Title: UBC-Nepal Expedition: Upper and Lower Limb Conduit Artery Shear Stress and Flow-Mediated Dilation on Ascent to 5050 m in Lowlanders and Sherpa

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Running title: Ascent Shear Stress and FMD in Lowlanders and Sherpa

Key words: flow-mediated dilation, hypoxia, shear stress

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Abbreviations

FMD, flow-mediated dilation; [Hb], hemoglobin concentration; HCT, hematocrit; MAP, mean arterial blood pressure; NO, nitric oxide; OSI, oscillatory shear index; S_aO_2, oxyhemoglobin saturation; P_aO_2, partial pressure of arterial oxygen; SS_{AUC}, shear stress area under the curve.

New and noteworthy (limit 75 words)

Upper and lower limb arterial shear stress and flow-mediated dilation (FMD) were assessed on matched-ascent from 1400m to 5050m in lowlanders and Sherpa. A shear stress pattern associated with vascular dysfunction/risk manifested in both limbs of lowlanders and Sherpa. FMD was impaired only in the upper limb of lowlanders. The findings indicate a limb-specific impact of high altitude trekking on FMD, and a vascular basis to acclimatization wherein endothelial function is protected in Sherpa on ascent.

Abstract

The study of conduit artery endothelial adaptation to hypoxia has been restricted to the brachial artery, and comparisons to highlanders have been confounded by differences in altitude exposure, exercise, and unknown levels of blood viscosity. To address these gaps, we tested the hypothesis that lowlanders, but not Sherpa, would demonstrate decreased mean shear stress and increased retrograde shear stress, and subsequently reduced flow-mediated dilation (FMD), in the upper and lower limb conduit arteries on ascent to 5050m. Healthy lowlanders (n=22, 28±6 years [mean±SD]) and Sherpa (n=12, 34±11 years) ascended over 10 days, with measurements taken on non-trekking days at 1400m (baseline), 3440m (day 4), 4371m (day 7), and 5050m (day 10). Arterial blood gases, blood viscosity, shear stress and FMD (duplex ultrasound of the brachial [BA] and superficial femoral [SFA] arteries) were acquired at each time-point. Ascent decreased mean and increased retrograde shear stress in the upper and lower limb of lowlanders and Sherpa. Although BA FMD decreased in the lowlanders from 7.1±3.9% to 3.8±2.8% at 5050m *versus* 1400m ($P<0.001$), SFA FMD was preserved. In the Sherpa, neither BA nor SFA FMD were changed upon ascent to 5050m. In lowlanders, the ascent-related exercise may favorably influence endothelial function in the active limb (SFA); selective impairment in FMD in the BA in lowlanders is likely mediated via the low mean or high oscillatory baseline shear stress. In contrast, Sherpa presented protected endothelial function, suggesting a potential vascular aspect of high altitude acclimatization/adaptation.

Introduction

Conduit artery shear stress profile plays a pivotal role in regulating local endothelial function. Evidence from cross-sectional studies (14), acute (9, 42, 43, 74, 82, 84, 88-91), and more prolonged interventions (84) suggests that augmented retrograde shear stress and low mean shear stress are associated with, and promote, conduit artery endothelial dysfunction. Over time, endothelial dysfunction may progress to atherosclerosis, which preferentially develops in arterial segments (i.e. superficial femoral artery) exposed to chronically low and oscillatory shear stress (3, 18, 96).

Recently, both acute and sustained hypoxia have been shown to increase brachial artery retrograde and decrease mean shear stress (47, 90), which may contribute to the observed reductions in brachial artery flow-mediated dilation (FMD). Indeed, during the process of acclimatization to hypobaric hypoxia (3800m), there appears to be a window wherein the endothelium is more susceptible to oscillatory shear stress (low time-averaged mean, high retrograde) – induced dysfunction compared to sea level (91). Thus, the previously reported reductions in FMD observed at high altitude (7, 16, 46, 90, 93) may have been influenced by alterations in conduit artery shear stress profile. To date, studies reporting shear stress and FMD at high altitude have only been performed in the brachial artery of lowlanders at varying durations of hypoxic exposure, while only one study has measured blood viscosity (90), a requisite parameter for the accurate calculation of shear stress.

Sherpa have adapted a physiological phenotype in response to living at high altitude for hundreds of generations under the constant evolutionary pressure of hypoxia (26). Tibetans, with

whom Sherpa share similar ancestry (13, 49), possess a high peripheral blood flow phenotype, promoting convective oxygen delivery (10). For instance, compared to lowlanders at sea level, Tibetans residing at 4200m present markedly elevated forearm blood flow, decreased vascular resistance, and increased exhaled and circulating nitric oxide (NO) metabolites (12, 25). A high-flow phenotype, as observed in Tibetan highlanders, may decrease the likelihood of developing adverse shear stress patterns at altitude, and thus serve as a protective mechanism to preserve conduit artery endothelial function via lowered downstream vascular resistance. Whether Sherpa exhibit this phenotype requires further investigation (15, 34, 73); previous reports suggest that Sherpa display similar brachial artery FMD to acclimatized lowlanders (46), but slightly lower when compared to lowlanders at sea level (15). However, the previous studies comparing Sherpa and lowlanders are confounded by differences in altitude exposure, exercise (i.e. mode of ascent), and an unknown blood viscosity, and hence FMD stimulus (i.e., shear stress = shear rate · viscosity (59, 60)).

The investigation of FMD in hypoxia has been restricted to the generally inactive arm brachial artery; however, this does not provide insight into active lower limb conduit artery endothelial function (57, 63, 81, 83). The superficial femoral artery exhibits lower baseline mean and higher retrograde shear stress than the brachial artery (56, 95), yet remains sensitive to shear stress profile-associated decrements in FMD (58, 68, 69, 74, 85, 87). The influence of ascent to high altitude on lower limb shear stress profile and FMD are unknown.

The purpose of this investigation was, for the first time, to characterize upper and lower limb conduit artery resting shear stress profile and FMD in lowlanders and Sherpa on ascent to

5050 m. We hypothesized that lowlanders would demonstrate decreased mean shear stress and increased retrograde shear stress, and subsequently reduced FMD, in both the upper and lower limb on ascent to 5050 m. In contrast, we hypothesized that Sherpa would present preserved shear stress patterns and FMD in upper and lower limbs on ascent to 5050 m. Elucidating whether a shear stress profile associated with vascular dysfunction/impairment manifests on active ascent with progressive hypoxemia may indicate a mechanistic link between environmental hypoxia and endothelial dysfunction. Moreover, comprehensive assessment of the conduit artery hemodynamic milieu in Sherpa may identify a vascular aspect of high altitude acclimatization or adaptation and further aid our understanding of adaptation to terrestrial altitude.

