictor capacity. Sherpa and Lowlander cohorts were compared at each site using the unpaired Mann Whitney U test, whilst the Wilcoxon Signed Rank Test was used to compare single cohorts between two different sites. The relationships between flux, oxygenation, flowmotion activity, site and skin temperature at each site were assessed individually using Spearman's rank correlation coefficient. A p-value of <0.05 was considered statistically significant for all analyses. Multivariable linear regression

models were developed to describe factors that were independently associated, with tissue RF and StO_2 as the dependent (outcome) variables. Factors that were entered into the model as explanatory variables, were chosen from the results of univariate analysis, and included skin temperature and flowmotion activity. To test whether there was an independent effect of group or site, these were included in the models as binary indicator variables.

RESULTS

A total of 144 participants underwent baseline testing - 83 Lowlanders and 61 Sherpas. Participant characteristics of the study groups are summarised in Table 1.
Lowlanders were significantly older, taller and heavier than the Sherpas ($p<0.001$). The ratio of males to females did not significantly differ between the two groups. All 144 study participants completed testing at NB, and 133 participants (77 Lowlanders 56 Sherpas) completed testing at EBC. The environmental conditions of the three measurement sites are presented in Table 2.

Physiological measurements

Values for physiological measurements, Hb and Hct are presented in Table 3. On ascent, similar values were seen in both cohorts except for RR which was higher in Sherpas at all sites. At EBC there were increases in SBP, DBP, MAP, HR, Hb, Hct and RR, and a decrease in SpO_2, when compared to baseline in both cohorts.

Microvascular blood flux and oxygenation measurements

Blood flux, reactivity and oxygenation data from the finger and forearm are summarised in Tables 4 and 5.

Resting blood flux and skin temperature. Sherpas had significantly higher RF measured at the finger and forearm at all altitudes compared to Lowlanders ($p<0.001$), with the exception of forearm RF at baseline. In both cohorts, exposure to high altitude at EBC resulted in a decrease in RF at the finger compared to baseline and NB ($p<0.001$). In the forearm, responses were more variable, and there was a statistically significant interaction effect between group and site on forearm RF ($F=10.9$, $p<0.0001$). Additionally, whist forearm RF in Sherpas decreased with increasing altitude ($p<0.0001$), Lowlanders demonstrated a transient increase at NB ($p=0.008$). Skin temperature, a major determinant of skin blood flow, measured at the finger was significantly greater in Sherpas than Lowlander at all sites ($p<0.001$). A similar trend was seen on ascent in both cohorts, with finger skin temperature lower at NB and EBC than at baseline ($p<0.001$). There was a positive association between forearm RF and skin temperature measured in both cohorts at baseline (Lowlanders, $r=0.37$ $p=0.0006$; Sherpas, $r=0.64$ $p<0.0001$) which was sustained at NB and EBC (all $r>0.35$, all $p<0.01$). A similar association was seen at the finger (data not shown).There was no correlation between finger or forearm RF and SpO_2, HR, MAP, Hb or Hct.

As mean MAP was shown to differ between groups and with site, and has an influence on resting microvascular perfusion, we estimated resting CVC. Resting CVC was significantly higher in the finger than forearm in both cohorts at all sites ($p<0.001$) (Tables 4 and 5). In both groups resting CVC showed similar trends to RF on ascent to altitude, with a strong independent effect of both group ($F=101$, $p<0.0001$) and

altitude site (F=20.3, p<0.001) on resting CVC at the finger (group*site F=1.6, p=0.199).

Resting tissue oxygenation. Tissue oxygenation parameters (oxyHb, deoxyHb and totalHb and StO_2) were measured simultaneously with blood flux at the forearm. There was a positive association at baseline between forearm RF and StO_2 (Lowlanders, r=0.62 p<0.0001; Sherpas, r=0.45 p=0.0004), and with oxyHb (Lowlanders, r=0.45 p=0.0035; Sherpas, r=0.36 p=0.005) in the two cohorts. This association was lost at altitude. There was an interaction between group and site (F=7.5, p=0.001), with StO_2 lower in Sherpas than Lowlanders at baseline and NB (p=0.0016 and p<0.0001), however, there was no significant difference in StO_2 between Sherpas and Lowlanders at EBC (p=0.432).

