Muscle Sympathetic Reactivity to Metabolic and Apneic Stress in High-Altitude Sherpa

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ABSTRACT

Lowland-dwelling populations exhibit persistent sympathetic hyperactivity at altitude that may alter vascular function. High altitude populations, such as Sherpa, exhibit greater peripheral blood flow in response to acute stress, suggesting Sherpas may exhibit lower sympathetic activity and reactivity to stress than Lowlanders. Muscle sympathetic activity (MSNA; microneurography) including frequency (bursts/min), incidence (bursts/100HB), amplitude (% of max burst), was measured at rest in Lowlanders (n=14; age=27±6yrs) at 344m and following a 8-9 days of graded ascent to 5050m. Sherpa (age=32±11yrs) were tested at 5050m (n=8). Neurovascular reactivity (i.e., change in MSNA patterns) was measured during maximal end-expiratory apnea, isometric hand-grip (IHG; 30% maximal voluntary contraction for 2 minutes) and post exercise circulatory occlusion (PECO; 3 minutes). Total normalized SNA (au/min) was calculated over 10 cardiac cycles during baseline and pre-volitional apnea breakpoint. Lowlander burst frequency (11±5 bursts/min to 30±7 bursts/min; Mean±SD; $p<0.001$) and burst incidence (25±13 bursts/100HB to 53±15 bursts/100HB; $p<0.001$) increased at 5050m. In contrast, Sherpas had lower burst frequency (23±11 bursts/min; $p<0.05$) and incidence (30±13 bursts/100HB; $p<0.05$) at 5050m. MSNA increases in Lowlanders and Sherpa during apnea at 5050m were significantly lower than Lowlanders at 344m (both $P<0.05$), with a possible sympathetic ceiling reached in Lowlanders at 5050m. MSNA increased similarly during the IHG/PECO in Lowlanders at 334m and 5050m altitude and Sherpa at 5050m. Sherpa demonstrate overall lower sympathetic activity and reactivity during severe stress. This may be a result of improved systemic hemodynamic function associated with evolutionary adaptations to permanent residency at altitude.

INTRODUCTION

The response to both acute (20, 22, 31, 32, 38) and chronic (e.g. altitude) (7, 15, 23, 26) reductions of oxygen availability in lowland dwelling populations is an increase in basal efferent sympathetic activity (SNA). This sympathoexcitation occurs in conjunction with local dilation to facilitate blood flow redistribution and oxygen delivery to critical tissues. Previous findings also show an augmented SNA response to additional stress (i.e. increased sympathetic reactivity) under acute hypoxic conditions (20, 35). Whether a similar potentiation occurs during chronic hypoxia is unknown. As Lowlanders exhibit heightened SNA at altitude, we propose a similar potentiation of sympathetic reactivity to additional stress. Furthermore, whether similar responses are evident in those exposed to long duration hypoxia, such as high altitude natives has not been studied. Nepalese/Tibetan Sherpa have resided at high altitude (>2500m) for thousands of years, allowing for unique evolutionary phenotypic adaptations under chronic hypoxia exposure. This includes cardiovascular adaptations that allow for increased oxygen delivery and metabolism at the local tissue (8, 11, 43). Limited data suggest that Sherpa exhibit an improved ability to increase systemic blood flow at altitude (11, 34), which we hypothesized may be in part through lower SNA reactivity to stress at altitude.

To address these hypotheses, we performed microneurography recordings of efferent post-ganglionic nerve activity in Lowland dwellers at low (344m) and high (5050m) altitudes and in a group of native Sherpa at altitude (5050m). This was complemented with brachial ultrasonography to determine the effect of heightened sympathetic stress on vascular function during stress in Sherpa and acclimatized Lowlanders.

METHODS

This study was carried out within the framework of the 2016 UBC Nepal Expedition to the Ev-K2-CNR Research Facility (5050m) (42). Though participants took part in a number of independent investigations, experiments were organized to ensure no contamination between studies, and each study addressed distinct *a priori* research questions. Baseline demographics, cardiovascular characteristics and heart rate responses to apnea have been previously reported from Lowlanders and Sherpa at altitude (4, 40). Metrics of basal sympathetic activity is reported in parallel to Simpson *et al.* (37). However, the current study focuses on novel independent analyses related to sympathetic reactivity.

Study Participants

Fourteen Lowlanders (27±6yrs; 2 female) and ten Sherpa (32±11yrs) from the Khumbu region of Nepal participated after providing informed written consent in their native language. Procedures were explained in Nepalese and English as needed, and were approved by the University of Alberta Biomedical Research Ethics Board, University of British Columbia Clinical Research Ethics Board, and Nepal Health Research Council. Participants were free of ventilatory, cardiovascular, metabolic, and neurological disorders as determined by a self-reported health history questionnaire. Four Sherpa were self-reported smokers (0.42±0.7 pack years).

Testing Location(s)

The ascent profile and testing schedules are outlined in Supplemental Figures 1,2, and 3. Pre-expedition testing of Lowlanders (n=14) was performed at 344m (Kelowna, Canada). To match the ascent profile and acclimatization process between groups, Sherpa were flown to Kathmandu, Nepal (1400 m), where they resided between 5-15 days. Data were successfully collected in a subset of Sherpa (n=5) in Kathmandu before both Lowlanders and Sherpa flew to Lukla, Nepal (2840m) and followed a 9-10 day ascent. One lowlander was administered oral acetazolamide (half life - 4 hrs) and another was administered an intramuscular injection of dexamethasone (half life – 3 hours) following 4 days arrival at 5050m for the treatment of acute mountain sickness; however, both were tested after a 48-hour washout. Sherpa were not on any medication and were tested on days 1-3 following arrival at 5050m, while Lowlanders were tested between days 1-10 (Supplemental figure 2).

Study Protocol

Following instrumentation, basal SNA and cardiovascular function was measured during 10 minutes of quiet rest. Sympathetic reactivity was subsequently assessed using two protocols: 1) a volitional end-expiratory apnea at functional residual capacity (24) and 2) isometric hand-grip (IHG) performed for 2 min followed by 3 min of post-exercise circulatory occlusion (PECO). Prior to apnea, an investigator paced the participants' breathing (2-3 breaths) to maintain rate and depth, while preventing hyperventilation. Participants were then instructed to "hold their breath for as long as possible. Participants performed IHG at 30% of their previously determined maximal voluntary contraction using handgrip dynamometer (Grip Force Transducer; ADInstruments, Australia). Immediately following 2 minutes of IHG, a manual blood pressure

cuff was inflated (>200mmHg) for 3 minutes to stimulate post-exercise ischemia while the limb was relaxed. The apnea protocol always preceded IHG/PECO protocol.

Experimental Measures

All participants were tested in the supine position. ECG (Lead II) and the arterial blood pressure waveforms (finger photoplethysmography; Finometer Pro, Finapres Medical Systems, Netherlands) were collected continuously at 1 KHz (ADInstruments, Chart Pro v8.3.1, Australia). Heart rate (HR) was calculated from the ECG R-R interval. Beat-by-beat cardiac output (CO) was calculated using the Model Flow algorithm and used to calculate total peripheral resistance (TPR = MAP/CO). Beat-by-beat mean (MAP), systolic (SBP) and diastolic (DBP) pressures were calculated from the arterial pressure waveform that was calibrated against manual sphygmometry.

Muscle Sympathetic Nerve Activity

Microneurography was used to directly measure efferent muscle sympathetic vasomotor nerve activity (MSNA) (13, 37, 39). A tungsten microelectrode (200μm diameter, 35 mm long, tapered to a 1-5 μm uninsulated tip) was inserted percutaneous into the peroneal (common fibular) nerve, with an uncoated tungsten reference electrode inserted subcutaneously 1-3 cm from the recording site. The recording electrode was manipulated until a pulse-synchronous bursting pattern was identifiable in response to apnea but not a loud noise (5). The raw MSNA signal was amplified (1000x pre-amplifier and 100x variable gain isolated amplifier), band pass filtered (700-2,000Hz), rectified, and integrated (decay constant 0.1s) to obtain a mean voltage neurogram

(model 662C-3; Iowa University Bioengineering; USA). Both raw and integrated signals were sampled at 10 KHz (ADInstruments, Chart Pro v8.3.1; Australia).

Vascular Ultrasonography

In a subset of Lowlanders (n= 8) and Sherpa (n = 4), ultrasonography was used to measure brachial artery (BA) diameter, Doppler velocity (BA_v), and flow (BA_F) in the non-exercising arm at baseline and during the IHG/PECO protocols (12 ~MHz linear array transducer; Vivid Q, GE Healthcare). Probe insonation-angle was kept constant (60°) across all tests. Video capture was used for recording vessel diameter (DVI2USB3.0; Epiphan Systems, Canada) and was stored off-line in audio video interleave format for future analysis with edge detection software (Brachial Analyzer, Medical Imaging Applications, USA). Image analysis of data was performed at 30Hz following visual confirmation (SAB) of the region of interest to ensure clearly distinguishable lumen walls. BA flow velocity waveforms were converted from Doppler audio signals (qDAT; Penn State, USA) (16) and stored offline at 1 KHz (ADInstruments, Chart Pro v8.3.1).

Data and Statistical Analysis

Baseline MSNA and cardiovascular data were averaged over ~10 minutes during quiet rest. MSNA bursts were identified using a semi-automated detection algorithm (Chart Pro 8.3.1) and confirmed by a trained observer (SAB). Baseline MSNA was quantified as burst frequency (bursts/min), incidence (bursts/100 HB), and normalized burst amplitude (% of maximal burst size at baseline) and area (area under the curve, [au]). For the apnea protocol, MSNA and cardiovascular data were analyzed from the final 10 cardiac cycles prior to volitional breakpoint.

Cardiovascular post-apnea nadir (S_pO_2, HR) and peak (SBP, DBP, MAP) responses were obtained in 10-15 seconds post breakpoint. MSNA bursts during the apnea were calculated as the burst area (area under the curve, [au]) during the last 10 cardiac cycles prior to volitional breakpoint. Burst area was normalized SNA (au/min) during baseline and apnea to account for variations in cardiac cycle length and burst width(4). In addition, the average likelihood (%) of a burst occurring during a given cardiac cycle for apneas was calculated across participants. Sympathetic reactivity to apnea was assessed as the increase in normalized burst area was compared between baseline and end-apnea. MSNA (frequency, incidence and normalized amplitude) and cardiovascular data (HR, blood pressure, TPR, and BA_F) were averaged during the last minute of both IHG and PECO. Sympathetic reactivity was assessed as the increase in MSNA from baseline to IHG and PECO.