Methods

Ethical approval

The Clinical Research Ethics Board of the University of British Columbia, the Queen's University Health Sciences Research Ethics Board, and the Nepal Health Research Council approved all experimental procedures and protocols in adherence with the principles of the Declaration of Helsinki (with the exception that this study was not registered in a public database). All lowlander participants gave written informed consent in English prior to participating. All Sherpa participants read an in-depth study information form, spoke with a Nepalese physician and provided written informed consent in Nepalese prior to participating. This study was part of the University of British Columbia - Nepal 2016 expedition (Willie *et al*. *In Review*). As such, participants took part in multiple studies conducted at the University of British Columbia – Okanagan (Kelowna, British Columbia; 344 m) and during three weeks at the

144 Ev-K2-CNR Pyramid Laboratory (Lobuche, Nepal; 5050 m). However, the *a priori* primary
145 research questions and outcome variables addressed in the current paper are novel and are
146 exclusively dealt with in this study.
147
148 *Participants*
149 The lowlander participants (n=22 [2 female], 28±6 years, 178±8 cm, 74±9 kg, BMI: 23±2
150 kg m^{-2} [mean±SD]) were free of cardiovascular, respiratory, and neurological disease, had not
151 been exposed to elevations >3000m for at least three months prior to the expedition, and were
152 nonsmokers. One lowlander was taking mesalamine (500 mg b.i.d.) for Crohn's disease, and one
153 was taking oral contraceptives. The Sherpa participants (n=12 [0 female], 35±12 years, 167±7
154 cm, 67±14 kg, BMI: 24±4 kg m^{-2}) were recruited from local villages in the Solukhumbu Valley
155 (elevation of residence: 3586±499m) and flew to Kathmandu prior to baseline testing (see
156 *Experimental Design*). Four Sherpa were current smokers, with an average of 1.3±1.1 pack-
157 years. The Sherpa were otherwise free of cardiovascular, respiratory, and neurological diseases.
158
159 *Experimental design*
160 Sherpa participants were flown to Kathmandu and were tested 5-15 days (median: 7)
161 after arrival. Lowlander participants spent 3-9 days in Kathmandu (1400 m) prior to flying to
162 Lukla (2860 m) with the Sherpa participants to begin the ascent to the EV-K2-CNR Pyramid
163 Research laboratory (5050 m). Ascent to the Pyramid Laboratory took place over a 9-day
164 trekking protocol without the use of acute mountain sickness prophylactics (i.e. acetazolamide).
165 Participants spent one night in Monjo (2800 m), three nights in Namche Bazaar (3440 m), one
166 night in Deboche (3820 m), and then three nights in Pheriche (4371 m) followed by the final

trekking day to 5050 m. Experimental measurements were conducted at 1400 m and on the day after arrival to 3440 m, 4371 m, and 5050 m. Participants refrained from strenuous exercise 24h before testing, caffeine and alcohol 12h prior, and were 6h fasted. After laying supine for a minimum of 20 minutes, reactive hyperemia FMD was performed simultaneously in the brachial and superficial femoral artery. Mean arterial blood pressure (MAP), heart rate, and arterial and venous blood samples were acquired as described below.

Experimental measurements

Following 10-minutes supine rest at each location during the ascent, arterial blood samples were taken from the radial artery. A 23-G self-filling catheter (SafePico, Radiometer, Canada) was advanced into the radial artery under local anesthesia (Lidocaine, 1.0%) and ultrasound guidance (Terason, uSmart 3300, USA). Approximately 1mL of blood was withdrawn anaerobically and immediately assessed using an arterial blood gas analyzer for the partial pressure of arterial oxygen (P_aO_2), oxyhemoglobin saturation (S_aO_2), hemoglobin concentration ([Hb]), and hematocrit (HCT) (i-STAT 1, Abbott Point of Care, Canada). Both MAP (2*diastolic blood pressure + systolic blood pressure) / 3) and heart rate (pulse rate) were calculated from the average of three automated measurements on the brachial artery (UA-767FAM, Life Source, Canada).

Venous blood (5 ml) was drawn into a Vacutainer® Blood Collection Tube (Becton, Dickinson and Company, USA) that contained lithium heparin. Blood viscosity was measured within 15 minutes of blood sample acquisition at a shear rate of 225 s^{-1} at 37°C with a cone-and-plate viscometer (Brookfield DV2T, Brookfield AMETEK, USA) (27).

Reactive hyperemia FMD was measured in the brachial and superficial femoral artery in adherence with internationally-accepted guidelines (31, 79). A one-minute recording of baseline arterial diameter and blood velocity was recorded, followed by a five-minute cuff occlusion (250 mmHg, brachial artery: distal to epicondyles; superficial femoral artery: proximal to knee). Vessel imaging was always performed proximal to the cuff. After cuff deflation, recording resumed for three minutes. All measurements were performed by the same experienced sonographers (J.C.T., R.L.H., and H.H.C.) with a 10 MHz multifrequency linear array probe (15L4 Smart Mark, Teratech, USA) attached to a high-resolution ultrasound machine (Terason usmart 3300 and Terason t3200, Teratech, USA). Standardized software approaches to acquire and analyze the Doppler ultrasound recordings were employed, as used extensively elsewhere (46, 82, 92). The angle of insonation for the acquisition of velocity was 60°. Screen capture of the ultrasound was saved as an audio video interleave file (Camtasia Studio, Techsmith Co, Ltd, USA) for future analysis using edge-detection software (94). A region of interest was placed around the highest quality portion of the B-mode longitudinal image of the artery and a second region of interest surrounded the Doppler strip to record blood velocity. The software automatically and continuously tracks the walls of the vessel and velocity trace within the regions of interest at a frequency of 30 Hz (94). Peak diameter was automatically detected using a moving window-smoothing function (smoothed median across time). The FMD was calculated as the absolute (mm) and relative (%) change from baseline to peak diameter.

Peak and total reactive hyperemia were acquired as indices of resistance vessel function (2, 33, 35, 52). Blood flow in the brachial and superficial femoral arteries was calculated as peak

envelope blood velocity / 2 * (π(0.5*diameter)^2). Peak reactive hyperemia was calculated as the greatest 3-second post-occlusion blood flow, and total reactive hyperemia was calculated as the area under the curve 3 minutes post-cuff deflation.

Shear stress was calculated as the product of shear rate (4*peak envelope blood velocity / arterial diameter) and whole blood viscosity at a shear rate of 225 s^{-1} (27). The FMD stimulus was quantified as the shear stress area under the curve (SS_{AUC}) from cuff deflation to peak diameter (60, 65). Antegrade and retrograde shear stress were calculated as shear stress in the positive (forward) and negative (backward) direction, respectively, and mean shear stress as the sum of antegrade and retrograde (time-averaged mean shear stress). The oscillatory shear index (OSI) was calculated as |retrograde shear stress| / (|antegrade shear stress| + |retrograde shear stress|) (54). Vascular resistance was calculated in the brachial and superficial femoral artery as MAP / blood flow.

