Articles in PresS. J Appl Physiol (January 26, 2017). doi:10.1152/japplphysiol.00970.2016

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

AUTHORS

Edward Gilbert-Kawai[1], Jonny Coppel[1], Jo Court[1], Jildou van der Kaaij[1], Andre Vercueil[1], Martin Feelish[1,3], Denny Levett[1-3], Monty Mythen[1], Michael P Grocott[1-3], and Daniel Martin[1] for the Xtreme Everest 2 Research Group.

AFFILIATIONS

1. University College London Centre for Altitude Space and Extreme Environment Medicine, UCLH NIHR Biomedical Research Centre, Institute of Sport and Exercise Health, First Floor, 170 Tottenham Court Road, London, W1T 7HA, UK

2. Anaesthesia and Critical Care Research Area, NIHR Respiratory Biomedical Research Unit, University Hospital Southampton NHS Foundation Trust, CE93, MP24, Tremona Road, Southampton, SO16 6YD, UK

3. Integrative Physiology and Critical Illness Group, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, CE93, MP24, Tremona Road, Southampton, SO16 6YD, UK

RUNNING HEAD

Sherpa microcirculation at high altitude

CORRESPONDING AUTHOR:

Copyright © 2017 by the American Physiological Society.

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

Edward Gilbert-Kawai

University College London Centre for Altitude Space and Extreme Environment Medicine, UCLH NIHR Biomedical Research Centre, Institute of Sport and Exercise Health, First Floor, 170 Tottenham Court Road, London, W1T 7HA, UK

e.gilbert@ucl.ac.uk

Tel: +44 2034472838

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

ABSTRACT

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.

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

106 microcirculatory blood flow and vessel density as a means to maintain oxygen
107 delivery to the peripheral tissues.

108

109 **MATERIALS AND METHODS**

110 **Participant selection**

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

121

122 **Study setting**

123 XE2 (29) was conducted from December 2012 to May 2013, and this was one
124 of the individual studies conducted on the research expedition. Sublingual
125 microcirculatory data were collected at three locations: 'Baseline testing' (BL),
126 'Everest Base Camp' (EBC) (5300m), and on descent in 'Kathmandu' (KTM)
127 (1300m). BL testing was conducted in London (LON) for Lowlanders (35m),
128 and in KTM (1300m) for Sherpas. Having departed from KTM, all participants
129 followed an identical ascent and descent profile. This consisted of a flight from

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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'.

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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/mm^2).

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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/m^2 [11.41-14.52] vs. 10.52 mm/m^2 [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 SpO_2).

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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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,

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

299 blood flow in the sublingual vessel was seen to reduce significantly in 12
300 lowland subjects (30), and similar data were recorded in a further 24 lowland
301 subjects on ascent to 5300m (28).

302 The Lowlander MFI data presented supports the findings of Martin et al. (28,
303 30) whereby flow decreased upon ascent to high altitude. In these
304 manuscripts it was theorised that the slowing microcirculatory blood flow
305 demonstrated could in fact be an adaptive response, applied to increase the
306 erythrocyte tissue transit time and improve oxygen diffusion. This is
307 conceivable since a prolonged course through the capillary network may
308 enhance offloading of oxygen in the presence of a reduced partial pressure
309 gradient between the capillary and mitochondria, particularly when cardiac
310 output is high, as is the case during exercise. Sherpas by contrast, seem to
311 utilise brisk flow to maintain localised oxygen delivery. This in turn may
312 explain the lower Hb concentration that this population demonstrate after
313 prolonged exposure to hypobaric hypoxia (4). Undoubtedly, increased Hb
314 concentration augments CaO_2, however, elevated Hct increases blood
315 viscosity, alters its rheology and at levels greater than 50% may decrease
316 cardiac output and oxygen delivery (43). Furthermore, elevated Hct
317 demonstrated in South American resident populations, are associated with an
318 increased prevalence of chronic mountain sickness and related embolic or
319 thrombotic events (33). In contrast, it seems Sherpas favour a blunted
320 erythropoetic response thereby allowing for brisk microvascular blood flow.