Results are reported as mean ± standard deviation (SD) with the exception of baseline burst amplitude, which is reported as median ± interquartile range (IQR) to account for differences in amplitude distribution between conditions. Multiple comparisons were assessed for all measurements using pre-planned contrasts of Lowlanders from low to high altitude (paired T-tests), and Lowlanders to Sherpa at high altitude (unpaired T-tests) with an adjusted alpha (α') value corrected for multiple comparisons (c). This was performed by adjusting the *a priori* alpha (α, 0.05) using the experiment-wise error rate (α_e) (4, 17):

$$a' = \frac{\alpha_e}{c}$$

$$\alpha_e = 1 - (1 - \alpha)^c$$

For normalized bursts amplitude, and vascular responses during baseline, IHG, and PECO protocols, a two ways repeated measures ANOVA compared the main and interaction effects in the distributions between conditions. To address the potential effect of duration at altitude on MSNA; a secondary analysis via Pearson's moment correlation analysis of dependent variables was performed in this study. Finally, ANCOVA analysis was used to control for duration at altitude. All statistical analyses were performed using Sigma Stat 3.13 (Systat Software, Chicago, IL).

RESULTS

Fourteen lowlanders were successfully tested at 344m and again at 5050m. Two Lowlanders reported having mild acute mountain sickness (AMS) on the day of testing (Lake Louise scores of 3). However, the data from these two subjects were comparable to the averaged responses and therefore included in the main analyses. Eight of the ten Sherpa who descended to Kathmandu were successfully tested at 5050m. No relationship was shown between duration after arriving at 5050m and MSNA responses in either Lowlanders (Burst Frequency r^2= 0.160, p= 0.065) or Sherpa (Burst Frequency r^2 = 0.001, p=0.937). Baseline cardiovascular and autonomic characteristics for both Sherpa and Lowlanders are listed in Table 1. SBP, DBP, MAP, CO, and TPR and SpO2 were not difference between lowlanders and Sherpa at 5050m

Baseline Neurovascular Characteristics in Sherpa and Lowlanders

Resting MSNA values for Sherpa and Lowlanders are exhibited in Figures 1 and 2. Lowlanders burst frequency tripled (11 ± 5 bursts/ min to 30 ± 7 bursts/ min; p<0.001) while burst incidence doubled (25 ± 13 bursts/100 HB to 50 ± 15 bursts/ 100 HB; p<0.001) following several days at high altitude. At 5050m, Sherpa had a lower burst frequency (23 ± 11 bursts/min;

183 $p<0.05$) and incidence (30 ± 13 bursts/100 HB; $p<0.05$) compared to Lowlanders at 5050m. At
184 altitude the distribution of normalized burst area was also shifted towards larger sized bursts in
185 Lowlanders (Figure 2), with the burst amplitude distributions in the Sherpa being similar to that
186 of Lowlanders at 5050m. After taking into account differences in both frequency and amplitude
187 distribution, total basal sympathetic activity was similar in Sherpa (1103 ± 520 au/min) and
188 Lowlanders at 5050m (1320 ± 520 au/min) with both being higher than observed in lowlanders
189 at low altitude (451 ± 206 au/min; $p<0.05$).
190 In, a subset of Sherpa (n=5) baseline sympathetic activity was collected in Kathmandu
191 (1400 m). At 1400 m, the subset of Sherpa exhibited a burst frequency (13 ± 3 bursts/min) and
192 incidence (21 ± 6 bursts/100 HB); these values were similar to that of Lowlanders at low altitude
193 (344m) (non-statistical observation). Although underpowered to perform complete statistical
194 analyses, these Sherpa had a lower burst Frequency ($P<0.05$) but not incidence ($P=0.198$)
195 compared to Sherpa tested at altitude. These data are included for completeness and descriptive
196 purposes (See Table 2).
197
198 **Sympathetic Reactivity to Apnea in Lowlanders and Sherpa**
199 Sympathetic neurovascular reactivity during apnea was assessed in 14 Lowlanders at low
200 and high altitude, and 8 Sherpa assessed at 5050m. At low altitude, Lowlanders had an apnea
201 duration of 30.4 ± 11.1 s (range 15-74s) which was reduced to 15.4 ± 5.3 s (range 9-27s)
202 ($P<0.001$) at 5050m. Lowlanders SpO_2 nadir post-apnea was 78 ± 7%. Sherpa apnea duration
203 (15.8 ± 2.6; Range 12-19s) and saturation (75 ± 5%) post-apnea were not different to that of
204 Lowlanders ($P = 0.84$).

Apnea across all groups and conditions produced a very robust increase in MSNA driven by changes in both burst occurrence and burst area (Figures 4 & 5). Apnea at low altitude was associated with a significant increase in MSNA in Lowlanders (normalized total area +31359 ± 30383 au/min compared to baseline; $P<0.01$). While apnea at altitude resulted in the largest burst augmentation and increase in burst occurrence (Figure 5), this occurred over a longer time due to a previously reported bradycardia [16]. Thus, the au/min response to apnea tended to be less at high altitude (+17711 ± 11018 au/min versus baseline; $p=0.063$). Interestingly, 5 out of the 14 Lowlanders had "prolongation" of sympathetic bursts during apnea at high altitude that did not represent normal burst firing characteristics. More specifically, the cyclical modulation of efferent bursts activity was altered at 5050m such that bursts became broader and less peaked, encompassing a larger portion of the cardiac cycle (Figure 3). In contrast, Sherpa sympathetic responses to apnea (+7708 ± 4312 au/min) were significantly lower than Lowlanders at low altitude ($P<0.05$) and at 5050m ($P<0.05$). Additionally, there were no observed cases of "prolonged" bursts in Sherpa neurograms.

Apnea resulted in a significant increase in mean blood pressure in all three groups; 34 ± 13 mmHg in Lowlanders at low altitude, 35 ± 20 mmHg in Lowlanders at 5050m and 23 ± 8 mmHg in Sherpa at 5050m (all $P<0.01$ with respect to baseline). The increase in MAP associated with apnea was smallest in Sherpa ($P<0.05$ when compared to Lowlanders at 334m). When these responses were considered together Sherpa had higher vascular responsiveness to sympathetic activation during apnea (3.70 ± 1.90 mmHg/au/min x 10^{-3}) compared to Lowlander at low (1.84 ± 1.17 mmHg/au/min x 10^{-3}, $P=0.009$) but not high (2.62 ± 1.81 mmHg/au/min x 10^{-3}, $P=0.227$) altitude.

Sympathetic and Vascular Reactivity to Isometric Hand Grip and Post Exercise Circulatory Occlusion

Sympathetic neurovascular reactivity during the IHG/PECO protocols was assessed in 14 Lowlanders at low and high altitude, and was successfully collected in 6 Sherpa 5050m. At altitude, Sherpa exhibited an overall lower burst frequency, incidence and total MSNA during the BL, IHG and PECO compared to acclimatized Lowlanders (Figure 6, each $P<0.001$). During IHG burst frequency (+17 ± 9, +18 ± 13, and +16 ± 12 bursts/min; all $P<0.001$), burst incidence (+14 ± 15, +5 ± 15, and +13 ± 13 bursts/100 HB; all $P<0.001$) and total MSNA (+1429 ± 893, +1247 ± 1178, and +1827 ± 1361 au/min) were elevated significantly in Lowlanders at 344m and 5050m, and Sherpa at 5050m respectively (Figure 6). No further increase in burst frequency or total MSNA occurred between IHG and PECO, although burst incidence climbed due to the concurrent return of heart rate to baseline during PECO (Figure 7). The increase in MSNA occurring with IHG/PECO was not different between groups.

While Sherpa tended to have an overall lower blood pressure, the changes in blood pressure responses to IHG and PECO were similar between groups, as was the change in total peripheral resistance (Figure 8). Thus, unlike the apnea protocol we did not detect significant differences in neurovascular reactivity during IHG / PECO stress with altitude (in Lowlanders) or between groups (at 5050m).

Forearm Blood Flow Reactivity in Lowlanders and Sherpa

Brachial artery blood flow was successfully analyzed in a subset of 8 Lowlanders and 4 Sherpa during the HG/PECO protocol. Basal blood flow was not different between groups,

250 although there was a significant main effect for brachial artery conductance ($P<0.05$), with
251 Lowlanders having a higher brachial artery conductance at altitude (Figure 8). The HG and
252 PECO protocols did not result in a change in contralateral blood flow or resistance (or
253 conductance). Consequently, no differences in reactivity were observed between groups, similar
254 to blood pressure results (above).

255

256 **DISCUSSION**

257 This study demonstrates that sympathetic neural activation (i.e. increases in MSNA) in
258 response to acute apneic stress appears lower in Lowlanders following acclimatization. However,
259 this is due to the unique nature of this stress. Nonetheless, Sherpa had a lesser response
260 compared to acclimatizing Lowlanders at 5050m and Lowlanders at low altitude (334m). This
261 lower MSNA response in Sherpa was offset by a greater vascular reactivity to sympathetic
262 activation. During isometric hand-grip and post-exercise circulatory occlusion, sympathetic
263 activation was observed to be much lower than the apneic stress, and no differences were noted
264 between groups with respect to sympathetic activation or vascular responses.

265 ***Basal MSNA at altitude***

266 The majority of MSNA studies involving hypoxia have been exclusively performed in
267 Lowlanders, where basal MSNA increases under acute hypoxia (minutes – hours) (32, 44) and
268 becomes further augmented during acclimatization (7, 10, 23, 26, 33). These previous findings
269 agree with the elevated burst frequency and incidence seen in our acclimatized Lowlanders. We
270 extend these findings by demonstrating lower MSNA for Sherpa under basal conditions. To date
271 there has only been one other published MSNA study performed in Bolivian highlanders. These

previous data indicate a similar basal MSNA in Andeans and acclimatized Lowlanders (following 50 days) at 4300m (23). However, the juxtaposition of our own data suggest differences in high-altitude populations due to potential differences in genetic expression (45) and phenotypic differences (2) that exist through generational residency at altitude.

MSNA responses to apneic and hand-grip / post-exercise circulatory occlusion stressors

Apnea proved to be a significant sympathetic stressor, both at low and high altitude. Although acclimatized Lowlanders demonstrated 100% burst occurrence and the greatest bust augmentation (i.e. increase in burst area), normalized total activity remained lower than that measured at low altitude. This was due to a previously reported bradycardia [16] that limits burst frequency. Thus, an apparent "sympathetic ceiling" may be reached during apnea in chronic hypoxic conditions. Despite similar apnea durations and desaturation in Sherpa compared to acclimatized Lowlanders, the Sherpa demonstrated a lower burst occurrence, lower burst augmentation and a lower total MSNA response to apnea. Importantly, this occurred without an apparent limitation on burst frequency (i.e. no bradycardia [16]). These data indicate that Sherpa are less responsive to apneic stress under the same hypoxic conditions as acclimatized lowlanders. This also suggests that Sherpa have a greater functional sympathetic reserve, whereby they may theoretically be able to increase MSNA more than observed in the current study. In contrast to apneic stress, the MSNA response to the IHG/PECO protocol was appreciably smaller (although not assessed statistically). Although a main effect of group was present, with acclimatized Lowlanders having the highest activity, all three groups exhibited similar increases in MSNA burst frequency, incidence, and total activity during IHG/PECO.