Statistics

All statistical analyses were performed using IBM SPSS 24 (International Business Machines Corp, USA). All data were analyzed using a linear mixed model with a compound symmetry co-variance structure with significance set at $P<0.05$ and are presented as mean ± SD. Two factor linear mixed models were performed with race (lowlander *versus* Sherpa) and elevation (repeated factors: Kathmandu [1400 m], Namche Bazaar [3440 m], Pheriche [4371 m], Pyramid [5050 m]) for all hematological and hemodynamic parameters. When significant main effects were detected, Bonferroni corrected post-hoc tests were used to make pairwise comparisons. To account for differences in FMD stimulus, testing was also performed with

236 SS_{AUC} included as a covariate (32). Furthermore, allometric scaling was performed to account for
237 differences in baseline diameter within and between individuals. Briefly, the diameter change on
238 a logarithmic scale (ln(peak diameter) - ln(baseline diameter)) was assessed as the outcome
239 variable in a linear mixed model with logarithmically-transformed baseline diameter included as
240 a covariate (5, 6).
241
242 **Results**
243 *Hematological and hemodynamic parameters*
244 P_aO_2, S_aO_2, and hematological parameters, and related p-values, are presented in Table 1.
245 Lowlanders and Sherpa demonstrated similar stepwise reductions in P_aO_2 and S_aO_2 on
246 progressive ascent to 5050 m. Lowlanders experienced an initial increase in [Hb] and HCT at
247 3440 m that persisted throughout ascent. Blood viscosity increased by 19±18%, 22±17%, and
248 29±16% at 3440 m, 4371 m, and 5050 m, respectively, compared to 1400 m in lowlanders. In the
249 Sherpa, [Hb], HCT, and viscosity were higher compared to lowlanders at 1400 m but were
250 unaltered during ascent.
251
252 Resting hemodynamic parameters and p-values are presented in Table 2. Lowlanders
253 demonstrated an increase in MAP on ascent while MAP was unchanged in the Sherpa. Brachial
254 and superficial femoral artery blood flow decreased at 3440 m compared to 1400 m and
255 remained decreased throughout ascent in both lowlanders and Sherpa. Forearm vascular
256 resistance was increased in lowlanders from 3440 m onward, while Sherpa forearm vascular
257 resistance was increased at 3440 m and 4371 m but decreased back towards baseline (i.e. 1400

m) levels at 5050 m. Leg vascular resistance was increased at 4371 m and 5050 m compared to 1400 m in lowlanders and Sherpa.

Shear stress pattern and magnitude

Brachial artery. The baseline brachial artery shear stress profile parameters and p-values are illustrated in Figure 1. Mean shear stress decreased (Figure 1A), while retrograde shear stress and OSI increased at 3440 m compared to 1400 m and remained altered throughout ascent in lowlanders and Sherpa (Figure 1C and D). Retrograde shear stress and OSI displayed a trend to be higher in lowlanders compared to Sherpa (P=0.085 and 0.073). Antegrade shear stress was lower (P<0.001) at 3440 m and 4371 m compared to 1400 m, but not at 5050 m in both groups (Figure 1B).

Superficial femoral artery. The baseline superficial femoral artery shear stress profile parameters and p-values are illustrated in Figure 2. The shear stress profile was similar between lowlanders and Sherpa throughout ascent. Mean shear stress decreased at 3440 m compared to 1400 m and remained decreased throughout ascent in both groups (Figure 2A). Antegrade shear stress was lower at 3440 m and 4371 compared to 1400 m, but not at 5050 m in both groups (Figure 2B). Retrograde shear stress was elevated at 5050 m compared to 1400 m, 3440 m, and 4371 m (Figure 2C), and OSI was greater at 4371 m and 5050 m compared to 1400 m and 3440 m in both groups (Figure 2D).

Flow-mediated dilation

Brachial artery. Brachial artery FMD parameters and p-values are presented in Table 3. Brachial artery FMD and p-values are displayed in Figure 3A. Absolute and relative (%) FMD were significantly decreased at 4371 m and 5050 m compared to 1400 m in lowlanders but not in Sherpa. At 5050 m, FMD in lowlanders was lower compared to Sherpa. The SS_{AUC} was unchanged in lowlanders at each elevation, while the SS_{AUC} was greater at 5050 m in Sherpa compared to lowlanders. Following inclusion of SS_{AUC} as a covariate, FMD remained reduced at 4371 m and 5050 m compared to 1400 m in lowlanders, while the difference between lowlanders and Sherpa at 5050 m was somewhat reduced (P=0.066). Allometric scaling to account for variation in baseline diameter did not change statistical interpretation. Peak reactive hyperemia was greater in Sherpa compared to lowlanders at 5050 m, whilst total reactive hyperemia decreased at 3440 m and 4371 m in both lowlanders and Sherpa but recovered at 5050 m only in Sherpa. The four Sherpa who were smokers did not show different brachial artery FMD compared to the eight who were not (smoking as a factor: P=0.642).

Superficial femoral artery. Superficial femoral artery FMD parameters and p-values are presented in Table 4. Superficial femoral artery FMD and p-values are displayed in Figure 3B. Absolute and relative (%) FMD were unchanged on ascent to 5050 m in lowlanders and Sherpa. Baseline and peak arterial diameter were larger in both groups at 5050 m compared to 1400 m. The SS_{AUC} was greater at 5050 m compared to 4371 m in both groups. Inclusion of SS_{AUC} as a covariate did not alter FMD statistical interpretation. Allometric scaling resulted in an effect of elevation (P=0.026), and post-hoc testing revealed that FMD was greater at 5050 m compared to 3440 m (P=0.029) in both groups. Allometric scaling also resulted in an effect of race (P=0.048), such that superficial femoral FMD was greater in lowlanders compared to Sherpa. Peak reactive

hyperemia was greater in lowlanders compared to Sherpa, and a main effect of elevation was observed such that peak reactive hyperemia was greater at 5050 m compared to 1400 m and 3440 m in lowlanders and Sherpa. Total reactive hyperemia was lower in Sherpa compared to lowlanders at 3440 m and 4371 m, but similar at 1400 m and 5050 m. The four Sherpa who were smokers did not show different superficial femoral artery FMD compared to the eight who were not (smoking as a factor: P=0.517).

Discussion

The present investigation sought to examine the impact of progressive hypoxic exposure during ascent to 5050 m on upper and lower limb conduit artery shear stress and FMD in lowlanders and Sherpa. The primary findings indicate impaired brachial artery FMD with ascent in lowlanders only and preserved superficial femoral artery FMD with ascent in both groups. This indicates the existence of distinct vascular acclimatization or adaptation wherein endothelial function is protected in Sherpa upon ascent to high altitude. While the ascent-related exercise may contribute to the preserved endothelial function in the active limb (superficial femoral artery), the selective impairment in FMD in the brachial artery might be mediated *via* the low mean or high retrograde shear stress during ascent in lowlanders.

Shear stress profile on ascent to high altitude

We advance the emerging evidence that hypoxia evokes a shear stress profile associated with vascular dysfunction/impairment (47, 90) by demonstrating increases in retrograde shear stress and reductions in mean shear stress in the brachial and superficial femoral artery of both

lowlanders and Sherpa on ascent to high altitude. These perturbations manifest rapidly on ascent and persist throughout the trek. Although Sherpa appeared to present a less oscillatory brachial artery shear stress profile (trend towards lower OSI and retrograde shear stress) compared to lowlanders on ascent, we did not observe markedly higher blood flow, as previously reported in Tibetans (25).

The mechanisms responsible for the development of baseline conduit artery shear stress profiles associated with vascular dysfunction/impairment in hypoxia have not been elucidated, but may involve sympathoexcitation, decreased NO bioavailability, or increased blood viscosity. Each of these influences vascular resistance, and consequently upstream shear stress profile. These putative mechanisms have also been implicated in hypoxia-associated reductions in FMD, however the discussion will concentrate on the observed findings on shear stress and FMD.

Sympathoexcitation has previously been shown to disrupt shear stress patterns (19, 61, 78). Hypobaric hypoxia increases resting sympathetic nerve activity and vascular resistance (17, 24, 30, 50), which may stimulate a high retrograde and low mean shear stress profile. In addition to sympathoexcitation, a predominantly vasoconstrictive shift in resistance vessel tone may promote retrograde shear stress (29). Although markers of NO-derived molecules are typically elevated upon ascent to high altitude (11, 41, 45), this is accompanied by an increase in vasoconstrictor bioavailability [i.e. endothelin-1 (21, 53)] in high altitude trekking conditions. This balance seems to favor vasoconstriction, especially in lowlanders, as blood pressure is typically elevated during ascent over time at altitude (62), and hence may contribute to the observed alterations in shear stress. In contrast to lowlanders, Sherpa did not display any change

in MAP on ascent, suggesting less ascent-related systemic vasoconstriction, perhaps contributing to the lower forearm vascular resistance compared to lowlanders at 5050 m.

