

Abnormal blood flow in the sublingual microcirculation at high altitude

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Abstract We report the first direct observations of deranged microcirculatory blood flow at high altitude, using sidestream dark-field imaging. Images of the sublingual microcirculation were obtained from a group of 12 volunteers during a climbing expedition to Cho Oyu (8,201 m) in the Himalayas. Microcirculatory flow index (MFI) was calculated from the moving images of microcirculatory red blood cell flow, and comparison was made between the baseline and high altitude measurements. Peripheral oxygen saturation (SpO_2) and Lake Louise scores (LLS) were recorded along with MFI. Our data demonstrate that there was a significant reduction in MFI from baseline to 4,900 m in small (less than 25 μm) and medium (26–50 μm) sized blood vessels ($P = 0.025$ and $P = 0.046$, respectively). There was no significant correlation between MFI and SpO_2 or MFI and LLS. Disruption of blood flow within microcirculatory may explain persistent abnormal oxygen flux to tissues following the normalisation of systemic oxygen delivery that accompanies acclimatisation to high altitude.

Keywords Hypoxia · Microcirculation · Altitude · Oxygen

Introduction

The delivery of oxygen to tissues results from the combined processes of convective flow within the systemic circulation and the microcirculation followed by diffusion along a partial pressure gradient towards metabolising cells. The microcirculation consists of the smallest vessels of the circulatory system, the arterioles, capillaries, and venules measuring less than 100 μm in diameter. Whilst adaptations of systemic oxygen delivery in response to chronic hypoxia at high altitude are well described, few studies have attempted to explore how the peripheral microcirculation adjusts to such a challenge. This is, at least in part, due to the technical difficulties of observing and quantifying the peripheral component of the oxygen flux process.

Recent advances in technology have facilitated in vivo visualisation of the human microcirculation (Groner et al. 1999; Slaaf et al. 1987). Sidestream dark-field (SDF) imaging allows direct examination of mucosal blood vessels using a hand-held microscope (Ince 2005). Tissue is illuminated by green light emitted from a concentric ring of flashing light emitting diodes (LEDs) at the tip of the microscope probe. The light has a wavelength of 548 nm, an isosbestic point within the absorptive spectra of oxy- and deoxyhaemoglobin, to maximize light absorption by haemoglobin regardless of its oxygenation status (Goedhart et al. 2007). Reflected light is captured on a charge-coupled camera device after passing through the lens that lies within the core of the light guide. The lens is optically isolated from the LEDs to prevent light reflected from the mucosal surface distorting images. The improvement in image quality over a preceding technique, orthogonal polarization spectral (OPS) imaging, has led to the widespread use of SDF imaging clinically (Goedhart et al. 2007;

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Ince 2005). The sublingual mucosa is commonly chosen as a region for clinical studies since no invasive procedures are necessary to access this part of the circulatory system (Boerma et al. 2005; De Backer et al. 2002, 2004; Trzeciak and Rivers 2005).

Animals studies have shown that exposure to chronic normobaric hypoxia in a laboratory setting resulted in impaired blood flow and reduced functional capillary density within the microcirculation of skeletal muscle (Saldivar et al. 2003). This finding is supported by data from other animal models (Fisher et al. 1992; Vicaut et al. 1987) but no similar data are available from human studies.

We hypothesised that blood flow in the sublingual microcirculation, quantified by the microcirculatory flow index (MFI) from SDF imaging, would be reduced in healthy volunteers exposed to environmental hypobaric hypoxic at high altitude.

Methods

Subject selection

Approval for this study was obtained from the University College London Committee on the Ethics of Non-NHS Human Research. Written informed consent was obtained from all participants. Data were collected from 12 subjects; 8 males and 4 females (mean age 34.4 years). Subjects, all members of a medical research expedition, were studied at baseline and increasing altitudes as they ascended a mountain in the Himalayas (Cho Oyu, 8,201 m). All subjects were medically screened prior to the expedition, by a doctor experienced in high altitude medicine, to confirm absence of intercurrent illness.

Study setting

Baseline studies of the sublingual microcirculation were obtained in London (75 m) or Kathmandu (Nepal, 1,300 m) depending on the availability of the subject. Further images were recorded at 4,900, 5,600 and 6,400 m after 7, 17 and 30 days at altitude, respectively. The relatively rapid ascent to 4,900 m over a period of 7 days followed by a slower ascent to 5,600 m and then 6,400 m was the result of expedition logistics.