321 The speed of microcirculatory blood flow *per se* may also be less important
322 than its nature. Maintaining a homogenous microcirculatory blood flow,
323 irrespective of the speed at which the contained blood may flow, could be

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

(>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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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 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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

398 the sublingual circulation is within the oral cavity, and as such it is regarded as
399 being at a similar temperature to one's core, the data demonstrated no
400 differences in the two cohorts' tympanic temperatures (Table 4). Other
401 potential confounding factors specific to the high altitude environment include
402 hydration status which in turn may affect Hct values, and thus alter blood
403 rheology. The effect of this potential confounding factor was minimised by
404 conducting studies at all altitudes after a period of overnight rest and ensuring
405 subjects had free access to oral fluids and were actively encouraged to drink
406 enough fluid to produce normal volumes of clear urine. Finally, ascent to
407 altitude may cause tissue oedema (27), which if occurring in the sublingual
408 mucosa could theoretically affect image quality and lead to false
409 measurements of flow and density. Baseline testing was conducted in London
410 for Lowlanders (35m), and in Kathmandu (1300m) for Sherpas due to
411 logistical restraints. The reasoning behind this was twofold. Firstly, it would
412 not have been pragmatic or financially viable to fly all Sherpas to London for
413 their baseline testing. Secondly, data from Caudwell Xtreme Everest 2007
414 (24) had failed to identify any significant differences in participants' physiology
415 between the sea level and Kathmandu (1300m) laboratories; thus we believed
416 it to be scientifically appropriate to use these two distinct locations for baseline
417 testing. Lastly the notable deficit in Sherpas tested on descent in Kathmandu
418 should be highlighted. Forty-six Sherpas were not tested in Kathmandu
419 having previously been tested at EBC. This was the result of logistical
420 constraints.
421

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

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.

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

FOOTNOTES

ACKNOWLEDGEMENTS

Members of the Xtreme Everest 2 Research Group are as follows: S Abraham, T Adams, W Anseeuw, R Astin, B Basnyat, O Burdall, J Carroll, A Cobb, J Coppel, O Couppis, J Court, A Cumptsey, T Davies, S Dhillon, N Diamond, C Dougall, T Geliot, E Gilbert-Kawai, G Gilbert-Kawai, E Gnaiger, M Grocott, C Haldane, P Hennis, J Horscroft, D Howard, S Jack, B Jarvis, W Jenner, G Jones, J van der Kaaij, J Kenth, A Kotwica, R Kumar BC, J Lacey, V Laner, D Levett, D Martin, P Meale, K Mitchell, Z Mahomed, J Moonie, A Murray, M Mythen, P Mythen, K O'Brien, I. Ruggles-Brice, K Salmon, A Sheperdigian, T Smedley, B Symons, C Tomlinson, A Vercueil, L Wandrag, S Ward, A Wight, C Wilkinson, S Wythe.

Members of the Xtreme Everest 2 Research Scientific Advisory Board: M 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.

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

Xtreme Everest 2 was supported by the Royal Free Hospital NHS Trust Charity, the Special Trustees of University College London Hospital NHS Foundation Trust, the Southampton University Hospital Charity, the UCL Institute of Sports Exercise and Health, The London Clinic, University College London, University of Southampton, Duke University Medical School, the United Kingdom Intensive Care Society, the National Institute of Academic Anaesthesia, the Rhinology and Laryngology Research Fund, The Physiological Society, Smiths Medical, Deltex Medical, Atlantic Customer Solutions and the Xtreme Everest 2 volunteer participants who trekked to Everest Base Camp.

Some of this work was undertaken at University College London Hospital-University College London NIHR Biomedical Research Centre, which received a proportion of funding from the United Kingdom Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme. Some of this work was undertaken at University Hospital Southampton-University of Southampton NIHR Respiratory Biomedical Research Unit, which received a proportion of funding from the United Kingdom Department of Health's National Institute for Health Research Biomedical Research Units funding scheme.

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

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

478 figures; EGK, MG and DM drafted the manuscript; all authors approved final
479 version of manuscript.

