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Baroreflex control of sympathetic vasomotor activity and resting arterial pressure at high altitude: insight from Lowlanders and Sherpa

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AUTHOR PROFILE

Lydia L Simpson completed her undergraduate BSc degree in Sport and Exercise Sciences at the University of Birmingham in 2014 and was awarded an MSc in Human and Applied Physiology from Kings College London in 2016. Lydia joined Bangor University in 2016 as a PhD researcher, under the supervision of Dr Jonathan Moore. Her research investigates the adaptation of the sympathetic nervous system to chronic high altitude hypoxia, with a specific focus on the sympathetic nervous system regulation of blood pressure.



KEY POINTS SUMMARY

- Hypoxia, a potent activator of the sympathetic nervous system, is known to increase muscle sympathetic nerve activity (MSNA) to the peripheral vasculature of native Lowlanders during sustained high altitude (HA) exposure.
- We show that the arterial baroreflex control of MSNA functions normally in healthy Lowlanders at HA, and that upward baroreflex resetting permits chronic activation of basal sympathetic vasomotor activity under this condition.
- The baroreflex MSNA operating point and resting sympathetic vasomotor outflow both are lower for highland Sherpa compared with acclimatizing Lowlanders; these lower levels may represent beneficial hypoxic adaptation in Sherpa.
- Acute hyperoxia at HA had minimal effect on baroreflex control of MSNA in Lowlanders and Sherpa, raising the possibility that mechanisms other than peripheral chemoreflex activation contribute to vascular sympathetic baroreflex resetting and sympathoexcitation.
- These findings provide better understanding of sympathetic nervous system activation and control of blood pressure during the physiological stress of sustained HA hypoxia.

ABSTRACT

Exposure to high altitude (HA) is characterized by heightened muscle sympathetic neural activity (MSNA); however, the effect on arterial baroreflex control of MSNA is unknown. Furthermore, arterial baroreflex control at HA may be influenced by genotypic and phenotypic differences between lowland and highland natives. Fourteen Lowlanders (10 male) and 9 male Sherpa underwent haemodynamic and sympathetic neural assessment at low altitude (Lowlanders, LA; 344m, Sherpa, KT; 1400m) and following gradual ascent to 5050m. Beat-by-beat haemodynamics (photoplethysmography) and MSNA (microneurography) were recorded lying supine. Indices of vascular sympathetic baroreflex function were determined from the relationship of diastolic blood pressure (DBP) and corresponding MSNA at rest (i.e. DBP 'operating pressure' and MSNA 'operating point'), and during a modified Oxford baroreflex test (i.e. 'gain'). Operating pressure and gain were unchanged for Lowlanders during HA exposure; however, the operating point was reset upwards (48 ± 16 vs 22 ± 12 bursts $\cdot 100\text{HB}^{-1}$, $P=0.001$). Compared to Lowlanders at 5050m, Sherpa had similar gain and operating pressure, but operating point was lower (30 ± 13 bursts $\cdot 100\text{HB}^{-1}$ $P=0.02$); MSNA burst frequency was lower for Sherpa (22 ± 11 versus 30 ± 9 bursts $\cdot \text{min}^{-1}$ $P = 0.03$). Breathing 100% oxygen did not alter vascular sympathetic baroreflex function for either group at HA. For Lowlanders, upward baroreflex resetting promotes heightened sympathetic vasoconstrictor activity and maintains blood pressure stability, at least during early HA exposure; mechanisms other than peripheral chemoreflex activation could be involved. Sherpa adaptation appears to favour lower sympathetic vasoconstrictor activity than Lowlanders for blood pressure homeostasis.

INTRODUCTION

The sympathetic nervous system is the ubiquitous controller of the cardiovascular system in humans, and thus plays a pivotal role in arterial pressure homeostasis (Guyenet, 2006). High altitude (HA) hypoxia is a major physiological stressor that is accompanied by a profound activation of muscle sympathetic nerve activity (MSNA), which is markedly greater than that observed during acute exposure to a similar hypoxic stimulus (Duplain *et al.*, 1999; Lundby *et al.*, 2017). Notably, sympathoexcitation is maintained for the duration of HA exposure, despite normalisation of resting arterial oxygen content to near sea-level values (Hansen & Sander, 2003; Lundby *et al.*, 2017). Furthermore, sympathetic activation is not reversed whilst breathing 100% oxygen, and persists for up to 3 days following descent to low altitude (Hansen & Sander, 2003; Mitchell *et al.*, 2018). These data suggest a form of neural “remodelling” associated with prolonged hypoxia.

Several studies have characterised MSNA and arterial pressure responses to sustained HA exposure in healthy Lowlanders (Hansen & Sander, 2003; Lundby *et al.*, 2017; Fisher *et al.*, 2018). Despite a greater probability of a burst of sympathetic vasoconstrictor activity at rest (i.e. 50% as opposed to 25%), any accompanying change in arterial pressure is relatively modest, at least for exposures lasting to 10-50 days. These observations imply chronic resetting of the neural vasoconstrictor reflexes which attempt to maintain blood pressure, presumably to balance local vasodilator mechanisms and secure haemodynamic stability. However, the arterial baroreflex control of sympathetic vasoconstrictor activity has never been investigated at HA.

Relatively little is known regarding the consequences of lifelong HA hypoxia on sympathetic activity and blood pressure regulation. A single microneurographic study found similar basal MSNA, but lower arterial pressure, for Bolivian highlanders compared to well-acclimatised Lowlanders (Lundby *et al.*, 2017); arterial baroreflex function was not tested. This suggests sustained sympathetic activation may be an evolutionary adaptation for those living permanently under HA hypoxia. However, distinct differences in physiological adaptation are known to exist between natives of the South American Andes, Himalaya plateau and Ethiopian highlands (Beall, 2006, 2007; Erzurum *et al.*, 2007), with the suggestion that the Sherpa (Himalayan) adaptation represents the most effective phenotype for chronic hypoxia (Gilbert-Kawai *et al.*, 2014; Horscroft *et al.*, 2017). However, due to a lack of microneurographic data for highlanders, other than Bolivians, it is unclear whether differences in the patterns of adaptation extends to sympathetic nervous system control of the cardiovascular system and arterial pressure homeostasis.