A significantly higher deoxyHb signal was present in Sherpas compared with Lowlanders at all altitudes (p=0.001). We explored the possibility that the differences in resting tissue StO_2 were due to differences in the rate of oxygen extraction/utilization in the tissue. At baseline, the oxygen unloading rate (negative on-slope of oxyHb signal over first 30 seconds of arterial occlusion) estimated in a subset from each cohort, was faster in Sherpas (-0.08 (-0.11,-0.07) $AU.s^{-1}$, n=43) (median, interquartile range) than Lowlanders (-0.05 (-0.08, -0.03) $AU.s^{-1}$, n=28) (p=0.0001). In Lowlanders, the oxygen unloading rate remained similar across the three altitude sites (p=0.1190), however, in Sherpas the rate of oxygen unloading slowed with ascent to altitude at EBC (-0.05 (-0.08, -0.04) $AU.s^{-1}$, n=43) (p=0.0019).

Microvascular constrictor response. The peripheral vasoconstrictor response to deep inspiration (IBH%) was influenced by both group (F=5.9, p=0.016) and site

(F=11.2, p<0.0001) (group*site F=7.1, p=0.001). In both cohorts, ascent resulted in a decline in the IBH% response (p<0.001), which was more marked in Lowlanders than Sherpas at both NB and EBC (p<0.001) (Table 4).

Microvascular dilator response. MF following occlusion was significantly greater in Sherpas than Lowlanders at all sites (p≤0.002) (Table 5), however, in both cohorts, the RH dilator response (MF normalised to RF) remained largely unchanged across the three sites (p=0.410).

Time series analysis of resting blood flux signals

We observed modulation of flowmotion activity in all three of the low frequency spectral bands in the RF signal measured both in the finger and forearm in the two cohorts with ascent to altitude (Figure 1).

Forearm: There was an interaction effect between group and site on flowmotion activity in the myogenic (F=7.6, p=0.004) and neurogenic (F=5.9, p=0.016) frequency bands. The relative spectral energy content of the myogenic frequency band around 0.1Hz (vasomotion) appeared greater in Sherpas than Lowlanders at baseline (p <0.001). While myogenic activity increased with ascent to EBC in Lowlanders (p<0.01), it remained relatively unchanged in Sherpas. The relative spectral power of the neurogenic band was greater in Sherpas than Lowlanders (p=0.016), and in both groups decreased with ascent to EBC (p<0.0001). The relative contribution of the endothelial spectral band was similar in both groups (p=0.079), and decreased with ascent to EBC (F=57.8, p<0.0001).

Finger: There was an interaction effect between group and site on flowmotion activity in the endothelial (F=6.0, p=0.003) and neurogenic (F=5.9, p=0.003) frequency bands.

The relative spectral power of the myogenic, neurogenic and endothelial bands was greater in Sherpas than Lowlanders on ascent to high altitude (all $p< 0.01$).

In a multiple linear regression model with RF at the arm as the dependent variable, and group, site, skin temperature, flowmotion activity and StO_2 as input variables, RF was independently associated with group, skin temperature, flowmotion activity in the myogenic frequency band and StO_2 (all $p<0.05$), which together statistically significantly predicted RF ($F=14.2$, $p=0.0001$, $R^2=0.174$, adjusted $R^2=0.160$). A similar model with StO_2 as the dependent variable, and group, site and RF as the input variables, significantly predicted StO_2 ($F=37.5$, $p=0.0001$, $R^2=0.237$, adjusted $R^2=0.230$) with all three variables adding statistically significance to the prediction ($p< 0.0001$).

DISCUSSION

This study is the first study to use combined LDF and WLRS to determine the effects of graded hypobaric hypoxia on the peripheral cutaneous microcirculation, in a large cohort of Sherpa and altitude-naïve Lowlander participants. Our findings show that Sherpas, when exposed to hypobaric hypoxia, demonstrate better preserved peripheral microcirculatory perfusion with sustained microvascular reactivity (vaso-dilator and -constrictor responses), when compared to altitude-naïve Lowlanders. While StO_2 was lower in Sherpas than Lowlanders, oxygen unloading rate (negative on-slope at the start of arterial occlusion) was faster, and deoxyHb levels higher in Sherpas at all altitudes. Spectral analysis of the blood flux signals revealed an enhanced myogenic (vasomotion) activity in Sherpas, that was unaffected

by ascent to EBC. The relative spectral power of the neurogenic and endothelial bands were also greater in Sherpas than Lowlanders at high altitude.

