Sympathetic Function in Sherpa and Lowlanders at High Altitude

by

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ABSTRACT

Introduction: Low altitude dwellers (Lowlanders) that ascend to altitude are met with the challenge of a chronically low oxygen environment (hypoxia). Muscle sympathetic nerve activity (MSNA) becomes augmented under hypoxia in an attempt to redistribute blood towards oxygen critical tissues. Previous work has shown that both basal MSNA and blood pressure progressively increase in Lowlanders over the span of several days at altitude. This sympathetic response may differ in native Sherpa, who exhibit an improved cardiovascular response at altitude relative to Lowlanders. However, no studies have measured MSNA in native Sherpa. Furthermore, the sympathetic response to additional stress (sympathetic reactivity) at altitude has not been previously been measured.

Hypothesis: We believe that Sherpa will exhibit lower basal sympathetic activity than acclimatized Lowlanders at high altitude. This will also correspond with lower sympathetic reactivity in Sherpa at altitude.

Methods: Microneurography was used to measure MSNA in Lowlanders (n= 14) at 344m and 5050m, while Sherpa (n = 8) were measured at 5050m. MSNA burst frequency (bursts/ min), burst incidence (bursts/100 hb), and burst amplitude (% of max burst) was collected during 10 minutes of supine rest. Sympathetic reactivity was measured via end-expiratory breath hold, which produces a strong sympathetic response. Sympathetic reactivity was quantified as burst area (au) and total normalized SNA (au/min) during the last 10 cardiac cycles of each breath hold in Lowlanders and Sherpa at both altitudes. In addition, total normalized area was calculated during the last 15 cardiac cycles of baseline for comparison with reactivity conditions.

Results: Ascent from low to high altitude saw an increase in Lowlander burst frequency (11±5 bursts/ min to 30±6 bursts/ min; Mean±SD; $p<0.001$) and burst incidence (25±13 bursts/ 100hb to 50±15 bursts/ 100hb; $p<0.001$), while Sherpas saw lower burst frequency (23±11 bursts/ min; $p<0.05$) and incidence (44±20 bursts/ 100hb; $p<0.05$) at 5050m. Burst amplitude was similar between both groups at altitude, though Lowlanders exhibited larger bursts at 5050m compared to 344m ($P<0.05$). Both groups showed an increase in MSNA during the last 5 cardiac cycles of breath holding prior to volitional breakpoint ($P<0.05$). However, burst area was lower in Sherpa compared to Lowlanders at high altitude ($P<0.05$) . In addition, the total normalized SNA and blood pressure responses were similar in Lowlanders between 344m and 5050m; while Sherpa exhibited a lower SNA and blood pressure response compared to Lowlanders at altitude.

Discussion: In the current study we were able to collect, for the first time, direct recordings of post-ganglionic muscle sympathetic activity in native Sherpa. These results demonstrate that Sherpas exhibit lower SNA at altitude compared to Lowlanders, despite no difference in blood pressure and resting arterial oxygen saturation. We also showed that sympathetic reactivity was lower in Sherpa at 5050m through the use of voluntary breath holds, despite a similar blood pressure during breath holding as Lowlanders. These findings suggest an increased vascular responsiveness within Sherpa that is able to maintain blood pressure similar to that of acclimatized Lowlanders under reduced sympathetic activation at altitude. In addition, the findings of a similar SNA response to breath holding in acclimatized, despite higher basal MSNA at altitude, suggest a sympathetic reserve becomes reduced at altitude.

PREFACE

This thesis is an original work by Stephen Busch. The research project, of which this thesis is a part, received ethics approval from the University of Alberta- Research Ethics Board, on December 7th 2016. The ethics project name was "Sympathetic Regulation at Altitude" (Pro00064195).

The research conducted for this thesis forms part of an international research collaboration led by Professor P.N. Ainslie at the University of British Columbia - Okanagan. Professors C.D. Steinback (University of Alberta), M. Stembridge (Cardiff Metropolitan University), and J.P. Moore (Bangor University) were the lead investigators for this particular study. The literature review in Chapter 2, summary techniques described in Chapter 3, and concluding analysis in Chapter 5 are my original work.