Therefore, the aims of this study were (1) to examine baroreflex regulation (resetting and gain) in healthy Lowlanders at 5050m, following 10 to 20 days of acclimatization and, (2) to compare arterial baroreflex function between acclimatizing Lowlanders and HA native Sherpa. Based upon previous reports for acute hypoxia (Halliwill & Minson, 2002; Halliwill *et al.*, 2003; Steinback *et al.*, 2009; Querido *et al.*, 2011), we hypothesized that the 'operating pressure' (i.e. diastolic blood pressure) and 'operating point' (i.e. MSNA burst incidence) of the vascular sympathetic baroreflex would shift to higher values, during HA acclimatisation, with no change in reflex 'gain' (i.e. slope). We further hypothesized that Sherpa, who cope extremely well with chronic hypoxia, would have lower arterial pressure and lower MSNA operating point, compared with Lowlanders.

METHODS

Ethical Approval

All testing procedures had Institutional Review Board approval from the University of British Columbia (H16-01297/H16-01028), University of Alberta, and Nepal Health Research Council. All participants were informed, using the native language, of the purpose and the risks involved with each procedure, and provided oral and written informed consent, in compliance with the latest revision of the *Declaration of Helsinki*, except for registration in a database.

Participants

Fourteen Lowlanders (twelve male; mean \pm SD: age, 27 ± 6 yrs; height, 1.77 ± 0.8 m; weight, 72.2 ± 10.1 kg) and nine male Sherpa (age, 33 ± 12 yrs; height, 1.68 ± 0.07 m; weight, 65.3 ± 10.3 kg) participated. All Sherpa were natives of the Khumbu valley (> 3440 metres). None of the participants had any history or symptoms of cardiovascular, respiratory, metabolic and neurological disease, and were not taking any prescription or over-the-counter medication during the time of participation. Five Sherpa were self-reported smokers (1-5 cigarettes per day). None of the Lowlanders experienced clinical acute mountain sickness (AMS) at the time of testing, as assessed by the Lake Louise questionnaire (LLQ score ≤ 3); however, one was tested 2 days following an intramuscular injection of dexamethasone (half-life, 3 hours). All participants abstained from caffeine and vigorous exercise for 12 hours prior to all testing session and arrived at the laboratory a minimum of 2 hours after a light meal.

Experimental design

This experiment was carried out within the framework of the 2016 UBC Nepal Expedition to the Ev-K2-CNR Research Facility (Willie *et al.*, 2018). Participants for the present study were also recruited for a number of other investigations. Therefore, care was taken to ensure that no overlap existed between any of the studies, and the present study addressed its own distinct *a priori* research question. Data collected during the testing sessions described in the following paragraph, but with separate *a priori* analyses, are presented elsewhere (Busch *et al.*, 2017).

All participants underwent two testing sessions. Pre-expedition, low altitude (LA), testing of Lowlanders was conducted at 344m (Kelowna, Canada, barometric pressure, 758 ± 8 mmHg). Pre-expedition testing of Sherpa was conducted at 1400m (Kathmandu [KT], Nepal; barometric pressure, 652 ± 3 mmHg); this was performed a minimum of 4 days following descent from their resident altitude. All of the HA testing was performed at 5050 m (barometric pressure, 431 ± 44 mmHg). These tests followed a gradual trek (i.e. 9 or 10 days), starting at 2860m with rest days at both 3400m and 4240m. Sherpa were studied on days 1-4 at 5050m (i.e. 10-14 days above 2860m), and Lowlanders were studied on days 1-10 (i.e. 10-20 days).

Measurements

Haemodynamics

Heart rate (HR) and beat-by-beat blood pressure (BP) were continuously recorded using Lead II electrocardiogram and finger photoplethysmography (Finometer Pro, Finapres Medical Systems BV, Amsterdam, Netherlands). Mean arterial pressure (MAP), and systolic (SBP) and diastolic (DBP) blood pressures, were calculated from

the arterial pressure waveform, which was calibrated against manual brachial artery pressure measurements. Cardiac output (CO) was estimated using the Model Flow algorithm and used to estimate total peripheral resistance ($TPR = MAP/CO$).

Peripheral capillary oxygen saturation (SpO_2) was determined using finger pulse oximetry (Nellcor, Medtronics, USA).

Muscle sympathetic nerve activity

Multi-unit MSNA was recorded from the peroneal (common fibular) nerve via microneurography (JPM/CDS) as previously described (Steinback & Shoemaker, 2012; Usselman *et al.*, 2015). MSNA signal was confirmed by pulse-synchronous activity that responded to end-expiratory apnea but not to startle stimuli or skin stroking (Delius *et al.*, 1972a, 1972b). Nerve signals were amplified (1000x pre-amplifier and 100x variable gain isolated amplifier), band pass filtered (700-2,000Hz) rectified (model 662C-3; Iowa University Bioengineering; USA) and integrated (decay constant 0.1s).

Experimental protocol

Basal sympathetic neural activity

Following arrival at the laboratory, subjects rested in the supine position and an antecubital venous cannula was inserted for subsequent drug administration.

Following instrumentation, acquisition of an acceptable MSNA signal and a period of stabilisation, 10 minutes of baseline data were recorded.

Arterial baroreflex function

Vascular sympathetic and cardiovagal baroreflex function was determined from the MSNA and R-R Interval (RRI) responses during arterial pressure perturbations induced by a single modified Oxford baroreflex test (Rudas *et al.*, 1999) during

ambient air breathing. Briefly, the modified Oxford test involved bolus injection of sodium nitroprusside (SNP), followed 90 seconds later by phenylephrine (PE). Prior to experimental testing, bolus doses of SNP and PE that evoked ~ 15 mmHg perturbations above and below resting BP were determined for each individual. The same relative dose ($\mu\text{g}\cdot\text{kg}^{-1}$) was administered at LA and HA for a given individual. Doses of vasoactive drugs administered and resultant blood pressure changes are shown in Table 1.

Arterial baroreflex-peripheral chemoreflex interaction

At LA, a modified Oxford test was also performed whilst breathing a gas mixture containing 11% oxygen (equivalent to 5050m), to increase peripheral chemoreceptor drive (acute hypoxia, AH). At both LA and HA, a single modified Oxford test was also performed whilst participants breathed 100% oxygen (LA + 100% O₂, HA + 100% O₂), to acutely eliminate peripheral chemoreceptor drive. Participants breathed each of the gas mixtures for a minimum of 5 minutes; as soon as a new steady state SpO₂ was achieved, the modified Oxford test was performed. No attempt was made to control ventilation or end tidal CO₂ during manipulation of peripheral chemoreceptor drive. At least 20 minutes separated the modified Oxford tests. The order of trials at LA were not randomized as persistent alterations in MSNA and vascular sympathetic baroreflex function have been shown following acute hypoxia stimulus (Querido *et al.*, 2011).

