Sublingual microcirculatory blood flow and vessel density in Sherpas at high altitude

AUTHORS

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RUNNING HEAD

Sherpa microcirculation at high altitude

CORRESPONDING AUTHOR:
Anecdotal reports suggest that Sherpa highlanders demonstrate extraordinary tolerance to hypoxia at high altitude despite exhibiting lower arterial oxygen content than acclimatised Lowlanders. This study tested the hypothesis that Sherpas exposed to hypobaric hypoxia on ascent to 5300m, develop increased microcirculatory blood flow as a means of maintaining tissue oxygen delivery. Images of the sublingual microcirculation were obtained from 64 Sherpas and 69 Lowlanders using incident dark field imaging. Serial measurements were obtained from participants undertaking an ascent from baseline testing (35m or 1300m) to Everest base camp (5300m), and following subsequent descent in Kathmandu (1300m). Microcirculatory flow index and heterogeneity index were used to provide indices of microcirculatory flow, whilst capillary density was assessed using small vessel density. Sherpas, when compared to Lowlanders, demonstrated significantly greater microcirculatory blood flow at Everest Base Camp, but not at baseline testing or on return in Kathmandu. Additionally, Sherpa blood flow exhibited greater homogeneity at 5300m and 1300m (descent) when compared to Lowlanders. Sublingual small vessel density was not different between the two cohorts at baseline testing or at 1300m, however, at 5300m Sherpas capillary density was up to 30% greater. These data suggest that Sherpas can maintain a significantly greater microcirculatory flow per unit time, and flow per unit volume of tissue at high altitude, when compared to Lowlanders. These findings support the notion that peripheral vascular factors at the microcirculatory level may be important in the process of adaptation to hypoxia.
NEW & NOTEWORTHY

Sherpa highlanders demonstrate extraordinary tolerance to hypoxia at high altitude, yet the physiological mechanisms underlying this remain unknown. In our prospective study, conducted on healthy volunteers ascending to Everest Base Camp (5300m), we demonstrated that Sherpas have a higher sublingual microcirculatory blood flow and greater capillary density at high altitude, when compared to Lowlanders. These findings support the notion that the peripheral microcirculation plays a key role in the process of long-term adaptation to hypoxia.

KEYWORDS
Hypoxia, Microcirculation, Altitude, Sherpa, Capillary

INTRODUCTION

Anecdotal reports suggest that Sherpa highlanders exhibit extraordinary tolerance to hypoxia at high altitude. Subjective demonstration of their remarkable exercise and endurance abilities may be readily observed by persons trekking and climbing in the Himalayan mountain regions. Having resided at high altitude for the last 500 generations (2), it is likely that these observations are underpinned by alterations in their genome, adapted through the process of natural selection driven by lifelong environmental exposure to hypobaric hypoxia. Whilst evidence of consequent downstream phenotypic alterations remains limited, intriguingly it has been demonstrated that Sherpas
exhibit a lower arterial oxygen content (CaO\(_2\)) when compared to Lowlanders who ascend to comparable altitudes (1, 4, 38, 44). It is thus conceivable that through the comparison of Lowlander and Highlander genotype-phenotype, one might uncover adaptive mechanisms that facilitate their apparent hypoxia tolerance.

The delivery of oxygen to metabolising tissues is a process of both convective flow within the systemic circulation, and diffusion along oxygen partial pressure gradients within the tissues. To date, studies have predominantly focused on the traditionally described aspects of acclimatisation, those involving the restoration of CaO\(_2\) and systemic oxygen delivery (DO\(_2\)) (4, 6, 15, 38). Whilst such studies have failed to provide a universally accepted explanation for hypoxia tolerance, little attention has been paid to the tissue components of the oxygen cascade. Within every tissue of the body, the microcirculation (anatomically described as blood vessels < 100 μm (26)) regulates localised blood flow to match micro-regional oxygen demand (9).