480

481

Downloaded from http://ajp.physiology.org/ by 10.220.33.6 on February 10, 2017

REFERENCES

1. **Adams WH, Strang LJ.** Hemoglobin levels in persons of Tibetan ancestry living at high altitude. *Proc Soc Exp Biol Med* 149: 1036-1039, 1975
2. **Aldenderfer MS.** Moving up in the world; archaeologists seek to understand how and when people came to occupy the Andean and Tibetan plateaus. *American Science* 91: 542-549, 2003
3. **Aykut G, Ince C, Boerma C.** Cytocam-IDF (incident dark field illumination) imaging for bedside monitoring of the microcirculation. *Intensive Care Medicine Experimental* 3: 1-10, 2015
4. **Beall CM, Reichsman AB.** Hemoglobin levels in a Himalayan high altitude population. *Am J Phys Anthropol* 63: 301-306, 1984
5. **Boerma EC, Mathura KR, van der Voort PH, Spronk PE, Ince C.** Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Crit Care* 9: R601-6, 2005
6. **Chen QH, Ge RL, Wang XZ, Chen HX, Wu TY, Kobayashi T, Yoshimura K.** Exercise performance of Tibetan and Han adolescents at altitudes of 3,417 and 4,300 m. *J Appl Physiol* 83: 661-667, 1997
7. **Creteur J, Vincent JL.** Monitoring the microcirculation in the critically ill patient: current methods and future approaches. *Intensive Care Med* 36: 1813-1825, 2010
8. **De Backer D, Hollenberg S, Boerma C, Goedhart P, Buchele G, Ospina-Tascon G, Dobbe I, Ince C.** How to evaluate the microcirculation: report of a round table conference. *Crit Care* 11: R101, 2007
9. **De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent JL.** Monitoring the microcirculation in the critically ill patient: current methods and future approaches. *Intensive Care Med* 36: 1813-1825, 2010
10. **Dobbe J SG, Atasever B, Van Zijderveld R, Ince C.** Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. *Medical and Biological Engineering and Computing* 46: 659-670, 2008
11. **Dubin A, Pozo MO, Casabella CA, Murias G, Palizas FJ, Moseinco MC, Kanoore Edul VS, Palizas F, Estenssoro E, Ince C.** Comparison of 6% hydroxyethyl starch 130/0.4 and saline solution for resuscitation of the microcirculation during the early goal-directed therapy of septic patients. *J Crit Care* 25: 659.e1-8, 2010
12. **Dubin A, Pozo MO, Ferrara G, Murias G, Martins E, Canullan C, Canales HS, Kanoore Edul VS, Estenssoro E, Ince C.** Systemic and microcirculatory responses to progressive hemorrhage. *Intensive Care Med* 35: 556-564, 2009
13. **Elbers PW, Ince C.** Mechanisms of critical illness--classifying microcirculatory flow abnormalities in distributive shock. *Crit Care* 10: 221, 2006
14. **Erzurum SC, Ghosh S, Janocha AJ, Xu W, Bauer S, Bryan NS, Tejero J, Hemann C, Hille R, Stuehr DJ, Feelisch M, Beall CM.** Higher blood flow and circulating NO products offset high-altitude hypoxia among Tibetans. *Proc Natl Acad Sci U S A* 104: 17593-17598, 2007