294 Previous studies in Lowlanders under acute hypoxic exposure demonstrate further MSNA
295 potentiation during dynamic exercise compared to normoxia. (20, 36) Since the ascent to
296 altitude causes further reductions in oxygen availability to the local tissue alongside concurrent
297 MSNA augmentation, we hypothesized that altitude would also be associated with an augmented
298 SNA response to IHG and PECO. However, the lack of difference in the response at low and
299 high altitudes argue that chronic hypoxic stress compounded with further metabolic activation
300 (albeit in one isolated limb during an isometric contraction) does not alter the MSNA response.
301 Therefore, MSNA reactivity to metaboreflex stress appears preserved in acclimatized
302 normotensive Lowlanders. We believe that this may be explained through several myogenic
303 adaptations during acclimatization that favor anaerobic metabolism under chronic hypoxia
304 exposure. With long-term exposure to altitude there has been previously noted reductions of
305 muscle oxidative capacity (18) associated with muscle atrophy and catabolism(3) in addition to a
306 shift away from FA enzyme oxidation during rest and exercise(30). These potential adaptations
307 during longer periods of residency at altitude would improve anaerobic metabolism, reduce
308 metabolic strain and overall sympathetic activation compared to exertion during an acute period
309 of hypoxia exposure. However, these adaptations may be time dependant, with shorter periods of
310 exposure not appearing to not show any significant changes in skeletal muscle function or
311 morphology (27). Therefore, any potential myogenic adaptations that may exist in the first week
312 of acclimatization for Lowlanders does not appear to directly affect efferent sympathetic
313 outflow. Previous data have indicated lower mitochondrial density(21), improved ATP to O_2
314 yield and greater energy production at a lower oxygen cost in Sherpa(19). Sherpa have also
315 previously demonstrated increased ability to augment femoral blood flow velocity post-
316 circulatory occlusion compared to Lowlanders (34). For these reasons, we hypothesized that

Sherpa would have a lower MSNA response to the IHG/PECO protocol. Counter to this hypothesis, we observed that Sherpa had a similar increase in MSNA during IHG/PECO. It is worth noting that MSNA was lower in Sherpa compared to acclimatized Lowlanders through baseline and IHG/PECO. Thus, the above noted mechanisms could still be involved in shifting the MSNA relationship lower, but keep the same gain of the response to metabolic stress.

Neurovascular Reactivity between Lowlanders and Sherpa at Altitude

During both apnea and IHG/ PECO reactivity protocols, there was no noted difference in pressor responses to sympathetic activation in Lowlanders at low or high altitudes, with exception to a lower TPR response under the PECO condition at altitude compared to sea level. Thus, the current study demonstrates that overall cardiovascular reactivity was preserved during altitude acclimatization 5050m. From the findings of both reactive conditions, Sherpa appeared to have a heightened vascular responsiveness during apnea, but not IHG/PECO. We postulate two explanations for this disparity. The first being the magnitude of the response to apnea was much larger than during the IHG/PECO protocol, and thus may have been more robust for identifying differences between groups. Second, as mean arterial pressure was the analyzed outcome of this incorporates total systemic changes in vascular resistance as well as any changes in cardiac output. Thus, the modest activation of sympathetic outflow to vascular within skeletal muscle may not have had a significant influence on mean pressure. The subset of data evaluating brachial artery blood flow support this hypothesis. Although our data do not support differences in the cardiovascular response to small muscle mass recruitment in Sherpa, we acknowledge previous data which suggests other cardiovascular adaptations in this population Sherpa have previously been shown to exhibit greater capillary density within skeletal muscle

(21) and improved ability to increase leg blood flow following 2 minutes of circulatory occlusion (34). Ezurum *et al.* (8) subsequently demonstrated that Tibetans have higher resting forearm blood flow and circulating NO by-products. These previous data support an improved dilatory capacity, but we believe that our data also support a lower resting sympathetic activity, a greater sympathetic reserve and greater vascular sensitivity to higher levels of sympathetic activity. Together, his may serve as an important control mechanism for redirected blood and oxygen during stress. Thus, Sherpa appear to have developed improved cardiovascular efficiency that does not rely to the same extent on sympathetic hyperactivity relative to acclimatized Lowlanders. Whether this is expressed through other high altitude populations, remains to be determined.

Perspectives and Considerations

An interesting finding for Lowlanders was the lower total absolute MSNA responses to apnea at high altitudes, despite basal MSNA being augmented at 5050m. As the apnea duration was shorter, in combination with a lower post-breakpoint SpO_2 (indicative of an increase chemoreceptor activation) (9, 41), it can be argued that apnea at altitude is a greater sympathetic stressor than it is at low altitude. However, the concurrent bradycardia which we have previously reported on [16] apparently limited sympathetic activation and could explain the appearance of abnormal MSNA burst patterns at 5050m. We believe this demonstrates a potential sympathetic "ceiling effect" may have developed in Lowlanders, where further stress does not produce additional MSNA activation. In other words, there is less MSNA reserve available for responding to acute stress at altitude. MSNA outflow is limited to an individuals' respective cardiac cycle, where a finite degree of sympathetic augmentation can occur during each R-R

363 interval (5, 25). Whether Sherpa truly have additional MSNA reserve available during apnea, or
364 simply reached their own respective sympathetic ceiling, cannot be confirmed due to us being
365 unable to obtain sympathetic reactivity in Sherpa at Kathmandu. However, the absence of
366 abnormal burst pattern and a lower average incidence of bursts in the cardiac cycles preceding
367 break-point supports this premise.
368
369 As previously mentioned there was an attempt was made to record MSNA in Sherpa at
370 Kathmandu to assess sympathetic de-acclimatization. MSNA was recorded on a subset of 5 (out
371 of 8) Sherpa who had descended and resided in Kathmandu (1400m) 5-15 days prior to testing.
372 Both Sherpa burst frequency (13 ± 3 bursts/ min) and incidence (21 ± 6 bursts/ 100 HB) was
373 similar to that of Lowlanders when tested at 344m (11 ± 5 bursts /min and 25 ± 13 bursts/ 100
374 HB). Although acknowledging the descriptive nature of these data, the present suggests that a)
375 altitude still represents a significant stress in high altitude natives, and b) the adaptations
376 observed may be specific to response under hypoxic conditions and do not persist in relative
377 normoxia. The latter findings likely differ from lowlanders since Hansen and Sander (15), who
378 reported persistently elevated burst frequency and incidence in lowlanders 3 days after return to
379 normobaric conditions following 4 weeks residency at 5260m. Our limited data from Sherpa
380 after ~1 week at low altitude therefore suggest that post hypoxia sympathetic activation
381 persistence may be shorter-lived than previously reported.
382
383 Though we report that MSNA is lower across both basal and reactivity conditions for
384 Sherpa, the specific mechanism that contributes to this overall lower MSNA response has not yet
385 been determined. During the transition between acute to chronic hypoxia exposure there is an

apparent time-dependant sensitization for the peripheral chemoreceptors that results in progressively heightened MSNA (6). This is supported by a higher basal MSNA previously observed by Hansen and Sander (15) and further confirmed by Lundby *et al.* (23). If this were true then it could be argued that attenuated chemoreflex sensitivity in Sherpa should explain their lower basal MSNA. However, chemoreceptor sensitization does not appear to explain the differences in MSNA observed between Lowlanders and Sherpa as the current consensus (including more recent publishing from this expedition) demonstrate a similar hypoxic ventilatory response between acclimatized Lowlanders and Sherpa (1, 4, 12). Therefore, the lower MSNA noted for Sherpa must be through a combination of other reflexes. These may include differences in long-term potentiation of central regulatory mechanisms, central resetting, or baroreflex-mediated changes between Lowlanders and Sherpa (14, 29, 44); the latter is addressed in a parallel paper by this research group (37). In general further research is needed in order to delineate underlying mechanisms that underpin autonomic and neurovascular control at high altitude in native Sherpa.

Limitations

As testing of Lowlanders and Sherpa occurred during the initial 10 days of being at 5050m, we acknowledge that the findings within this study may be in part influenced by the respective date individuals were tested at 5050m. As previously stated we assessed the potential covariate of duration through Pearson's correlation and follow up ANCOVA analysis. However, when examining baseline sympathetic function, and both sympathetic and vascular reactivity between groups, there was no relationship following correction for the day they were tested at 5050m. Although there may exist a gradual increase in sympathetic activity following prolonged

durations at altitude, this does not appear to have affected our results. Furthermore, Lundby *et al.* (23) also showed that MSNA was similar in acclimatized Lowlanders between days 10 and 50 at 4100 m. MSNA therefore does not appear to increase further following several days at altitude, though the exact period is currently undefined. However, we also acknowledge that degree of sympathetic activation may be dose dependant with regards to the specific severity of hypoxic exposure (32). Whether MSNA shows further augmentation over the span of days-weeks should be considered for future studies.

The use of voluntary apnea is a simple model of assessing muscle autonomic reactivity as it evokes both a quick and large sympathetic response. However, apnea tolerance can be objectively difficult to assess as duration can be affected by several factors including previous repetitive practice overall tolerance to apneic stress between individuals (28). This raises the question of whether Sherpa truly demonstrated a maximal apnea at altitude. However, two main considerations make us believe that Sherpa performed apneas maximally. First, all experimental procedures and manoeuvres were explained to Sherpa in Nepali and trials were repeated if there was any confusion. Second, Sherpa had both a similar apnea duration and drop in SpO_2 to that of Lowlanders.

Conclusion

In conclusion, the study demonstrates that overall MSNA activity and reactivity to stress is lower in Sherpa at altitude; although, the specific mechanisms that governs this difference in MSNA between acclimatized Lowlanders and Sherpa is uncertain. With regards to sympathetic reactivity of acclimatized Lowlander, we demonstrate a potential "sympathetic ceiling" in which only a finite degree of MSNA augmentation is possible. We propose that the observation of an

apparent sympathetic plateauing observed in Lowlanders indirectly supports a potentially greater sympathetic reserve at altitude for Sherpa, though our findings from Kathmandu are underpowered to confirm this. Altered responses to altitude in Sherpa may be a beneficial adaptation to residency at altitude that prevents chronic hypertensive states to increased physical demands during daily life at altitude.