Data analyses

All haemodynamic data were sampled at 1KHz using a commercial data acquisition software (LabChart Pro v 8.3.1, AD Instruments, Sydney, Australia) and stored for offline analysis. The raw MSNA signal was sampled at 10 KHz. Multi-unit bursts of

MSNA were identified using a semi-automated detection algorithm (Chart Pro 8.3.1) and confirmed by a trained observer (SAB/CDS). To account for vascular sympathetic baroreflex latency, MSNA data were shifted backwards (Average; 1.32 ± 0.07 s) so that the peak of each sympathetic burst coincided with the diastolic period that initiated it (Usselman *et al.*, 2015). Burst amplitude data were normalised by assigning a value of 100 to the largest burst observed during baseline and calibrating all other bursts against this value. Resting MSNA was quantified as burst frequency ($\text{burst} \cdot \text{min}^{-1}$), burst incidence ($\text{burst} \cdot 100\text{HB}^{-1}$), mean burst amplitude (a.u) and total activity (mean burst amplitude x burst frequency [$\text{au} \cdot \text{min}^{-1}$]).

Vascular sympathetic baroreflex gain was estimated from the relationship between DBP and MSNA burst probability during a modified Oxford test. DBP was used because MSNA correlates more closely with DBP than SBP (Sundlof & Wallin, 1978). All DBP values were assigned to a 3 mmHg bin to reduce the statistical impact of respiratory related oscillations (Eckberg & Eckberg, 1982). The percentage of cardiac cycles associated with a burst of MSNA (ranging from 0-100%) was calculated for each DBP bin to give values of burst probability (Usselman *et al.*, 2015). Non-linear saturation and threshold regions, if present, were excluded through visual inspection of data points by agreement of two observers (LLS/JPM). The slope of the linear relationship was determined by weighted linear regression analysis, and this value provided an index of vascular sympathetic baroreflex gain. Only slopes with (i) at least five data points and (ii) $R \geq 0.5$ were included in the group mean data (Hart *et al.*, 2011; Taylor *et al.*, 2015). Vascular sympathetic baroreflex gain for rising and falling pressures were not determined independently. The vascular sympathetic baroreflex operating point was taken as the average values for

DBP and MSNA burst incidence during the resting period immediately prior to the modified Oxford test.

Cardiovagal baroreflex gain was estimated from the relationship between SBP and RRI during each modified Oxford test. SBP was used as it correlates more closely with RRI than DBP (Sundlof & Wallin, 1978). Values were averaged over 3mmHg SBP bins. Baroreflex delays were accounted for by associating SBP values with either the concurrent heartbeat (when resting RRI >800msec) or subsequent heartbeat (when resting RRI <800msec) (Eckberg & Eckberg, 1982). Saturation and threshold regions were excluded through visual inspection of data (LLS/JPM). Slopes were determined by weighted linear regression analysis and only slopes with at least five data points and $R \geq 0.8$ were included in the group mean data. To minimize the potential effects of hysteresis, we restricted data analysis to the rising arm of SBP during the modified Oxford test (Hunt & Farquhar, 2005). The cardiovagal baroreflex operating point was taken as the average value for SBP and RRI during the resting period immediately prior to the modified Oxford test.

Statistical analyses

The effects of HA acclimatization in Lowlanders were assessed using paired t-tests, whereas differences between Lowlanders and Sherpa at HA and Sherpa at HA and Lowlanders at LA were assessed using independent t-tests. The effects of manipulating peripheral chemoreceptor drive on baroreflex function at LA (AH, LA + 100% O₂) and HA (HA + 100% O₂) were assessed using paired t-tests. Multiple t-tests were chosen to maximize the number of subjects included in statistical analyses. *A priori* alpha was adjusted, using the experiment-wise error rate, to correct for multiple comparisons (Busch *et al.*, 2017). All statistical analyses were

performed using Prism 7.03 (GraphPad software, USA) and statistical significance was set at $P < 0.05$ *a priori*. Group data are reported as means (\pm SD).

RESULTS

Resting haemodynamics, basal sympathetic neural activity, and arterial baroreflex function

Examples of MSNA and haemodynamic data recorded in one Lowlander and one Sherpa, under each of the experimental conditions, are presented in Figure 1. In Lowlanders ($n=14$), SpO_2 was decreased and HR was increased with HA acclimatization, whereas CO, TPR, and MAP were similar compared to LA. All parameters of MSNA were greater in Lowlanders at HA compared to LA (Table 2).

Due to technically challenging conditions during pre-expedition testing at KT, resting sympathetic neural activity could only be obtained in 4 out of 9 Sherpa, and 1 of these 4 Sherpa was not re-tested at HA (Table 2). At HA, Sherpa ($n=8$) and Lowlanders had a similar SpO_2 and resting haemodynamics; however, Sherpa exhibited significantly lower MSNA burst frequency than Lowlanders, with no difference in mean burst amplitude. Compared to Lowlanders at LA, Sherpa had lower SpO_2 and higher HR, but similar CO, TPR and MAP. Sherpa exhibited significantly greater MSNA burst frequency, and mean burst amplitude, versus Lowlanders at LA.

Data from ten Lowlanders were included in the comparisons of sympathetic and cardiovagal baroreflex function, as baroreflex slopes for four participants did not fulfil the inclusion criteria. HA acclimatization had no effect on the baroreflex diastolic operating pressure for Lowlanders, but the MSNA operating point (burst incidence) was increased compared to LA. Vascular sympathetic baroreflex gain (i.e. slope)

was not different at LA and HA (Figure 2). HA acclimatization resulted in a downward shift of the cardiovascular baroreflex, reflected by a reduction in RRI, with no change in prevailing SBP; this was accompanied by a reduction in reflex gain (Figure 3).