Increases in blood viscosity, likely mediated through high altitude-associated reduction in plasma volume (64, 77), may be contributing to the observed increases in vascular resistance in lowlanders (70), and consequently the detrimental upstream shear stress profile. The inverse relationship between blood viscosity and blood flow (23) suggests that antegrade, and therefore, mean shear stress may be reduced under conditions of increasing blood viscosity. Future investigation of the potential mechanisms (i.e. sympathoexcitation, vasodilator/vasoconstrictor balance, blood viscosity) responsible for invoking shear stress profiles associated with vascular dysfunction and risk at high altitude in lowlanders, and whether Sherpa are insensitive to such perturbations, are warranted.

Flow-mediated dilation on ascent to high altitude

Brachial artery. Brachial artery FMD was reduced by 24±38% and 29±76% at 4371 m and 5050 m, respectively, compared to 1400 m in lowlanders. Similar reductions in brachial artery FMD have been reported in lowlanders during trekking expeditions in the Himalayas (7, 46), however hypobaric hypoxia is not always accompanied by a reduced brachial artery FMD (16, 36, 71, 91, 92). These discrepancies may relate to mode of ascent (i.e. passive or active) (16, 66), duration and severity of hypoxia (16, 47), prophylaxis treatment for acute mountain sickness (46), or differences in FMD shear stress stimulus (SS_{AUC}) (7, 46, 91). For the first time, we measured blood viscosity on ascent to calculate shear stress, and observed similar SS_{AUC} at each altitude, suggesting that the reductions observed in brachial artery FMD were not due to a

372 blunted stimulus. Indeed, without measuring blood viscosity, SS_{AUC} would have been
373 underestimated by 20-30%, highlighting the importance of measuring blood viscosity in
374 conditions where it is expected to change. The observation that decreases in mean shear stress
375 and increases in OSI preceded reductions in brachial artery FMD in lowlanders suggests that
376 these disruptions may be contributing to the reduction in FMD.
377
378 In contrast to the FMD decline with ascent observed in lowlanders, brachial artery FMD
379 was maintained in Sherpa throughout ascent, and was greater than lowlander brachial artery
380 FMD at 5050 m. Previous investigations have reported similar brachial artery FMD in Sherpa
381 compared to acclimatized lowlanders at 5050 m (46), and that Sherpa tested at 2600m or 3800m
382 had lower brachial artery FMD compared to lowlanders tested at sea level (15). We advance
383 these findings by demonstrating that when assessed at a lower elevation, Sherpa present similar
384 brachial artery FMD to lowlanders. The trend of a lesser perturbation in retrograde shear stress
385 and OSI on ascent, and potential rectification of mean and antegrade shear stress at 5050 m may
386 mitigate the endothelial insult, preserving FMD. However, whether Sherpa are less sensitive to
387 oscillatory shear stress perturbations than lowlanders is unclear.
388
389 Upper limb total reactive hyperemia displayed somewhat similar trends to brachial artery
390 FMD, such that Sherpa presented similar resistance vessel function at 5050 m and 1400 m, and
391 greater resistance vessel function at 5050 m compared to lowlanders. However, in contrast to
392 FMD which demonstrated no impairment in this group, Sherpa experienced blunted reactive
393 hyperemia during the first part of the ascent (3440 m and 4371 m). Reactive hyperemia is
394 mediated by a myriad of pathways (20), thus identifying the primary insult for impairment or the

mechanism of recovery from 3440 m to 5050 m in Sherpa remains elusive. Taken together, lowlanders experienced more pervasive (conduit and resistance vessel) and persistent impairment of upper limb vascular function on ascent to 5050 m. These functional differences may indicate a vascular -basis to high altitude adaptation or accelerated acclimatization in Sherpa. This may be clinically relevant for the maintenance of cardiovascular health with altitude exposure as both FMD and reactive hyperemia are predictive markers of cardiovascular risk and events (2, 37, 52, 67).

Superficial femoral artery. Despite developing increased retrograde shear stress and decreased mean shear stress, superficial femoral artery FMD was preserved throughout ascent to 5050 m in both lowlanders and Sherpa. This occurred in contrast to the marked reduction in brachial artery FMD observed in lowlanders. The preserved superficial femoral artery FMD may be explained by the lesser relative disruption to shear stress (pooled reduction in mean shear stress effect size: 0.88 for arm, 0.67 for leg [Cohen's d]) and/or a protective effect conferred by the greater level of lower limb exercise throughout the trek.

The superficial femoral artery presents lower resting mean shear stress and higher retrograde shear compared to the brachial artery (56, 95). Although previous studies administering an intervention to further reduce mean shear stress (58, 68, 69, 85) or increase retrograde shear in the superficial femoral artery have observed a reduction in FMD (74, 88, 89), the perturbations were greater than the changes we observed on ascent. With respect to a vasoprotective effect of trekking, prior exercise prevents shear-mediated impairments in FMD (8, 55, 58). Further, two weeks of treadmill and cycle training improves popliteal artery FMD

418 (86), thus it is possible that there was some localized training stimulus in the exercised limb that
419 protected or offset the detrimental resting shear stress profile and other harmful stimuli (i.e.
420 redox imbalance) present in hypobaric hypoxia. Although rhythmic lower limb exercise, such as
421 walking, incurs brachial artery shear stress patterns associated with atheroprotective endothelial
422 adaptations (80), exercise in hypoxia exacerbates the increase in retrograde shear rate in the
423 inactive limb (38-40, 44, 92), providing further support for limb-specific influences of high
424 altitude trekking on FMD. Whether passive ascent differentially influences lower limb conduit
425 artery FMD is unclear thus we cannot conclude that the trekking was responsible for the
426 preserved lower limb FMD. Further, although participants (lowlanders and Sherpa) trekked
427 together as a group at a conservative pace in an effort to control for the combined stimulus of
428 exercise and hypoxia, there may have been interindividual variability in relative hiking intensity
429 and hence training stimuli. More concerted investigations on the potential vasoprotective effects
430 of exercise during progressive ascent to high altitude should control for exercise intensity, which
431 influences training-induced adaptations in FMD at sea level (4).
432
433 Lower limb peak, but not total, reactive hyperemia was lower in Sherpa compared to
434 lowlanders. However, peak and total reactive hyperemia were preserved or enhanced at 5050 m
435 compared to 1400 m in both Sherpa and lowlanders. The preserved or enhanced resistance vessel
436 function may be a localized training effect due to trekking, as discussed above for lower limb
437 conduit artery function, however improvements in lower limb reactive hyperemia are atypical in
438 training studies involving young, healthy participants at sea level (22, 75, 86). Therefore,
439 whether hypoxemia, or the combination of hypoxemia and trekking, positively influences lower
440 limb resistance vessel function is presently unclear.