Observation of the sublingual microcirculation

The sublingual microcirculation was visualised using the Microscan SDF camera (Microvision Medical, Amsterdam, The Netherlands). All images were obtained by a single investigator, experienced in this technique (DM). Subjects were required to rest for 10 min with a closed mouth prior

to the investigation. The investigator then positioned and focused the SDF camera under their tongue whilst the subject held their mouth in a semi-closed position in order to help to steady the camera. Studies were made during the day and subjects sheltered from any extremes of temperature and light. Once a clear image had been obtained a sequence of 10-s duration was recorded onto a digital video camera (Sony MV960). Four such recordings were made from different areas of the sublingual region each time a subject underwent investigation. Images were stored on digital video tapes then converted into an electronic computer compatible format (DV-AVI) for analysis.

Other physiological variables recorded

Peripheral arterial oxygen saturation (SpO₂) was measured for each subject at the time of investigation using a pulse oximeter (Onyx 9500, Nonin, USA). A symptom diary incorporating a validated scoring system for assessing the presence of altitude related illness, the Lake Louise Score (LLS), was completed daily (Roach 1993). Details of any medications being taken were also recorded.

Analysis and scoring of microcirculatory video images

Two assessors blinded to subject identifiers, altitude of measurement and physiological co-variables, independently assessed each stored video image. The 10-s video image was 'looped' on a computer screen and blood flow in small (<25 μm), medium (26–50 μm) and large (51–100 μm) blood vessels were graded according to a previously described semi-quantitative score, the MFI (Boerma et al. 2005; De Backer et al. 2007). Flow was graded as 0 for no flow, 1 for intermittent flow, 2 for slow flow and 3 for continuous/normal flow. The analysis screen was divided into four equal quadrants using a superimposed fine grid and flow was assessed separately in each quadrant. The mean of the values from each quadrant was then calculated for each category of vessel size for each film. A subject's overall MFI for small, medium and large vessels was generated from the mean values of each of the four films taken at any one altitude.

Analysis plan

Baseline MFI was compared to MFI at the three higher altitudes (4,900, 5,600 and 6,400 m). MFI is ordinal data and was analysed using a non-parametric test (Wilcoxon Signed Ranks test). Comparison between MFI and other variables such as LLS and SpO₂ was performed using non-parametric correlation (Spearman's rank correlation coefficient). A *P* value lower than 0.05 was considered to be statistically significant.

Results

A complete data set of microcirculatory images was obtained from all 12 subjects at baseline and 4,900 m. Images were also obtained in subgroups ascending higher, with nine sets of images at 5,600 m and four at 6,400 m. The SDF camera recorded images faultlessly at the maximum altitude (6,400 m). Typical images captured from the same subject at baseline (75 m) and high altitude (4,900 m) can be seen in Figs. 1 and 2, respectively. An example of video footage of sublingual microcirculatory blood flow from the same subject at baseline (75 m) and 6,400 m on Cho Oyu can be found on <http://www.sdfimaging.net/dsmsealevelmicrocirc.wmv> and <http://www.sdfimaging.net/dsm6400metermicrocirc.wmv>.

Median and interquartile ranges (Q25 and Q75) for small vessel MFI were 3.00 (3.00–3.00) at baseline, 2.88 (2.47–2.95) at 4,900 m, 3.00 (2.80–3.00) at 5,600 m and 2.33 (2.00–2.75) at 6,400 m. For medium sized vessels, these values were 3.00 (3.00–3.00), 3.00 (2.57–3.00), 3.00 (2.71–3.00) and 2.50 (2.00–3.00), respectively. Mean (\pm SD) SpO₂ was 97.5% (\pm 0.8) at baseline, 83.8% (5.3) at 4,900 m and 80.8% (\pm 4.3) at 5,600 m. No SpO₂ values were obtained at 6,400 m due to equipment failure. A LLS was obtained from 10 of the 12 subjects at 4,900 m and 6 of the 9 subjects at 5,600 m. The median LLS at 4,900 m was 3.0 whilst at 5,600 m, it was 0.5.

Box plots for MFI in small and medium sized vessels at baseline, 4,900 and 5,600 m are shown in Figs. 3 and 4, respectively. Large sized vessels were removed from the final analysis as so few were visualised, even at baseline, that analysis was not possible (66.6% of subjects had no large vessels visible at baseline). Figure 5 depicts individual changes in MFI from baseline to 4,900 m.