Modified Oxford tests were performed successfully in 7 out of 8 Sherpa investigated at HA; no baroreceptor tests were performed in KT. At HA, the diastolic operating pressure for Sherpa was similar to that of Lowlanders, but the MSNA operating point was lower for Sherpa. There was no difference in vascular sympathetic baroreflex gain (Figure 2). Furthermore, when compared to Lowlanders at LA, operating pressure, operating point and vascular sympathetic baroreflex gain for Sherpa were similar. The cardiovascular baroreflex gain in Sherpa was similar to that for Lowlanders at HA, but less than that of Lowlanders at LA. The cardiovascular baroreflex operating SBP was similar for Sherpa, compared to Lowlanders at both HA and LA, whereas RRI was similar to Lowlanders at HA, but lower than that at LA (Figure 3). In Sherpa, cardiovascular baroreflex gain was similar to that of Lowlanders at HA, but less than that of Lowlanders at LA.

Arterial baroreflex-peripheral chemoreflex interaction at low altitude

Due to the loss of MSNA signals in three participants, the data analyses for arterial baroreflex-peripheral chemoreflex interactions at LA are for eleven Lowlanders (Table 3). AH reduced SpO₂, increased HR, MAP and CO, and decreased TPR. MSNA burst frequency was unchanged, but total MSNA was increased, due to an augmented burst amplitude. Vascular sympathetic baroreflex gain was reduced during AH, with no change in baroreflex diastolic operating pressure, or the MSNA operating point. Administration of 100% oxygen at LA had no effect on baseline

haemodynamics, MSNA burst frequency, burst amplitude, or indices of vascular sympathetic and cardiovagal baroreflex function.

Arterial baroreflex-peripheral chemoreflex interaction at high altitude

As a result of MSNA signal losses, these comparisons were performed for nine Lowlanders and four Sherpa (Table 4). For Lowlanders exposed to 100% O₂, SpO₂ and MAP increased, and HR decreased, with no effect on any other baseline haemodynamics or MSNA. Vascular sympathetic baroreflex function gain was also unchanged. There was no change in cardiovagal baroreflex gain. For Sherpa breathing 100% oxygen, SpO₂ increased and HR decreased with no effect on other baseline haemodynamics. MSNA burst frequency, burst incidence, total activity and total MSNA were unchanged; however, mean burst amplitude decreased. Vascular sympathetic baroreflex gain was unchanged. Breathing 100% oxygen reduced RRI, with no change in cardiovagal baroreflex gain.

DISCUSSION

Principal novel findings are as follows: (1) Baroreflex control of MSNA is preserved in Lowlanders following 10-20 days at HA; (2) The operating point of the vascular sympathetic baroreflex is upwardly reset with no change in operating DBP for Lowlanders at HA; (3) Sherpa have lower basal MSNA burst frequency compared to Lowlanders at HA, but similar resting blood pressure; (4) Sherpa have similar vascular sympathetic baroreflex gain, but a lower operating point when compared with Lowlanders at HA. Finally, (5) eliminating peripheral chemoreceptor drive at HA

did not influence the vascular sympathetic baroreflex operating point or gain for both Lowlanders and Sherpa. Taken together, these findings provide important new insight into reflex control of the vasoconstrictor drive and blood pressure at HA, and highlight a novel adaptation in Sherpa.

Sympathoexcitation at high altitude

Following 10-20 days of HA exposure, we observed an almost three-fold increase in MSNA burst frequency for Lowlanders at 5050m; this is consistent with previous microneurographic studies at HA (Hansen & Sander, 2003; Lundby et al., 2017). Furthermore, for the first time, we demonstrate that the basal MSNA burst frequency of Sherpa is lower than that of Lowlanders at this altitude, despite similar peripheral oxygen saturation in both groups. Our observation for Sherpa contrasts with that of the only previous study of highlanders (Lundby *et al.*, 2017), which found that Bolivian Aymara had basal MSNA that was comparable with Lowlanders after 10 and 50 days of HA exposure. Although many factors may influence basal sympathetic outflow, the present study raises the importance of ethnicity. The divergent pathways of physiological adaptation observed in geographically distinct HA populations might extend to sympathetic nervous system activation, whereby adaptation in Sherpa appears to favour lower basal sympathetic activity. However, we also found that basal MSNA for Sherpa at HA was higher than that for Lowlanders at LA. Furthermore, for three Sherpa studied 4 days following descent to 1440m, basal MSNA burst frequencies were approximately 30% lower than those observed when they were re-tested at 5050m. Taken together these findings suggest that hypoxia remains a significant physiological stressor for Sherpa despite generations of adaptation and lifelong exposure.

Remarkably, resting MAP for Lowlanders was similar at LA and HA, despite the significantly elevated basal MSNA at 5050m. Moreover, Sherpa and Lowlanders exhibited similar MAP at 5050m, even though Sherpa had markedly less basal MSNA. This may reflect differences in the release of vasoactive substances and vascular sensitivity to these factors. It is possible that α adrenergic receptor sensitivity is reduced in Lowlanders during prolonged HA hypoxia, meaning that they require more MSNA to produce the same vascular response. That the same dose of phenylephrine administered during the modified Oxford test elicited a smaller pressor response for lowlanders at HA than at LA, supports this notion. Furthermore, Sherpa may possess a greater vascular responsiveness to sympathetic vasoconstrictor drive, meaning that the vascular effect of a burst of neural activity is greater. However, characterisation of a dose-response relationship to vasoactive substances would be required to confirm these possibilities.

Arterial baroreflex function at high altitude

Our data indicate an upward resetting of the vascular sympathetic baroreflex in Lowlanders at 5050m. This occurred without a change in the ability of the reflex to increase or decrease MSNA in response to a baroreceptor challenge i.e. the gain was unchanged. Furthermore, the ability of the baroreflex to regulate MSNA in Sherpa and Lowlanders is similar, but the likelihood of a burst of MSNA at a given diastolic pressure is lower for Sherpa. Vascular sympathetic baroreflex function at HA had not been assessed prior to this study. Previous reports of heightened MSNA burst incidence (Hansen & Sander, 2003; Lundby *et al.*, 2017; Fisher *et al.*, 2018) indirectly support an upward resetting of the vascular sympathetic baroreflex operating point for Lowlanders exposed to chronic HA hypoxia. However, in contrast to this study, previous studies found an increase in resting MAP accompanied higher

MSNA burst incidence (Hansen & Sander, 2003; Lundby *et al.*, 2017; Fisher *et al.*, 2018). This may be due to methodological differences across studies in relation to the ascent profile, physical activity levels whilst at altitude, and the final elevation achieved. In addition, a temporal relationship may exist between elevated sympathetic vasomotor activity and MAP in Lowlanders. Arterial baroreflex resetting and heightened sympathetic outflow initially may be homeostatic during early acclimatisation; however, over time other cardiovascular changes and alterations in constricting and dilating factors acting on the vasculature (Calbet *et al.*, 2014; Bruno *et al.*, 2016) could contribute to elevated MAP at HA. We suggest that future studies at HA should incorporate serial measurements of arterial baroreflex control of MSNA and other factors that modulate arterial pressure.