Interpretation of results

In the finger, RF decreased to a lesser extent on ascent to 5300m in Sherpas compared with Lowlanders (21% vs. 36% relative to baseline, $p<0.001$). These data, albeit obtained using a different technique at a different site, are consistent with those reported using sidestream dark field (SDF) imaging to observe sublingual microcirculatory flow in Lowland subjects during ascent to EBC (Martin et al. 2010). Our data also demonstrated a transient increase in RF in Lowlanders at 3500m, suggesting that in Lowlanders regulatory mechanisms initially respond to the hypoxic challenge by increasing microcirculatory flow; a response not seen, or ameliorated, in Sherpas. This response is further supported by a recent study using LDF, which demonstrated an increase in forearm blood flux of healthy Lowlanders at 6 and 12 hours of exposure to normobaric hypoxia (FiO_2 equivalent to 4500m altitude) (Treml et al. 2018). However, as the degree of hypoxia increases at higher altitudes, possible dysregulation and disruption of microcirculatory flow may occur, affecting Lowlanders to a greater degree. Our findings suggest that Sherpas preserve microcirculatory flow and function at high altitude to a greater degree than Lowlanders and are therefore relatively protected from the apparent dysregulation occurring in Lowlanders as a component of acclimatisation to hypoxia. Moreover, our data implies that physiological differences in vasomotor and neurovascular control mechanisms may, in part, explain this disparity.

Effective perfusion and oxygen delivery in Sherpas may be modulated by myogenic activity within the skin microvascular bed. Myogenic activity (vasomotion), which has

been shown to be closely associated with effective oxygen delivery and to increase in hypoxia (Thorn et al. 2016; Schmidt et al. 1993: Bertuglia et al. 1991), was greater in Sherpas than Lowlanders at baseline in the forearm and at high altitude in the finger. In both vascular beds myogenic activity in Sherpas remained relatively constant with ascent to EBC. This contrasts with Lowlanders in whom arm myogenic activity was increased under hypobaric hypoxic conditions. In the context of critical illness, dysregulated vasomotion is thought to participate in the development of pathophysiological states (Nilsson et al. 2003). An increase in vasomotion is observed in reduced perfusion states such as sepsis and cardiopulmonary bypass and has been closely associated with worsening multiple organ dysfunction and mortality (Young et al. 1995; Podgoreanu et al. 2002; Knotzer et al. 2007). This increase in vasomotion, evident in Lowlanders on exposure to altitude, may be a rescue response of the skin microvasculature to impaired oxygen utilization of the skin tissue. Sherpas meanwhile, appear able to sustain an appropriately high level of vasomotion without evidence of dysregulation.

The vasodilator response is mediated by endothelium dependent vasorelaxation. There was a trend towards reduced RH in Lowlanders with ascent to high altitude and flowmotion activity in the endothelial band was greater at altitude in both forearm and finger vascular beds of Sherpas. Consistent with our findings in skin are those of Treml et al (Treml et al. 2018) using LDF, who found that Lowlanders, on exposure to normobaric hypoxia, demonstrated a reduced hyperaemic response following occlusion. Moreover, reduced skeletal muscle microvascular reactivity has been demonstrated in Lowlanders ascending to EBC using near infrared spectroscopy (NIRS) (Martin et al. 2013). Clinically, impaired microvascular reactivity is seen in

tissue hypo-perfusion states such as sepsis and has been associated with worse clinical outcomes (Neviere et al. 1996). Sherpas by contrast, seem to demonstrate preserved endothelial control and microvascular dilator capacity suggestive of a beneficial adaptation, enabling a more effective response to micro-regional oxygen demand at altitude, for instance during exercise, and repaying any oxygen debt more quickly.