There was a significant reduction in MFI within small and medium sized vessels between baseline and 4,900 m

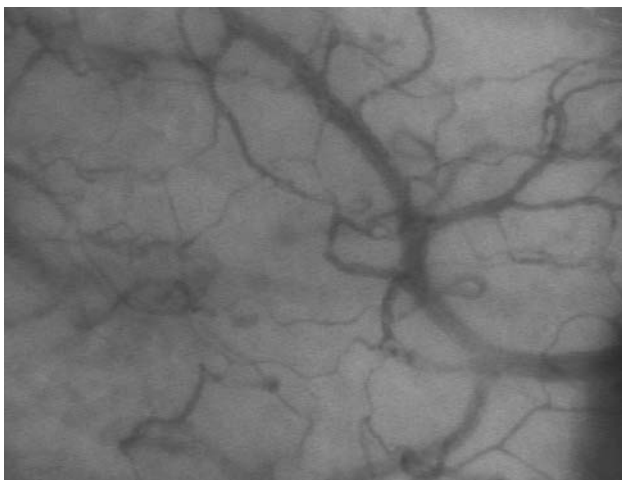


Fig. 1 Typical still image captured from video footage of sublingual microcirculatory blood flow at baseline (75 m)

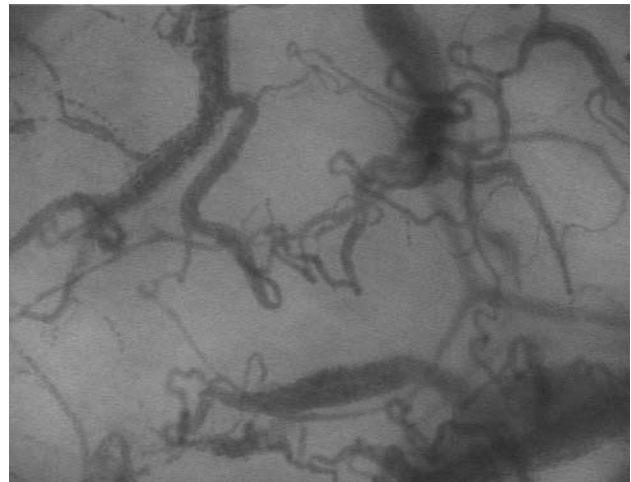


Fig. 2 Typical still image captured from video footage of sublingual microcirculatory blood flow at altitude (4,900 m)

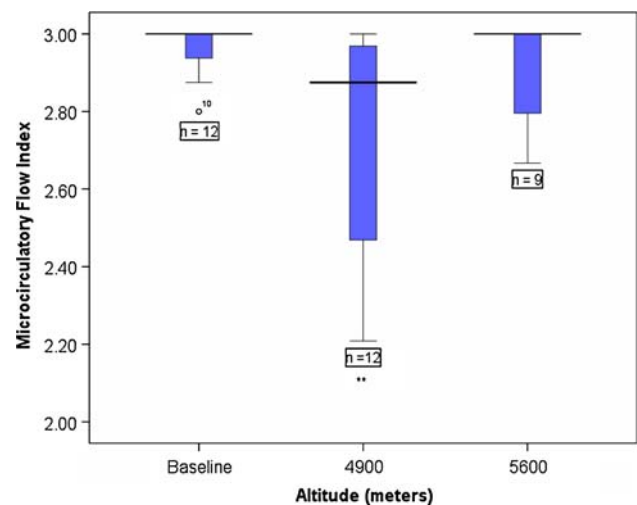


Fig. 3 Boxplots of small vessel (<25 μ m) microcirculatory flow index at baseline, 4,900 and 5,600 m. Boxplots were generated from the MFI five number summaries; maximum and minimum represented by the outer horizontal whiskers, first (Q25) and third (Q75) quartiles by the shaded box and the median by the horizontal line transecting the box. The number '10' at baseline indicates the subject number of a distinct outlier. **Significantly different from baseline ($P < 0.05$)

($P = 0.025$ and $P = 0.046$, respectively) but not between baseline and 5,600 m or baseline and 6,400 m. SpO₂ was significantly lower than baseline at 4,900 m ($P = 0.002$) and 5,600 m ($P = 0.008$), however there was no difference between SpO₂ at 4,900 and 5,600 m ($P = 0.261$). There was no significant difference in the LLS between 4,900 and 5,600 m ($P = 0.496$). At 4,900 m, three subjects were taking paracetamol, one of whom was taking aspirin and another individual was taking ibuprofen. No medication was being taken at 5,600 m and no data were available for 6,400 m.