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DISCOLSURES

None

FIGURE CAPTIONS

FIGURE 1. *B*aseline burst frequency (bursts/min) and incidence (burst/100 HB) in Lowlanders at 344m (n=14), 5050m (n=14), and Sherpa at 5050m (n=8). Both burst frequency and incidence was significantly increased at 5050m in Lowlanders. Both burst frequency and incidence were higher in Lowlanders at 5050m versus Sherpa at 5050m. Burst incidence was similar between Lowlanders at low altitude and Sherpa at 5050m. * Significantly different from Lowlanders at low altitude, $P<0.05$; † Significantly different from Sherpa at high altitude, $P<0.05$.

FIGURE 2: Histograms representing normalized burst amplitude distribution during baseline. Frequencies are represented as a percent of bursts for a given size. *Panel A,* Lowlander (n=14) distributions at low (344m, red dotted line) altitude plotted against high (5050m, solid grey bars) altitude. *Panel B,* Lowlander (n=14) distribution at high (5050m, blue dotted line) altitude plotted against Sherpa (n=8) at high (5050m,solid white bars) altitude. Burst amplitude showed a main effect ($P<0.05$) shift in Lowlanders towards larger sized bursts at altitude that was similar to Sherpa.

FIGURE 3: Integrated neurogram demonstrating sympathetic activity during baseline (left) and reactivity during apnea (right). Apnea shows last 10 cardiac cycles obtained prior to volitional breakpoint. *Panel A,B,C.* Example of Neurogram from the same male at 355m (A) and 5050m (B) against Sherpa at 5050m (C). Apnea at altitude caused prolonged burst periods and loss of characteristic burst "peak" in Lowlanders . However, these prolonged burst remained contained with a cardiac cycle. Sherpa did not develop prolonged burst firing patterns at 5050m.

FIGURE 4: Integral burst area (delta % change relative to baseline, mean± SD; denoted as "#A") and incidence of bursts (% of individuals who exhibited a burst during the respective cardiac cycle, denoted as "#B") within Lowlanders (n.=14) and Sherpa (n=8) during apnea. Values represent 10 cardiac cycles

prior to volitional breakpoint (indicated by dashed red line along with mean apnea duration). *Panel 1,2*, Lowlanders at 344m (blue) and 5050m (orange), *Panel 3,* Sherpa at 5050m (red). Maximum integrated burst area was determined as the highest response during the 10 cardiac cycles for each participant. Burst incidence was calculated as the number of individuals (represented as a %) exhibiting a burst during that respective cardiac cycle. Lowlanders showed an increase in sympathetic activity prior to apnea breakpoint at both low and high altitude ($P<0.05$). Sherpa exhibited a smaller increase in burst area ($P<0.05$) versus Lowlanders at 5050m. The incidence of bursts in Lowlanders prior to volitional breakpoint was 100% while incidence of bursts in Sherpa was 88%. * Significant difference from respective baseline $P<0.05$; † Significantly different from Lowlanders at high altitude, $P<0.05$.

FIGURE 5: Bar graph (Mean± SD) representing the absolute changes from baseline in normalized total peak SNA (au/min, solid bars) and change in mean arterial pressure (mmHg, hatched bars) during apnea. The normalized SNA represents the sum of SNA across the duration of 10 cardiac cycles. All groups exhibited a significant increase in MSNA and blood pressure. However, acclimatized Lowlanders (5050m) and Sherpa (5050m) exhibited a smaller change in total SNA compared to Lowlanders at low altitude (334m). Acclimatized Lowlanders (5050m) and Sherpa (5050m) also had smaller blood pressure response compared to Lowlanders at low altitude (334m). * Significant increase with respect to baseline, $P<0.05$; † Significantly different from other groups, $P<0.05$, ‡ Significantly different from Lowlanders at 334m only, $P<0.05$.

FIGURE 6: Line graph representing absolute burst frequency (Denoted as "A"; bursts/min), incidence, (Denoted as "B"; bursts / 100 cardiac cycles) and total sympathetic activity (Denoted as "C"; au) in Lowlanders at low altitude (n=14; white circle), high altitude (n=14; black circle), and Sherpa at high altitude (n=6; black triangle) during the isometric handgrip and occlusion protocol. Lowlanders at exhibited both an interactive and main effect in burst Frequency ($P<0.001$), and incidence ($P<0.001$). Sherpa exhibited an overall lower burst frequency, incidence and total MSNA during the BL, IHG and PECO compared to acclimatized Lowlanders (each $P<0.001$). During IHG burst frequency (all $P<0.001$), burst incidence (all $P<0.001$) and total MSNA (all $P<0.001$) were elevated significantly in Lowlanders at 344m and 5050m, and Sherpa at 5050m respectively. No further increase in burst frequency or total MSNA occurred between IHG and PECO, although burst incidence climbed due to the concurrent return of heart rate to baseline during PECO (Figure 7). The increase in MSNA occurring with IHG/PECO was not different between groups.

FIGURE 7. Line graph representing absolute Heart Rate (Denoted as "A"; bpm), Mean Arterial Pressure, (Denoted as "B"; mmHg) and Total Peripheral Resistance (Denoted as "C"; mmHg/L/min) in Lowlanders at low altitude (n=14; white circle), high altitude (n=14; black circle), and Sherpa at high altitude (n=6; black triangle) during the isometric handgrip and occlusion protocol. Though Sherpa tended to have an overall lower blood pressure ($P<0.001$), the changes in blood pressure responses to IHG and PECO were similar between groups, as was the change in total peripheral resistance. Thus, unlike the apnea protocol we did not detect significant differences in neurovascular reactivity during IHG / PECO stress with altitude (in Lowlanders) or between groups (at 5050m).

Figure 8. Line graph representing absolute Brachial Artery Flow (Denoted as "A"; mL/min), Brachial Artery Resistance , (Denoted as "B"; mmHg/mL/min) and Brachial Artery Conductance (Denoted as "C";mmHg/L/min) in Lowlanders at low altitude (n=8; white circle), high altitude (n=8; black circle), and Sherpa at high altitude (n=4; black triangle) during the isometric handgrip and occlusion protocol. Basal blood flow was not different between groups, although there was a significant main effect for brachial artery conductance ($P<0.05$), with Lowlanders having a higher brachial artery conductance at altitude (Figure 8). The HG and PECO protocols did not result in a change in contralateral blood flow or resistance (or conductance). Consequently, no differences in reactivity were observed between groups, similar to blood pressure results (Figure 7).

REFERENCES

1. **Beall CM, Strohl KP, Blangero J, Williams-Blangero S, Almasy LA, Decker MJ, Worthman CM, Goldstein MC, Vargas E, Villena M, Soria R, Alarcon AM and Gonzales C.** Ventilation and hypoxic ventilatory response of Tibetan and Aymara high altitude natives. *Am.J.Phys.Anthropol.* 104: 4: 427-447, 1997.

2. **Beall CM.** Two routes to functional adaptation: Tibetan and Andean high-altitude natives. *Proc.Natl.Acad.Sci.U.S.A.* 104 Suppl 1: 8655-8660, 2007.

3. **Boyer SJ and Blume FD.** Weight loss and changes in body composition at high altitude. *J.Appl.Physiol.Respir.Environ.Exerc.Physiol.* 57: 5: 1580-1585, 1984.

4. **Busch SA, Davies HE, Van Diepen S, Simpson LL, Sobierajski F, Riske L, Stembridge M, Ainslie PN, Willie CK, Hoiland RL, Moore JP and Steinback CD.** Chemoreflex Mediated Arrhythmia during Apnea at 5050m in Low but not High Altitude Natives. *J.Appl.Physiol.(1985)* 2017.

5. **Delius W, Hagbarth KE, Hongell A and Wallin BG.** General characteristics of sympathetic activity in human muscle nerves. *Acta Physiol.Scand.* 84: 1: 65-81, 1972.

6. **Dempsey JA, Powell FL, Bisgard GE, Blain GM, Poulin MJ and Smith CA.** Role of chemoreception in cardiorespiratory acclimatization to, and deacclimatization from, hypoxia. *J.Appl.Physiol.(1985)* 116: 7: 858-866, 2014.

7. **Duplain H, Vollenweider L, Delabays A, Nicod P, Bartsch P and Scherrer U.** Augmented sympathetic activation during short-term hypoxia and high-altitude exposure in subjects susceptible to high-altitude pulmonary edema. *Circulation* 99: 13: 1713-1718, 1999.

8. **Erzurum SC, Ghosh S, Janocha AJ, Xu W, Bauer S, Bryan NS, Tejero J, Hemann C, Hille R, Stuehr DJ, Feelisch M and Beall CM.** Higher Blood Flow and Circulating NO Products Offset High-Altitude Hypoxia among Tibetans. *Proc.Natl.Acad.Sci.U.S.A.* 104: 45: 17593-17598, 2007.

9. **Eyzaguirre C and Lewin J.** Chemoreceptor activity of the carotid body of the cat. *J.Physiol.(Lond.)* 159: 2: 222-237, 1961.

10. **Fisher JP, Fluck D, Hilty MP and Lundby C.** Carotid chemoreceptor control of muscle sympathetic nerve activity in hypobaric hypoxia. *Exp.Physiol.* 2017.

11. **Gilbert-Kawai E, Coppel J, Court J, van der Kaaij J, Vercueil A, Feelisch M, Levett D, Mythen M, Grocott MP, Martin D and Xtreme Everest 2 Research Group.** Sublingual microcirculatory blood flow and vessel density in Sherpas at high altitude. *J.Appl.Physiol.(1985)* 122: 4: 1011-1018, 2017.

12. **Hackett PH, Reeves JT, Reeves CD, Grover RF and Rennie D.** Control of breathing in Sherpas at low and high altitude. *J.Appl.Physiol.Respir.Environ.Exerc.Physiol.* 49: 3: 374-379, 1980.

13. **Hagbarth KE and Vallbo AB.** Pulse and respiratory grouping of sympathetic impulses in human muscle-nerves. *Acta Physiol.Scand.* 74: 1: 96-108, 1968.

14. **Halliwill JR, Morgan BJ and Charkoudian N.** Peripheral Chemoreflex and Baroreflex Interactions in Cardiovascular Regulation in Humans. *J.Physiol.* 552: Pt 1: 295-302, 2003.

15. **Hansen J and Sander M.** Sympathetic neural overactivity in healthy humans after prolonged exposure to hypobaric hypoxia. *J.Physiol.* 546: Pt 3: 921-929, 2003.

16. **Herr MD, Hogeman CS, Koch DW, Krishnan A, Momen A and Leuenberger UA.** A real-time device for converting Doppler ultrasound audio signals into fluid flow velocity. 298: 5: H1626-H1632, 2010.