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

15. **Ge R.** Comparisons of oxygen transport between Tibetan and Han residents at moderate altitude. *Wilderness & Environmental Medicine* 6: 391-400, 1995
16. **Genzel-Boroviczeny O, Christ F, Glas V.** Blood transfusion increases functional capillary density in the skin of anemic preterm infants. *Pediatr Res* 56: 751-755, 2004
17. **Gilbert-Kawai E, Coppel J, Bountziouka V, Ince C, Martin D.** A comparison of the quality of image acquisition between the incident dark field and sidestream dark field video-microscopes. *BMC Med Imaging* 16: 10, 2016
18. **Gilbert-Kawai E, Sheperdigian A, Adams T, Mitchell K, Feelisch M, Murray A, Peters M, Gilbert-Kawai G, Montgomery H, Levett D, Kumar R, Mythen M, Grocott M, Martin D.** Design and conduct of Xtreme Everest 2: An observational cohort study of Sherpa and lowlander responses to graduated hypobaric hypoxia. *F1000Res* 4: 90, 2015
19. **Gilbert-Kawai ET, Milledge JS, Grocott MP, Martin DS.** King of the mountains: Tibetan and Sherpa physiological adaptations for life at high altitude. *Physiology (Bethesda)* 29: 388-402, 2014
20. **Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG.** Orthogonal polarization spectral imaging: a new method for study of the microcirculation. *Nat Med* 5: 1209-1212, 1999
21. **Ince C.** Sidestream dark field imaging: an improved technique to observe sublingual microcirculation. *Critical Care* 9: 72-75, 2005
22. **Kayser B, Hoppeler H, Claassen H, Cerretelli P.** Muscle structure and performance capacity of Himalayan Sherpas. *J Appl Physiol* 70: 1938-1942, 1991
23. **Kingma BR, Frijns AJ, Saris WH, van Steenhoven AA, van Marken Lichtenbelt WD.** Cold-induced vasoconstriction at forearm and hand skin sites: the effect of age. *Eur J Appl Physiol* 109: 915-921, 2010
24. **Levett DZ, Martin DS, Wilson MH, Mitchell K, Dhillon S, Rigat F, Montgomery HE, Mythen MG, Grocott MP.** Design and conduct of Caudwell Xtreme Everest: an observational cohort study of variation in human adaptation to progressive environmental hypoxia. *BMC Med Res Methodol* 10: 98, 2010
25. **Levett DZ, et al.** The role of nitrogen oxides in human adaptation to hypoxia. *Scientific Reports* 1: 109-113, 2011
26. Levick R, *Cardiovascular Physiology: An introduction*. Butterworth-Heinemann, 2009.
27. **Maggiorini M, Buhler B, Walter M, Oelz O.** Prevalence of acute mountain sickness in the Swiss Alps. *BMJ* 301: 853-855, 1990
28. **Martin DS, Goedhart P, Vercueil A, Ince C, Levett DZH, Grocott MPW.** Changes in sublingual microcirculatory flow index and vessel density on ascent to altitude. *Experimental Physiology* 95: 880-891, 2010
29. **Martin DS, Gilbert-Kawai E, Levett DZ, Mitchell K, Kumar Bc R, Mythen MG, Grocott MP.** Xtreme Everest 2: unlocking the secrets of the Sherpa phenotype? *Extrem Physiol Med* 2: 30, 2013
30. **Martin DS, Ince C, Goedhart P, Levett DZ, Grocott MP.** Abnormal blood

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

flow in the sublingual microcirculation at high altitude. *Eur J Appl Physiol* 106: 473-478, 2009

31. **Massey MJ, Larochelle E, Najarro G, Karmacharla A, Arnold R, Trzeciak S, Angus DC, Shapiro NI.** The microcirculation image quality score: development and preliminary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. *J Crit Care* 28: 913-917, 2013
32. **McGill HCJ.** Smoking and the pathogenesis of atherosclerosis. *Adv Exp Med Biol* 273: 9-16, 1990
33. **Monge C, Leon-Velarde F.** Physiological adaptation to high altitude: oxygen transport in mammals and birds. *Physiol Rev* 71: 1135-1172, 1991
34. **Parthasarathi K, Lipowsky HH.** Capillary recruitment in response to tissue hypoxia and its dependence on red blood cell deformability. *Am J Physiol* 277: H2145-57, 1999
35. **Petersen SM, Greisen G, Hyttel-Sorensen S, Hahn GH.** Sidestream dark field images of the microcirculation: intra-observer reliability and correlation between two semi-quantitative methods for determining flow. *BMC Med Imaging* 14: 14, 2014
36. **Pozo MO, Kanoore Edul VS, Ince C, Dubin A.** Comparison of different methods for the calculation of the microvascular flow index. *Crit Care Res Pract* 2012: 102483, 2012
37. **Sakr Y, Chierego M, Piagnerelli M, Verdant C, Dubois MJ, Koch M, Creteur J, Gullo A, Vincent JL, De Backer D.** Microvascular response to red blood cell transfusion in patients with severe sepsis. *Crit Care Med* 35: 1639-1644, 2007
38. **Samaja M, Veicsteinas A, Cerretelli P.** Oxygen affinity of blood in altitude Sherpas. *J Appl Physiol* 47: 337-341, 1979
39. **Schneider A, Greene RE, Keyl C, Bandinelli G, Passino C, Spadacini G, Bonfichi M, Arcaini L, Malcovati L, Boiardi A, Feil P, Bernardi L.** Peripheral arterial vascular function at altitude: sea-level natives versus Himalayan high-altitude natives. *J Hypertens* 19: 213-222, 2001
40. **Thompson-Torgerson CS, Holowatz LA, Flavahan NA, Kenney WL.** Cold-induced cutaneous vasoconstriction is mediated by Rho kinase in vivo in human skin. *Am J Physiol Heart Circ Physiol* 292: H1700-5, 2007
41. **Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, Arnold RC, Colilla S, Zanotti S, Hollenberg SM.** Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Ann Emerg Med* 49: 88-98, 98.e1, 2007
42. **Trzeciak S, Rivers EP.** Clinical manifestations of disordered microcirculatory perfusion in severe sepsis. *Crit Care* 9 Suppl 4: S20-6, 2005
43. **Winslow RM, Monge CC, Brown EG, Klein HG, Sarnquist F, Winslow NJ, McKneally SS.** Effects of hemodilution on O2 transport in high-altitude polycythemia. *J Appl. Physiol* 59: 1495-1502, 1985