There was a significant negative correlation between small vessel MFI and LLS at 5,600 m but not at 4,900 m

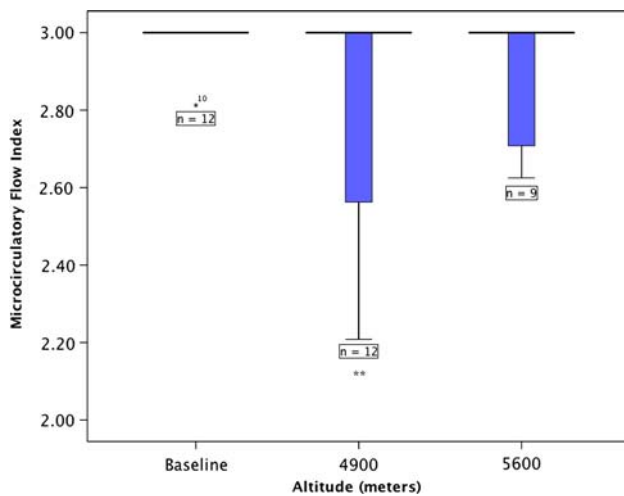


Fig. 4 Boxplot of medium vessel (26–50 μm) microcirculatory flow index at baseline, 4,900 and 5,600 m. See Fig. 3 for main legend. The number '10' at baseline indicates the subject number of a distinct outlier. **Significantly different from baseline ($P < 0.05$)

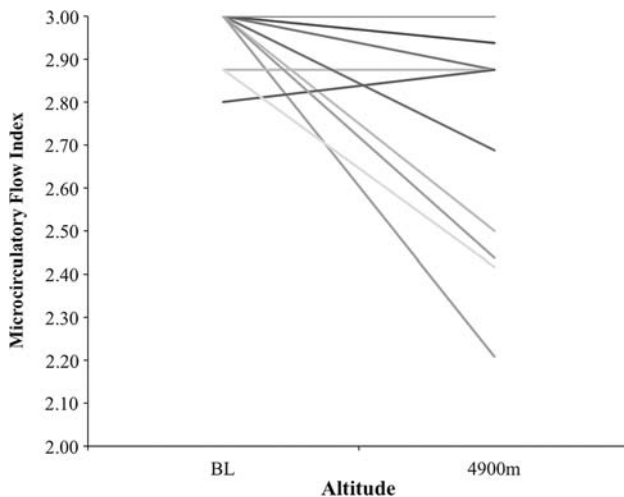


Fig. 5 Graph depicting individual reduction in small vessel microcirculatory flow index from baseline (BL) to 4,900 m in the cohort of 12 subjects. Two of the plotted lines cannot be seen as they follow the same changes as another individual (from 3.00 at BL to 3.00 at 4,900 m)

nor with medium vessel MFI at either altitude. There was no correlation between MFI and SpO_2 or between LLS and SpO_2 at high altitude.

Discussion

This is the first report of direct visualisation of the sublingual microcirculation at high altitude. We have demonstrated that it is possible to use SDF imaging equipment in a remote environment and obtain good quality digital video images from which the microcirculation can be quantitatively

assessed. These data showed a significant reduction in MFI at high altitude (4,900 m) when compared with baseline in small (<25 μm) and medium (26–50 μm) sized blood vessels. The results also show a further reduction in MFI within small and medium vessels at extreme altitude (6,400 m). Such is the degree of abnormal blood flow in the SDF films collected from subjects at 6,400 m in this study, that it is clearly noted even by untrained observers (clips can be seen online at <http://www.sdfimaging.net/dsmsealevelmicrocirc.wmv> and <http://www.sdfimaging.net/dsm6400metermicrocirc.wmv>). Although difficult to see on still images, the higher blood flow in baseline images can be seen as blurring of red blood cells within the vessels (Fig. 1) which is not as apparent in a still frame from 4,900 m in the same subject (Fig. 2).

We speculate that the surprising lack of difference between baseline and 5,600 m may be due to the subjects being better acclimatised (adapted) at this altitude. There was a 10-day period between measurements taken at 4,900 and 5,600 m following a relatively rapid ascent to 4,900 m. This idea is supported by a trend towards a lower median LLS at 5,600 m than at 4,900 m (0.5 and 3.0, respectively). Small and medium vessel MFI at 6,400 m (median values of 2.33 and 2.50, respectively) was dramatically reduced in comparison with baseline (median values of 3.00 and 3.00, respectively). However, this did not meet the criterion for statistical significance within the small group in whom data were available at 6,400 m ($n = 4$), likely due to the small sample size at this altitude (type I error).

