Enhanced flow-motion complexity of skin microvascular perfusion in Sherpas and lowlanders during ascent to high altitude

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ABSTRACT

An increased and more effective microvascular perfusion is postulated to play a key role in the physiological adaptation of Sherpa highlanders to the hypobaric hypoxia encountered at high altitude. To investigate this, we used Lempel-Ziv complexity (LZC) analysis to explore the spatiotemporal dynamics of the variability of the skin microvascular blood flux (BF) signals measured at the forearm and finger, in 32 lowlanders (LL) and 46 Sherpa highlanders (SH) during the Xtreme Everest 2 expedition. Measurements were made at baseline (BL) (LL: London 35m; SH: Kathmandu 1300m) and at Everest base camp (LL and SH: EBC 5,300m). We found that BF signal content increased with ascent to EBC in both SH and LL. At both altitudes, LZC of the BF signals was significantly higher in SH, and was related to local slow-wave flow-motion activity over multiple spatial and temporal scales. In SH, BF LZC was also positively associated with LZC of the simultaneously measured tissue oxygenation signals. These data provide robust mechanistic information of microvascular network functionality and flexibility during hypoxic exposure on ascent to high altitude. They demonstrate the importance of a sustained heterogeneity of network perfusion, associated with local vaso-control mechanisms, to effective tissue oxygenation during hypobaric hypoxia.

INTRODUCTION

Sherpas highlanders (SH) are known to demonstrate considerable tolerance to hypobaric hypoxia, however, the mechanisms behind this adaptation are not well understood[1]. Previous studies have shown that SH exhibit a lower arterial oxygen content than lowlanders (LL) who ascend to comparable altitudes[2-4], and thus it has been suggested that the mechanisms that facilitate SH apparent hypoxia tolerance reside, in part, within the microcirculation[5-8]. Adaptive SH microcirculatory mechanisms that are not evident in LL include a sustained microvascular perfusion and dilator capacity measured in the skin[8,9] and a higher microvascular flow index (MFI) and reduced perfusion heterogeneity index (HI) in the small (<25 μm) vessels of the sublingual mucosa[7,8].

Blood flow within a microcirculatory network is highly variable and its distribution and magnitude determined by local and regional metabolic demand. Local control of blood flow ensures matching of perfusion to spatially varying oxygen demand, thereby achieving efficient oxygen delivery[10,11]. Regulation of microvascular perfusion is predominately

achieved through changes in network conductance, modulated at a local level by endothelial, neurogenic, and myogenic regulatory activity[12]. Together, these activities determine the cyclic oscillations of arteriolar diameter (vasomotion) that are related to changes in blood flow distribution in the microvascular networks (flow-motion)[13]. The relative contribution of this rhythmic, low frequency oscillatory activity, as well as higher frequency cardiac and respiratory rhythms to microvascular perfusion has been assessed using spectral analysis of blood flux (BF) signals obtained using non-invasive laser Doppler fluximetry in the skin[14]. This has led to the suggestion that measurement of flow-motion activity may provide an early indicator of declining function (for review see[53]). In SH, the sustained network perfusion seen on exposure to hypobaric hypoxia has been associated with an enhanced low frequency flow-motion activity that was not evident in LL[8,9]. However, the extent to which such changes in local low-frequency oscillatory activity contributes to the observed variability in microvascular blood flow under conditions of hypobaric hypoxia[7,8] remains unexplored; as does the extent to which such oscillatory activity may be beneficial to tissue oxygenation under such conditions[11,16,17].

Nonlinear complexity measures have been extensively used to assess the variability or loss of randomness of bio-signals notably electroencephalograms[18] and electrocardiograms[19]. The spatiotemporal variability of these signals reflect the physiological adaptability of the system and are established biomarkers of overall health status[20]. We and others have used non-linear analysis, such as Lempel-Ziv (LZ) complexity[21], to determine the information content of microcirculatory blood flow signals as a metric of perfusion variability[22,23]. To date, these measures have confirmed a declining regularity and randomness of microcirculatory BF signals in both a primate model of diabetes[25] and in humans with or at risk of cardiovascular disease (CVD) in whom microvascular dysfunction is evident[26]. To what extent the variability in the BF signals arising from the cumulative activity (and temporal variation) of all the processes modulating microvascular blood flow differs between SH and LL, and with ascent to altitude, has yet to be explored; as does the impact of the complexity of the BF signal on effective tissue oxygenation.

The processes that regulate the cardiovascular system operate across multiple temporal scales. Consequently, multiscale nonlinear analysis of complex oscillatory signals such as heart rate variability has been used, in addition to traditional time- and frequency-domain analysis, as a tool to enhance diagnosis and risk stratification[26]. The rhythmical oscillatory processes that determine flow-motion, and that operate across differing time-scales in the range ~0.6-100 seconds, have similarly been explored using multiscale non-linear analysis of the laser Doppler BF signal [22,23,27]. To date these studies have shown multiscale

complexity analysis to be a good predictor of the variability of the signal over multiple time scales[22,23,27] and that they may provide novel mechanistic insight into the extent to which the BF signal is modulated by the different frequency bands under differing (patho)physiological conditions [24,25].

The primary objective of the current study was to investigate the complexity of the oscillatory rhythms in microvascular blood flow and tissue oxygenation signals obtained in the skin of individuals ascending to 5300m as participants of the XE2 research expedition[28], using non-linear uni- and multi-scale approaches. We hypothesised that non-linear approaches will provide novel information on the processes by which SH preserve superior peripheral microcirculatory perfusion at altitude[7-9]. We also sought to explore whether an enhanced complexity of the BF signal was associated with the more efficient oxygen delivery postulated to play a key role in the physiological adaptation of Sherpas to hypobaric hypoxia[5-8].

Microvascular blood flux was measured non-invasively using laser Doppler fluximetry at two skin sites; that of the well characterised ventral forearm under both endothelium dependent and neurovascular control, and that of the finger pulp in which BF is largely determined by the abundantly present arteriovenous anastomoses under sympathetically mediated constrictor tone[29]. The analysis of signals from both beds allowed us to explore the differential impact of local flow-motion activity on the complexity of microvascular perfusion.

MATERIALS AND METHODS

Study Participants

The signals analysed are from measurements made in 32 lowlanders (LL) all of whom were born and lived below 1000m, and were not from a native high altitude population, and 46 Sherpa highlanders (SH). These 78 individuals represent a subset of the 144 participants of the Xtreme Everest 2 research expedition (XE2)[28] in whom we have previously reported the effects of hypobaric hypoxia on microvascular blood flow in the time and spectral domains[7]. Approval of the study design, risk management plan and protocol were obtained from both the University College London Research Ethics Committee (Ref: 3750/002) and the Nepal Health Research Council (NHRC) (1334). The study was performed to the standards set by the Declaration of Helsinki, except for registration in a database. All participants provided written informed consent for participation in the studies.