Exposure to high altitude also increases sympathetic outflow via the stimulation of chemoreceptors to modulate the direct vasodilatory effects of hypobaric hypoxia (Rostrup et al. 1998; Hansen et al. 2003). In both Sherpas and Lowlanders, ascent resulted in an attenuation of the vasoconstrictor response to deep inspiratory breath-hold as overall systemic sympathetic tone increased. This attenuation of the response was greater in Lowlanders, indicative of decreased autonomic control of microvascular flow due to the high levels of basal sympathetic activation. Conversely, Sherpas are able to preserve the vasoconstrictor response at altitude. This may be due to an adaptive modulation of sympatho-vagal activity through which Sherpas can better regulate flow, allowing them to stay in a hypobaric atmosphere at lower temperatures without excessive autonomic stress (Passino et al. 1996). Despite this, increased local flowmotion activity in the neurogenic band was seen in the resting finger blood flux signals of Sherpas at altitude, which is inconsistent with previous data observing reduced 0.1Hz sympathetic activity in Himalayan high altitude dwellers compared with sea-level dwellers (Bernadi, 2007).

Despite significant differences in microvascular flux and function, our data showed little distinction in forearm tissue oxygenation between the cohorts at high altitude. As

anticipated, StO_2 and oxyHb were positively correlated with resting forearm blood flux and decreased on exposure to altitude in both cohorts. While Sherpas appeared to have a lower StO_2 than Lowlanders at baseline and NB, the oxygen unloading efficiency and deoxyHb levels were greater in Sherpas than Lowlanders indicative of a protective mechanism during hypobaric hypoxia. The ability of Sherpas to unload oxygen more effectively, demonstrated by greater deoxyHb levels, may be because they are better able to regulate and vary flow to meet local demand. Greater variability in patterns of blood flowmotion are thought to give rise to a more effective microvascular network with an increased adaptability to a physiological or pathological challenge (Butcher et al. 2013). In computational models, chaotic capillary activity has been shown to promote more efficient tissue oxygenation in skeletal muscle than regular rhythmic patterns of vasomotion (Pradhan et al. 2007). Declining spontaneous variation in flowmotion activity may be deleterious to health and has been identified in cardiovascular and metabolic disease (Clough et al. 2016). Figure 1 demonstrates the changes in flowmotion control in both cohorts on ascent to altitude. Sherpas appear to maintain a greater variability of flowmotion on ascent to EBC, especially in the finger microvascular bed. We speculate that this may provide Sherpas with improved network functionality and flexibility. Further work is necessary to investigate the statistical complexity of the blood flux signals, determining how much the blood flux signal differs from a random sequence (Kuliga et al. 2018; Tigno et al. 2009).

Questions arising from the study

Is the skin as an oxygen sensor in humans? The skin has an extensive vasculature, which is known to be responsive to shifts in oxygen availability (Durand et al. 1969; Minson et al. 2003). It has been implicated in the acute responsiveness to hypoxia and

suggested that in humans it may play a role in the long term adaptation to hypobaric hypoxia through local modulation of blood flow (Pucci et al. 2012). Skin also acts as a dynamic oxygen sensor directly modifying the cardiovascular response to systemic hypoxia in rodents (Cowburn et al. 2017). By undertaking regression modelling, we aimed to explore the independence of the association between StO_2, resting blood flux, altitude and study cohort and to explore the effects of potential confounders. We found that group, site and RF predicted approximately 25% of the variance in tissue oxygenation (adjusted R^2 =0.230, p= 0.0001).

Could these mechanisms be important in critical illness? The vasomotor and neurovascular mechanisms identified appear to play a dynamic role in maintaining and preserving oxygen homeostasis. Understanding the underlying mechanisms of hypoxia tolerance is of direct relevance to clinicians caring for critically ill patients in which tissue hypoxaemia is commonplace, especially as the practice of normalising arterial oxygen content has shown to be harmful in some circumstances (Damiani et al. 2014; Helmerhorst et al. 2015). The mechanisms identified in this study may therefore offer some protection against the cellular hypoxia present in critical illness, and further work is necessary to establish whether similar evidence of sustained vasomotor and neurovascular control can be identified in the setting of longstanding hypoxaemia, and if this is associated with clinical outcome.

Study Strengths and Limitations

This study uses LDF combined with white light reflectance spectroscopy to study the microcirculation and flowmotion control in an indigenous high altitude population on exposure to high altitude. A large number of Sherpa and Lowlander participants were

tested following a standardised ascent profile to EBC, which supports the notion that differences between participants reflect inter-individual variability in hypoxic adaptation, as opposed to variability in hypoxic exposure. Additionally, our study also examined well-characterised physiological variables and perturbations in peripheral blood flow using a widely recognised non-invasive technique such that the data obtained from Sherpas and Lowlanders could therefore provide a reference against which to further investigate microvascular control, and its relationship to tissue oxygenation, in the context of sustained hypoxia.