441

442 *Methodological considerations*

443 For the first time, in the largest sample size to date for this type of field investigation, we
444 assessed blood viscosity, shear stress patterns, and upper and lower limb FMD on matched
445 ascent to 5050 m in lowlanders and Sherpa. However, this field research study protocol had
446 several limitations that are subsequently addressed. We did not assess endothelium-independent
447 vasodilation (i.e. sublingual nitroglycerine). A decrease in endothelium-independent vasodilation
448 has been observed at 5050 m in lowlanders compared to sea level, while Sherpa endothelium-
449 independent vasodilation was not different from that of acclimatized lowlanders (46). Thus, it is
450 possible that a decrease in vascular smooth muscle function contributed to the reduction in FMD
451 with hypoxia. Although Sherpa spent 5-15 days at 1400 m, and although arterial blood gases
452 were comparable to the lowlanders, that time is likely insufficient to fully de-acclimatize.
453 Hemoglobin mass in lowlanders reverts to pre-ascent levels 1-2 weeks after descent (72, 76), and
454 Tibetans who have spent >2 years at sea level have been shown to possess lower [Hb] compared
455 to lowlanders (48). However, from 3440 m onward, hematological parameters were similar
456 between lowlanders and Sherpa. Whether high altitude natives who have migrated to sea level
457 possess a similarly preserved FMD on ascent is unknown; Tibetans remain protected against
458 acute mountain sickness on re-ascent to high altitude even after residing at sea level for 7 years
459 (28), thus physiological adaptation traits may remain intact even after prolonged absence from
460 high altitude. To elucidate whether the preserved FMD in Sherpa is an adaptation, future
461 investigation should assess whether Sherpa who have resided at sea level for one or two
462 generations exhibit protected FMD on ascent to high altitude. Similarly, investigating whether

first or second generation native lowlanders living at high altitude possess protected vascular function upon reascent would provide an ideal model of acclimatization *versus* adaptation.

Our measurements of resting shear stress represent a single snapshot that may not be representative of the cumulative alteration in shear stress profile on ascent. For instance, there is reason to speculate that retrograde shear stress would be enhanced during trekking and sleep on ascent. Hypoxic exercise exacerbates the increase in retrograde shear and OSI in the inactive limb compared to normoxic exercise (38-40, 44, 92), which may contribute to the limb-specific effect of high altitude trekking on FMD. Further, a case study has reported neurogenic retrograde flow in the brachial artery during obstructive sleep apnea (51). Central sleep apnea (and sleep disruption) is highly prevalent on ascent to high altitude (reviewed in: (1)), and whether this elicits concomitant rises in vascular resistance and alterations in conduit artery shear stress profile is unknown. Thus, capturing shear stress profile in a diverse array of common conditions encountered on ascent (notably during exercise and sleep) may serve to comprehensively characterize conduit artery shear stress in this environment. Lastly, this investigation was performed on healthy lowlanders and Sherpa who work as trekking guides, and the findings cannot be generalized to other populations.

Conclusions

For the first time in a relatively large sample size, we have characterized upper and lower limb conduit artery shear stress profile and FMD on ascent to 5050 m in lowlanders and Sherpa. Lowlanders displayed a selective reduction in upper limb FMD on ascent that was preceded by

an increase in blood viscosity, vascular resistance, and a decrease in mean shear stress and an increase in OSI. By contrast, Sherpa displayed preserved upper limb FMD, potentially due to a lesser disruption in blood viscosity, vascular resistance, and shear stress profile. Lower limb FMD was preserved amongst lowlanders and Sherpa, suggesting a potentially vasoprotective influence of trekking on the exercised limb vascular function. Collectively, these findings highlight limb-specific effects of high altitude trekking on vascular function in lowlanders and a generalized (upper and lower limb) vasoprotective acclimatization or adaptation response in Sherpa.

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Additional Information

Competing interests

None.

Author contributions

J.C.T., R.L.H., M.S., C.K.W., and P.N.A. conceived and designed research; J.C.T., R.L.H., H.H.C., C.A.H., M.S., C.K.W., C.G., and D.B.M. performed experiments; J.C.T. analyzed data; J.C.T., R.L.H., H.H.C., C.A.H., M.S., C.G., D.B.M., K.E.P., and P.N.A. interpreted results of experiments; J.C.T. prepared figures; J.C.T. and P.N.A. drafted manuscript; J.C.T., R.L.H., H.H.C., C.A.H., M.S., C.G., D.B.M., K.E.P., and P.N.A. edited and revised manuscript; J.C.T., R.L.H., H.H.C., C.A.H., M.S., C.G., D.B.M., K.E.P., and P.N.A approved final version of manuscript.

Funding

This study was supported by the Natural Sciences and Engineering Research Council of Canada (P.N.A.), the Canadian Foundation for Innovation and a Canada Research Chair (P.N.A.). J.C.T. was supported by a Natural Sciences and Engineering Research Council of Canada Doctoral CGS award and a Queen’s University Graduate Dean's Travel Grant for Doctoral Field Research.

Acknowledgments

This study was performed within the framework of the UBC International Research Expedition to Nepal. We thank the research station staff for friendly accommodation. We are grateful to the members of the UBC International Research expedition to the Ev-K2 CNR pyramid laboratory for invaluable help with organization and implementation of this research study.

809 **Figure 1.** Brachial artery mean shear stress (SS; A), antegrade shear stress (B), retrograde shear
810 stress (C), and oscillatory shear index (D) in lowlanders (open circles) and Sherpa (filled circles)
811 on ascent to 5050 m (meters above sea level; m.a.s.l.). The shaded bars represent the mean. *,
812 P<0.05 *versus* 1400 m; §, P<0.05 *versus* 1400 m and 5050 m.
813
814 **Figure 2.** Superficial femoral artery mean shear stress (SS; A), antegrade shear stress (B),
815 retrograde shear stress (C), and oscillatory shear index (D) in lowlanders (open circles) and
816 Sherpa (filled circles) on ascent to 5050 m (meters above sea level; m.a.s.l.). The shaded bars
817 represent the mean. *, P<0.05 *versus* 1400 m; †, P<0.05 *versus* 1400 m and 3440 m; ‡, P<0.05
818 *versus* 1400 m, 3440 m, and 4371 m; §, P<0.05 *versus* 1400 m and 5050 m.
819
820 **Figure 3.** Brachial artery (A) and superficial femoral artery (B) flow-mediated dilation (FMD)
821 on ascent to 5050 m (meters above sea level; m.a.s.l.) in lowlanders (open circles) and Sherpa
822 (filled circles). The shaded bars represent the mean. *, P<0.05 *versus* 1400 m; †, P<0.05 *versus*
823 lowlanders at 5050 m.
824

Table 1. Oxygen tension (P_aO_2), oxyhemoglobin saturation (S_aO_2), and hematological parameters on ascent to 5050 m in lowlanders and Sherpa.