Tissue Blood Flux and Oxygenation Signal Capture

Skin microvascular blood flux (BF) and tissue oxygenation (OXY) signals, and skin temperature were recorded simultaneously at the forearm using a combined laser Doppler fluximetry (LDF) and white light reflectance spectroscopy probe (CP1T-1000 LDF™/OXY™/temperature probe, Moor Instruments Ltd, Axminster, UK) placed approximately 10 cm proximal to the wrist. A second combined LDF™/temperature probe (VP1T, Moor, Axminster, UK) was placed on the pulp of the index finger. Probe position was recorded with a photograph and permanent marker pen to ensure use of the same anatomical site in subsequent measurements as previously described[7].

Signals were collected at baseline (BL) in London for LL (35m), and in Kathmandu (1300m) for SH, and at Everest Base Camp (EBC) (5300m) as described previously[8]. Measurements were made on the morning of day 2 after arrival at EBC after an 11 day trek from Lukla (2800m). Participants lay in a supine position during signal capture with their non-dominant arm resting at heart level. Full details of the environmental and physiological measurements made at each altitude are given elsewhere[7].

Signal Analysis

All signals were captured at a 40Hz sampling rate using the manufacturer's software (moorVMS-PC software, Moor Instruments Ltd, Axminster, UK) and a continuous 10 min sample of each signal extracted for analysis in the time and spectral domain. The signals analysed were resting skin blood flux (BF, in arbitrary perfusion units, PU) and oxygenated Hb (oxyHb, in arbitrary units, AU). We elected to focus on the oxyHb output as the prime tissue oxygenation signal for the complexity analysis as suggested by our previous studies[22].

The complexity of the BF and oxyHb signals arising from rhythmical flow-motion activity was explored using non-linear LZ complexity (LZC) analysis[21] to determine how much the BF and oxyHb signals differed from a random sequence of finite symbolic sequences derived from the time series[20,30]. The signals were represented as a binary string using delta encoding whereby a zero is recorded if a value is less than the previous value in the time series and a one when it is greater than that previously[22,23]. The LZC is a measure of the information content, or effort to describe, a signal and is the length of the shortest instruction set required to reconstruct it without loss of information. A random signal would have high complexity as there are no rules that define it whereas a periodic signal would have low complexity as the same terms are repeated continually. LZC analysis does not require the

signal to be strongly stationary, unlike chaos-based entropy analysis and thus the signal can be normalised to the length of the sample window[31]. Exhaustive LZC, where the signal is decomposed into all the instructions required to reproduce it, was then calculated for each 40 second epoch to determine the complexity lower bound. A complexity index (LZC index) was calculated as the mean of the 15 × 40 second epochs for each sampled signal to provide an index of the dynamic activity modulating the BF and oxyHb signals.

The physiological processes that modulate flow-motion and determine the information content of the BF signals operate at frequencies ranging from 0.001Hz to 2Hz[14] . They also appear to vary with altitude[8,9]. To take account of these multiple, and potentially varying, process scales we measured LZC in multiple time-scales (MLZC) using a coarse-graining approach[25,32,45]. MLZC was explored between scales τ = 1-12, as the maximum frequency of interest is governed by an upper limit of heart rate of 1.6 Hz and Nyquist frequency twice this or 3.2 Hz which corresponds to scale τ = 12. To determine the association between the complexity of the BF signal and the mechanisms underlying flow-motion we calculated the Spearman correlations for relative power spectral density of the endothelial (0.0095-0.02 Hz), sympathetic (0.02-0.06 Hz), myogenic (0.06-0.15 Hz), respiratory (0.15-0.4 Hz), and cardiac (0.4-1.6 Hz)[14] spectral bands and LZC measured at each time scale (MLZC).

Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 25.0 (IBM, USA). Data were tested for Gaussian distribution using the Kolmogorov-Smirnov test and visual inspection of histograms. As the BF signal data did not show a normal distribution, data are presented as median (95%CI). SH and LL cohorts at BL and EBC were compared using two-way ANOVA. We used Mann Whitney U test and Wilcoxon Signed Rank test to compare single cohorts between BL and EBC. The relationships between BF, oxyHb and network perfusion complexity at each site were assessed individually using Spearman's rank correlation coefficient. A p-value of <0.05 was considered statistically significant for all analyses. We used multivariable linear regression models to explore factors that were independently associated, with microvascular network perfusion heterogeneity and oxygenation as the dependent (outcome) variables. Explanatory variables included in the models were skin temperature, age and gender, with site included as a binary indicator variable.

RESULTS

Artefact-free skin BF and tissue oxygenation signals (**Fig 1**) of sufficient length for LZC analysis at both BL and EBC, were obtained in 32 LL (16M/16F), age 46(14)y, BMI 24.3(3.3) kg/m^2 (mean(SD)) and 46 SH (23M/23F), age 28(6)y ($p=0.0001$, SH vs LL) and BMI 23.8(3.4) kg/m^2 ($p>0.05$, SH vs LL) who represented a subset of the 144 participants from the Xtreme Everest 2 research expedition (XE2)[28].

The time domain characteristics of microcirculatory BF signals from the current subset of XE2 individuals used for complexity analysis are summarised in **Table 1**. Resting skin BF (PU) at the forearm of the 46 SH decreased with ascent to EBC, while that of the 32 LL remained unchanged, as reported previously in the full cohort[8]. In both sub-groups, resting BF at the finger decreased on ascent to EBC compared to BL (both $p<0.001$). Skin temperature varied between groups and with ascent to EBC but was notably higher at the finger in SH at both BL and EBC (Table 1). In both groups resting BF were positively associated with skin temperature at BL at the forearm (LL, $r= 0.370$ $p=0.037$; SH, $r= 0.577$ $p<0.0001$) and at the finger (LL, $r= 0.436$ $p=0.013$; SH, $r=0.764$ $p<0.0001$). This association was lost at EBC in LL ($p>0.05$ for forearm and finger), but not in SH (forearm $r=0.261$ $p=0.021$; finger $r=0.643$ $p>0.0001$).

Peripheral oxygen saturation (SpO_2) declined with ascent to EBC in both LL and SH ($p<0.001$), as did tissue oxygenation (StO_2) measured at the forearm using VMSOXY ($p<0.001$)[8].