There are also a number of limitations with our study. Firstly, LDF assesses blood flux over a small volume (around $1mm^3$) of tissue. As flux measured represents the aggregate flow in several vessels of variable size, the inherent regional heterogeneity of cutaneous perfusion contributes to poor reproducibility of the technique, as does placement of the LDF/WLRS probe. A second limitation of our study is the potential confounding effect of environmental and skin temperature. Cutaneous vasoconstriction is the initial thermoregulatory response to defend against cold exposure (Thompson-Torgerson et al. 2007) and thus any disparities in temperature, could lead to changes in microvascular flow independent of hypoxia. The temperature of the London laboratory where baseline measurements were performed in Lowlanders was lower than that in Kathmandu where Sherpas were studied. It is therefore probable that this contributed to the lower forearm and finger fluxes seen in Lowlanders at baseline. Both cohorts were exposed to the same laboratory conditions at NB and EBC. At these sites skin temperature measured at the finger was higher in Sherpas, as was blood flux, which suggests that the physiological differences seen at NB and EBC are independent of external temperature.

Further limitations also include potential recruitment bias, and the different altitude for baseline testing in Sherpas (1300m) and Lowlanders (35m). Recruitment was performed through open advertisement, such that participants were therefore self-selecting, and thus the study population may not be truly representative of an average Sherpa and Lowlander population. Roughly equal numbers of participants were compared, with a similar gender ratio in each group, however participants were markedly younger in the Sherpa cohort. However, we could find no independent effect of age on blood flux or StO_2 in our regression models. Baseline testing was conducted in London (35m) for Lowlanders and Kathmandu (1300m) for Sherpas primarily due to logistical restraints. It is therefore possible, that the differing altitudes at the two sites (Table 2) may have affected microcirculatory flow, however data from the Caudwell Xtreme Everest 2007 expedition did not identify any demonstrable differences in participants' physiology between sea level and Kathmandu laboratories (Levett et al. 2010), and thus, we felt it justified to use these two distinct locations for baseline normoxia testing.

Finally, we were unable to explore the effects of hypocapnia on microvascular reactivity in this study. The current consensus is that Sherpas hypoxic ventilatory response is in keeping with acclimatized lowlanders (Gilbert-Kawai et al. 2014). However, the hypoxic ventilatory response was not measured in this study, and at present we are unable to assess local CO_2 levels in the skin. It is plausible that increasing hypocapnia at altitude induces changes in the microcirculation and that this may contribute to the observed differences seen between sites.

CONCLUSION

Physiological differences in local microvasculature vasomotor and neurovascular control may play a key role in Sherpa adaptation to high altitude hypobaric hypoxia, through providing a means to maintain local perfusion and tissue oxygenation. Together these data suggest that peripheral tissues play an important physiological role in the cardiovascular adaptation to hypoxia, and that this role is better developed in native altitude dwellers than altitude-naïve lowlanders.

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COMPETING INTERESTS

No competing interests were disclosed.

AUTHOR CONTRIBUTIONS

EGK, MM, DL, KM, MG, GC and DM were involved in the conception and design of the study. TD, EGK, SW and PM performed experiments. TD, EGK, SW and GC analysed the data. TD, EGK and GC interpreted the results. TD, EGK, GC and DM prepared the first draft of the manuscript. All authors were involved in the revision of the draft manuscript and have approved the final content. All authors agree to be accountable for all aspects of the work in ensuring questions relating to the accuracy and integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify are listed.