Chapter 4 of this thesis is in the process of being published as S.A. Busch, J.P. Moore, M. Stembridge, L.L. Simpson, F. Sobierajski, L. Riske, P.N. Ainslie, C.D. Steinback, "Reduced Sympathetic Activity and Reactivity in High-Altitude Sherpa". I contributed to the conception of work and am responsible for data collection, data analysis, and composition of the manuscript. Co-authors listed have contributed to either i.) conception or design of work (CDS, MS, JPM), ii.) acquisition, analysis, or interpretation of data for the work (FS, LR, LS, CDS, JPM, MS), or iii.) drafting the work or revising it critically for important intellectual content (PNA, CDS, JPM, and MS). All persons listed have read and approved of the final version of the manuscript.

DEDICATION

The thesis you are about to read is the product of my work and time at the University of Alberta. However, the following chapters would not have been possible without the individuals and groups listed below. Their support cannot be acknowledged enough towards the completion of this degree.

To my supervisor, Dr. Craig Steinback; thank you for allowing me to be apart of such an amazing lab. Your hand on approach and willingness to supervise me has allowed for the development of skills that I will use not only in an academic sense, but also in life. My experiences in Edmonton, and halfway around the world, have allowed me to grow both academically and independently. Our output during the last 2.5 years is a testament to your mentoring and drive. It is for all of these things that I am extremely grateful.

I would like to thank everyone involved within this study. This includes all members of the Nepal 2016 expedition. To Drs. Jonathan Moore and Mike Stembridge, and Ms. Lydia Simpson; I could not ask for a livelier and more positive team to have been oxygen starved with. I continue to be thankful for your willingness to go through countless reviews of my manuscripts (and many more to come). I would like to especially thank Dr. Philip Ainslie, whose guidance during my undergraduate opened the doors to a much larger world. In addition, I would like to thank my lab mates in PADL and the NVHL. Specifically, Dr. Margaret Davenport, Rachel Skow, Graeme Purdy, Frances Sobierajski, Marina James, and Laurel Riske. Their contributions, feedback, insight into academic life, and positive energy towards my work have been indispensible. I look forward to celebrating more publications with you all.

I want to especially thank my family, whom have supported and nurtured this scientific endeavor since I was a child. My mother and father continue to be a source of inspiration while keeping me objective and grounded. To my father especially, who has always ensured that I was indeed “scientific”.

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To Weronika; your love and support has been a source of strength for me well before this Masters' started, and will continue to make me a better person. The challenges and achievements we have experienced together in these past few years are a testament to the bond that we share. The future ahead will continue to be a journey of unknowns that take us further away from our comfort zones. Though it is through these bricks of uncertainty that we will build our memories of the past, and dreams for the future.

… I'm also sorry for the countless hours of sleep you lost to my nocturnal typing, data analysis, and figure making.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABP	Arterial Blood Pressure
ATP	Adenosine triphosphate
BP	Blood Pressure
Ca^{2+}	Calcium Ion
cAMP	Cyclic Adenosine Monophosphate
CM	Calmodulin
CNS	Central Nervous System
CO_2	Carbon Dioxide
CVLM	Caudal Ventrolateral Medulla
DBP	Diastolic Blood Pressure
ESV	End Systolic Volume
FiO_2	Fraction of inspired Oxygen Content
FRC	Functional Residual Capacity
Hb	Hemoglobin
Hc	Hematocrit
HPV	Hypoxic Pulmonary Vasoconstriction
HR	Heart Rate
IML	Intermediolateral Cell Column
IP_3	Inositol Triphosphate
K^+	Potassium Ion
MAP	Mean Arterial Pressure
MSNA	Muscle Sympathetic Nerve Activity
NADPH	Nicotinamide Adenine Dinucleotide Phosphate

NO	Nitric Oxide
NPY	Neuropeptide- Y
NTS	Nucleus Tractus Solitaires
$PaCO_2$	Partial Pressure of Arterial Carbon Dioxide Content
PaO_2	Partial Pressure of Arterial Oxygen Content
P_AO_2	Partial Pressure of Alveolar Oxygen Content
PO_2	Partial Pressure of Oxygen
PiO_2	Partial Pressure of Inspired Oxygen
PIP2	Phosphatidylinositol bisphosphate
PG	Prostaglandin
Q	Cardiac Output
ROS	Reactive Oxygen Species
RVLM	Rostral Ventrolateral Medulla
SaO_2	Arterial Oxygen Saturation
SAR	Slow Adapting Pulmonary Stretch Receptor
SpO_2	Peripheral Capillary Oxygen Saturation
SBP	Systolic Blood Pressure
SNA	Sympathetic Nerve Activity
SNS	Sympathetic Nervous System
SV	Stroke Volume
VLM	Ventrolateral Medulla