17. **Hinkle DE, Wiersma W and Jurs SG.** Multiple-Comparison Procedures. In: *Applied Statistic for the Behavioral Sciences*Anonymous . New York: Houghton Mifflin Company, 2003, p. 370-390.

18. **Hoppeler H, Howald H and Cerretelli P.** Human muscle structure after exposure to extreme altitude. *Experientia* 46: 11-12: 1185, 1990.

19. **Hoppeler H and Vogt M.** Muscle tissue adaptations to hypoxia. *J.Exp.Biol.* 204: 18: 3133-3139, 2001.

20. **Katayama K, Ishida K, Iwamoto E, Iemitsu M, Koike T and Saito M.** Hypoxia augments muscle sympathetic neural response to leg cycling. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 301: 2: R456-64, 2011.

21. **Kayser B, Hoppeler H, Claassen H and Cerretelli P.** Muscle structure and performance capacity of Himalayan Sherpas. *J.Appl.Physiol.(1985)* 70: 5: 1938-1942, 1991.

22. **Leuenberger U, Gleeson K, Wroblewski K, Prophet S, Zelis R, Zwillich C and Sinoway L.** Norepinephrine clearance is increased during acute hypoxemia in humans. *Am.J.Physiol.* 261: 5 Pt 2: H1659-64, 1991.

23. **Lundby C, Calbet J, van Hall G, Saltin B and Sander M.** Sustained sympathetic activity in altitude acclimatizing lowlanders and high-altitude natives. *Scand.J.Med.Sci.Sports* 2017.

24. **Macefield VG and Wallin BG.** Modulation of muscle sympathetic activity during spontaneous and artificial ventilation and apnoea in humans. *J.Auton.Nerv.Syst.* 53: 2-3: 137-147, 1995.

25. **Macefield VG, Elam M and Wallin BG.** Firing properties of single postganglionic sympathetic neurones recorded in awake human subjects. 95: 1–2: 146-159, 2002.

26. **Mitchell KM, Bradbury KE, Posch AM, Beidleman BA, Fulco CS, Muza SR and Charkoudian N.** Influence of recent altitude exposure on sea level sympathetic neural & hemodynamic responses to orthostasis. *Auton.Neurosci.* 210: 18-23, 2018.

27. **Murray AJ.** Energy metabolism and the high-altitude environment. *Exp.Physiol.* 101: 1: 23-27, 2016.

28. **Parkes MJ.** Breath-holding and its breakpoint. *Exp.Physiol.* 91: 1: 1-15, 2006.

29. **Querido JS, Wehrwein EA, Hart EC, Charkoudian N, Henderson WR and Sheel AW.** Baroreflex control of muscle sympathetic nerve activity as a mechanism for persistent sympathoexcitation following acute hypoxia in humans. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 301: 6: R1779-85, 2011.

30. **Roberts AC, Butterfield GE, Cymerman A, Reeves JT, Wolfel EE and Brooks GA.** Acclimatization to 4,300-m altitude decreases reliance on fat as a substrate. *J.Appl.Physiol.(1985)* 81: 4: 1762-1771, 1996.

31. **Rowell LB, Johnson DG, Chase PB, Comess KA and Seals DR.** Hypoxemia raises muscle sympathetic activity but not norepinephrine in resting humans. *J.Appl.Physiol.(1985)* 66: 4: 1736-1743, 1989.

32. **Saito M, Mano T, Iwase S, Koga K, Abe H and Yamazaki Y.** Responses in muscle sympathetic activity to acute hypoxia in humans. *J.Appl.Physiol.(1985)* 65: 4: 1548-1552, 1988.

33. **Sander M.** Does the Sympathetic Nervous System Adapt to Chronic Altitude Exposure? *Adv.Exp.Med.Biol.* 903: 375-393, 2016.

34. **Schneider A, Greene RE, Keyl C, Bandinelli G, Passino C, Spadacini G, Bonfichi M, Arcaini L, Malcovati L, Boiardi A, Feil P and Bernardi L.** Peripheral arterial vascular function at altitude: sea-level natives versus Himalayan high-altitude natives. *J.Hypertens.* 19: 2: 213-222, 2001.

35. **Seals DR, Johnson DG and Fregosi RF.** Hypoxia potentiates exercise-induced sympathetic neural activation in humans. *J.Appl.Physiol.* 71: 3: 1032-1040, 1991.

36. **Seals DR, Johnson DG and Fregosi RF.** Hypoxia potentiates exercise-induced sympathetic neural activation in humans. *J.Appl.Physiol.(1985)* 71: 3: 1032-1040, 1991.

37. **Simpson LL, Busch SA, Oliver SJ, Ainslie PN, Stembridge M, Steinback CD and Moore JP.** Baroreflex control of sympathetic vasomotor activity and resting arterial pressure at high altitude: insight from Lowlanders and Sherpa. *J.Physiol.* 2019.

38. **Steinback CD and Kevin Shoemaker J.** Differential regulation of sympathetic burst frequency and amplitude following acute hypoxia in humans. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 303: 6: R633-8, 2012.

39. **Steinback CD and Kevin Shoemaker J.** Differential regulation of sympathetic burst frequency and amplitude following acute hypoxia in humans. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 303: 6: R633-8, 2012.

40. **Tymko MM, Tremblay JC, Steinback CD, Moore JP, Hansen AB, Patrician A, Howe CA, Hoiland RL, Green DJ and Ainslie PN.** UBC-Nepal Expedition: Acute alterations in sympathetic nervous activity do not influence brachial artery endothelial function at sea-level and high-altitude. *J.Appl.Physiol.* 2017.

41. **Vidruk EH, Olson EB,Jr, Ling L and Mitchell GS.** Responses of single-unit carotid body chemoreceptors in adult rats. *J.Physiol.* 531: Pt 1: 165-170, 2001.

42. **Willie CK, Stembridge M, Hoiland RL, Tymko MM, Tremblay JC, Patrician A, Steinback C, Moore J, Anholm J, Subedi P, Niroula S, McNeil CJ, McManus A, MacLeod DB and Ainslie PN.**

633 UBC-Nepal Expedition: An experimental overview of the 2016 University of British Columbia Scientific
634 Expedition to Nepal Himalaya. *PLoS One* 13: 10: e0204660, 2018.

635 43. **Wu TY, Liu FY, Hu L, Wei CY, Wang ZG, Ouzhou-Loubu, Cu CY, Bianba, Qi XB and Su B.**
636 Hematological parameters in high altitude residents: Tibetan natives versus Han migrants. *Zhongguo Ying*
637 *Yong Sheng Li Xue Za Zhi* 30: 6: 516-525, 2014.

638 44. **Xie A, Skatrud JB, Puleo DS and Morgan BJ.** Exposure to hypoxia produces long-lasting
639 sympathetic activation in humans. *J.Appl.Physiol.(1985)* 91: 4: 1555-1562, 2001.

640 45. **Xing G, Qualls C, Huicho L, Rivera-Ch M, Stobdan T, Slessarev M, Prisman E, Ito S, Wu H,**
641 **Norboo A, Dolma D, Kunzang M, Norboo T, Gamboa JL, Claydon VE, Fisher J, Zenebe G,**
642 **Gebremedhin A, Hainsworth R, Verma A and Appenzeller O.** Adaptation and mal-adaptation to
643 ambient hypoxia; Andean, Ethiopian and Himalayan patterns. *PLoS One* 3: 6: e2342, 2008.

TABLE 1: Participant demographics and metrics of basal neuro-cardiovascular function in lowlanders (at 344m and 5050m) and Sherpa (at 5050m).

	LOWLANDERS		**SHERPA**
	344m (N = 14)	5050m (N = 14)	5050m (N = 8)
Subject Demographics			
Age (years)	27±6	27± 6	32±13
Height (m)	1.77±0.8	1.77± 0.08	1.68±0.08
Weight (kg)	72.2±10.1	69.4± 8.6	63.7±10.1
BMI (kg/m^2)	23.1±2.8	22.2±2.5	22.8±3.5
Resting Cardiovascular Function			
Heart Rate (bpm)	61 ± 15	70 ± 15*	71± 5
SPO_2 (%)	98 ± 1	83 ± 3	83 ± 4
Systolic Pressure (mmHg)	119 ± 9	113 ± 13	111 ± 9
Diastolic Pressure (mmHg)	66 ± 7	70 ± 10	65 ± 8
Mean Pressure (mmHg)	84 ± 8	86 ± 11	84 ± 9
Cardiac Output (L/min) ♦	5.9 ± 1.8	5.5 ± 1.4	6.0 ± 1.7
Total Peripheral Resistance ♦	15 ± 4	17 ± 4	16 ± 7
Resting Sympathetic Function			
Burst Frequency (burst min^{-1})	11 ± 5	30 ± 7*	23 ± 11*†
Burst Incidence (burst 100 HB^{-1})	25 ± 13	53 ± 15*	30 ± 13*†
Burst Amplitude (A.U.) •	42.1± 22.2	46.7±7.9	46.3± 19.1

♦ Derived from Model Flow calculation.

•Burst amplitude calculated as median and interquartile range

* Significantly different from Lowlanders tested at low altitude (344m); $p < 0.05$.

† Significantly different from Lowlanders tested at high altitude (5050m); $p<0.05$.

TABLE 2: Participant demographics and metrics of basal neuro-cardiovascular function in Sherpa (at 1400m).

	SHERPA (1400m)
	1400m (n = 5)
Subject Demographics	
Age (years)	28 ± 7
Height (m)	1.67 ± 0.08
Weight (kg)	62.5 ± 10.7
BMI (kg/m^2)	22.7 ± 4.6
Resting Cardiovascular Function	
Heart Rate (bpm)	65 ± 8
SPO_2 (%)	96 ± 2
Systolic Pressure (mmHg)	137 ± 13
Diastolic Pressure (mmHg)	81 ± 10
Mean Pressure (mmHg)	102 ± 11
Cardiac Output (L/min) ♦	5.1 ± 1.2
Total Peripheral Resistance ♦	21 ± 5
Resting Sympathetic Function	
Burst Frequency (burst/ min)	13 ± 3
Burst Incidence (burst / 100 HB)	21 ± 6
Burst Amplitude (A.U.) •	69.5 ± 19.3

♦ Derived from Model Flow calculation.