LZ complexity of microcirculatory blood flux and oxygenation signals increases with hypobaric hypoxia

The complexity of the BF and oxyHb signals arising from oscillatory flow-motion activity was determined using non-linear LZ complexity (LZC) analysis. Examination of the BF signals obtained from SH and LL at BL and on ascent to EBC, revealed that the information content of the BF signals remained relatively constant over the 15 epochs (600s) analysed in both groups under normoxia and hypobaric hypoxia. As previously, it was thus deemed appropriate to additionally present the complexity of each signal as a mean LZC index[22,23]. **Fig 2** shows that the complexity of resting BF signals measured at the forearm and finger in SH and LL remained relatively constant over the 15 epochs sampled, at both BL and EBC. LZC index (mean LZC of 15 epochs) of the BF signal in both the forearm and finger microvascular beds was significantly influenced by group (forearm: $p=0.0001$, $F=15.6$; finger: $p<0.0001$, $F=59.9$), and site (forearm: $p=0.0013$, $F=10.7$; finger $p=0.0025$, $F=9.5$). Forearm

BF LZC index increased in SH with ascent to EBC (BL vs EBC p<0.0031), and at EBC was significantly greater than that of LL (SH vs LL p<0.0001) (**Fig 3)**. There was no association between forearm BF LZC index and resting BF or skin temperature at BL or EBC in SH or LL.

LZC index of the finger BF signal was higher in SH than LL at both BL and EBC (both p<0.0001), but only increased with ascent to EBC in LL (p=0.0413) (**Fig 3**). In SH, finger BF LZC index was positively associated with RF (p<0.001 at both BL and EBC) and skin temperature at both altitudes (p=0.001 at both BL and EBC). No such association was seen in LL.

Neither age nor sex were independently associated with BF LZC index in SH. However, in LL age was negatively associated with forearm BF LZC index (r=-0.491 p=0.004).

We found no significant difference in LZC index of the oxyHb signals measured at the forearm between LL and SH, at either BL or EBC (p>0.05) (**Fig 4**). However, in SH forearm BF LZC index was positively associated with the LZC index of the oxyHb signal at both BL (r=0.414 p=0.005) and EBC (r=0.307 p=0.004). No such association was found in LL at BL (p=0.672), and only approached significance at EBC (r=0.353 p=0.065).

Changes in multiscale LZC (MLZC) at altitude are associated with flow-motion activity

The LZC of the BF signals at the forearm was computed over 12 scales for all LL and SH at BL and EBC (**Fig 5)**. As the scale length increased, so too did LZC, with better separation seen at certain scales. In both SH and LL, the largest differences between the LZC values at BL and EBC were for time scales >10 in both forearm and finger microvascular beds (p<0.05).

Fig 6 shows the association of the power in the five spectral bands to LZC forearm and finger BF complexity at each scale for the two groups at each altitude. There were clear differences in the associations between LZC and spectral power in the five bands both between SH and LL, and between BL and EBC in the forearm and finger BF signals. In LL, cardiac activity negatively associated with forearm BF LZC at all scales at both altitudes. Power in the respiratory band was positively associated with LZC up to scale at BL, but did not reach significance at EBC. In the low frequency bands, the myogenic activity negatively associated with LZC at EBC over scales τ =1-12 (40 - 3.34 Hz sample rate). No significant association was found with power in the neurogenic activity band, and LZC at any scale at either BL or EBC in LL. In SH, there was no association between LZC and spectral power in any band at BL. At EBC, there was a significant negative association between myogenic

activity and forearm BF LZC, and a positive association between neurogenic activity and BF LZC across scales τ = 2-11. No association between forearm BF LZC and the high frequency activity bands was seen at either altitude in SH.

Similar trends were seen in the association between spectral power in the five bands and LZC in the finger BF signals. However, the only associations between BF LZC and spectral power that reached significance in the finger were in LL at BL, where cardiac activity negatively associated with BF LZC, and myogenic activity negatively associated with BF LZC at all scales (**Fig 6**).

DISCUSSION

We have shown that the LZ complexity of the skin microvascular blood flux signals, measured in participants of the XE2 research expedition[28], increased with ascent to EBC (5300m). We observed that SH exhibited a greater level of network complexity and hence capacity for heterogeneous flow distribution than LL, at both BL and EBC. We also found clear differences in the influence of local flow-motion activity on the information content, and hence complexity of the BF signal, between SH and LL on ascent to altitude. Finally, we have shown that in SH, the increased complexity of microvascular network perfusion is associated with an enhanced complexity of the simultaneously sampled oxyHb signals, suggestive of an improved ability in SH to match O_2 demand to local O_2 delivery under conditions of hypobaric hypoxia.

We have previously shown using time and spectral domain signal analysis that SH, when exposed to hypobaric hypoxia, demonstrated superior preservation of peripheral microcirculatory perfusion compared to LL and that physiological differences in local microvasculature myogenic (vasomotor) and neurogenic control may play a key role in that adaptation of SH to high altitude hypobaric hypoxia by sustaining local perfusion and tissue oxygenation. However, a robust and consistent mechanistic description of flow dynamics within the microcirculation cannot be achieved using time domain analysis (in either the resting state or during haemodynamic perturbation) or frequency domain analysis methods alone[15].

Non-linear complexity-based analysis applied to BF signals derived from the peripheral vasculature has been shown to yield additional, and much deeper, understanding of the loss of system flexibility and to enhance risk assessment[25,27]. In the current study, LZC index

306 was higher in SH than LL in both forearm and finger BF signals (**Fig 2** and **3**). It also
307 increased to a greater extent in SH than LL on ascent to altitude. LZC of the BF signal has
308 previously been shown to decline in individuals with or at risk of CVD[25] and in a primate
309 model of diabetes[24]. Similarly, Frisbee et al[32], using chaotic network attractor analysis to
310 explore the spatial and temporal shift in perfusion distribution at successive arteriolar
311 bifurcations within skeletal muscle, have shown an imbalanced and temporally stable
312 distribution of flow through the microvascular network in rodent models of increasing
313 metabolic and CV disease risk. Our current findings suggest that the increase in capacity
314 for variability in the BF signal seen in SH is indicative of an enhanced adaptive capacity at
315 altitude and thus may indicate more effective autoregulation within the microvascular
316 system[22,33,34].
317
318 Distributive alterations and heterogeneity of flow within microvascular networks are critical to
319 an adequate tissue oxygenation[10,11] and oscillatory fluctuations in microvascular network flow
320 have been shown to ensure a more effective tissue oxygenation than would be obtained with
321 a steady blood flow[16]. While we report no change in LZC of the oxyHb signal with ascent to
322 EBC nor differences between SH and LL, we did observe a positive association between the
323 LZC index of forearm BF and LZC index of oxyHb signals in SH, measured simultaneously
324 at the forearm, at both BL and EBC. We additionally found that in SH the enhanced
325 complexity of network perfusion measured at both altitudes was positively associated
326 ($r=0.252$ $p=0.019$) with the previously reported microvascular oxygen unloading rate,
327 measured in the same individuals[8]. Such a relationship was not seen in LL ($p=0.761$). The
328 distribution of O_2 in tissue depends on microvascular network structure and flow and
329 haematocrit distributions, which are all markedly heterogeneous[10]. Disturbed capillary flow
330 patterns have been shown to limit the efficacy of oxygen extraction even in the absence of
331 changes in mean flow[35]. While we are unable to demonstrate a causal relationship between
332 microvascular blood flow, complexity of the perfusion signals and tissue oxygenation, such a
333 relationship would appear consistent with a beneficial adaptation in SH whereby enhanced
334 variability in flow-motion activity gives rise to more effective O_2 unloading.
335
336 The increase in LZC index derived from laser Doppler BF signals from the skin with ascent
337 to EBC, and the differences in variability of the signals between SH and LL, appear at first to
338 be contrary to the decrease in flow heterogeneity index (HI) reported by Gilbert-Kawai et al[7]
339 derived from direct observation of the movement of red blood cells in the buccal mucosal
340 microcirculatory bed using incident dark-field imaging. In this study of 64 SH and 69 LL from
341 XE2, Gilbert-Kawai et al report that in SH sublingual blood flow increases on ascent to high
342 altitude in a ‘’uniform and homogenous manner’’ in vessels <25 µm. By contrast they report