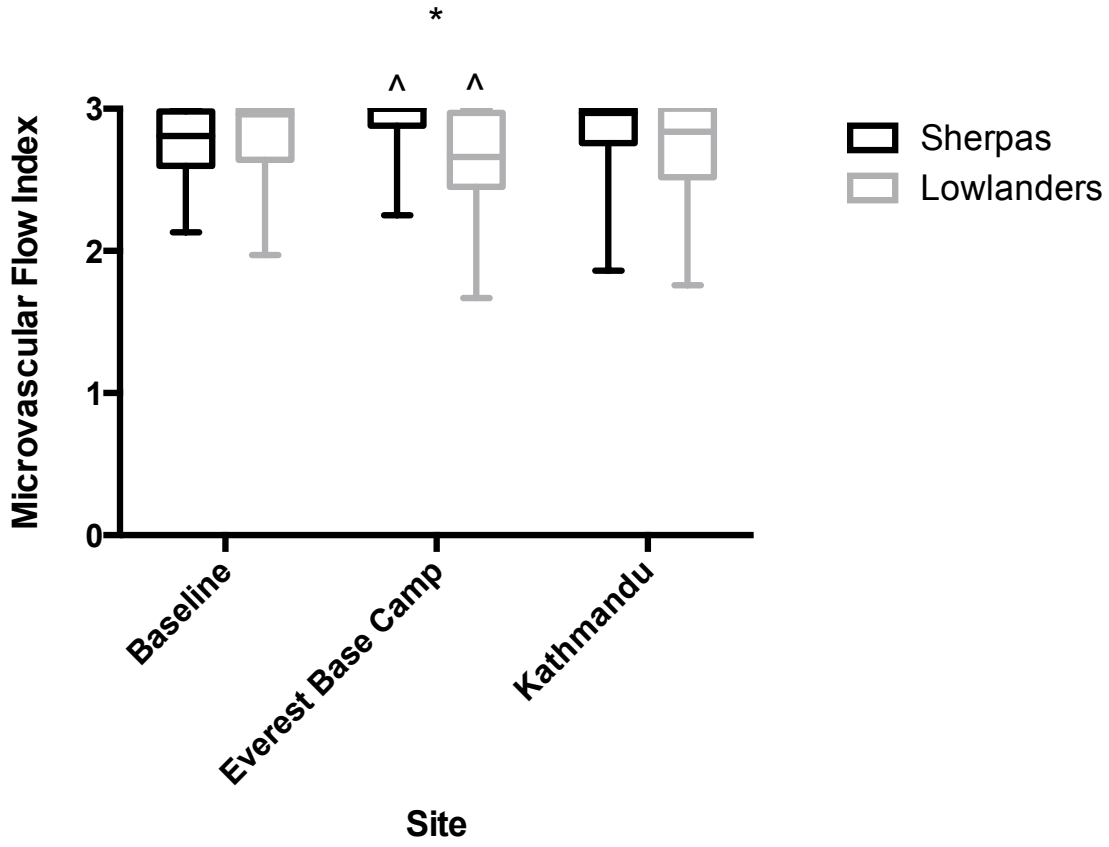
Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

44. **Wu T, Wang X, Wei C, Cheng H, Wang X, Li Y, Ge-Dong, Zhao H, Young P, Li G, Wang Z.** Hemoglobin levels in Qinghai-Tibet: different effects of gender for Tibetans vs. Han. *J Appl.* 98: 598-604, 2005