•Burst amplitude calculated as median and interquartile range

FIGURE 1

657

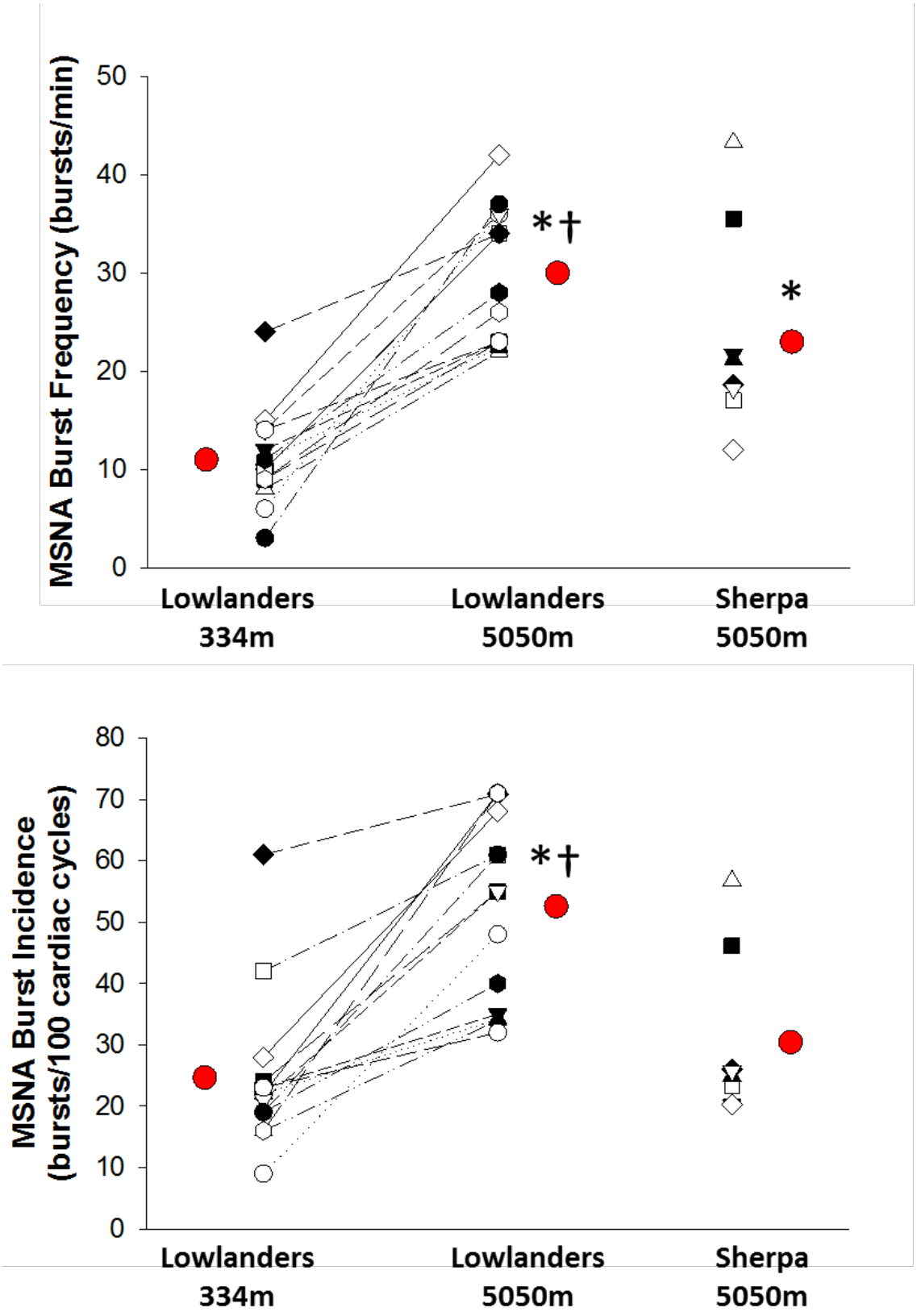

MSNA Burst Frequency (bursts/min)
0
10
20
30
40
50
*†
*
Lowlanders 334m
Lowlanders 5050m
Sherpa 5050m
MSNA Burst Incidence (bursts/100 cardiac cycles)
0
10
20
30
40
50
60
70
80
*†
Lowlanders 334m
Lowlanders 5050m
Sherpa 5050m

FIGURE 2

658

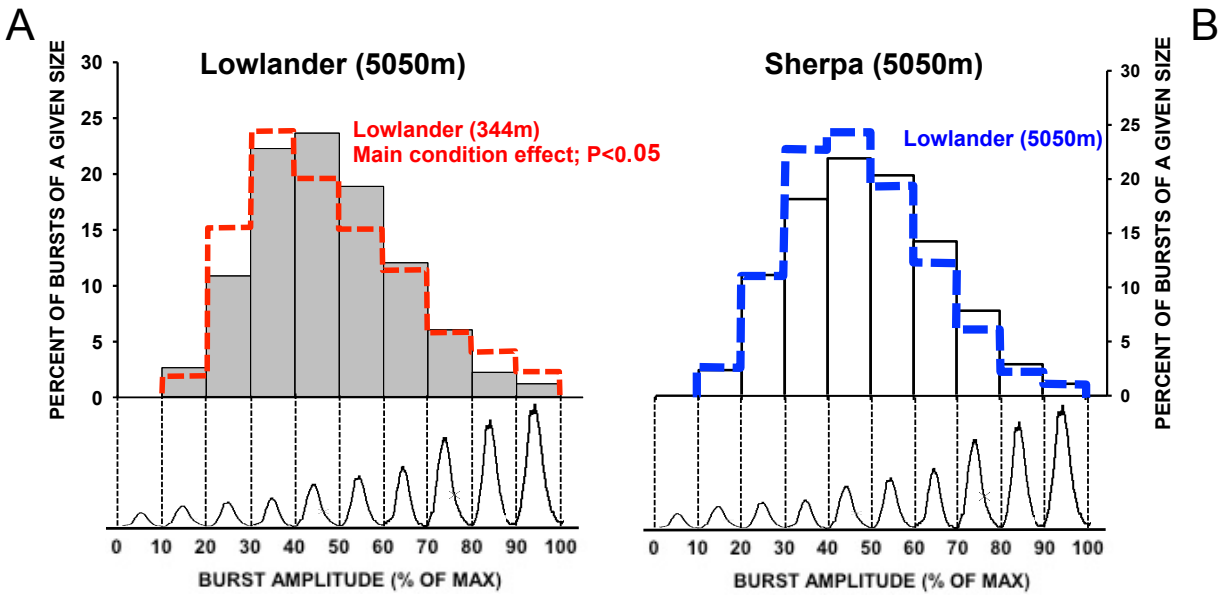

A
B
Lowlander (5050m)
Sherpa (5050m)
Lowlander (344m)
Main condition effect; P<0.05
Lowlander (5050m)
PERCENT OF BURSTS OF A GIVEN SIZE
BURST AMPLITUDE (% OF MAX)
0 10 20 30 40 50 60 70 80 90 100
0 5 10 15 20 25 30