CHAPTER 1- INTRODUCTION

The sympathetic nervous system (SNS) is a subdivision of the autonomic nervous system associated with unconscious control of the "fight-or-flight" response. During periods of heightened stress, an increase in SNS activation promotes several different cardiovascular responses to maintain homeostatic balance throughout the body. For example, direct sympathetic innervation to the heart promotes greater cardiac contractility and rhythm, while sympathetic-mediated vasoconstriction of smooth muscle increases blood pressure (49). The SNS can regulate cardiovascular function through either direct innervation of the vasculature (via post-ganglionic nerve impulses), or through the release of neurohormonal messengers from the adrenal medulla into the blood. It is through these two routes of activation which allow for a robust cardiovascular response towards environmental stressors. For example, the sympathetic nervous system becomes highly active within individuals who ascend to high altitude (>2500m), where the challenge of chronically reduced oxygen availability (hypoxia) places considerable cardiovascular and metabolic strain through oxyhemaglobin desaturation (hypoxemia) and tissue de-oxygenation (183). The body acclimatizes to altitude by increasing alveolar ventilation and several sympathetic-induced cardiovascular responses that maintain oxygen delivery. The ventilatory adjustments have been covered in great detail previously (3, 66, 129, 139). However, the heightened sympathetic vasomotor response at altitude has been studied considerably less.

The potentiation of sympathetic activity at altitude is a homeostatic response towards chronic tissue deoxygenation. Though the specific mechanisms that leads to further potentiation of MSNA remains unclear, it is in part facilitated through progressive peripheral chemoreflex sensitization (38, 40, 69, 147). MSNA increases over the span of several days at altitude (40, 69), gradually restoring vascular resistance and redistribution of blood towards oxygen dependant organs through peripheral vasoconstriction. The dose dependent increase in SNA at altitude has previously been observed through several measurements, most of which is through whole body plasma catecholamine analysis (52, 96, 115-

117). Although this method has previously been used to great extent in field studies, catecholamine studies exhibit considerable variation in findings relative to the degree of hypoxic stress. Furthermore, catecholamine measures are not considered time sensitive, thus do not represent an appropriate quantification of time-dependant SNA to acute hypoxia (143, 147). Another method of measuring SNA is through direct neural recordings of post-ganglionic muscle sympathetic nerve activity (MSNA) (65). The advantage of this measure is that MSNA allows for direct, real-time measurement of SNA that occurs when the central nervous system is communicating with the peripheral vasculature. The MSNA response to hypoxia has previously been show to increase in Lowlanders under acute lab settings (33, 67, 71, 88, 96, 121, 145, 164, 193) and field studies at altitude (40, 50, 69, 103), with the degree of MSNA augmentation dependant on the strength of the hypoxic stimuli.

Sympathetic hyperactivity is observed in Low altitude dwellers (Lowlanders) that ascend to altitude (147). However, it still remains unclear if individuals that permanently reside at altitude (High altitude dwellers) also demonstrate chronically elevated SNA. Evolutionary adaptations may exist within groups that have resided at altitude for hundreds of generations to favour improved autonomic function under chronic hypoxic stress. One such group that has been of considerable interest amongst researchers are the native Sherpa/ Tibetans of the Himalayas. Sherpa are renowned for their ability to function at extreme altitudes, often being recruited as guides on expeditions to the worlds' highest peaks. It is their unique tolerance to high altitude that has drawn many studies to demonstrate numerous beneficial adaptations that favour improved cardiovascular function (55, 66, 80, 86, 154, 168) relative to Lowlanders. Previous cardiovascular measurements (20, 44, 54, 195, 197) suggest a lower SNA response within Sherpa, though no studies have directly measured SNA. As such, there exists a large gap in the current knowledge relating to autonomic regulation within high altitude populations.