	Kathmandu (1400 m)	Namche Bazaar (3440 m)	Pheriche (4371 m)	Pyramid (5050 m)
P_aO_2 (mmHg)				
Lowlander	77±7	52±4*	48±4†	41±4‡
Sherpa	75±8	52±5*	47±4†	41±4‡
		Elevation P<0.001 Race P=0.523 Interaction P=0.693		
S_aO_2 (%)				
Lowlander	95±1	87±3*	84±3†	79±5‡
Sherpa	95±2	87±3*	82±5†	77±5‡
		Elevation P<0.001 Race P=0.152 Interaction P=0.809		
[Hb] (g dL^{-1})				
Lowlander	13.6±0.8	14.5±0.7*	14.4±0.5*	14.5±0.7*
Sherpa	14.8±0.9†	14.5±0.7	14.8±0.9	14.8±0.7
		Elevation P=0.012 Race P=0.017 Interaction P=0.001		
HCT (%)				
Lowlander	40±2	43±2*	42±2*	43±2*
Sherpa	44±3†	43±2	44±3	44±2
		Elevation P=0.010 Race P=0.020 Interaction P=0.001		
Viscosity (cP)				
Lowlander	3.76±0.47	4.41±0.29*	4.53±0.42*	4.81±0.39†
Sherpa	4.32±0.40§	4.46±0.67	4.63±0.58	4.62±0.29
		Elevation P<0.001 Race P=0.249 Interaction P=0.002		

[Hb], hemoglobin concentration; HCT, hematocrit. *, P<0.05 *versus* 1400 m; †, P<0.05 *versus* 1400 m, and 3440 m; ‡, P<0.05 *versus* 1400 m, 3440 m, and 4371 m; §, P<0.05 *versus* lowlanders at 1400 m.

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Table 2. Hemodynamic parameters in the brachial and superficial femoral artery of lowlanders and Sherpa on ascent to 5050 m.

	Kathmandu (1400 m)	Namche Bazaar (3440 m)	Pheriche (4371 m)	Pyramid (5050 m)
Mean arterial pressure (mmHg)				
Lowlander	85±6	93±9*	94±9*	100±10*
Sherpa	95±9†	101±11†	101±10†	97±5
		Elevation P<0.001 Race P=0.021 Interaction P=0.002		
Brachial artery blood flow (ml min^{-1})				
Lowlander	67±38	33±14*	34±15*	34±20*
Sherpa	75±49	33±16*	36±21*	56±36*
		Elevation P<0.001 Race P=0.157 Interaction P=0.335		
Forearm vascular resistance (mmHg (ml min^{-1})$^{-1}$)				
Lowlander	1.75±1.23	3.19±1.49*	3.19±1.36*	3.66±1.88*
Sherpa	1.81±1.10	3.45±1.48*	3.73±2.38*	2.32±1.30†§
		Elevation P<0.001 Race P=0.771 Interaction P=0.010		
Superficial femoral artery blood flow (ml min^{-1})				
Lowlander	89±32	64±30*	59±23*	60±42*
Sherpa	95±84	56±25*	41±31*	65±37*
		Elevation P<0.001 Race P=0.696 Interaction P=0.398		
Leg vascular resistance (mmHg (ml min^{-1})$^{-1}$)				
Lowlander	1.12±0.54	1.75±0.82	1.79±0.75*	2.39±2.04*
Sherpa	1.54±1.02	2.06±0.70	2.87±1.17*	2.14±1.32*
		Elevation P<0.001 Race P=0.177 Interaction P=0.088		

*, P<0.05 *versus* 1400 m; †, P<0.05 *versus* 4371 m; §, P<0.05 *versus* lowlanders at the same elevation.

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Table 3. Brachial artery flow-mediated dilation (FMD) parameters and peak reactive hyperemia in lowlanders and Sherpa on ascent to 5050 m.

	Kathmandu (1400 m)	Namche Bazaar (3440 m)	Pheriche (4371 m)	Pyramid (5050 m)
Baseline Diameter (mm)				
Lowlander	4.23±0.55	4.17±0.52*	4.20±0.55	4.19±0.55
Sherpa	4.37±0.29	4.15±0.35*	4.15±0.40	4.25±0.37
	Elevation P=0.032 Race P=0.836 Interaction P=0.205			
Peak Diameter (mm)				
Lowlander	4.52±0.52	4.40±0.51*	4.39±0.55*	4.34±0.52
Sherpa	4.62±0.27	4.39±0.35*	4.37±0.41*	4.53±0.38
	Elevation P=0.001 Race P=0.691 Interaction P=0.134			
Absolute FMD (mm)				
Lowlander	0.29±0.14	0.23±0.11	0.19±0.10*	0.15±0.10*
Sherpa	0.24±0.12	0.23±0.11	0.23±0.10	0.28±0.08†
	Elevation P=0.076 Race P=0.266 Interaction P=0.005			
SS_{AUC} (au)				
Lowlander	985±373	953±300	823±265	908±330
Sherpa	1083±311	858±231	960±521	1204±446†§
	Elevation P=0.039 Race P=0.233 Interaction P=0.049			
SS_{AUC}-corrected FMD (%)				
Lowlander	7.0±2.8	5.6±2.8	5.1±2.8*	3.9±2.8*
Sherpa	5.3±2.8	6.0±2.8	5.5±2.7	5.8±2.8
	Elevation P=0.151 Race P=0.718 Interaction P=0.029			
Time to peak FMD (s)				
Lowlander	52±15	49±15	44±14	53±22
Sherpa	50±10	46±10	44±11	46±13
	Elevation P=0.241 Race P=0.325 Interaction P=0.739			
Peak reactive hyperemia (ml min^{-1})				
Lowlander	341±81	307±92	295±91	306±94
Sherpa	372±90	304±103‡	295±80‡	389±106†
	Elevation P<0.001 Race P=0.304 Interaction P=0.025			
Total reactive hyperemia (l)				
Lowlander	22.3±7.4	15.3±6.4*	13.7±5.9*	12.1±6.2*
Sherpa	22.9±5.7	14.2±7.2‡	14.6±5.9‡	23.5±9.8§
	Elevation P<0.001 Race P=0.082 Interaction P<0.001			

*, P<0.05 *versus* 1400 m; †, P<0.05 *versus* 3440 m; ‡, P<0.05 *versus* 1400 m and 5050 m; §, P<0.05 *versus* lowlander at the same elevation.

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Table 4. Superficial femoral artery flow-mediated dilation (FMD) parameters and peak reactive hyperemia in lowlanders and Sherpa on ascent to 5050 m.

	Kathmandu (1400 m)	Namche Bazaar (3440 m)	Pheriche (4371 m)	Pyramid (5050 m)
Baseline Diameter (mm)				
Lowlander	6.47±0.57	6.54±0.58	6.75±0.65	6.83±0.61*
Sherpa	6.38±0.96	6.32±0.69	6.47±0.85	6.64±0.93*
	Elevation P=0.014 Race P=0.346 Interaction P=0.714			
Peak Diameter (mm)				
Lowlander	6.77±0.59	6.88±0.60	7.13±0.63	7.24±0.59†
Sherpa	6.69±1.01	6.53±0.68	6.78±0.82	6.97±0.92†
	Elevation P=0.001 Race P=0.215 Interaction P=0.399			
Absolute FMD (mm)				
Lowlander	0.31±0.15	0.34±0.17	0.38±0.19	0.41±0.23
Sherpa	0.31±0.08	0.21±0.09	0.31±0.11	0.33±0.12
	Elevation P=0.080 Race P=0.069 Interaction P=0.379			
SS_{AUC} (au)				
Lowlander	597±326	790±298	686±175	787±251‡
Sherpa	662±326	617±284	486±209	732±371‡
	Elevation P=0.028 Race P=0.275 Interaction P=0.103			
SS_{AUC}-corrected FMD (%)				
Lowlander	4.9±2.6	5.1±2.6	5.7±2.6	6.0±2.6
Sherpa	4.9±2.6	3.5±2.6	4.6±2.6	5.1±2.5
	Elevation P=0.210 Race P=0.129 Interaction P=0.556			
Time to peak FMD (s)				
Lowlander	54±22	57±23	56±20	57±24
Sherpa	55±18	59±24	47±26	51±25
	Elevation P=0.676 Race P=0.603 Interaction P=0.620			
Peak reactive hyperemia (ml min^{-1})				
Lowlander	829±243	955±235	953±275	1069±286†
Sherpa	769±270	691±242	755±211	913±290†
	Elevation P=0.002 Race P=0.019 Interaction P=0.230			
Total reactive hyperemia (l)				
Lowlander	38.2±13.1	44.4±16.8	41.1±13.3	44.6±18.1
Sherpa	37.5±19.8	30.3±11.5†	28.3±13.8§	43.4±15.9‡
	Elevation P=0.026 Race P=0.095 Interaction P=0.038			

*, P<0.05 *versus* 1400 m; †, P<0.05 *versus* 1400 m and 3440 m; ‡, P<0.05 *versus* 4371 m; §, P<0.05 *versus* lowlander at the same elevation.