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TABLES & FIGURES

Table 1: Demographic summary of participants

	Sherpas	**Lowlanders**
Number	61	83
Gender (Males/Females)	28/33	39/44
Age (years)	27.9 (6.9)	38.8 (13.1)*
Height (cm)	160 (6)	172 (10)*
Weight (kg)	61.1 (9.0)	71.8 (13.0)*

Data are expressed as mean value (± standard deviation)

* Significant difference demonstrated between cohorts

Table 2: Laboratory environmental conditions

Laboratory	Altitude (m)	Barometric pressure (kPa)	Temperature (°C)	Humidity (%)	PO_2 (mmHg)
London	35	100.6 (0.2)	16.9 (1.8)	35.4 (6.5)	157.5
Kathmandu	1300	86.8 (0.4)	23.8 (3.4)	47.4 (15.7)	135.8
Namche Bazaar	3500	66.5 (0.3)	13.9 (3.1)	72.1 (8.1)	103.5
Everest Base Camp	5300	53.0 (0.2)	12.9 (8.2)	37.8 (17.5)	82.5

Barometric pressures, temperature and humidity are mean (± standard deviation) values recorded during laboratory testing in the field. PO_2 = calculated from barometric pressures assuming FIO_2 0.209.

Table 3: Values for physiological observations, haemoglobin concentration and haematocrit

	Lowlanders (n=83)			Sherpas (n=61)		
	Baseline	**NB**	**EBC**	**Baseline**	**NB**	**EBC**
HR	64 (57-71)	72a (64-84)	77a,b (68-87)	70c (62-75)	74a (67-84)	87a,b,c (80-94)
SBP (mmHg)	126 (114-135)	126a (119-141)	129a (123-141)	121 (115-128)	120c (115-125)	125a,b,c (116-135)
DBP (mmHg)	77 (73-85)	83a (74-89)	87a,b (80-91)	83 (75-87)	83 (79-88)	90a,b,c (83-96)
MAP (mmHg)	95 (88-101)	98a (91-105)	102a (95-107)	96 (88-101)	95 (91-98)	101a,b (95-110)
SpO_2 (%)	99 (98-99)	90a (88-93)	80a,b (75-83)	98c (96-98)	90a (89-92)	79a,b (76-82)
Hb (g/l)	139 (129-151)	148a (134-158)	156a,b (145-168)	137 (127-150)	146a (134-160)	151a,b (140-165)
Hct (%)	43 (40-47)	46a (43-49)	50a,b (45-53)	43 (41-46)	45a (43-49)	49a,b (46-52)
RR	12 (10-14)	13a (11-16)	15a,b (12-19)	16c (14-18)	17a,c (14-20)	20a,b,c (18-22)

Data are expressed as median (interquartile range)

Baseline = (London for Lowlanders (35m) and Kathmandu (1300m) for Sherpas),

NB = Namche Bazaar (3500m), EBC = Everest Base Camp (5300m).

HR = heart rate, SBP = Systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, SpO_2 = peripheral oxygen saturations, Hb = haemoglobin concentration, Hct = haematocrit, RR = respiratory rate

a = Significant difference demonstrated for that cohort between relevant site and baseline

b = Significant difference demonstrated for that cohort between relevant site and NB

c = Significant difference demonstrated between cohorts at that site

Table 4: Combined skin blood flux and temperature, and tissue oxygenation measurements at the forearm in Lowlanders and Sherpas on ascent to Everest Base Camp (EBC) . All 144 participants (83 Lowlanders and 61 Sherpas) completed testing at baseline and Namche Bazaar (NB) and 133 participants (77 Lowlanders 56 Sherpas) completed testing at EBC.

	Lowlanders			**Sherpas**		
	Baseline	**NB**	**EBC**	**Baseline**	**NB**	**EBC**
			Blood Flux			
Resting flux (RF)	10.0	15.0a	13.1b	14.9c	12.5c	11.3a,c
(PU)	(7.9-16.0)	(11.8-24.3)	(10.8-17.4)	(10.6-19.6)	(9.0-17.7)	(8.5-15.4)
Maximal flux (MF)	55.4	78.5a	59.8b	68.4c	78.9	59.1
(PU)	(43.4-85.3)	(61.2-101.0)	(48.0-80.8)	(48.6-101.0)	(58.4-101.0)	(48.2-85.8)
RH response	4.4	3.6	3.5 a	3.7	4.8a,c	4.2b,c
	(2.7-6.5)	(2.7-5.1)	(2.1-4.7)	(2.6-4.88)	(3.55-6.9)	(3.2-5.7)
Skin temperature	27.7	27.4a	28.5a,b	31.4c	26.9a	28.3a,b
(^{o}C)	(27-28.3)	(26.3-28.5)	(26.8-29.7)	(30.5-32.4)	(25.4-28.2)	(27.4-30.1)
Resting CVC	0.11	0.16a	0.13b	0.17c	0.13c	0.11a
(PU/mmHg)	(0.09-0.18)	(0.12-0.24)	(0.11-0.17)	(0.11-0.21)	(0.10-0.19)	(0.08-0.19)
Peak CVC	0.59	0.79a	0.63b	0.74	0.83	0.58b
(PU/mmHg)	(0.45-0.95)	(0.65-1.04)	(0.46-0.79)	(0.51-1.05)	(0.62-1.10)	(0.43-0.88)
			Tissue Oxygenation			
StO_2	42	45	28a,b	35c	33c	27a,b
(%)	(36-46)	(38-50)	(23-34)	(31-41)	(29-40)	(24-31)