The majority of MSNA studies demonstrate augmentation of basal sympathetic function at altitude in Lowlanders. However, previous findings demonstrate that the sympathetic response to other

stressors (known as "sympathetic reactivity") may also be further augmented at altitude (50). The extent of this remains unclear though. Voluntary breath holds have been used previously to evoke both a brief and large MSNA response while requiring minimal movement, thus maintaining nerve sites more successfully (19, 39, 76, 106, 112, 167). Therefore, the use of voluntary breath holding may be a simple method of evoking significant sympathetic stress independent of hypoxic challenge at altitude.

1.1 Objectives

In the fall of 2016 we assessed MSNA in Lowlanders and Sherpa at the EV-K2-CNR research facility (5050m) in the Khumbu Valley near Mount Everest. Previous microneurography recordings of acclimatized Lowlanders show augmented MSNA (40, 50, 69, 103) at altitude. However, we wanted to determine if the sympathetic response to additional stress was also augmented. We determined sympathetic reactivity through the performance of a voluntary breath hold, which have previously shown large increases in MSNA (76, 106, 161). Since no SNA studies to our knowledge have been performed in native Sherpa; we also measured MSNA to determine sympathetic activity and reactivity in Sherpa relative to acclimatized Lowlanders.

1.2 Research Questions and Hypotheses

Acclimatized Lowlanders demonstrate progressive sympathoexcitation under chronic hypoxia. However, sympathetic reactivity in Sherpa and Lowlanders at altitude still remains unknown. Although this study is exploratory, we have proposed three main questions, with a stated hypothesis below each respective question. They are as follow:

(I) Do Sherpa exhibit a lower degree of basal sympathetic activity than acclimatized Lowlanders at altitude?

We believe that Sherpa will demonstrate lower basal sympathetic function than Lowlanders at high altitude.

(II) Does sympathetic reactivity to additional stress (ie. voluntary breath holding) differ between low and high altitude in Lowlanders?

We expect to see greater augmentation of MSNA during volitional breath holding at high altitude when compared to low altitude.

(III) Does sympathetic reactivity differ between Lowlanders and Sherpa at altitude?

Though not previously explored, we expect to see lower sympathetic reactivity in Sherpa.

1.3 Study Significance

As of this point, the exact role of sympathetic hyperactivity at altitude has not been elucidated. However, the degree of sympathetic hyperactivity at altitude may translate to an individual's ability to tolerate stress under chronic hypoxia. More specifically, understanding SNA regulation between Lowlanders and Sherpa will allow for us to differentiate individuals who are better suited towards travelling and residing at altitude (40). The following study was performed following several days at an altitude of 5050m. Therefore, we believe this represents a more accurate depiction of sympathetic hyperactivity within acclimatized Lowlanders and Sherpa when compared to other studies that simulate hypoxia (either through titration of FiO_2 or hypobaric chamber studies). The popularity of adventure holidays is increasing, with an estimated 100 million Lowlanders worldwide trekking in the mountains each year (22). Many jobs also exist that require native Lowlanders to either rapidly ascent, or temporarily reside, at high altitude (14, 67). Lowlanders have a reduced work capacity at altitude due to reduced oxygen availability (110). Therefore, the ability of Lowlanders effectively redistribute oxygen towards working muscles, while maintaining oxygenation towards critical organs, becomes highly relevant with regards to sympathetic reactivity. This also may uncover the potential health risks of chronic sympathoexcitation at altitude on overall vascular dysfunction, persistent hypertension, and risk of sudden cardiac death (10, 23, 38). Finally, determining sympathetic function in native Sherpa will provide novel information with regards to autonomic function of high altitude dwelling residents.

CHAPTER 2- LITERATURE REVIEW

The following review will address SNA augmentation under hypoxia (both acute and chronic). The mechanisms and pathways related to SNA will be discussed, including the corresponding effects on the cardiovascular system. MSNA studies under acute and chronic hypoxia will be reviewed in Lowlanders. Current indirect findings associated with SNA in high altitude populations will be specifically addressed towards Tibetans and Nepalese Sherpa, who originated from similar nomadic tribes of the Tibetan Plateau. Since voluntary breath holds will be used to measure sympathetic reactivity, a brief overview of the mechanisms governing MSNA during breath holding will also be discussed.