The secondary effects of increased ventilation at HA may complicate the effects of hypoxia on baroreflex control of the heart (Angell James & De Burgh Daly, 1969; Eckberg *et al.*, 1980). Nevertheless, we determined how the cardiovagal component of the arterial baroreflex was affected in this study. At HA, cardiovagal baroreflex gain was similar for Lowlanders and Sherpa, but we observed that the gain for Lowlanders was reduced compared with at LA. Interestingly, acute hyperoxia at 5050m did not reverse this reduction in gain for Lowlanders. Taken together, our data suggest that altitude acclimatization has differential effects on the responsiveness of the vascular sympathetic and cardiovagal limbs of the arterial baroreflex.

Vascular sympathetic baroreflex-peripheral chemoreflex interactions

For Lowlanders exposed to AH, there was no change in basal MSNA burst frequency, although there was a modest increase in mean burst amplitude and thus a modest increase in total activity. This implies that MSNA burst frequency and

amplitude can be regulated independently of each other, as previously suggested (Kienbaum *et al.*, 2001; Salmanpour *et al.*, 2011; Steinback & Shoemaker, 2012). Furthermore, the operating point of the vascular sympathetic baroreflex was not significantly different during AH, while the gain was reduced. These findings for AH contrast with those for HA, and suggest different mechanisms contribute to activation of central sympathetic outflow during acute and chronic hypoxic exposure. Vascular sympathetic baroreflex resetting in Lowlanders at 5050m was not reversed during acute administration of 100% oxygen. Furthermore, MSNA burst frequency was not reduced, a finding that is consistent with previous studies that attempted to reduce peripheral chemoreflex drive at HA (Hansen & Sander, 2003; Fisher *et al.*, 2018). Therefore, mechanisms other than the peripheral chemoreflex likely play a role in vascular sympathetic baroreflex resetting at HA. Interestingly, the peripheral chemoreflex may be more important in mediating HA sympathoexcitation in Sherpa. Although the vascular sympathetic baroreflex operating point was not changed during 100% oxygen administration, we observed a reduction in mean burst amplitude and total activity. This possibility, however, requires further investigation.

Experimental Considerations

This is the first study to record sympathetic neural discharges from Sherpa at HA. However, technically challenging conditions in Kathmandu limited the study to only four participants at a lower elevation. Furthermore, around half of Sherpa were light to moderate smokers and it is reported that tobacco smoking leads to increased basal MSNA and attenuates vascular sympathetic baroreflex sensitivity. However, smoking status was not a significant covariate for any indices in this study. Compared with Lowlanders, Sherpa were naive to the microneurographic technique, and may have experienced some anxiety during testing. Thus, we cannot rule out an

overestimation of resting MSNA for Sherpa. Ascent to 5050m was gradual, to facilitate acclimatization. However, it was not possible for groups of participants to arrive on separate days to minimize any confounding effects of the varying time course of acclimatization once at 5050m. While we acknowledge that a difference between day 10 and day 20 may have influenced our results, our analysis indicates that test day was not a significant covariate. We did not assess vascular sympathetic baroreflex gain to rising and falling pressure independently and we acknowledge that this fails to take baroreflex hysteresis into account (Rudas *et al.*, 1999). A change in baseline MSNA may have influenced responsiveness of the vascular sympathetic baroreflex to both rising and falling pressures in an equal but opposite manner (Hart *et al.*, 2011).

The mechanisms by which sustained HA acclimatization produce baroreflex resetting and chronic sympathoexcitation require elucidation. Our findings suggest factors other than the peripheral chemoreflex play a role. We acknowledge that relative hypovolemia (Ryan *et al.*, 2014), systemic inflammation and oxidative stress (Lewis *et al.*, 2014), erythropoietin production (Oshima *et al.*, 2018), and changes in intracranial pressure (Schmidt *et al.*, 2018), all might influence sympathetic outflow in HA hypoxia. Furthermore, sympathetic activation in response to elevated pulmonary artery pressure has been shown in experimental animals (Moore *et al.*, 2011).

CONCLUSIONS

We demonstrate highly effective arterial baroreflex control of sympathetic vasomotor activity in healthy humans during sustained hypoxia. Chronic resetting of the vascular sympathetic baroreflex supports elevated vasoconstrictor drive in Lowlanders during early acclimatisation to HA, but without an increase in resting

arterial pressure. Sherpa, by comparison, have a lower vascular sympathetic baroreflex operating point and lower vasoconstrictor drive, but similar vascular resistance and arterial pressure. For Lowlanders, vascular sympathetic baroreflex resetting and heightened sympathetic activity may protect against orthostatic hypotension at high altitude. In contrast, Sherpa may have adapted to high altitude to require lower sympathetic outflow for homeostatic control of blood pressure. Such a difference may represent another example of a beneficial hypoxic adaptation in this highland population.

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ADDITIONAL INFORMATION

Competing Interests

None

Author Contributions

Testing was conducted at the Centre for Heart, Lung and Vascular Health, University of British Columbia, Kelowna, Canada, and Ev-K2-CNR Research Facility, Khumbu Valley, Nepal. JPM, CDS, MS and PNA contributed to conception and design of the work. LLS, JPM, CDS, MS, SAB contributed to acquisition and analysis of the data. LLS, JPM, MS, CDS, PNA, and SJO contributed to the interpretation of the data and writing and critical revision of the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons included as an author qualify for authorship, and all those who qualify for authorship are listed.

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Authors Translational Perspectives

Sustained activation of the sympathetic nervous system and reduced vascular sympathetic baroreflex responsiveness are features of several disease states involving chronic systemic hypoxemia. Together, chronic sympathetic nervous system activation and reduced vascular sympathetic baroreflex responsiveness facilitate elevated arterial pressure and hemodynamic instability in these populations. Our data demonstrate that sustained HA hypoxia, a model of chronic systemic hypoxemia independent of co-morbidity, is accompanied by sustained chronic sympathoexcitation. Notably, however, vascular sympathetic baroreflex responsiveness is preserved. Chronic resetting of vascular sympathetic baroreflex, and hence sympathoexcitation, at HA are important for blood pressure homeostasis in acclimatized lowlanders and well-adapted Sherpa. Furthermore, mechanisms acting independently of the peripheral chemoreflex appear to be involved in HA sympathoexcitation. This raises an intriguing possibility that these mechanisms could overlap with those that activate the sympathetic nervous system in disease states.