45. **Yuruk K, Almac E, Bezemer R, Goedhart P, de Mol B, Ince C.** Blood transfusions recruit the microcirculation during cardiac surgery. *Transfusion* 51: 961-967, 2011

Downloaded from http://ajp.physiology.org/ by 10.220.33.6 on February 10, 2017

Figure 1. Small vessel (<25μm) microvascular flow index (MFI) in Sherpas and Lowlanders on ascent to and descent from high altitude

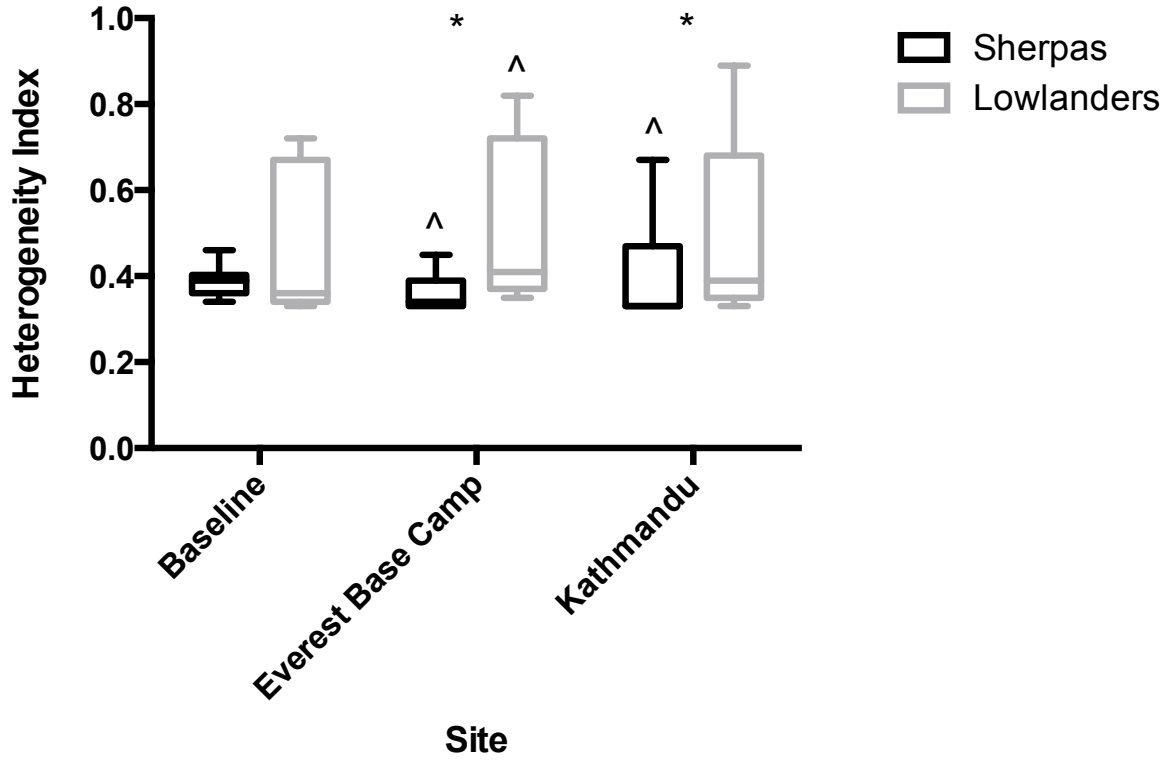


* = Significant difference demonstrated between cohorts at that site.