OxyHb	6.7	9.3	6.5b	10.0c	8.7	7.0a,b
(AU)	(5.0-11.0)	(6.8-10.9)	(4.3-8.7)	(6.8-13.5)	(6.4-11.3)	(5.2-9.2)
DeOxyHb	9.3	10.7	15.9a,b	14.8c	16.9c	17.3a
(AU)	(7.7-16.0)	(8.3-13.2)	(12.7-19.4)	(12.5-19.5)	(11.6-19.1)	(14.3-23.4)
TotalHb	16.6	20.4	22.2	22.1c	25.0c	24.5
(AU)	(13.5-26.7)	(16.7-24.2)	(17.5-27.5)	(17.5-30.5)	(22.2-29.3)	(19.9-32.1)

Data are expressed as median (interquartile range)

Baseline = (London for Lowlanders (35m) and Kathmandu (1300m) for Sherpas),

NB = Namche Bazaar (3500m), EBC = Everest Base Camp (5300m).

PU = perfusion units, RH = reactive hyperaemia, CVC = cutaneous vascular conductance, AU = arbitrary units, StO_2 = tissue oxygen saturation, OxyHb = oxyhaemoglobin, DeoxyHb = deoxyhaemoglobin, TotalHb = total haemoglobin.

a = Significant difference demonstrated for that cohort between relevant site and baseline

b = Significant difference demonstrated for that cohort between relevant site and NB

c = Significant difference demonstrated between cohorts at that site

Table 5: Skin blood flux and skin temperature measurements at the finger in Lowlanders and Sherpas on ascent to Everest Base Camp (EBC). All 144 participants (83 Lowlanders and 61 Sherpas) completed testing at baseline and Namche Bazaar (NB) and 133 participants (77 Lowlanders 56 Sherpas) completed testing at EBC.

	Lowlanders			**Sherpas**		
	Baseline	**NB**	**EBC**	**Baseline**	**NB**	**EBC**
RF (PU)	44 (18-176)	163a (52-355)	28a,b (18-56)	278c (220-341)	324c (209-409)	219a,c (116-283)
Skin temperature (ºC)	23.6 (22.5-25.9)	22.3a (20.6-24.8)	20.7a (19.5-25.1)	31.9c (29.8-33.0)	27.3a,c (23.0-30.2)	27.4a,c (24.3-30.2)
Vasoconstrictor Response (%IBH)	84.5b (75-92)	72.3a (62-86)	52.9a,b (41-70)	83.3 (66-91)	75.1a (51-86)	72.6a,c (48-80)

Data are expressed as median (interquartile range)

Baseline = (London for Lowlanders (35m) and Kathmandu (1300m) for Sherpas),

NB = Namche Bazaar (3500m). , EBC = Everest Base Camp (5300m).

PU = perfusion units, IBH = response to inspiratory breath hold

a = Significant difference demonstrated for that cohort between relevant site and baseline

b = Significant difference demonstrated for that cohort between relevant site and NB

c = Significant difference demonstrated between cohorts at that site

Figure 1. Relative power spectral density of low frequency flow motion activity in A. finger and B. forearm skin resting blood flux signal in Lowlanders (n=76) (black symbols) and Sherpas (n=56) (grey symbols) on ascent to Everest Base Camp.

Data are mean SD. * p for site. Baseline = (London for Lowlanders (35m) and Kathmandu (1300m) for Sherpas), NB = Namche Bazaar (3500m) , EBC = Everest Base Camp (5300m).