As the final step in the convective portion of the oxygen cascade, from where oxygen diffuses into the surrounding tissues, alterations in the microvasculature may disrupt the balance between oxygen supply and demand at a cellular level, thereby acting as a ‘bottleneck’ in the oxygen cascade. Accordingly, this potential limiting factor may be reduced or obviated by maintaining adequate microcirculatory flow per unit time and/or per unit volume of tissue (functional capillary density), and thus the microcirculation should be considered important to the development of hypoxia tolerance (25). This study tested the hypothesis that Sherpas exposed to hypobaric hypoxia on ascent to 5300m, demonstrate increased
microcirculatory blood flow and vessel density as a means to maintain oxygen
delivery to the peripheral tissues.

MATERIALS AND METHODS

Participant selection

Approval for this study was obtained both by the University College London
Research Ethics Committee, and the Nepal Health Research Council (NHRC)
as part of the Xtreme Everest 2 (XE2) research expedition (18). Healthy
Sherpa and Lowlander volunteers were recruited and written consent was
obtained from all participants. Sherpas were defined as being direct
descendants of Nepali Sherpas (for at least two generations), drawn from
communities in the Solukhumbu and Rolwaling valleys. Lowlanders were
recruited in the UK, they were not descendants from a native high altitude
population (e.g. Tibetan, Andean, Ethiopian), and all were born and lived
below 1000m.

Study setting

XE2 (29) was conducted from December 2012 to May 2013, and this was one
of the individual studies conducted on the research expedition. Sublingual
microcirculatory data were collected at three locations: ‘Baseline testing’ (BL),
‘Everest Base Camp’ (EBC) (5300m), and on descent in ‘Kathmandu’ (KTM)
(1300m). BL testing was conducted in London (LON) for Lowlanders (35m),
and in KTM (1300m) for Sherpas. Having departed from KTM, all participants
followed an identical ascent and descent profile. This consisted of a flight from
KTM to an altitude of 2800m, followed by an 11 day trek to EBC. A total of three nights were then spent at 5300m, before descent to KTM in 5 days.

**Observation of the sublingual microcirculation**

The sublingual microcirculation was visualised using the Cytocam incident dark field (IDF) imaging video-microscope (Braedius Medical, Huizen, The Netherlands) (3). Prior to its use in the study, thorough assessment of this new video-microscope and its automated analysis software was undertaken. Results demonstrated that firstly the IDF-camera provided improved image acquisition of human sublingual microcirculation when compared to the sidestream dark field (SDF) video-microscope (17). The camera uses polarised green light (wavelength 548nm) to illuminate the observed tissue. This light corresponds to one of the isobestic points of oxy- and deoxy-haemoglobin, and thus ensures optimal absorption by red blood cells within the microvasculature regardless of oxygenation status (20). Absorption of light by haemoglobin, but not by surrounding tissue, creates a distinct contrast of dark and light colour respectively, and red blood cells moving through the mucosal microcirculation thus appear as dark globules moving along the axis of flow.

At each measurement point, participants were required to rest for ten minutes in the supine position before any images were obtained. Images were subsequently obtained following the standard operating guidelines of Trzeciak et al. (41), whereby the investigator positioned and focused the IDF camera under participant’s tongue. Ten seconds of video footage was then digitally...
recorded onto the computer where images were stored for later analysis. This
process was repeated on each participant until five good quality recordings
had been acquired from separate areas of the sublingual region. Studies were
conducted during the day, and subjects were sheltered from any extremes of
temperature. All images were obtained by one of three researchers, all of
whom were experienced in using the IDF video-microscope.