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

Fig 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

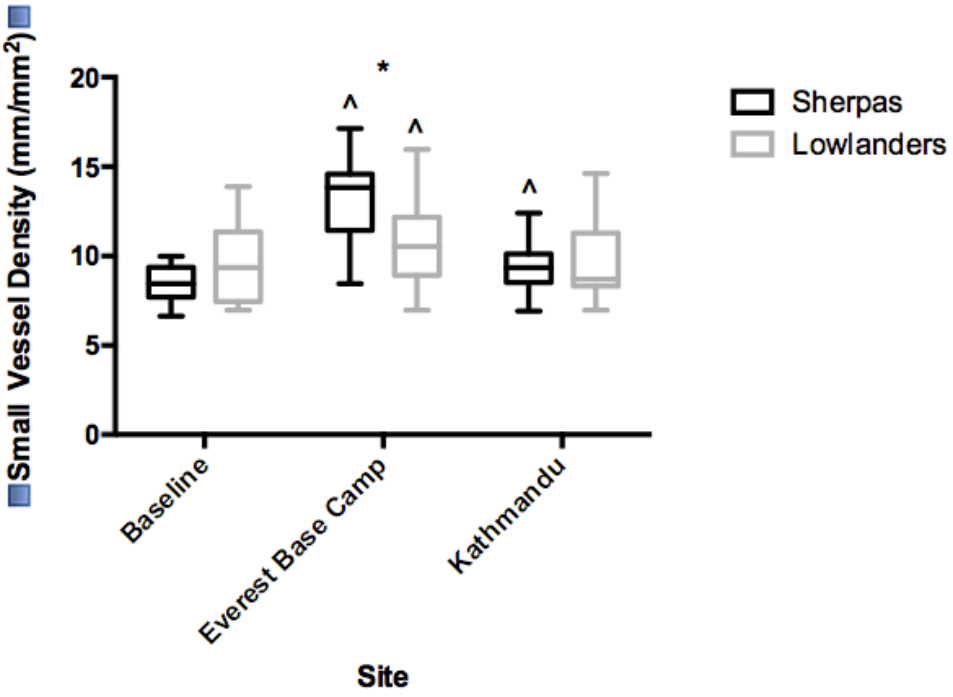


* = Significant difference demonstrated between cohorts at that site.

^ = 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.