that in LL microvascular flow decreased, but observed that this decrease was ''not in a uniform manner, such that it became heterogeneous in nature''. HI is calculated using a semi-quantitative technique, as the highest site flow velocity minus the lowest site flow velocity divided by the mean of the flow velocities across all sublingual vessels imaged[36]. HI is thus indicative of variations in red blood cell flow velocities across a series of individual capillaries, while LZC index is a measure of the algorithmic complexity of the time series data of the laser Doppler blood flux signal and yields a measure of the variability or predictability of the oscillatory signal. As such, these indices cannot be compared. It should not be neglected that capillary density has been reported to increase under conditions of hypobaric hypoxia[1], and that while Gilbert-Kawai et al[7] report that sublingual small vessel density was not different between the SH and LL at BL testing, capillary density was up to 30% greater in SH at EBC. Taken together, these data from the buccal mucosa suggest that SH maintain a significantly greater microcirculatory flow per unit time and flow per unit volume of tissue than LL at high altitude; an anticipated consequence of which might be greater information content of the BF signals and consequently a higher LZC.

From a signal perspective, variability in BF arises from the cumulative activity of all the processes modulating BF and their temporal variation. Fluctuations in microcirculatory flow occur at different frequencies related to local endothelial (0.0095-0.02Hz), sympathetic (0.02-0.06Hz) activity, myogenic activity in the vessel wall (0.060.15Hz), and modulation by respiratory (0.15-0.4Hz), and heart (0.4-1.6Hz) rates[14]. Examination of the information content of the BF signals from SH and LL revealed a clear and significant different in LZC between the two groups on ascent to altitude that becomes more pronounced at certain timescales (or sampling rates). Furthermore, by examining the association of the individual spectral bands associated with flow-motion with the different time-scales in MLZC, our data provide strong evidence that the influence of the modulators of flow-motion activity differs between SH and LL and with ascent to altitude. The Spearman correlations between the power bands of the forearm BF signals and LZC across multiple time scales shown in **Fig 6** showed that in LL there was a significant contribution of cardiac power compared with SH, at both BL and EBC. Consequently, the BF signal had proportionally more periodic content in LL and the complexity of the signal was reduced as the information content falls. Previous studies in altitude-naive LL and high altitude residents have shown that autonomic function, heart rate variability and respiration rate are differentially affected at high altitude, with LL showing sympathetic activation to modulate the direct vasodilatory effects of hypobaric hypoxia[37,38]. Heart rate variability is also known to contribute to complexity of the BF signal[39,40] and cardiac rhythm to be modulated by respiratory oscillation[41]. This coupling of

379 the two HF components offers a possible partial explanation as to why the MLZC increases
380 with scale in both SH and LL (**Fig 5**).
381
382 The lower frequencies associated with flow-motion (that generally contain most of the power
383 in the signal) also contributed to signal variability. We report a marked uprating of the
384 association between neurogenic power with LZC of BF measured at the forearm at high
385 altitude, which is positively associated in SH. This appears consistent with the preservation
386 of vasoconstrictor response and enhanced neurogenic activity reported in SH at altitude
387 reported previously[8]. Such changes are indicative of an adaptive modulation of sympatho-
388 vagal activity through which SH can better regulate flow, allowing them to stay in a hypobaric
389 atmosphere at lower temperatures without excessive autonomic stress[42]. These adaptations
390 may also contribute to the higher skin temperatures measured in SH. We saw no significant
391 uprating of the association between neurogenic power and LZC of BF at any scale
392 measured at the forearm of LL at high altitude.
393
394 Myogenic activity (vasomotion) is closely associated with effective oxygen delivery[17,43,44] and
395 has been shown to increase in hypobaric hypoxia[8,9]. Consistent with this we observed an
396 increased association of the myogenic power band with LZC of the blood flux signal over
397 multiple time scales in both SH and LL, under conditions of hypobaric hypoxia at EBC. The
398 association of myogenic power with BF LZC was negative, consistent with its periodic
399 nature. While the effect of flow-motion on the transport of oxygen to tissue 'is highly
400 complicated'[45], an increase in the lower frequencies of flow-motion and particularly in
401 vasomotion has been shown to give rise to transients in the partial pressure of O_2 (PO_2) to
402 substantially increase the volume of oxygenated tissue and to oxygenate tissue domains
403 which under steady-state conditions would remain anoxic[11]. Indeed, mathematical modelling
404 suggests that vasomotion activity can change oxygen delivery to tissues by up to eightfold
405 under certain conditions[46]. Clinically, it has been shown that vasomotion is increased in
406 patients with mild peripheral arterial occlusive disease (PAOD) and that those patients with
407 flow-motion had significantly higher tissue oxygen levels than those without, despite similar
408 blood flow[47]. Our data are also consistent with the increase in vasomotion seen in reduced
409 perfusion states such as sepsis and cardiopulmonary bypass and in multiple organ
410 dysfunction and mortality[48-50]. Thus, the increased strength of the association of the
411 myogenic power band activity with LZC, is consistent with vasomotion being an important
412 modulator of gaseous exchange under conditions of hypobaric hypoxia, when the
413 microcirculation may tend towards a critical state.
414

We found little association between forearm BF LZC and the power of the endothelial frequency band in either LL or SH. While the strength of the association in SH was greater at EBC than at BL, it only reached significance at the highest sampling frequency of 40 Hz (τ =1). Thus, while endothelium-mediated flow-motion might be expected to enhance network perfusion and tissue oxygenation, we can as yet draw few conclusions on the role of the endothelium-attributed flow-motion activity within the skin microvasculature at altitude.