**Analysis and scoring of microcirculatory video images**

IDF data analysis was conducted by two researchers using the AVA 3.0
microcirculatory analysis software (MicroVision Medical, Amsterdam,
Netherlands) (10). To avoid observer bias during analysis of microcirculatory
films, investigators were blinded to both the study location and cohort identity
by assigning random codes to identify films. To assess for inter-observer
variability, the two observers evaluated a selection of IDF videos (30 films).
Each video was only deemed appropriate for analysis if it adhered to the
‘microcirculation image quality scoring system’ (31), whereby stability,
illumination, duration, focus, content, and pressure artefact are assessed.
Videos were subsequently corrected for background variation, image contrast
optimised, and to compensate for movement artefact, all video images
underwent image stabilisation by the analysis software. After initial automated
vessel detection, every film was checked visually, whereupon incorrectly
identified blood vessels were deleted, and undetected vessels were drawn
manually. Additionally, incorrectly disconnected segments of vessels were
‘chained’, and erroneously connected segments were ‘unchained’. 
In keeping with the consensus statement set out in 2007 (8), the mean score from each of the five measurements recorded at each altitude was used. IDF variables measured included the microvascular flow index, heterogeneity index and vessel density.

i) Microvascular Flow Index (MFI). The magnitude of microvascular perfusion is commonly evaluated by a semi-quantitative scoring system referred to as the MFI (5, 12). The MFI is based on the determination of the average or predominant flow type in the field of view at a given time point. It is quantified using an ordinal scale as follows: 0 = no flow, 1 = intermittent flow, 2 = sluggish flow, 3 = continuous flow. The ‘vessel by vessel’ approach to MFI calculation was utilised (11, 12) in this study, in which the mean value of the MFIs in each individual vessel is calculated. This approach has been best shown to correlate with both the erythrocyte velocity and the proportion of perfused small vessels (36), and furthermore demonstrates the closest intra-observer reliability for vessel detection and flow classification (35).

ii) Heterogeneity Index (HI). The flow HI provides information relating to the presence of microcirculatory distributive alterations and shunting (13). It is calculated as the highest site flow velocity (i.e. the MFI) minus the lowest site flow velocity, divided by the mean flow velocity of all sublingual sites at that time point (42).

iii) Vessel Density. Microcirculatory density is assessed as the vessel density. The total length of the vessel is divided by the total surface of the analysed vessel (mm/mm²).
In each instance, both ‘small vessel density’ (<25 μm diameter) and ‘large vessel density’ (>25 μm diameter) values are reported. Whilst the former relates to capillaries (and thus contribute principally to organ perfusion and are arguably the vessel of greatest significance), the latter are reported as they are used as a quality control measure to ensure that excessive pressure was not used in obtaining the videos.

**Physiological measurements**

Haemoglobin concentration (Hemocue AB, Hemocue, Sweden) and haematocrit values (Sigma 1-14 microcentrifuge, Sigma, Germany) were obtained from whole blood samples. Peripheral oxygen saturation (Nonin Onyx 9500, Nonin Medical Inc, Minnesota, USA), heart rate, and blood pressure (Omron M3H, Moron Healthcare, Japan) were recorded after ten minutes seated at rest. Mean arterial pressure was calculated from the systolic and diastolic values. Participant’s tympanic temperature was measured from the ear canal (Braun 4020, Kronberg, Germany).

**Statistical analysis**

All data were assessed for normality. A Shapiro Wilk’s test (P>0.05), and visual inspection of their histograms, normal Q-Q plots, and box plots showed that the data were not normally distributed. Non-parametric tests were therefore used for statistical analysis with values summarised as median and interquartile ranges. Related samples Friedman’s Two-Way Analysis of Variance by Ranks tests (more than two sites) and related samples Wilcoxon
Signed Rank Test (between two sites) with Bonferroni correction applied were used to assess the effect of hypoxia on the peripheral microcirculation. Sherpa and Lowlander cohorts were compared using the unpaired Mann Whitney U test. Data were presented as Box-Whisker plots. The relationship between microcirculatory flow and other physiological variables were assessed individually using Spearman’s Rank correlation coefficient ($r$). Inter-observer variability for analysis of the IDF images was assessed by calculating the intra-class correlation coefficient. All statistical calculations were performed on SPSS version 21 (IBM, USA), and a $p$-value of <0.05 was taken to indicate statistical significance.