FIGURE 3

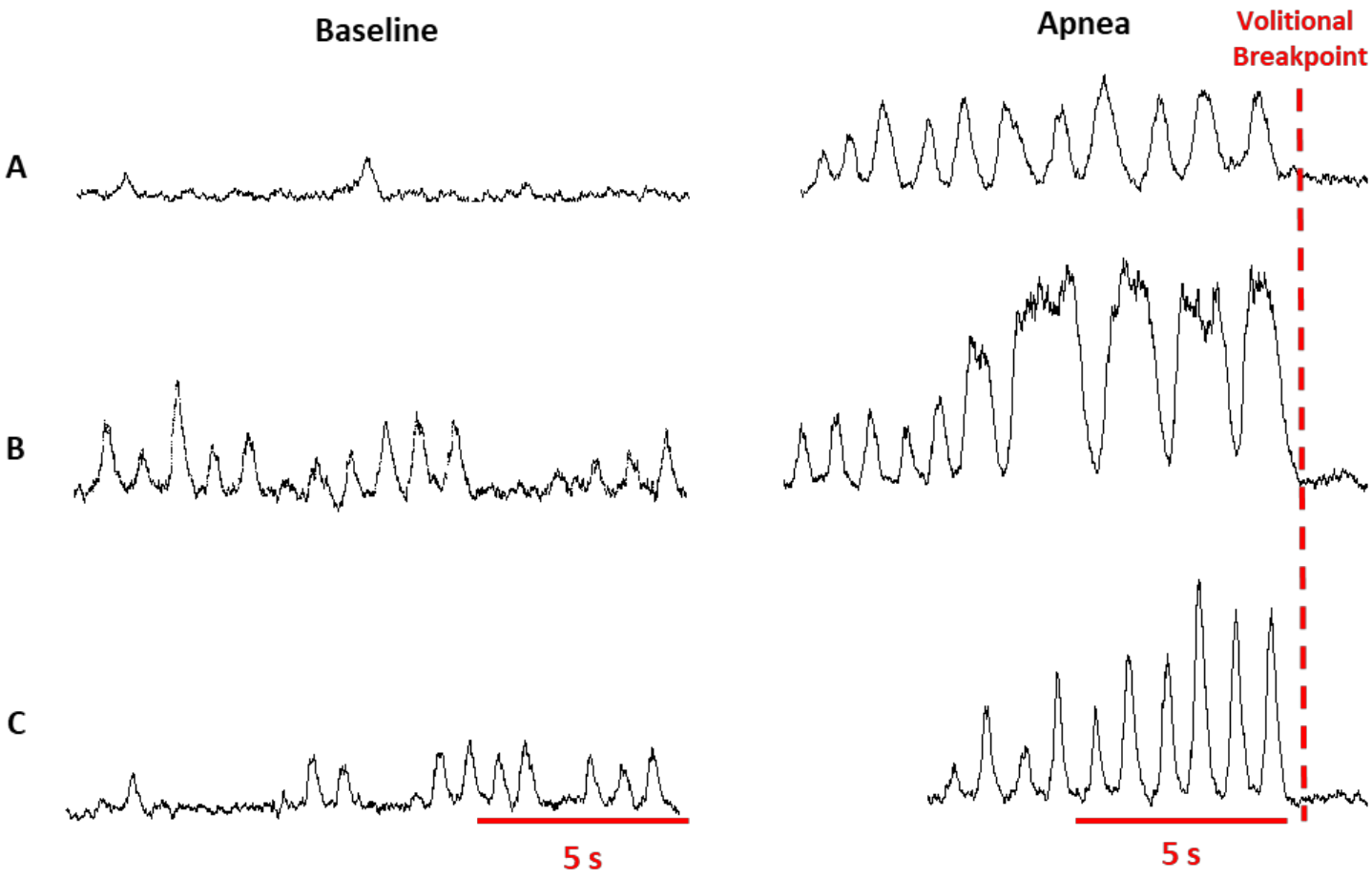


FIGURE 4

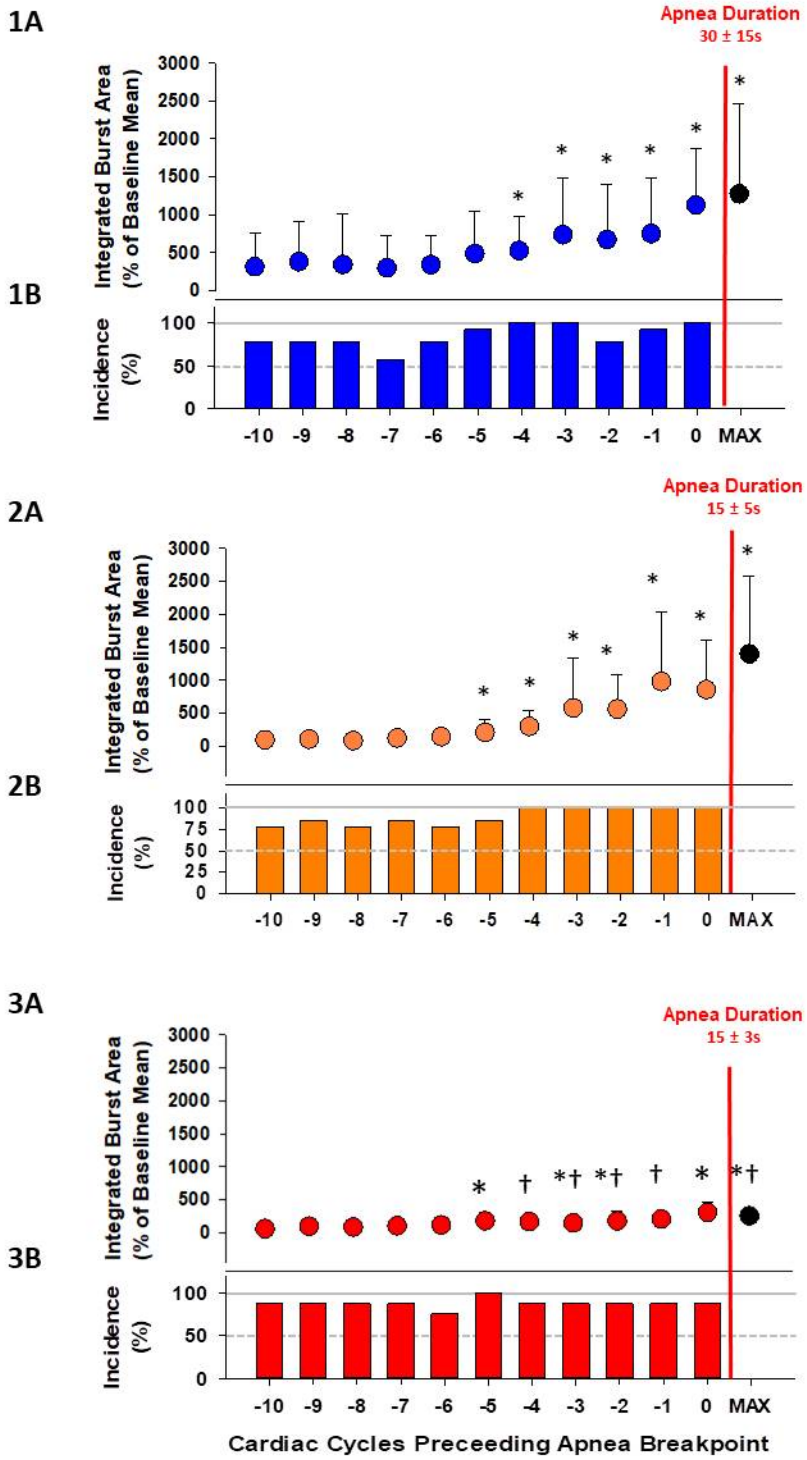

1A
Apnea Duration
30 ± 15s
Integrated Burst Area (% of Baseline Mean)
3000
2500
2000
1500
1000
500
0
1B
Incidence (%)
100
50
0
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 MAX
2A
Apnea Duration
15 ± 5s
Integrated Burst Area (% of Baseline Mean)
3000
2500
2000
1500
1000
500
0
2B
Incidence (%)
100
75
50
25
0
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 MAX
3A
Apnea Duration
15 ± 3s
Integrated Burst Area (% of Baseline Mean)
3000
2500
2000
1500
1000
500
0
3B
Incidence (%)
100
50
0
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 MAX
Cardiac Cycles Preceeding Apnea Breakpoint

FIGURE 5

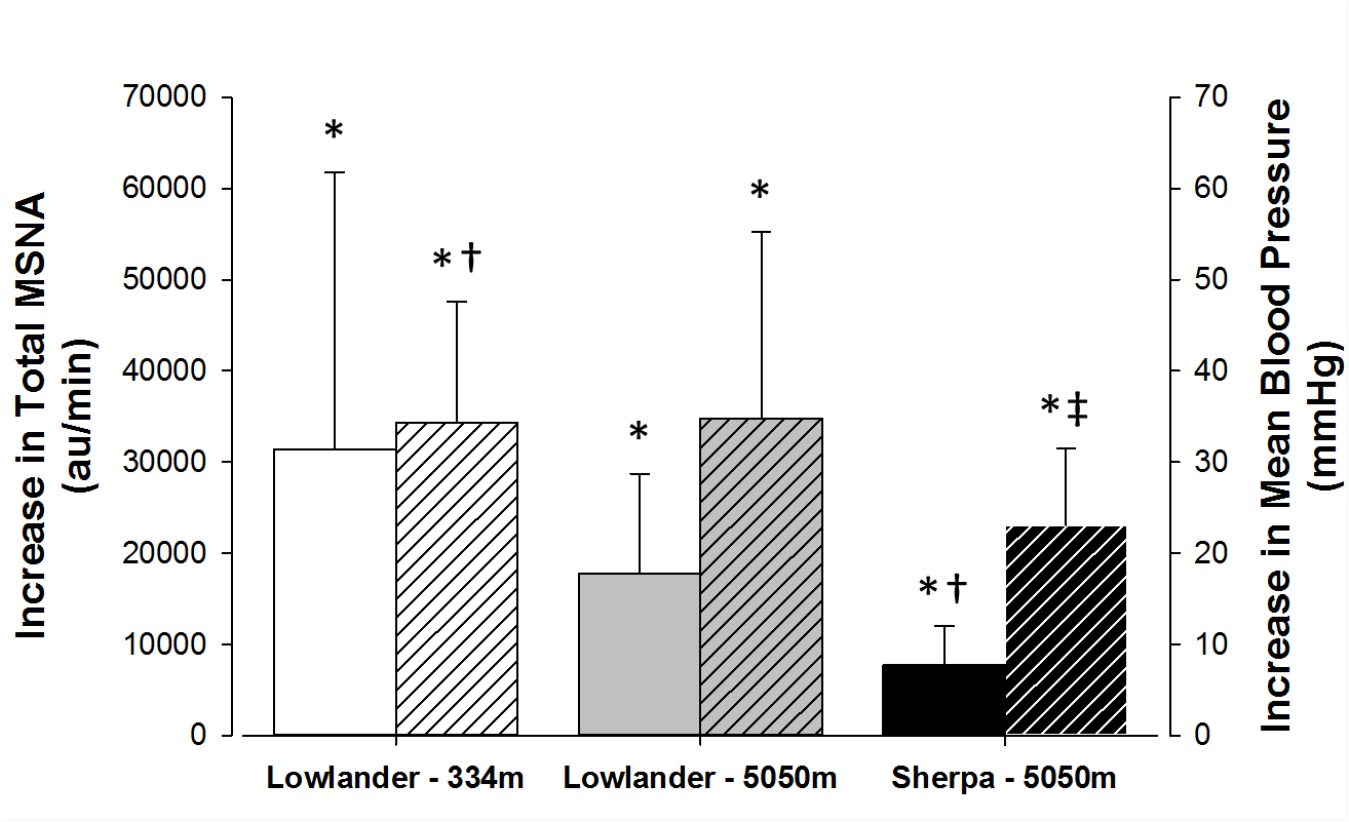

Increase in Total MSNA (au/min)
70000
60000
50000
40000
30000
20000
10000
0
Increase in Mean Blood Pressure (mmHg)
70
60
50
40
30
20
10
0
*
*†
*
*
*†
*‡
Lowlander - 334m
Lowlander - 5050m
Sherpa - 5050m