FIGURES

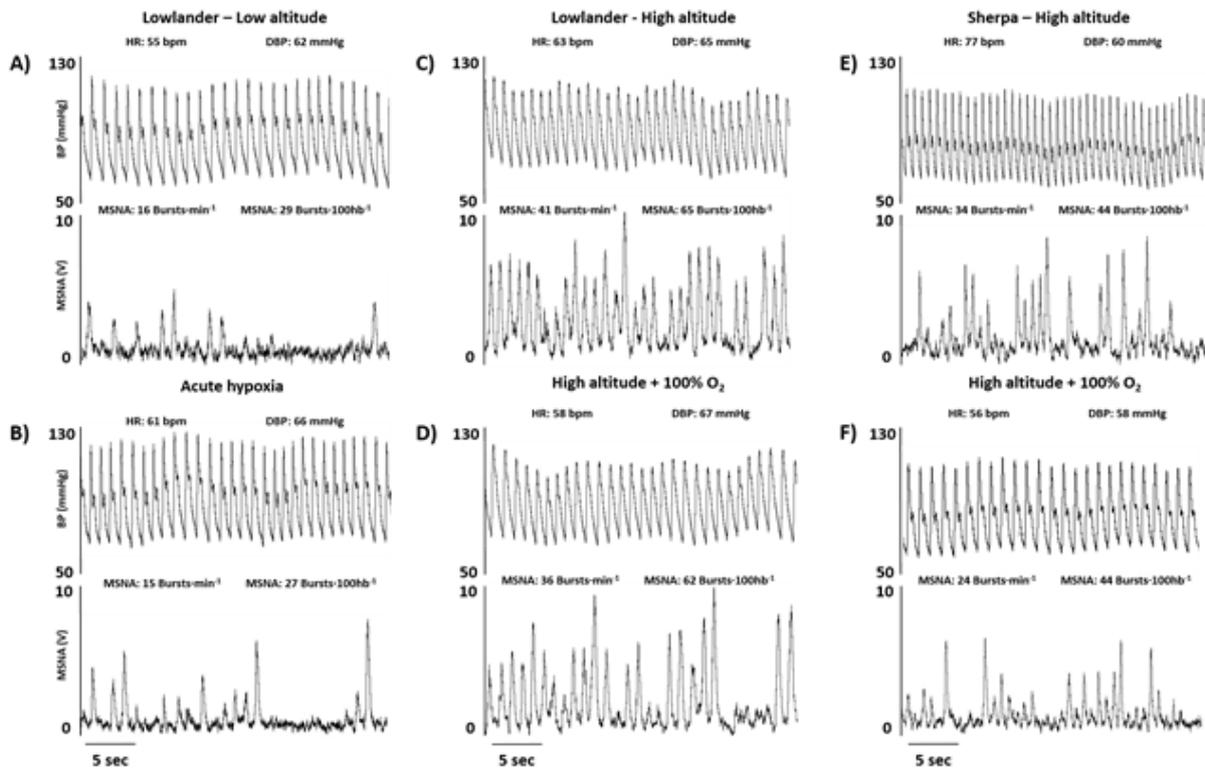


Figure 1.

LEGENDS

Figure 1. Example recordings of muscle sympathetic nerve activity (MSNA) and blood pressure (BP) from one representative Lowlander (aged 29 years) at A) Low altitude, B) during acute hypoxia, C) following 8 days at high altitude, D) during 100% oxygen breathing at high altitude and from one representative Sherpa (aged 26 years) E) following 3 days at high altitude and F) during 100% oxygen breathing at high altitude.

Figure 2.

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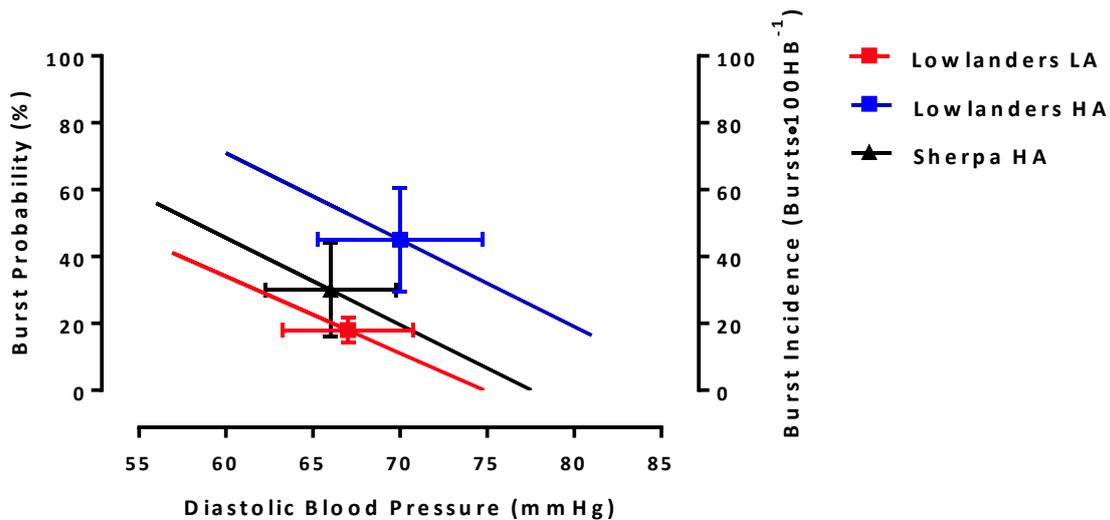


Figure 2. Vascular sympathetic baroreflex function: group average regressions between MSNA burst probability and DBP in Lowlanders (n=10) at 344m (LA) and 5050m (HA) and in Sherpa at HA (n=7). The operating points are indicated by symbols and error bars (mean \pm SD). MSNA operating point was significantly elevated in Lowlanders at HA, relative to Lowlanders at LA. MSNA operating point was lower in Sherpa relative to Lowlanders at HA, and similar to Lowlanders at LA. Operating DBP were similar. This indicated an upward resetting of the vascular sympathetic baroreflex following ascent to HA in Lowlanders. The slopes of the relationships were similar in Lowlanders at LA and HA (-2.3 ± 0.7 vs -2.6 ± 1.2 ; $P=0.33$) and similar in Sherpa at HA (-2.6 ± 0.9) compared to Lowlanders at both HA ($P=0.98$) and LA ($P=0.99$). This indicated no differences in vascular sympathetic baroreflex gain.