RESULTS

Of the 133 participants (64 Sherpas and 69 Lowlanders) who underwent baseline testing (BL) testing, 131 (63 Sherpas and 68 Lowlanders) completed testing at Everest base camp (EBC), and 83 (17 Sherpas and 66 Lowlanders) in Kathmandu (KTM). The demographics of the participants are shown in Table 1, and the information relating to the laboratory environments in Tables 2 and 3. At each altitude, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), haemoglobin concentration (Hb), haematocrit (Hct), peripheral oxygen saturation (SpO$_2$), and core temperature were similar between the two cohorts (Table 4).

MFI and HI were used to provide indices of microcirculatory flow. The MFI for small vessels (<25μm diameter) did not differ between Sherpas and Lowlanders at BL (2.81 [2.60–2.98] vs. LL 2.96 [2.62-3.00] respectively), or in KTM (2.97 [2.75-3.00] vs. 2.84 [2.52-3.00]), however, at EBC Sherpas had a
significantly higher MFI (3.00 [2.88 -3.00] vs. 2.66 [2.45-2.97]); (p < 0.001) (Figure 1). The MFI for large vessels (>25μm diameter) did not differ between Sherpas and Lowlanders at any of the three measurement points. There was no difference in the small vessel HI between Sherpas or Lowlanders at BL (0.386 [0.336-0.402] vs. 0.359 [0.336-0.667]), however, Lowlander values were significantly greater than Sherpa values at both EBC (0.408 [0.374-0.724] vs. 0.341 [0.333-0.390]); (p < 0.001), and on descent to KTM (0.392 [0.352-0.667] vs. 0.333 [0.333-0.470]); (p = 0.010) (Figure 2).

Small vessel density (<25 μm diameter) was not different between the two cohorts at BL, or in KTM, but Sherpas had a significantly greater small vessel density at EBC (13.83 mm/m² [11.41-14.52] vs. 10.52 mm/m² [8.90-11.34]); p = 0.047) (Figure 3). There was no difference between Sherpas’ and Lowlanders’ large vessel density (>25 μm diameter) at any site.

There was no correlation between either small vessel MFI, HI, or vessel density, and any of the measured physiological variables (Hb, Hct, HR, SBP, DBP, MAP, and SpO₂).

Inter-observer variability in IDF image analysis was assessed between two investigators using the intra-class correlation coefficient. A strong correlation was demonstrated, 0.89 (95%CI 0.83-0.96).

**DISCUSSION**

This study demonstrated differences between Sherpa and Lowlander microcirculatory responses to sustained hypobaric hypoxia at high altitude. Whilst no difference in microcirculatory blood flow and capillary density was
seen between cohorts in normoxia (BL), upon exposure to hypoxia Sherpas demonstrated significantly greater values for both indices. Hypoxia caused Sherpas to increase both microcirculatory blood flow and capillary density, whilst Lowlanders decreased flow, but increased density, however, to a lesser extent than the Sherpas (Figure 4). On descent to KTM, the relative increase in vessel densities for both cohorts persisted, however, blood flow returned to previous baseline values.

Numerous studies have attempted to determine the genetic and physiological differences between the indigenous high altitude Sherpa (and Tibetan) people, and those who live at low altitude (19). Few of these studies however, have revealed any marked differences that might explain how this high altitude population not only live, but seemingly thrive, so effectively under conditions of chronic environmental hypoxia. In 2007, Erzurum et al (14) explored the possibility that peripheral blood flow was an important determinant in long-term adaptation to hypoxia. Venous occlusion plethysmography (VOP) was utilised to measure blood flow in the forearm of 88 Tibetans at 4200m and 50 sea level residents at 206m. Their results demonstrated Tibetans to have more than double the forearm blood flow than American controls. Whilst these results supported earlier works relating to blood flow (39), and skeletal muscle capillary density (22), notably the data obtained using VOP in Erzurum’s study relates to total blood flow in the forearm as opposed to that in the microcirculation per se. The first description of in vivo microcirculatory changes on ascent to high altitude, coincided with the introduction of sidestream dark field imaging (21). On ascent to 4900m,
blood flow in the sublingual vessel was seen to reduce significantly in 12 lowland subjects (30), and similar data were recorded in a further 24 lowland subjects on ascent to 5300m (28).