This study has several strengths, not least the large and sex balanced group size. While we saw no independent effect of sex or BMI on our outcome measures of complexity in either SH or LL, we did observe a negative effect of age on BF LZC index in LL but not in SH. LL (mean age 46(14)y) were significantly older than the SH (28(6)y) (p=0.0001, SH vs LL) in this subset of participants from XE2, and it is probable that increasing age constitutes a risk factor for declining heterogeneity of microcirculatory network perfusion[51]. A similar age-related reduction in signal complexity has been reported using nonlinear measures applied to cardiac signals[52] and signals derived from large blood vessel (pulse wave velocity)[53].

To the best of our knowledge, this study is the first to analyse the effect of hypobaric hypoxia at high altitude on the microcirculation using complexity-based measures applied to skin microvascular LD blood flux signals. We have previously demonstrated that complexity-based measures can differentiate both between haemodynamic states[22,23] and between groups of individuals at increasing risk of developing microvascular dysfunction[25]. The current study extends and strengthens the utility of these approaches to widely varying cohorts.

We analysed signals from two skin sites that allowed us to explore the differential impact of local flow-motion activity on network flow heterogeneity. Skin is a major thermoregulatory organ sensitive to environmental temperature. The temperature of the London laboratory where baseline measurements were performed in lowlanders was lower than that in Kathmandu where Sherpas were studied. It is therefore probable that this contributed to the lower forearm and finger fluxes seen in Lowlanders at baseline. While both cohorts were exposed to the same laboratory conditions at EBC, finger BF and skin temperature remained significantly higher in SH than LL. This suggests that the physiological differences in SH and LL seen at EBC are independent of external temperature. However, the variations in skin temperature across the cohorts and altitudes may be expected to differentially influence relative flow-motion activity and signal complexity[23] as will differences in haematocrit that have been reported previously[5-7] and which may influence the laser Doppler signal[8].

The paucity of significant associations between LZC and spectral power across the five frequency bands measured at the finger was unexpected, as LZC of the BF signals measured at both the forearm and the finger were higher in SH than LL and both influenced by ascent to altitude. It was also unexpected as skin BF at the finger is largely determined by arteriovenous anastomoses under vasoconstrictor sympathetic control[29] and we have previously shown that ascent to EBC results in differential changes in vasoconstrictor responses and in local flow-motion activity in the low frequency bands in the resting blood flux signals in SH and LL[8].

In exploring the associations between LZC and spectral power of the BF signals, we used fixed non-overlapping intervals to define the spectral bands as described previously by Stefanovska and colleagues[14]. It is unlikely that the boundaries of these frequency intervals remain constant across a cohort of individuals, or for a given individual, under changing conditions of physiological stress. State-dependent fluctuations in frequency intervals may thus give rise to different patterns in complexity within, and across, the cohorts studied. It could be interesting to explore further the impact of time-varying spectral band boundaries on signal complexity, and how this may inform a mechanistic interpretation of the heterogeneity of microvascular network perfusion.

CONCLUSIONS

These data confirm that synchronicity of rhythms in the modulators of microcirculatory blood flux signals, assessed using non-linear complexity analysis, contributes to the heterogeneity of microvascular perfusion. They also go some way to describe the changes in the activity of local and systemic physiological mechanisms that modulate the potentially beneficial adaptation response seen in SH under conditions of hypobaric hypoxia. Together these data suggest that peripheral tissues play an important physiological role in the cardiovascular adaptation to hypoxia, and that this role is better developed in native altitude dwellers than in lowlanders.

In future, it is possible that a combination of time, frequency and complexity analysis will yield a deeper understanding of the loss of system flexibility that may prevent the microvascular networks from adapting to an imposed stressor and provide further insight into the parameters that influence this.

DATA AVAILABILITY STATEMENT

Data supporting this study are openly available from the University of Southampton repository at https://doi.org/10.5258/SOTON/D1076.

ACKNOWLEDGEMENTS

Xtreme Everest 2 is a research project coordinated by the Xtreme Everest Oxygen Research Consortium, collaboration between the UCL Centre for Altitude, Space, and Extreme Environment Medicine, the Centre for Human Integrative Physiology at the University of Southampton and the Duke University Medical Centre. We thank the Xtreme Everest 2 Research Group for its contribution (Members of the group were 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).

COMPETING INTERESTS

MPG serves on the medical advisory board of Sphere Medical Ltd.DSM, MPG and MGM are directors of Oxygen Control Systems Ltd. MPG, DSM, DZHL, MGM & KM serve on the board of the Xtreme Everest Oxygen Research Consortium and MPG and KM serve as trustees of the Xtreme Everest charity.

AUTHOR CONTRIBUTION

EGK, MM, DL, KM, DM and MPG were involved in the conception and design of the study. TD, EGK, KM, DL, SW performed experiments. TD, EGK, DC, MT, AJC and GFC analysed the data. DC, MT, AJC and GFC interpreted the results. DC, MT, AJC and GFC prepared the first draft of the manuscript. All authors were involved in the revision of the draft

manuscript and have approved the final content. All authors agree to be accountable for all aspects of the work in ensuring questions relating to the accuracy and integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify are listed.

FUNDING

No grant funding supported this work; financial contributions were provided by the following organisations: 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 Biomedical Research Centre, which received a proportion of funding from the United Kingdom Department of Health's National Institute for Health Research Biomedical Research Centre's funding scheme. Some of this work was undertaken at University Hospital Southampton, University of Southampton 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. MPG is part funded by the National Institute of Health Research Senior Investigator Scheme.

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677 **TABLES**

678

679 **Table 1.** Summary of peripheral oxygen saturation (SpO_2) and skin blood flux and
680 oxygenation recorded in the time domain at the forearm and finger in lowlanders (LL) and
681 Sherpa highlanders (SH) at baseline (BL) and Everest base camp (EBC, 5300 m). Data are
682 median (95% confidence interval) from n=32 LL and n=46 SH. * BL vs EBC within group, †
683 LL vs SH within site. SpO_2 (%) = peripheral oxygen saturation (%) (Nonin Onyx 9500, Nonin
684 Medical Inc, Minnesota, USA); Skin BF (PU), = resting laser Doppler skin blood flux
685 (perfusion units); StO_2 (%) = tissue oxygen saturation (%); oxyHb (AU) = oxygenated
686 haemoglobin (arbitrary units); Skin Temp (°C) = skin temperature (°C) (moorVMS-OXY,
687 Moor Instruments Ltd, Axminster, UK).