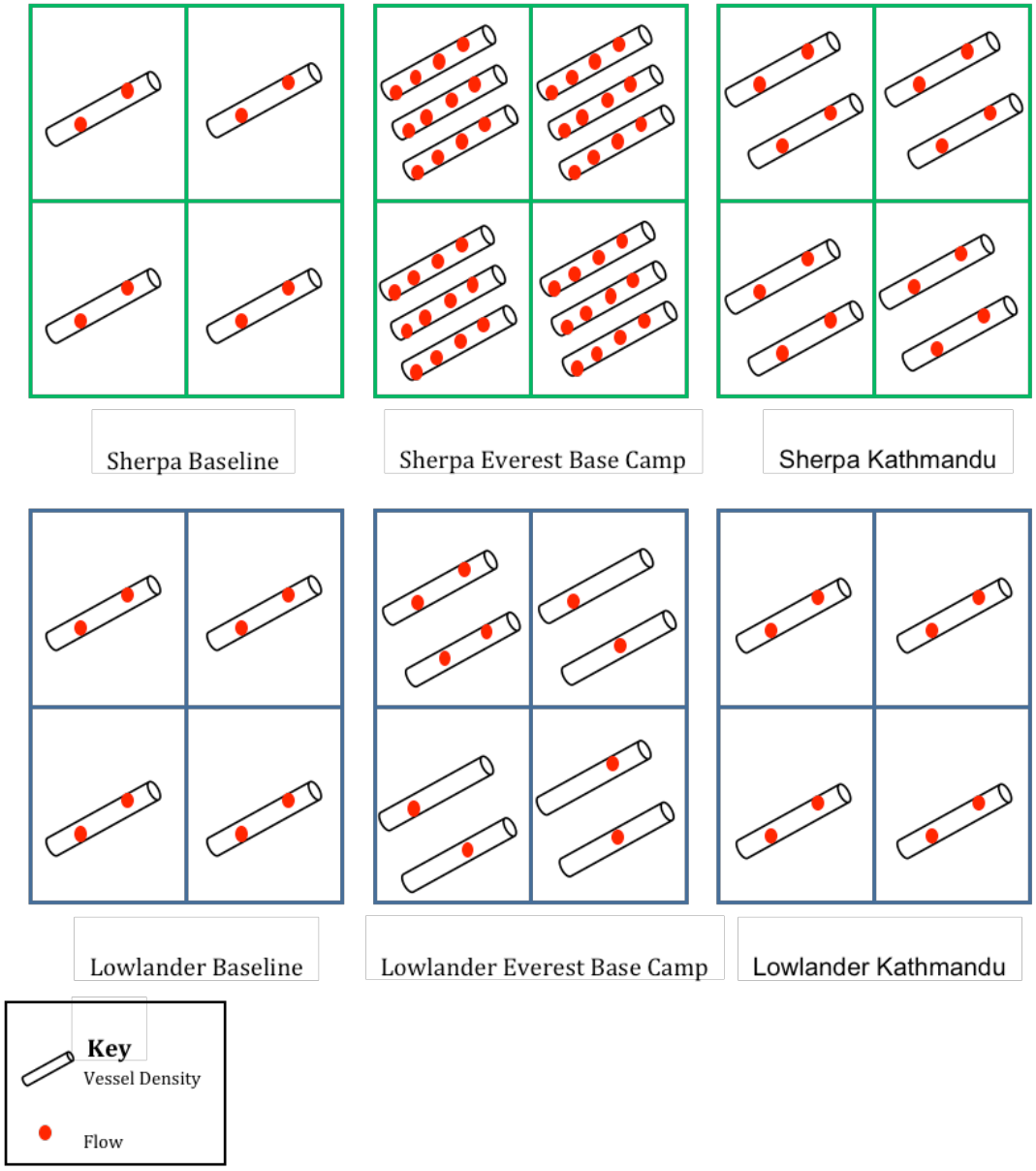


Fig 4. On ascent to high altitude, Sherpas are seen to increase dramatically both their small vessel density, and microvascular flow in a uniform and homogenous manner. On re-exposure to normoxia, flow returns to previous baseline values, but whilst vessel density decreases, it still remains greater 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

	Sherpas	**Lowlanders**
Number	64	69
Gender (% male)	47%	39%
Age (years)	27.9 (±6.9)	41.3 (±13.9)
Height (cm)	160 (±6)	171 (±10)
Weight (kg)	71.1 (±13.5)	61.3 (±8.9)
Smokers	14 (±21%)	6 (±8.6%)

Values for age, height, weight and smokers are presented as mean value (± standard deviation).

Table 2: Laboratory environmental conditions

Laboratory	**Altitude (m)**	**Barometric pressure (kPa)**	**Temperature (°C)**	**Humidity (%)**	**PO_2 (kPa)**
London	35	100.6 (±0.2)	16.9 (±1.8)	35.4 (±6.5)	21.0
Everest Base Camp	5300	53.0 (±0.2)	12.9 (±8.2)	37.8 (±17.5)	11.0
Kathmandu	1300	86.8 (±0.4)	23.8 (±3.4)	47.4 (±15.7)	18.1

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

	Baseline^		**Everest Base Camp**		**Kathmandu (Descent)**	
	Sh	**LL**	**Sh**	**LL**	**Sh**	**LL**
Laboratory temperature, °C	16.9 (±1.8)	22.6 (±3.2)	12.6 (±8.4)	12.9 (±7.9)	23.8 (±3.3)	24.1 (±3.1)
P_IO_2, kPa	16.8	19.8	9.8	9.8	16.8	16.8

^ 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

Laboratory	HR		SBP (mmHg)		DBP (mmHg)		MAP (mmHg)		SpO_2 (%)		Hb (g/l)		Hct (%)		Core temperature (°C)	
	Sh	LL	Sh	LL	Sh	LL	Sh	LL	Sh	LL	Sh	LL	Sh	LL	Sh	LL
Baseline	69 (10)	64 (9)	121 (10)	127 (19)	81 (10)	79 (10)	94 (9)	95 (13)	97 (1)	98 (1.2)	137 (16)	141 (14)	43 (4.5)	43 (3.9)	36.3 (0.5)	36.3 (0.4)
Everest Base Camp	87 (10)	77 (14)	125 (13)	132 (16)	89 (11)	86 (8)	101 (11)	101 (10)	78 (5)	79 (5.3)	151 (17)	153 (20)	49 (4.5)	48 (5.7)	36.2 (0.6)	36.1 (0.7)
Kathmandu	75 (13)	68 (12)	112 (8)	122 (15)	75 (9)	80 (8)	87 (8)	95 (10)	95 (7)	97 (1.4)	140 (13)	145 (20)	43 (3.4)	46 (5.8)	36.1 (0.4)	36.2 (0.5)

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 (SpO_2), haemoglobin concentration (Hb), haematocrit (Hct), and core temperature at each laboratory.

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017

Downloaded from http://jap.physiology.org/ by 10.220.33.6 on February 10, 2017