The Lowlander MFI data presented supports the findings of Martin et al. (28, 30) whereby flow decreased upon ascent to high altitude. In these manuscripts it was theorised that the slowing microcirculatory blood flow demonstrated could in fact be an adaptive response, applied to increase the erythrocyte tissue transit time and improve oxygen diffusion. This is conceivable since a prolonged course through the capillary network may enhance offloading of oxygen in the presence of a reduced partial pressure gradient between the capillary and mitochondria, particularly when cardiac output is high, as is the case during exercise. Sherpas by contrast, seem to utilise brisk flow to maintain localised oxygen delivery. This in turn may explain the lower Hb concentration that this population demonstrate after prolonged exposure to hypobaric hypoxia (4). Undoubtedly, increased Hb concentration augments CaO₂, however, elevated Hct increases blood viscosity, alters its rheology and at levels greater than 50% may decrease cardiac output and oxygen delivery (43). Furthermore, elevated Hct demonstrated in South American resident populations, are associated with an increased prevalence of chronic mountain sickness and related embolic or thrombotic events (33). In contrast, it seems Sherpas favour a blunted erythropoetic response thereby allowing for brisk microvascular blood flow.

The speed of microcirculatory blood flow per se may also be less important than its nature. Maintaining a homogenous microcirculatory blood flow, irrespective of the speed at which the contained blood may flow, could be
crucial to tissue perfusion. In this study, ascent to EBC was associated with a
fall in the HI in Sherpas, and an increase in Lowlanders, such that a
significant difference is evident between cohorts at high altitude. A lower HI
equates to more homogenous flow, and the importance of this may be
highlighted in the clinical setting where dysregulated, heterogeneous
microvascular flow is a fundamental mechanism through which tissue hypoxia
occurs in sepsis [7]. In either case, whether the important determinant of
tissue oxygenation relates to the speed of blood flow, and / or the
homogenous nature of its flow, Sherpas demonstrate superiority in both.

The descent data observed in this study are also novel. The fact that MFI
values were similar between the two cohorts at BL and KTM, suggests that
the physiological basis underpinning Sherpas’ ability to maximise
microcirculatory blood flow at altitude is transient and hypoxia-dependent. We
do however appreciate that only a small number of Sherpas were studied on
their return to Kathmandu, and thus we are cautious in our interpretation of
these data.

The data presented illustrating the effects of hypoxic exposure on Sherpa
capillary density is the first of its kind. Whilst no difference in small vessel
density was evident between cohorts at BL, these data demonstrate that
Sherpas have a substantial capacity to increase their capillary numbers. An
increase in sublingual vessel density on ascent to altitude has been previously
reported by Martin et al [28]. That said, in his study conducted on 21
Lowlanders ascending to 5300m, it was not the density of small vessels
(<25μm) which altered at high altitude, but rather that of the larger vessels
(>25 μm). Whilst the actual values reported by Martin et al for small vessel density were similar to those seen above, our data contrast with prior data where we found Lowlanders to increase their small vessel density on ascent to 5300m (Figure 3) (p=0.020), whilst their large vessel density did not change. Whilst both studies used a very similar ascent profile, the discrepancy between our findings may be due to the increased statistical power of this study, and / or the fact that we used an IDF video-microscope as opposed to the SDF video-microscope (17).