2.1 Introduction to Hypobaric Hypoxia

Oxygen (O_2) is essential for normal cellular function and metabolism. As such, tight regulation of O_2 transportation within the body allows for proper organ function and biological processes to occur. This process of regulation starts during inspiration, where ambient air is inhaled, warmed, and moistened to the conditions within the lung. Once in the lung, O_2 diffuses from the pulmonary alveoli and binds with hemoglobin bound red blood cells and plasma that fill the pulmonary capillaries. This begins a long journey from the pulmonary capillaries that travels through the heart, arteries, arterioles, and finally capillary beds of the target tissue. O_2 diffuses across the cellular membrane between the capillary beds and respective cells of the target tissue, where it is utilized for the cellular metabolism of adenosine triphosphate (ATP) (94). Oxygen not utilized for metabolism continues to travel through the capillaries alongside newly produced CO_2 from cellular metabolism in the form of mixed venous blood. This cycle of O_2 transportation is continuous, with each breath introducing fresh O_2 to the body. However, the pressure of O_2 (PO_2) transported through the body is not uniform. Rather, PO_2 becomes progressively reduced in a cascade effect (82) (Figure 1) that consists of several limiting steps. Each of these steps see a gradual reduction in O_2 content through passive diffusion limitations within the alveolar/capillary

membrane, ventilation perfusion mismatch between the alveoli and pulmonary capillaries, hemoglobin concentration and binding of O_2 within the blood, and diffusion/ rate of O_2 usage in the cellular mitochondria (95, 151, 172). Yet despite the respective limitations at each step of the cascade (94), there is sufficient O_2 availability for cellular metabolism and organ function in healthy populations under normoxic conditions. This holds true during periods of heightened stress (ie. exercise), where increased cellular metabolism is matched by sympathetic-mediated increases in ventilation and redistribution of oxygenated blood towards the working muscle, thus maintaining oxygenation throughout the body under heightened stress.

The ample availabity of O_2 at lower elevations is challenged under hypobaric hypoxia, which reduces whole body O_2 availability. Hypobaric hypoxia is separate to other forms hypoxia seen within clinical conditions, where low blood O_2 content (hypoxemia) are due to disease-related limitations at specific points of the O_2 cascade (ie. Obstructive Sleep Apnea) (124, 160). Hypobaric hypoxia promotes hypoxemia through drastic reductions in atmospheric pressure that affect the partial pressure of inspired O_2 (PiO_2). An example of this is with low altitude dwelling populations (Lowlanders) who trek to high altitude, where the reduced PiO_2 corresponds to a downward shift in the O_2 cascade (181) (Figure 1). Depending on the severity of hypoxia, drastic tissue de-oxygenation can be detrimental on organs such as the brain, which requires constant O_2 availability for normal metabolic and cellular function (18). Inappropriate adaptive responses by the body at altitude can promote maladaptation through organ dysfunction and altitude related illness (100, 182). Therefore, minor reductions of O_2 availability are met with drastic physiological adjustments to minimize tissue de-oxygenation during acclimatization. This is facilitated through an increase in activation of the autonomic nervous system, which promotes greater alveolar ventilation and sympathetic recruitment of the cardiovascular system. Ventilation acts as the primary response to increase O_2 tension at altitude. However, alveolar (P_AO_2) and arterial (PaO_2) oxygen content remains lower in acclimatized Lowlanders relative to sea level (87, 182). Although less understood, cardiovascular adjustments also optimize O_2 redistribution towards critical tissues (66, 147).