688

Median (95%CI)	**Lowlanders N=32**		**Sherpa Highlanders N=46**	
	BL	**EBC**	**BL**	**EBC**
SpO_2 (%)	98.0(98,99)	77.5*(74,81)	97.2(97,98)	79.1*(78,80)
Skin BF forearm (PU)	11.5(9.1,14.4)	14.1(12.4,15.7)	15.2†(13.2,19.2)	11.2*†(9.2,13.1)
StO_2 forearm (%)	40.9(37.0,45.7)	27.1*(24.4,32.0)	36.3†(34.2,38.2)	28.4*(24.5,30.8)
oxyHb forearm (AU)	6.5(5.6,8.9)	5.9(4.6,8.3)	8.7†(7.3,10.6)	7.2*(5.8,8.7)
Skin BF finger (PU)	46(26,84)	34(21,52)	283†(247,327)	232† (178,278)
Skin Temp forearm (°C)	27.6(26.8,28.2)	28.5(27.6,29.5)	31.4†(30.6,32.7)	28.2*(27.2,30.7)
Skin Temp finger (°C)	23.9(22.7,25.7)	23.7(19.6,25.3)	32.0† (30.0,33.1)	28.2*†(23.6,30.5)

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690

691 **FIGURE LEGENDS**

692

693 **Figure 1.** Examples of (A) skin BF and oxyHb signals from the forearm and (B) skin BF
694 signals from finger measured in the time domain of an individual lowlander and Sherpa
695 highlander at baseline (BL, black) and Everest base camp (EBC, 5300 m, blue).

696

697 **Figure 2.** Lempel-Ziv complexity (LZC) of skin BF signal measured over 15 epochs at the
698 finger and forearm at baseline (BL, black) and Everest base camp (EBC, 5300 m, blue).
699 Mean data (±SEM) are shown as solid lines for n=32 lowlanders and n=46 Sherpa
700 highlanders. Significant difference between groups, *p<0.05, +p<0.001

701

702 **Figure 3.** Lempel-Ziv complexity (LZC) index of skin BF signals measured at the forearm
703 and finger in n=32 lowlanders and n=46 Sherpa highlanders at baseline (BL, black) and
704 Everest base camp (EBC, 5300 m, blue). Data are median and 95%CI. LZC index (mean
705 LZC of 15 epochs) of the BF signal in both the forearm and finger microvascular beds was
706 significantly influenced by group (forearm: p=0.0001, F=15.6; finger: p<0.0001, F=59.9), and
707 site (forearm: p=0.0013, F=10.7; finger p=0.0025, F=9.5). ns not significant, *p =0.0003 ,
708 **p<0.0001

709

710 **Figure 4.** Lempel-Ziv complexity (LZC) index of skin oxyHb signals measured at the forearm
711 in n=32 lowlanders and n=46 Sherpa highlanders at baseline (BL, black) and Everest base
712 camp (EBC, 5300 m, blue). Data are median and 95%CI. There was no significant difference
713 between groups.

714

715 **Figure 5.** Multiscale Lempel-Ziv complexity (MLZC) measured between scales τ = 1-12 of
716 skin BF signal at baseline (BL, black) and Everest base camp (EBC, 5300 m, blue).at the
717 forearm and finger at baseline (BL, black) and Everest base camp (EBC, 5300 m, blue).
718 Data are mean ± SEM of n=32 lowlanders and n=46 Sherpa highlanders. Significant
719 difference between groups, *p<0.05, +p<0.001

720

721 **Figure 6.** Spearman correlations between the flow-motion spectral power bands
722 corresponding to endothelial (0.0095-0.02Hz) (black), neurogenic (0.02-0.06Hz) (green),

723 myogenic (0.06-0.15Hz) (purple), respiratory (0.15-0.4Hz) (blue), and cardiac (0.4-1.6Hz)
724 (red) activity and multiscale Lempel-Ziv complexity (MLZC) of the (A) forearm and (B) finger
725 skin blood flow signals in n=32 lowlanders and n=46 Sherpa highlanders at baseline (BL)
726 and Everest base camp (EBC, 5300 m). Effective sampling rate = 40Hz/scale. *p<0.05
727 +p<0.01.

728 **FIGURES**

729

730 **Figure 1.** Examples of (A) skin BF and oxyHb signals from the forearm and (B) skin BF
731 signals from finger measured in the time domain of an individual lowlander and Sherpa
732 highlander at baseline (BL, black) and Everest base camp (EBC, 5300 m, blue).

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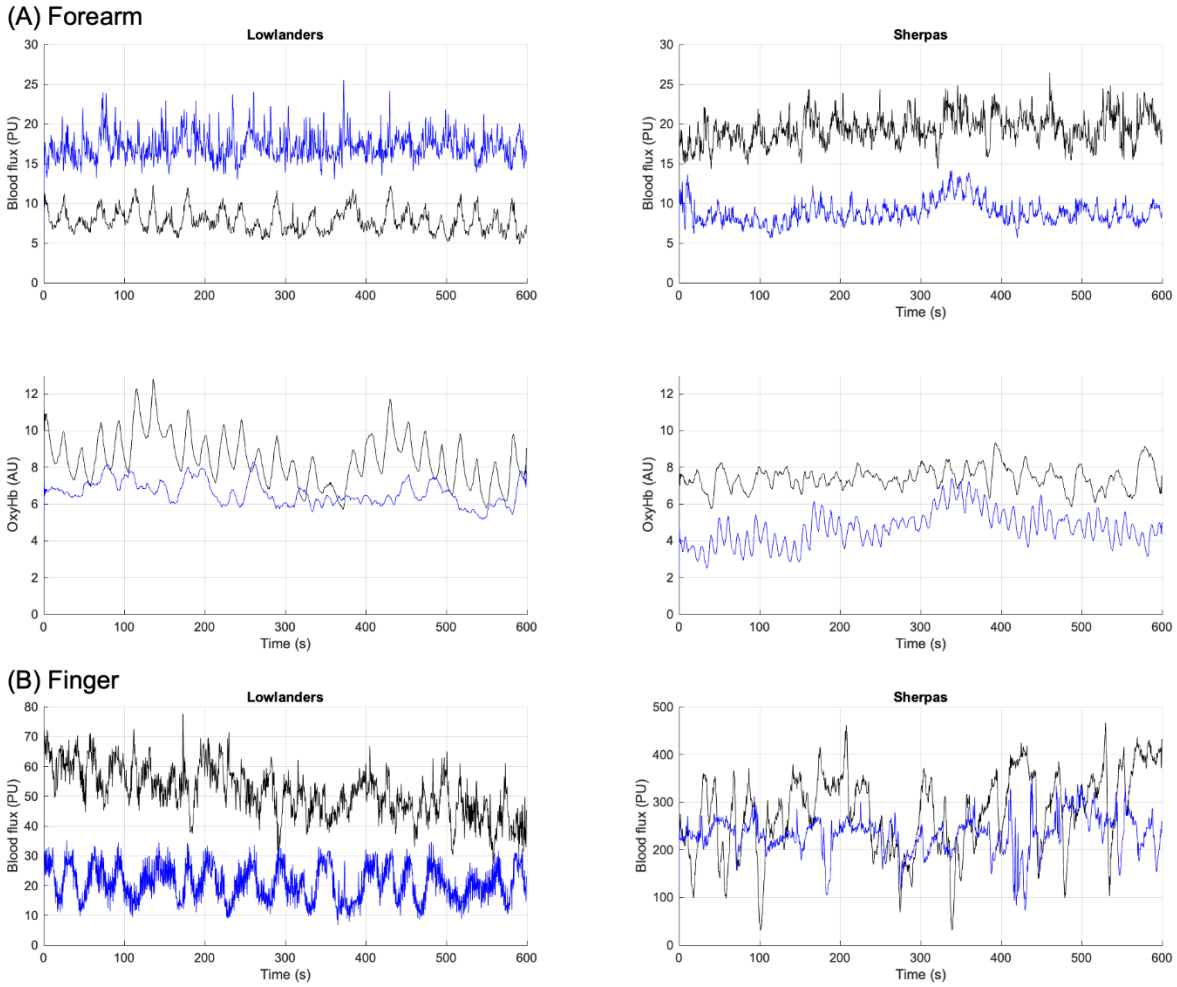