Whilst both cohorts demonstrated increased capillary density on ascent to high altitude, Sherpas did so to a much greater degree. At EBC, their capillary network was approximately 30% denser than Lowlanders. Vessel recruitment due to elevated Hb and Hct might have accounted for the rise in capillary density in both groups (16, 34, 37, 45). These values however, were similar between the two cohorts upon arrival at EBC, so it seems unlikely that this explains the observed difference between them, unless Sherpas have a much larger un-recruited (and thus unseen) reservoir in normoxia. This is certainly plausible, and as with flow, it is likely that the difference is ultimately underpinned by genetic differences.

This is the first study of Sherpa microcirculation on ascent to, and descent from high altitude. A large number of participants were studied, and over 98% successfully ascended to EBC following an identical ascent profile. This matched ascent profile along with serial measurements controls for variability
of exposure to hypoxia, and thereby enables valid inter-individual comparison of hypoxia responses whilst amplifying the signal to noise ratio (24). The newly released Cytocam video-microscope was used to obtain images of the sublingual microcirculation, and our assessment of it prior to the expedition demonstrated its superior capabilities regarding image acquisition compared to its predecessor SDF imaging (17). Unfortunately, no validation of the camera in a hypobaric hypoxic environment was performed prior to the expedition, and this is a limitation of the study. Further limitations include potential recruitment bias, confounding factors within laboratories, the different altitude for baseline testing in Sherpas (1300m) and Lowlanders (35m), and the small number of Sherpas tested on descent. Whilst recruitment was through open advertisement and word of mouth, the participants were self-selecting by virtue of this research expedition involving opportunistic observation of individuals with a desire to visit the study environment, and thus may not be truly representative of a ‘normal’ Sherpa or Lowlander population. The demographic data (Table 1) demonstrates that approximately equal numbers of participants were compared, with a similar gender ratio in each group, however, the age of participants was markedly lower in the Sherpa cohort, whilst the percentage of smokers was higher. Smoking is known to affect the vasculature and could thus be a confounding factor in the results (32). Despite our best efforts to minimise temperature differences between laboratories, disparities were still seen. This could affect microvascular flow due to cold-induced vasoconstriction (23, 40). There were, however, no significant differences between the environmental temperatures both cohorts were exposed to within each individual laboratory (Table 3). Additionally, as
the sublingual circulation is within the oral cavity, and as such it is regarded as
being at a similar temperature to one’s core, the data demonstrated no
differences in the two cohorts’ tympanic temperatures (Table 4). Other
potential confounding factors specific to the high altitude environment include
hydration status which in turn may affect Hct values, and thus alter blood
rheology. The effect of this potential confounding factor was minimised by
conducting studies at all altitudes after a period of overnight rest and ensuring
subjects had free access to oral fluids and were actively encouraged to drink
enough fluid to produce normal volumes of clear urine. Finally, ascent to
altitude may cause tissue oedema (27), which if occurring in the sublingual
mucosa could theoretically affect image quality and lead to false
measurements of flow and density. Baseline testing was conducted in London
for Lowlanders (35m), and in Kathmandu (1300m) for Sherpas due to
logistical restraints. The reasoning behind this was twofold. Firstly, it would
not have been pragmatic or financially viable to fly all Sherpas to London for
their baseline testing. Secondly, data from Caudwell Xtreme Everest 2007
(24) had failed to identify any significant differences in participants’ physiology
between the sea level and Kathmandu (1300m) laboratories; thus we believed
it to be scientifically appropriate to use these two distinct locations for baseline
testing. Lastly the notable deficit in Sherpas tested on descent in Kathmandu
should be highlighted. Forty-six Sherpas were not tested in Kathmandu
having previously been tested at EBC. This was the result of logistical
constraints.
In conclusion, this study suggests that adaptation to hypoxia in the Sherpa sublingual microcirculation involves increasing both microcirculatory blood flow and capillary density. In turn, teleological reasoning would suggest that this results in a greater oxygen delivery both per unit time, and per unit volume of tissue. It remains unclear whether these microvascular alterations are restricted to the sublingual microcirculation, or what underlying biochemical and physiological factors facilitate the changes in blood flow and vessel density, and further work is required to explore these questions.
FOOTNOTES

ACKNOWLEDGEMENTS

Members of the Xtreme Everest 2 Research Group are as follows:


Members of the Xtreme Everest 2 Research Scientific Advisory Board:

Feelisch, E Gilbert-Kawai, M Grocott (chair), M Hanson, D Levett, D Martin, K Mitchell, H Montgomery, R Moon, A Murray, M Mythen, M Peters.