Figure 3.

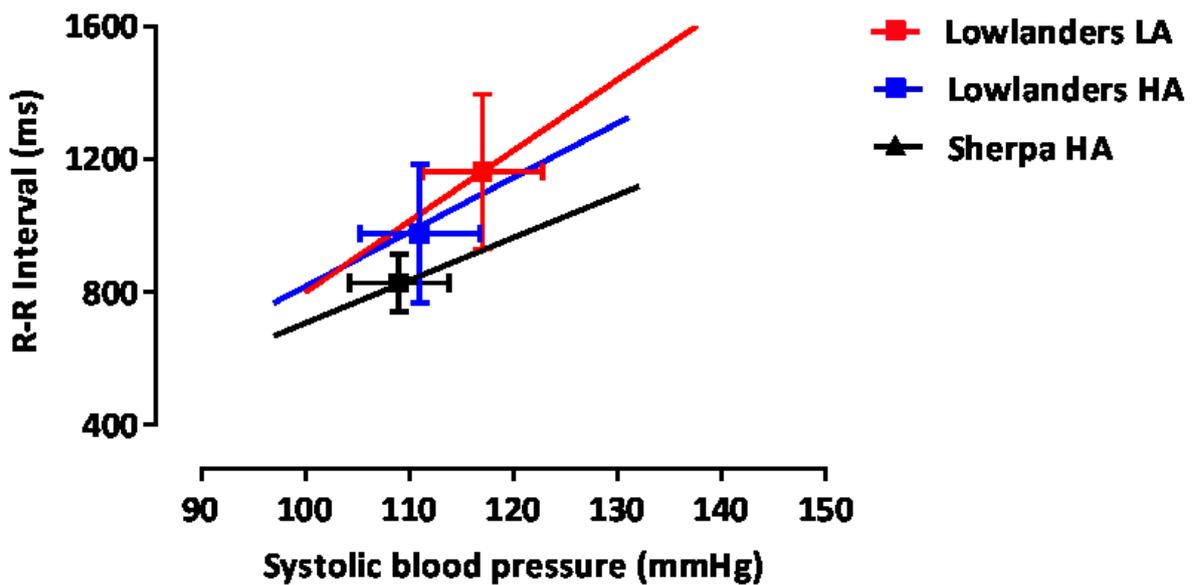


Figure 3. Cardiovascular baroreflex function: group average regressions between RRI and SBP in Lowlanders (n=10) at 344m (LA) and 5050m (HA) and in native Sherpa at HA (n=7). The operating points are indicated by symbols and error bars (mean \pm SD). RRI significantly decreased in Lowlanders at HA, relative to Lowlanders at LA, but was similar in Sherpa relative to Lowlanders at HA. Operating SBP were similar. This indicated a downward (RRI) resetting of the cardiovascular baroreflex in Lowlanders following ascent to HA. The slope of the relationship, was less steep in Lowlanders at HA (16.2 ± 8.2) versus LA (20.6 ± 5.0 ; $P=0.007$), indicating a reduction in cardiovascular baroreflex gain following ascent to HA in Lowlanders. The slope of the relationship between SBP and RRI was similar in Sherpa at HA (12.9 ± 5.4 ; $P=0.60$) relative to Lowlanders at HA; indicating no differences in reflex gain. Compared to Lowlanders at LA, operating SBP was similar, but RRI was significantly smaller in Sherpa at HA, and the slope of the relationship was less steep ($P=0.01$)

Table 1. Doses of Sodium nitroprusside (SNP) and Phenylephrine (PE) administered in Lowlanders at 344m (LA) and 5050m (HA) and Sherpa at HA and the resultant blood pressure changes.

	Lowlanders			Sherpa	
	LA (n=14)	HA (n=14)	<i>P</i> Value	HA (n=8)	<i>P</i> Value
SNP dose ($\mu\text{g}\cdot\text{kg}^{-1}$)	1.71 \pm 0.53	1.72 \pm 0.39	-	1.60 \pm 0.40	-
PE dose ($\mu\text{g}\cdot\text{kg}^{-1}$)	2.27 \pm 0.21	2.29 \pm 0.24	-	2.10 \pm 0.20	-
SNP blood pressure decrease (mmHg)	15 \pm 6	15 \pm 6	0.78	14 \pm 4	0.9
PE blood pressure increase (mmHg)	19 \pm 9	11 \pm 3	0.02	15 \pm 4	0.15
Total blood pressure changes (mmHg)	35 \pm 11	26 \pm 8	0.02	28 \pm 6	0.15

	Lowlanders			KT (n=4)	Sherpa		
	LA (n=14)	HA (n=14)	P Value		HA (n=8)	HA vs Lowlanders HA	HA vs Lowlanders LA
Haemodynamic variables							
SpO ₂ (%)	98 ± 1	82 ± 3	0.001	96 ± 1	81 ± 4	0.6	0.001
Heart rate (bpm)	54 ± 10	64 ± 13	0.006	63 ± 7	73 ± 7	0.16	0.002
Mean arterial pressure (mmHg)	84 ± 8	85 ± 10	0.84	92 ± 3	84 ± 9	0.84	0.9
Cardiac output (L·min ⁻¹)	5.2 ± 1.0 [♦]	5.2 ± 1.2 [♦]	0.46	4.9 ± 1.2	5.8 ± 1.7	0.42	0.41
Total Peripheral Resistance (mmHg·L·min ⁻¹)	16.7 ± 3.3 [♦]	17.5 ± 3.9 [♦]	0.94	19.8 ± 4.9	16.3 ± 6.8	0.73	0.9
Muscle sympathetic Nerve Activity							
Burst frequency (bursts·min ⁻¹)	11 ± 5	30 ± 9	0.001	11 ± 2	22 ± 11	0.05	0.003
Burst incidence (bursts·100HB ⁻¹)	22 ± 12	48 ± 16	0.001	18 ± 6	30 ± 13	0.02	0.16
Mean burst amplitude (a.u)	43 ± 8	50 ± 5	0.02	49 ± 7	53 ± 4	0.2	0.003
Total activity (a.u·min ⁻¹)	461 ± 194	1508 ± 548	0.001	521 ± 140	1168 ± 540	0.2	0.002

Table 2. Haemodynamic and basal MSNA variables at 344m (LA), 1400m (KT) and 5050m (HA). Data are presented as mean (± SD). [♦] Cardiac Output and Total Peripheral Resistance for Lowlanders, n = 10. Note: No intragroup comparison for Sherpa, HA *versus* KT, as only 3 were tested at both altitudes.