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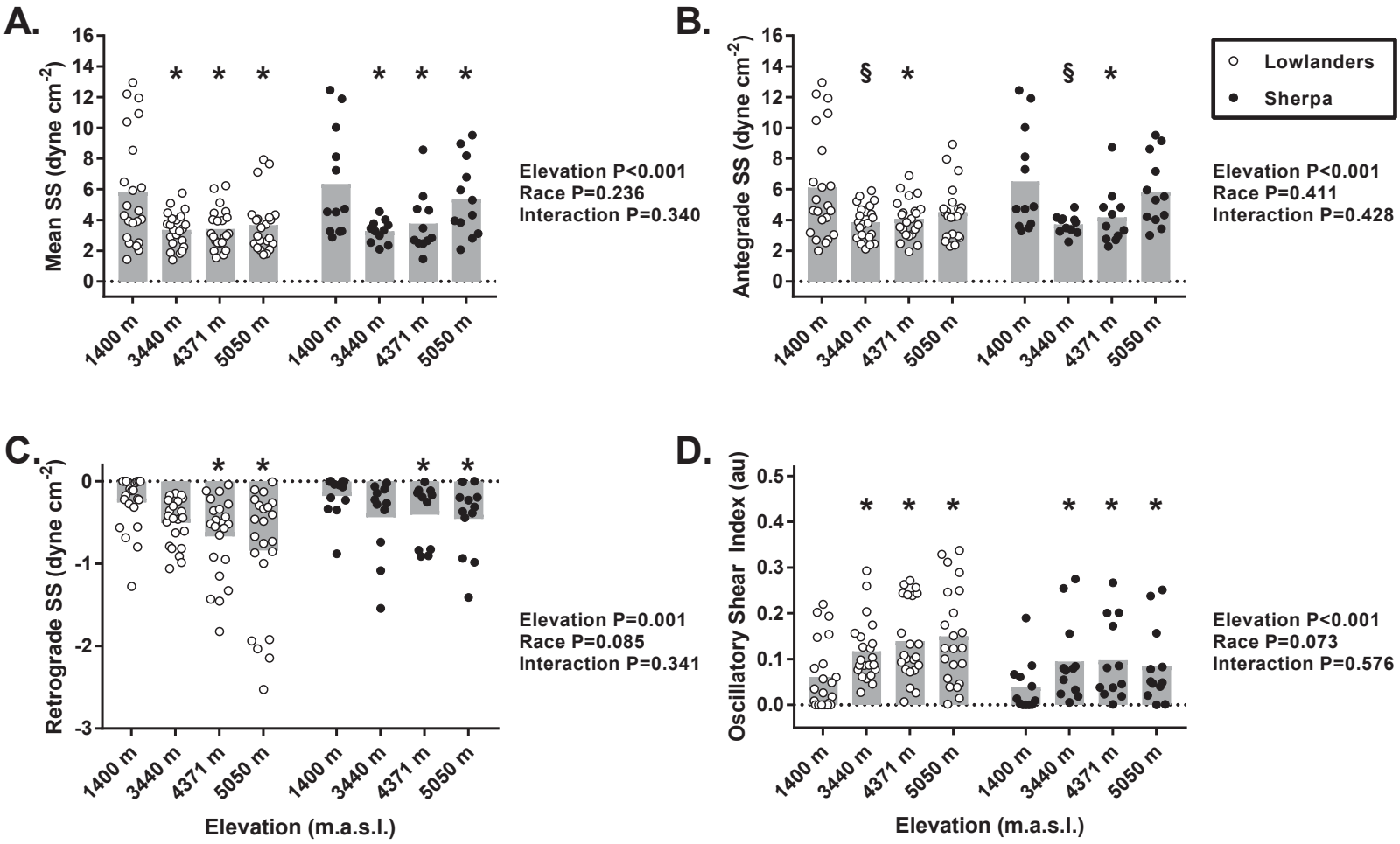
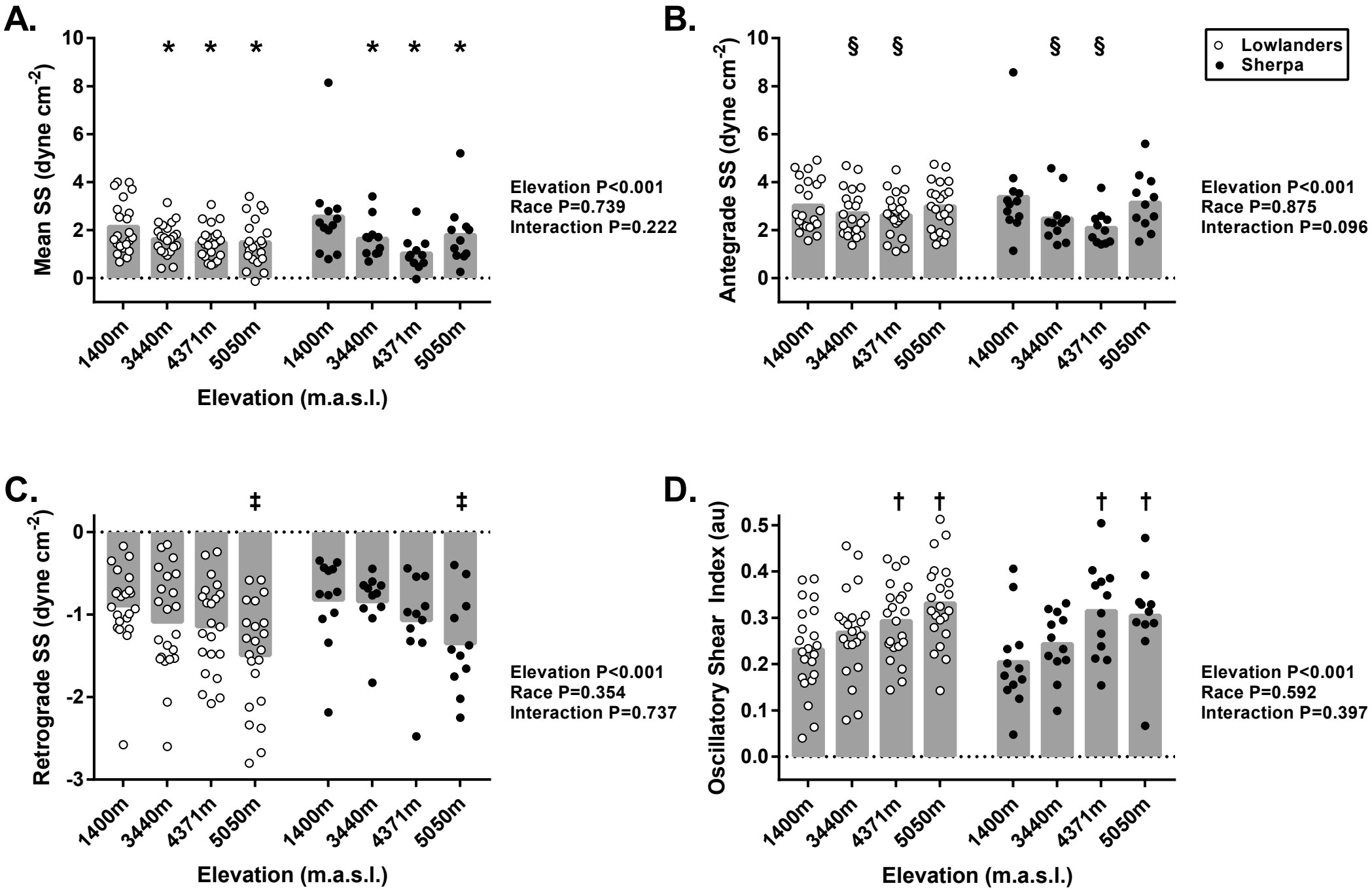
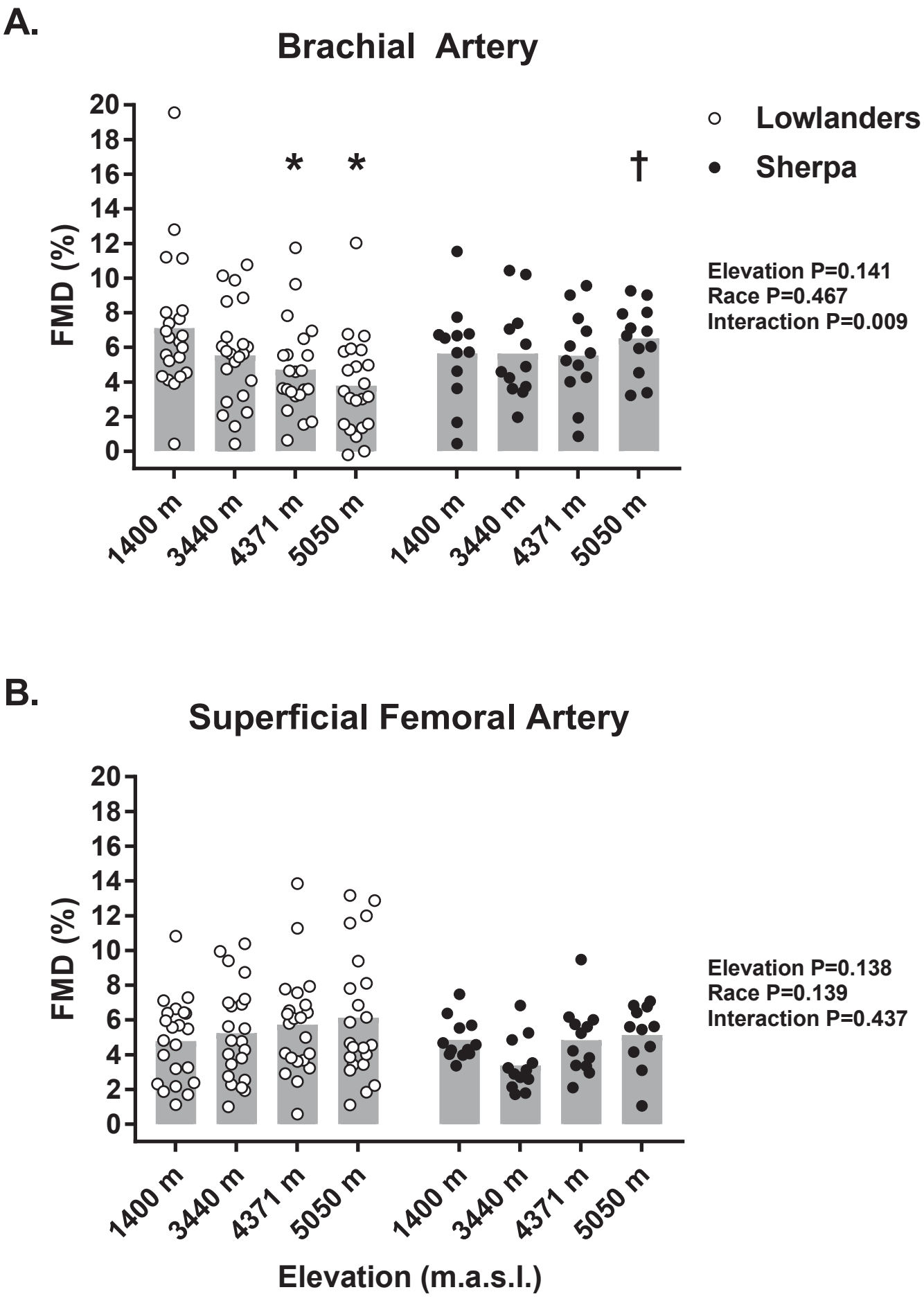