734

735 **Figure 2.** Lempel-Ziv complexity (LZC) of skin BF signal measured over 15 epochs at the
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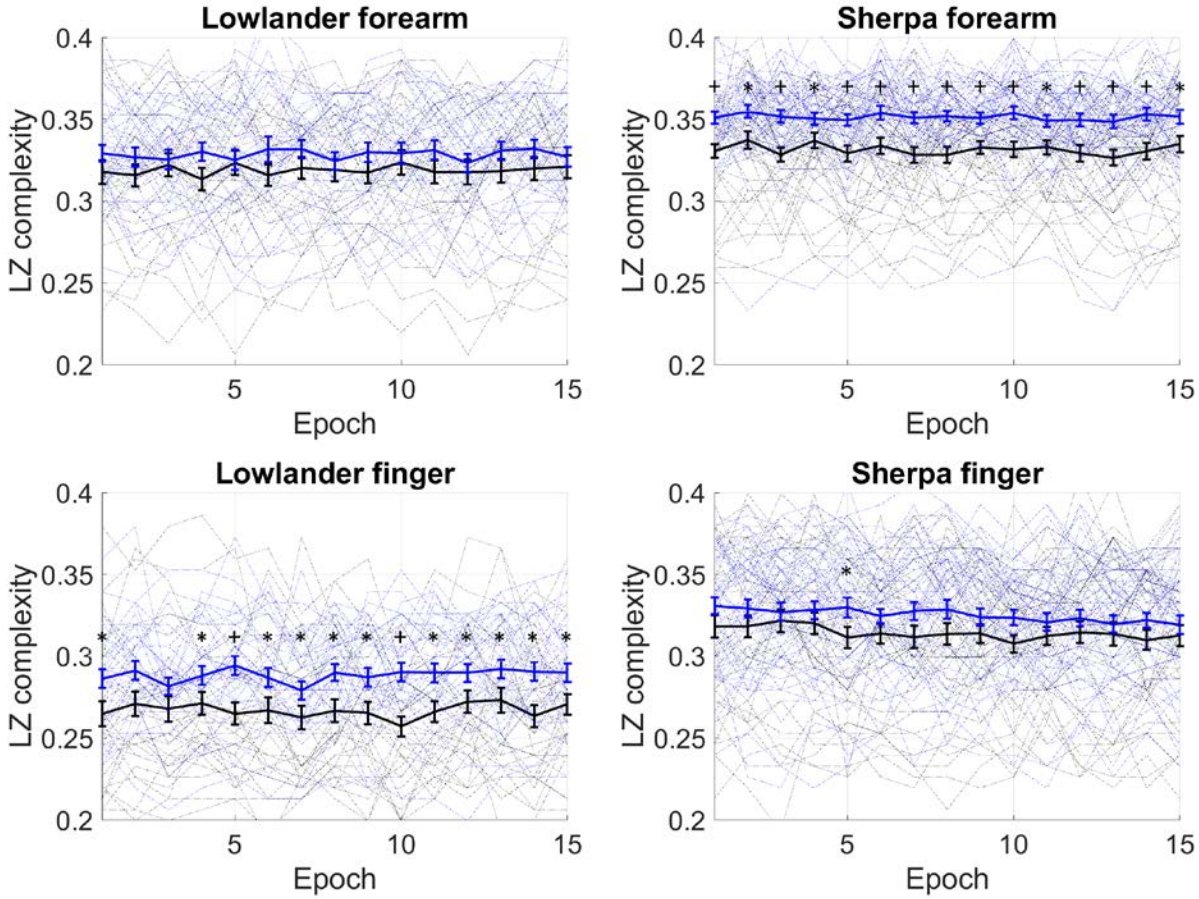

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742

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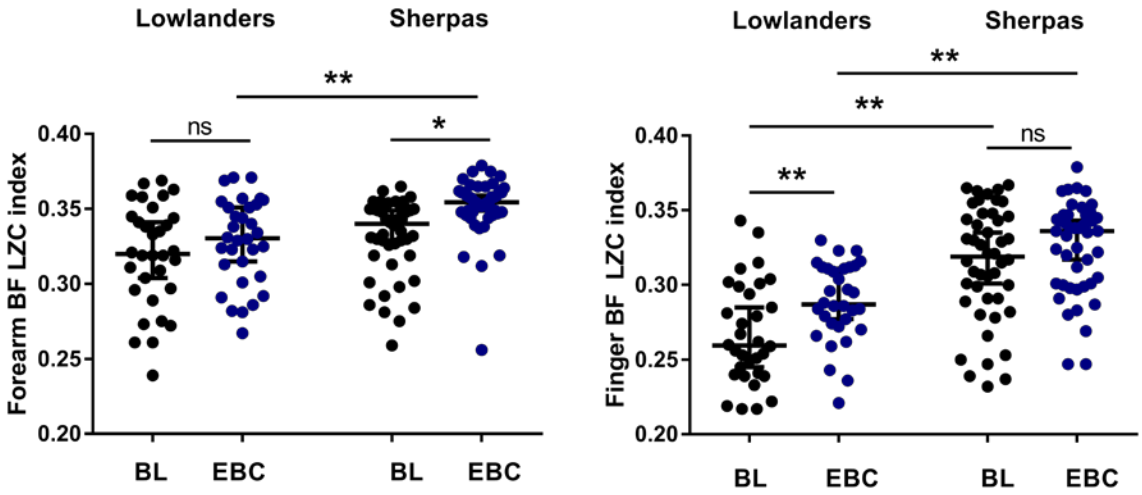