GRANTS AND DISCLOSURES

Xtreme Everest 2 is a research project coordinated by the Xtreme Everest Hypoxia Research Consortium, a collaboration between the University College London Centre for Altitude, Space, and Extreme Environment Medicine, the Centre for Human Integrative Physiology at the University of Southampton and Duke University Medical Centre.
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AUTHORS CONTRIBUTIONS

EGK, MG and DM were involved in the conception and design of the study, EGK, JC, JC, JVK, AND AV performed experiments; EGK and JC analyzed data; EGK and DM interpreted results of experiments; EGK and DM prepared
figures; EGK, MG and DM drafted the manuscript; all authors approved final
version of manuscript.
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**Figure 1.** Small vessel (<25µm) microvascular flow index (MFI) in Sherpas and Lowlanders on ascent to and descent from high altitude

<table>
<thead>
<tr>
<th>Site</th>
<th>Microvascular Flow Index</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>0.0</td>
</tr>
<tr>
<td>Everest Base Camp</td>
<td>1.0</td>
</tr>
<tr>
<td>Kathmandu</td>
<td>0.0</td>
</tr>
</tbody>
</table>

- * = Significant difference demonstrated between cohorts at that site.
- ^ = Significant difference demonstrated for that cohort between relevant site and BL.

Fig 1. Ascent to high altitude caused Sherpa microvascular flow index (MFI) to increase from Baseline, whilst Lowlanders’ decreased (^). A significant difference is demonstrated between Sherpa and Lowlanders small vessel MFI at Everest Base Camp (*).
**Figure 2.** Small vessel (<25μm) heterogeneity index in Sherpas and Lowlanders on ascent and descent from high altitude

* = Significant difference demonstrated between cohorts at that site.

^ = Significant difference demonstrated for that cohort between relevant site and BL.

Fig 2. Sherpas heterogeneity index (HI) was seen to decrease at Everest Base Camp (EBC) and Kathmandu (KTM) compared to Baseline (BL), whilst Lowlanders increased at EBC (^). A significant difference was seen between cohorts HI at EBC and KTM (*).
**Figure 3.** Small vessel (<25μm) density in Sherpas and Lowlanders on ascent and descent from high altitude

* = Significant difference demonstrated between cohorts at that site.

^ = Significant difference demonstrated for that cohort between relevant site and BL.

Fig 3. Sherpas small vessel density (VD) can be seen to increase at Everest Base Camp (EBC), and remains higher than Baseline values on return to Kathmandu (KTM) (^). Lowlanders VD increases at EBC (^), but then returns to Baseline values on return to KTM. At EBC, Sherpas demonstrate a significantly larger VD compared to Lowlanders (*).
Figure 4: Depiction of the changes in sublingual small vessel density and microvascular flow occurring on ascent to, and descent from, high altitude.

Fig 4. On ascent to high altitude, Sherpas are seen to increase dramatically both their small vessel density, and microvascular flow in a uniform and homogenous manner. On re-exposure to normoxia, flow returns to previous baseline values, but whilst vessel density decreases, it still remains greater than initial baseline values. Lowlanders also increase their small vessel density on ascent to high altitude but to a far lesser extent than Sherpas. Their microvascular flow decreases, however not in a uniform manner such that it has become heterogenous in nature. On re-exposure to normoxia, both vessel density and flow return to baseline values.
**Table 1:** Demographic summary of participants