FIGURE 6

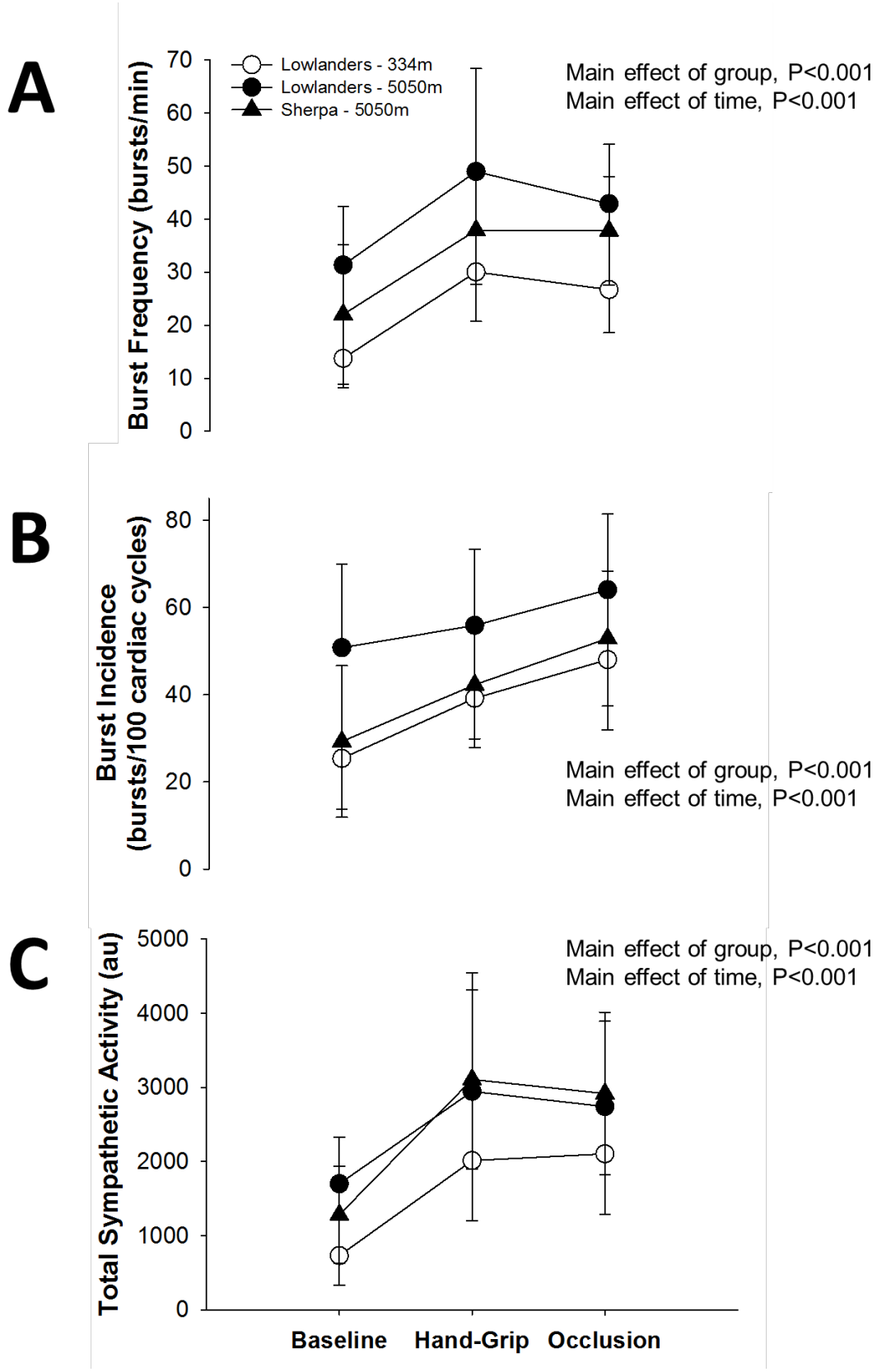

A
Lowlanders - 334m
Lowlanders - 5050m
Sherpa - 5050m
Main effect of group, P<0.001
Main effect of time, P<0.001
Burst Frequency (bursts/min)
0
10
20
30
40
50
60
70
B
Burst Incidence (bursts/100 cardiac cycles)
0
20
40
60
80
Main effect of group, P<0.001
Main effect of time, P<0.001
C
Total Sympathetic Activity (au)
0
1000
2000
3000
4000
5000
Main effect of group, P<0.001
Main effect of time, P<0.001
Baseline
Hand-Grip
Occlusion

FIGURE 7

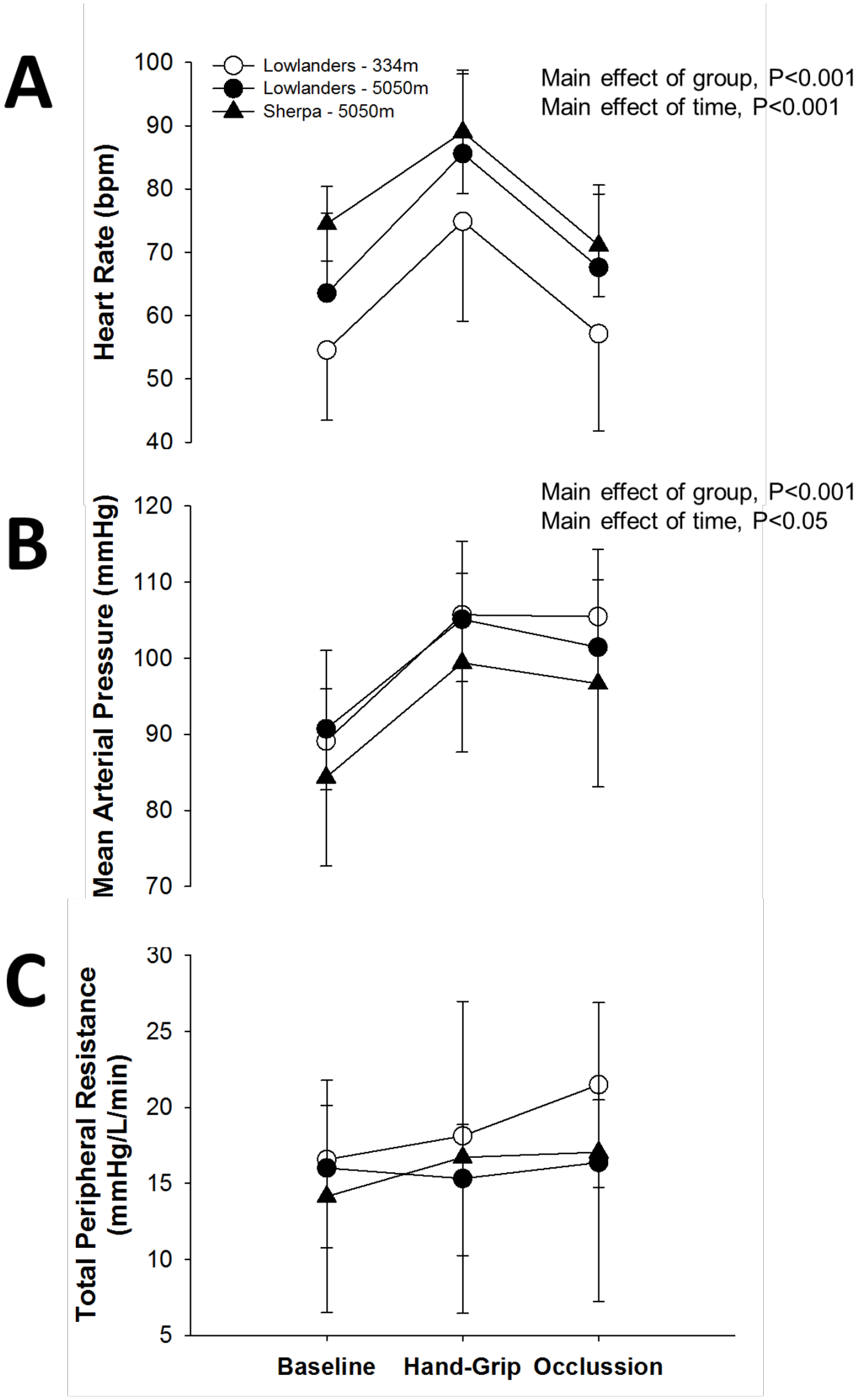
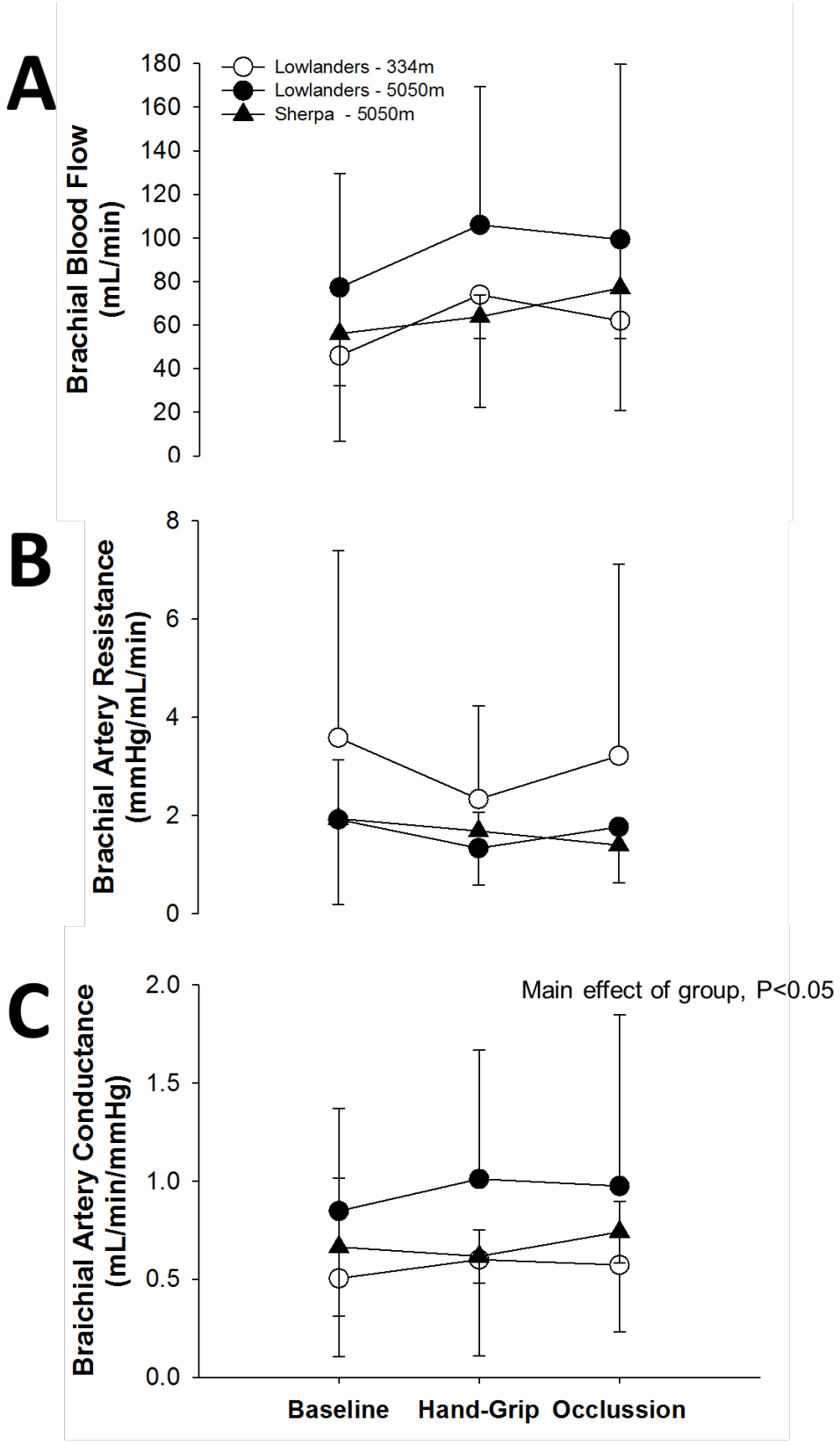

A
Lowlanders - 334m
Lowlanders - 5050m
Sherpa - 5050m
Main effect of group, P<0.001
Main effect of time, P<0.001
Heart Rate (bpm)
100
90
80
70
60
50
40
B
Main effect of group, P<0.001
Main effect of time, P<0.05
Mean Arterial Pressure (mmHg)
120
110
100
90
80
70
C
Total Peripheral Resistance (mmHg/L/min)
30
25
20
15
10
5
Baseline
Hand-Grip
Occlussion

FIGURE 8


A
Brachial Blood Flow (mL/min)
180
160
140
120
100
80
60
40
20
0
Lowlanders - 334m
Lowlanders - 5050m
Sherpa - 5050m
B
Brachial Artery Resistance (mmHg/mL/min)
8
6
4
2
0
C
Braichial Artery Conductance (mL/min/mmHg)
2.0
1.5
1.0
0.5
0.0
Main effect of group, P<0.05
Baseline
Hand-Grip
Occlusion

659