A.
Mean SS (dyne cm-2)
16
14
12
10
8
6
4
2
0
*
1400 m
3440 m
4371 m
5050 m
Elevation P<0.001
Race P=0.236
Interaction P=0.340
B.
Antegrade SS (dyne cm-2)
§
*
Lowlanders
Sherpa
Elevation P<0.001
Race P=0.411
Interaction P=0.428
C.
Retrograde SS (dyne cm-2)
0
-1
-2
-3
Elevation (m.a.s.l.)
Elevation P=0.001
Race P=0.085
Interaction P=0.341
D.
Oscillatory Shear Index (au)
0.5
0.4
0.3
0.2
0.1
0.0
Elevation (m.a.s.l.)
Elevation P<0.001
Race P=0.073
Interaction P=0.576


A.
Mean SS (dyne cm-2)
10
8
6
4
2
0
*
1400m
3440m
4371m
5050m
Elevation (m.a.s.l.)
Elevation P<0.001
Race P=0.739
Interaction P=0.222
B.
Antegrade SS (dyne cm-2)
§
Lowlanders
Sherpa
Elevation P<0.001
Race P=0.875
Interaction P=0.096
C.
Retrograde SS (dyne cm-2)
0
-1
-2
-3
‡
Elevation P<0.001
Race P=0.354
Interaction P=0.737
D.
Oscillatory Shear Index (au)
0.5
0.4
0.3
0.2
0.1
0.0
†
Elevation P<0.001
Race P=0.592
Interaction P=0.397


A.
Brachial Artery
FMD (%)
20
18
16
14
12
10
8
6
4
2
0
*
*
†
Lowlanders
Sherpa
Elevation P=0.141
Race P=0.467
Interaction P=0.009
1400 m
3440 m
4371 m
5050 m
1400 m
3440 m
4371 m
5050 m
B.
Superficial Femoral Artery
FMD (%)
20
18
16
14
12
10
8
6
4
2
0
Elevation P=0.138
Race P=0.139
Interaction P=0.437
1400 m
3440 m
4371 m
5050 m
1400 m
3440 m
4371 m
5050 m
Elevation (m.a.s.l.)