<table>
<thead>
<tr>
<th></th>
<th>Sherpas</th>
<th>Lowlanders</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>64</td>
<td>69</td>
</tr>
<tr>
<td><strong>Gender (% male)</strong></td>
<td>47%</td>
<td>39%</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>27.9 (±6.9)</td>
<td>41.3 (±13.9)</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>160 (±6)</td>
<td>171 (±10)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>71.1 (±13.5)</td>
<td>61.3 (±8.9)</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>14 (±21%)</td>
<td>6 (±8.6%)</td>
</tr>
</tbody>
</table>

Values for age, height, weight and smokers are presented as mean value (± standard deviation).
Table 2: Laboratory environmental conditions

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Altitude (m)</th>
<th>Barometric pressure (kPa)</th>
<th>Temperature (°C)</th>
<th>Humidity (%)</th>
<th>PO_2 (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>London</td>
<td>35</td>
<td>100.6 (±0.2)</td>
<td>16.9 (±1.8)</td>
<td>35.4 (±6.5)</td>
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</tr>
<tr>
<td>Everest Base Camp</td>
<td>5300</td>
<td>53.0 (±0.2)</td>
<td>12.9 (±8.2)</td>
<td>37.8 (±17.5)</td>
<td>11.0</td>
</tr>
<tr>
<td>Kathmandu</td>
<td>1300</td>
<td>86.8 (±0.4)</td>
<td>23.8 (±3.4)</td>
<td>47.4 (±15.7)</td>
<td>18.1</td>
</tr>
</tbody>
</table>

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: Laboratory temperature and partial pressure of oxygen according to study cohort

<table>
<thead>
<tr>
<th></th>
<th>Baseline^</th>
<th>Everest Base Camp</th>
<th>Kathmandu (Descent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sh</td>
<td>LL</td>
<td>Sh</td>
</tr>
<tr>
<td>Laboratory temperature, °C</td>
<td>16.9 (±1.8)</td>
<td>22.6 (±3.2)</td>
<td>12.6 (±8.4)</td>
</tr>
<tr>
<td>PIO₂, kPa</td>
<td>16.8</td>
<td>19.8</td>
<td>9.8</td>
</tr>
</tbody>
</table>

^ Baseline testing for Lowlanders (LL) was in London, and baseline testing for Sherpas (Sh) was in Kathmandu. Both cohorts were tested in Kathmandu on descent. Values are mean (± standard deviation).
Table 4: Physiological variables for participants during the study

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>HR</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>SpO₂ (%)</th>
<th>Hb (g/l)</th>
<th>Hct (%)</th>
<th>Core temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sh</td>
<td>LL</td>
<td>Sh</td>
<td>LL</td>
<td>Sh</td>
<td>LL</td>
<td>Sh</td>
<td>LL</td>
</tr>
<tr>
<td>Baseline</td>
<td>69 (10)</td>
<td>64 (9)</td>
<td>121 (19)</td>
<td>81 (10)</td>
<td>79 (10)</td>
<td>94 (9)</td>
<td>95 (13)</td>
<td>97 (1)</td>
</tr>
<tr>
<td>Everest Base Camp</td>
<td>87 (10)</td>
<td>77 (14)</td>
<td>125 (16)</td>
<td>89 (11)</td>
<td>86 (8)</td>
<td>101 (11)</td>
<td>101 (10)</td>
<td>78 (5)</td>
</tr>
<tr>
<td>Kathmandu</td>
<td>75 (13)</td>
<td>68 (12)</td>
<td>112 (15)</td>
<td>75 (9)</td>
<td>80 (8)</td>
<td>87 (8)</td>
<td>95 (10)</td>
<td>95 (7)</td>
</tr>
</tbody>
</table>

Fig 4. The mean (± standard deviation) values for Sherpa (Sh) and Lowlander (LL) heart rate (HR), Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), peripheral oxygen saturations (SpO₂), haemoglobin concentration (Hb), haematocrit (Hct), and core temperature at each laboratory.