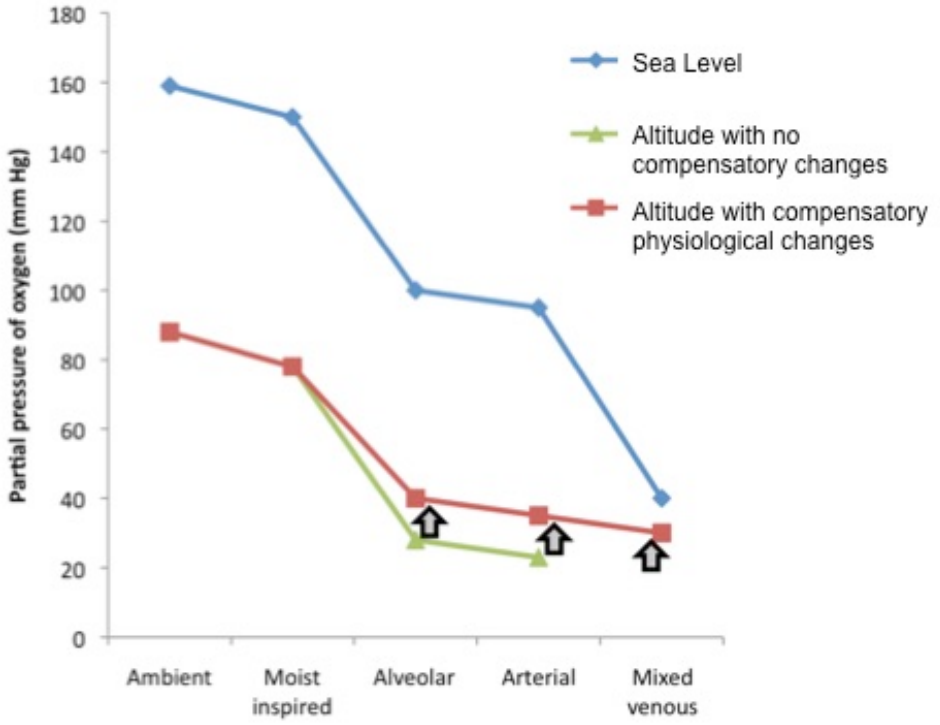

Figure 1. Partial pressure of O_2 (PO_2) through the O_2 cascade at sea level and altitude. O_2 transportation sees a progressive reduction at each step of the cascade. At altitude, an overall reduction in ambient PO_2 causes a downward shift across the whole cascade. Without both cardiovascular and respiratory compensation there would be a further reduction in O_2 tension alongside greater risk of organ dysfunction and altitude related illness. Arrows indicate minor increases in alveolar (P_AO_2) and arterial (PaO_2) content due to compensatory mechanisms associated with acclimatization, improving whole body oxygen tension to various organs. Adapted from *West* (184)

2.2 Hypobaric Hypoxia Induced Vasodilation of the Local Vasculature

The cardiovascular response to hypoxia, in addition to maintaining O_2 tension, may offset the degree of hypoxia-induced dilation seen within the local vasculature (73). Under normoxic conditions a local dilatory response, such as seen during exercise, is an adaptive response within working muscle under heightened O_2 demand (27, 71, 138). This is often limited to the working muscle, where vasoconstriction of non-essential organs during exercises still occurs through increased SNA. However, acute hypoxia sees a whole body dilatory response throughout the peripheral vasculature (minus the pulmonary vasculature which experiences constriction under hypoxia) relative to the reduction in PaO_2. This initially lowers total peripheral resistance (TPR) and blood pressure, while increasing blood flow to O_2 deprived local tissue (2, 177, 189). Several mechanisms of local hypoxic vasodilation have previously been examined, including greater expression of nitric oxide (NO) and prostaglandin (Pg) expression within the endothelial lining (17, 113). These both inhibit calcium ion concentrations within the smooth muscle that surround blood vessels, thus lowering the ability to vasoconstrict. These two pathways are also believed to complement each other, with greater release of Pg increasing NO synthesis. However, the specific mechanisms contributing toward the expression of NO/ prostaglandins remains unknown. Several others pathways have been suggested to play a minor role in promoting hypoxia mediated vasodilation. These include acetylcholine and ATP/ NO release from red blood cells under hypoxia (5). These pathways promote a strong vasodilatory response on the local vasculature that conflict with increased sympathetic activation at altitude.