	Lowlanders				
	LA (n=11)	AH (n=11)	P Value	LA + 100% O ₂ (n=9)	# P Value
Haemodynamic variables					
SpO ₂ (%)	98 ± 1	84 ± 4	0.001		
Heart Rate (bpm)	53 ± 10	68 ± 15	0.001	56 ± 11	0.55
Mean Arterial Pressure (mmHg)	84 ± 7	89 ± 5	0.05	88 ± 5	0.09
Cardiac Output (L·min ⁻¹)	5.1 ± 1.0 [*]	6.7 ± 1.8 [*]	0.002	5.7 ± 0.9	0.27
Total Peripheral Resistance (mmHg·L·min ⁻¹)	16.8 ± 3.5 [*]	14.2 ± 3.7 [*]	0.01	15.9 ± 2.7	0.67
Muscle sympathetic Nerve Activity					
Burst frequency (bursts·min ⁻¹)	11 ± 4	13 ± 6	0.43	11 ± 6	0.9
Mean burst amplitude (a.u)	40 ± 7	52 ± 13	0.01	46 ± 14	0.06
Total activity (AU·min ⁻¹)	502 ± 183	789 ± 489	0.06	549 ± 305	0.49
Baroreflex Function					
Vascular sympathetic baroreflex gain (%·mmHg ⁻¹)	-2.7 ± 1.0	-1.9 ± 0.6	0.02	-2.3 ± 0.9	0.35
Diastolic operating pressure (mmHg)	67 ± 7	70 ± 4	0.09	70 ± 9	0.14
Burst incidence operating point (bursts·100HB ⁻¹)	20 ± 6	19 ± 8	0.68	20 ± 9	0.77
Cardiovagal baroreflex gain (ms·mmHg ⁻¹)	21.2 ± 8.4	23.5 ± 16.2	0.51	27.2 ± 17.4	0.24
Systolic operating pressure (mmHg)	117 ± 10	123 ± 7	0.12	121 ± 10	0.32
R-R Interval operating point (ms)	1164 ± 226	933 ± 248	0.001	1177 ± 245	0.78

Table 3. Manipulation of peripheral chemoreceptor drive in Lowlanders at 344m (LA). Data are presented as mean (± SD). ^{*}Cardiac Output and Total Peripheral Resistance, n=10. [#]Intragroup comparison for 9 participants, LA *versus* LA + 100 % O₂.

	Lowlanders			Sherpa		
	HA (n=9)	HA + 100% O ₂ (n=9)	P Value	HA (n=4)	HA + 100% O ₂ (n=4)	P Value
Haemodynamic variables						
SpO ₂ (%)	82 ± 4	97 ± 2	0.001	82 ± 5	99 ± 1	0.008
Heart rate (bpm)	70 ± 12	61 ± 8	0.01	74 ± 6	62 ± 5	0.02
Mean arterial pressure (mmHg)	88 ± 9	93 ± 9	0.008	78 ± 8	79 ± 11	0.79
Cardiac output (L·min ⁻¹)	5.4 ± 1.0	5.2 ± 1.0	0.60	6.2 ± 1.7	5.7 ± 1.8	0.34
Total Peripheral Resistance (mmHg·L·min ⁻¹)	17.1 ± 3.7	18.3 ± 4.1	0.26	14.4 ± 7.4	16.5 ± 10.7	0.26
Muscle sympathetic Nerve Activity						
Burst frequency (bursts·min ⁻¹)	30 ± 10	27 ± 11	0.35	22 ± 8	17 ± 6	0.14
Mean burst amplitude (a.u)	50 ± 5	46 ± 13	0.36	53 ± 5	46 ± 6	0.01
Total activity (a.u·min ⁻¹)	1495 ± 614	1289 ± 729	0.18	1158 ± 330	786 ± 250	0.08
Baroreflex Function						
Vascular sympathetic baroreflex gain (%·mmHg ⁻¹)	-2.6 ± 1.2	-2.5 ± 1.0	0.16	-2.8 ± 1.2	-3.0 ± 1.3	0.69
Diastolic operating pressure (mmHg)	72 ± 9	74 ± 10	0.06	63 ± 8	65 ± 12	0.34
Burst incidence operating point (bursts·100HB ⁻¹)	44 ± 16	45 ± 16	0.62	29 ± 10	29 ± 12	0.93
Cardiovascular baroreflex gain (ms·mmHg ⁻¹)	21.5 ± 5.5	21.2 ± 11.4	0.92	13.2 ± 3.5	18.4 ± 9.1	0.20
Systolic operating pressure (mmHg)	113 ± 11	119 ± 9	0.01	103 ± 7	111 ± 12	0.10
R-R interval operating point (ms)	882 ± 129	994 ± 139	0.006	790 ± 64	960 ± 80	0.04

Table 4. Manipulation of peripheral chemoreceptor drive in Lowlanders and Sherpa at 5050m (HA). Data are presented as mean (± SD).

Table 1. Doses of Sodium nitroprusside (SNP) and Phenylephrine (PE) administered in Lowlanders at 344m (LA) and 5050m (HA) and Sherpa at HA and the resultant blood pressure changes.

Table 2. Haemodynamic and basal MSNA variables at 344m (LA), 1400m (KT) and 5050m (HA). Data are presented as mean (\pm SD). \blacklozenge Cardiac Output and Total Peripheral Resistance for Lowlanders, n = 10. Note: No intragroup comparisons for Sherpa, HA *versus* KT, as only 3 were tested at both altitudes.

Table 3. Manipulation of peripheral chemoreceptor drive in Lowlanders at 344m (LA). Data are presented as mean (\pm SD). \blacklozenge Cardiac Output and Total Peripheral Resistance, n=10. $\#$ Intragroup comparison for 9 lowlanders, LA *versus* LA + 100 % O₂.

Table 4. Manipulation of peripheral chemoreceptor drive in Lowlanders and Sherpa at 5050m (HA). Data are presented as mean (\pm SD).