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752

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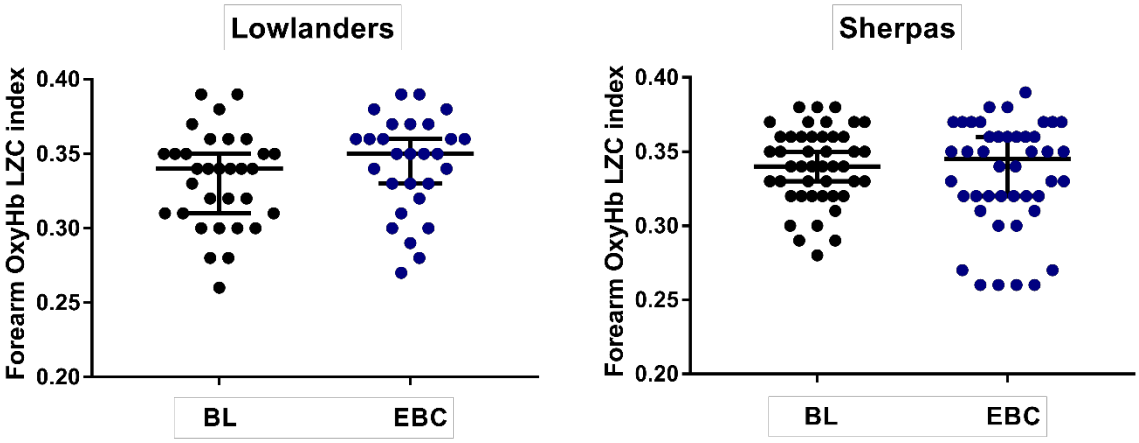

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760

761 **Figure 5.** Multiscale Lempel-Ziv complexity (MLZC) measured between scales τ = 1-12 of
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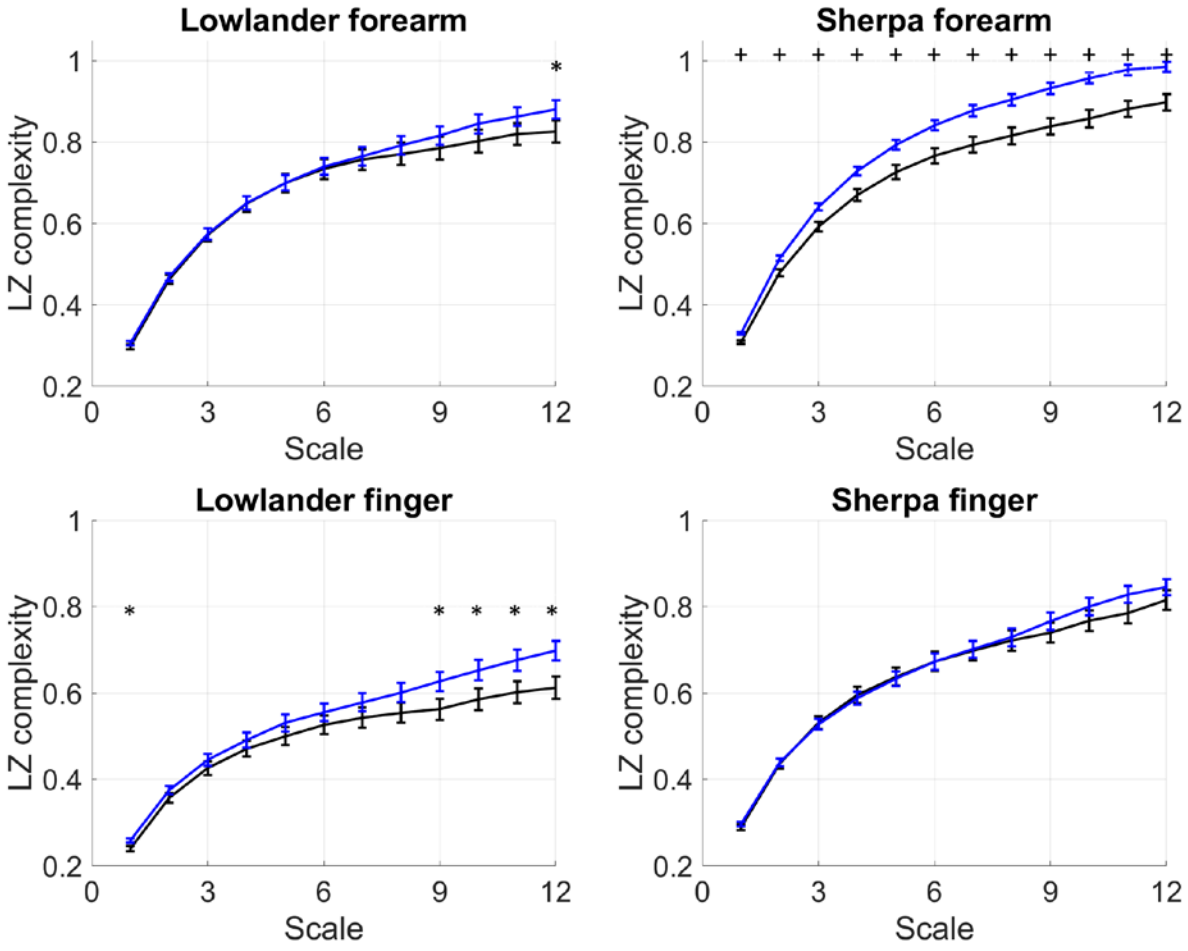

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Figure 6. Spearman correlations between the flow-motion spectral power bands corresponding to endothelial (0.0095-0.02Hz) (black), neurogenic (0.02-0.06Hz) (green), myogenic (0.06-0.15Hz) (purple), respiratory (0.15-0.4Hz) (blue), and cardiac (0.4-1.6Hz) (red) activity and multiscale Lempel-Ziv complexity (MLZC) of the (**A**) forearm and (**B**) finger skin blood flow signals in n=32 Lowlanders and n=46 Sherpa highlanders at baseline (BL) and Everest base camp (EBC, 5300 m). Effective sampling rate = 40Hz/scale. *p<0.05 +p<0.01.