2.3 Cardiovascular Response to Acute Hypoxia

Initial exposure to acute hypoxia unconsciously triggers a rapid response by the autonomic nervous system (170). The sympathetic division becomes highly active in order to activate central (cardiac) and peripheral (peripheral vasoconstriction) components of the cardiovascular system. The initial cardiovascular response occurs in order to reduce tissue de-oxygenation, where the degree of hypoxia corresponds to a respective increase in SNA outflow(147). Greater sympathoadrenal activity

results in an increased cardiac response (via greater epinephrine mediated β-adrenergic receptor activity) and peripheral vasoconstriction (via elevated norepinephrine binding to α-adrenergic receptors) (190). The increases in heart rate (HR) under hypoxia also drive an increase in cardiac output (Q). Stroke volume (SV), only mildly contributes to due to lower end-systolic volumes (ESV) and faster myocardial contraction velocity (2). The increase in Q is dependant on the respective increase in HR at altitude. Grollman *et al*. (57) classically demonstrated upwards of a 40% increase in Q following several days at 4300m, while Vogel (177) saw no change in Q below an altitude of 700m. This increase in HR is suspected to occur due to elevations of SNA outflow. This may be further facilitated through parasympathetic withdrawal, as previously seen with the lack of abolishment in hypoxia-induced HR elevation following beta blockade (141).

The observed reduction in systemic pressure and vascular resistance under acute hypoxia (38, 190) is due to an imbalance of local peripheral vasodilatory factors (see section 2.2) relative to sympathetic activation. Vasodilation is evident in all vascular beds with exception of pulmonary vascular beds, which undergo hypoxic pulmonary vasoconstriction (72, 73). The reductions in peripheral resistance under acute hypoxia are duration specific, with small decreases of resistance and mean arterial pressure (MAP) becoming evident following several hours of exposure (53, 177). However, vasodilatory dominated balance may be short-lived, with previous findings of circulating catecholamines suggesting a shift towards greater SNA within several hours to promote a hypertensive state (190). Thus, the initial sympathetic mediated increase help to sustain Q and minimize reductions in TPR during the initial period of hypoxia exposure.

2.4 Cardiovascular Response to Chronic Hypoxia

Chronic exposure to hypoxia (several days-weeks) sees additional cardiovascular adaptations that attempt to restore PaO_2 towards normoxic levels. Increased hematocrit concentration, oxyhemaglobin

saturation, ventilatory acclimatization, and SNA mediated vasoconstriction (2) are some of the adaptations associated with acclimatization. These adaptations are incapable of returning PaO_2 back to normoxic levels, but rather see small improvements in O_2 content relative to the initial exposure period. These adaptations in acclimatized individuals allow for small improvements of functional capacity at altitude. The degree of basal HR elevation is altitude dependent in that higher elevations see a larger magnitude of HR elevation (4, 89, 177). Though the respective mechanisms governing this elevation remain unclear, current speculation suggests a shift in balance between vagal and sympathetic tone (148). The depression of SV continues though reduced plasma volume (123), which further reduces Q (2). The early reduction of resting SV has been recorded as early as two days following hypoxia exposure (92) and gradually declines for up to 1 week, where stabilization may prevent further reductions (4). However, the degree of SV reduction is proportional to the elevation achieved with ascent to higher elevations seeing greater SV loss (136). The reduced Q also appears to be affected through lower left ventricular diastolic filling (169) rather than reduced ventricular contractility during systole (4, 169).

Progressively greater augmentation of SNA occurs over a period of several days at altitude (40, 69, 147). This may explain a similar increase in systemic and peripheral vasoconstrictive response over several days at altitude. Forearm blood flow becomes reduced, while TPR and MAP increases (26, 180). Further reductions in leg blood flow have been observed under supplemental oxygen following several weeks at altitude, suggesting the tight linking between tissue oxygenation and peripheral blood flow. Therefore, the local vasodilatory response during acute hypoxia appears to diminish through an elevated sympathetic response combined with improvements in oxygenation capability (greater red cell mass and concentration) to the local tissue. This translates to a gradual increase in systemic at altitude (2, 15, 177, 189). These findings of increased arterial pressure at 5000m do not appear to translate under extreme altitude (>7000m), as no further increases in blood pressure or systemic resistance have been observed (59). This may be in part due to the large magnitude of hypoxia promoting a tonic vasodilatory state in the vasculature.