This pilot study was limited by a number of constraints; primarily the small number of subjects available for investigation. Furthermore, data collection was incomplete at altitude due to the inevitable logistical and medical problems prevalent on high altitude expeditions. Ideally, a complete data set during ascent would have been obtained. Due to the constraints of field investigations, data collection occurred at altitudes convenient to the expedition rather than altitudes selected for physiological purposes. Finally, the small number of co-variables measured limited interpretation of the observations of abnormal microcirculation.

The analysis of film clips displaying microcirculatory blood flow is a new and evolving field. Interpretation of microcirculatory moving images relies on subjective quantification of flow and validity in the reporting of results is essential. Several methods have been used to report findings and debate continues as to which is the most appropriate (De Backer et al. 2007). Although the calculation of MFI relies on subjective assessment of flow by trained observers, it has been shown to be a robust technique with high Kappa coefficient scores for interrater variability (Boerma et al. 2005). Computer software has recently been developed to automate the analysis of microcirculation film images, but as yet has not been validated for this purpose (Dobbe et al. 2008). Furthermore,

the current absence of a reliable continuous measure of flow from microcirculatory films precludes the use of parametric statistics.

One explanation for the observed reduction in microcirculatory blood flow might be the rise in haematocrit observed with chronic exposure to high altitude (Winslow et al. 1984). However, we were unable to measure haemoglobin or haematocrit during this study in order to confirm this hypothesis. At the time of recording images subjects had been at altitude (above 1,300 m) for: 7 days at 4,900 m, 17 days at 5,600 m and 30 days at 6,400 m; adequate time for their haematocrit to rise significantly from sea level values. Results from this study suggest that changes in MFI were not directly related to individual SpO₂ or the presence of AMS identified by the LLS; the lack of statistical significance could again be due to a type I error.

The sublingual circulation receives the majority of its blood supply from the lingual artery, a branch of the external carotid artery. However, the sublingual circulation shares an embryological origin with the splanchnic mucosa. Therefore, it is not clear which major circulatory system the sublingual circulation represents. The value of monitoring this area of the peripheral circulation is demonstrated by the fact that early changes in the sublingual microcirculatory flow are a sensitive and specific predictor of poor outcome in septic critically ill patients (Sakr et al. 2004; Trzeciak and Rivers 2005). Although the observed reduction in median small vessel MFI between baseline and 6,400 m in this study may appear small (3.00–2.33), this can be compared to a group of critically ill patients with abdominal sepsis whose median MFI was 2.08 on the day of diagnosis and 2.66 after 3 days of active treatment (Boerma et al. 2007). In this latter study, two patients had died by day 3 of the study, demonstrating the severity of systemic illness associated with impaired microvascular blood flow.

Stagnant hypoxia may occur in tissues as a result of reduced microcirculatory blood flow and consequent failure of oxygen mass transfer. Furthermore, disparity of oxygen supply and demand at a microvascular level could lead to heterogeneous tissue oxygenation and cellular hypoxia (Ince and Sinaasappel 1999). The observations reported in this study were restricted to the sublingual microcirculation in resting subjects. However, if the changes reported were a systemic phenomenon we speculate that a similar reduction in MFI may be occurring in skeletal muscle, as previously noted by a reduction in microvascular red blood cell velocity in hypoxic animal models (Saldivar et al. 2003). Persistence of this phenomenon during exercise at altitude may contribute to the well-documented persistent reduction in exercise capacity and oxygen consumption that accompanies prolonged altitude exposure (Cerretelli 1976; Pugh 1964; Sutton et al. 1988; West et al. 1983).

Disruption of blood flow within the microcirculation may therefore be a key component in understanding performance limitation at altitude and this merits further investigation.

Conclusion

This first study of the sublingual microcirculation at high altitude demonstrates reduced flow in small and medium sized vessels at 4,900 m when compared to baseline. Further work to thoroughly explore these observations and the mechanisms and consequences of disordered microcirculatory flow at altitude is required.

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Conflict of interest statement C. Ince is, besides a professor at the Academic Medical Center (AMC), the chief scientific officer of a company called MicroVision Medical. MicroVision Medical is a university-based company dedicated to the development of optical spectroscopic tools for study of the microcirculation and tissue oxygenation, among which is the Microscan. In this context C. Ince holds patents and shares.

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