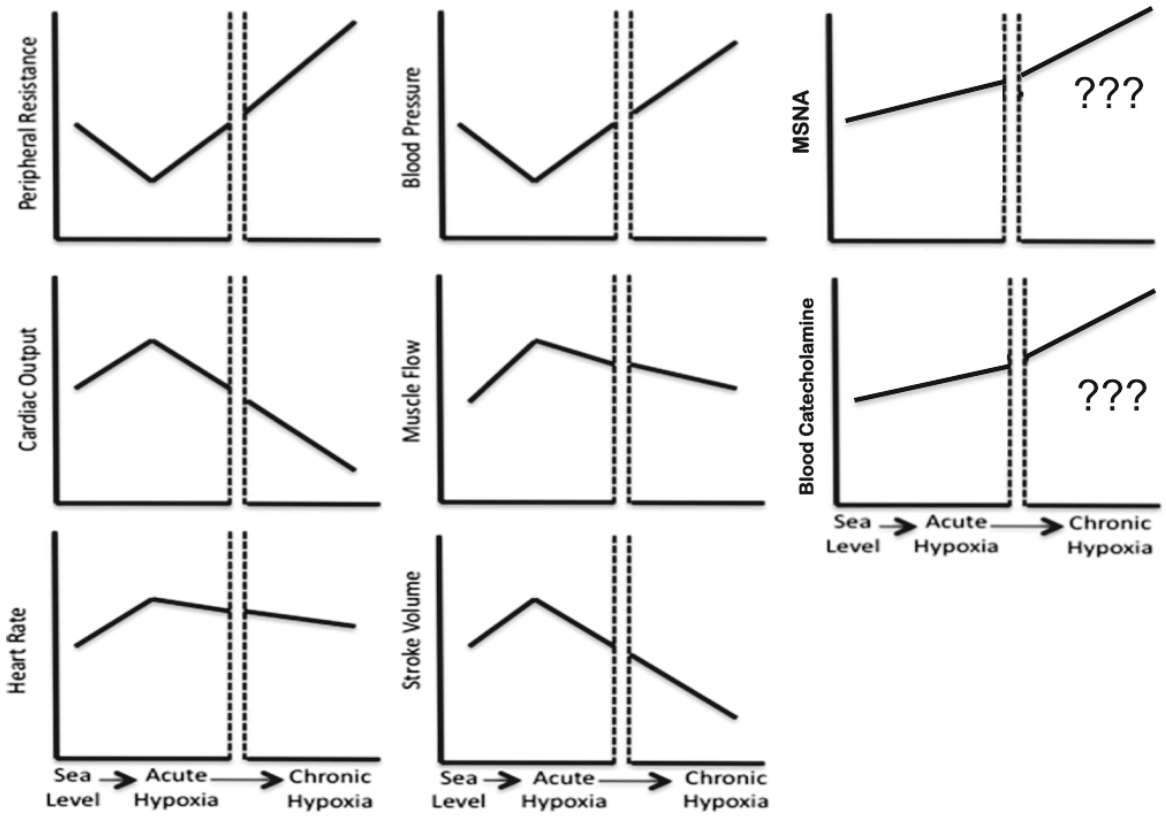

Figure 2. Cardiovascular response during acute and chronic altitude exposure in lowlanders. Initial reductions in PiO_2 promote sympathetic augmentation to combat the direct local vasodilatory effects of hypoxia while maintaining tissue oxygenation through heightened cardiac function and peripheral vasoconstriction. Initial exposure sees a progressive rise in cardiac (via elevated HR and SV) and a decrease in TPR and blood pressure, which corresponds to an increase in muscle blood flow. Overtime a gradual increases in SNA outflow counteracts the hypoxia-induced local vasodilation, which sees an increase in TPR and MAP. When combined with reduced Q, the improved transient time of blood flow sees an overall reduction within muscle blood flow during chronic exposure. Although the shift from acute to chronic hypoxia promotes further SNA augmentation, the degree of augmentation remains less well understood in regards to all these responses. Modified from *Baggish et al.* (2)

2.5 Sympathetic Activation to Hypobaric Hypoxia

Hypoxia is a strong stimulator of SNA, which allows for quick recruitment of the cardiovascular system in response to decreases of PaO_2 (114). The change in SNA during these periods is often measured through one of several methods. The majority of studies have previously measured the whole body SNA response through analysis of blood and urine catecholamine levels, and catecholamine spillover. The third is through the measurement of efferent postganglionic sympathetic nerve discharges (known as muscle sympathetic nerve activity; MSNA). These methods have been used extensively to measure the SNA response under hypoxia (67, 69, 96, 115, 117, 121, 144, 145, 159, 163, 190, 193). In addition, these methods correlate well together under acute hypoxia conditions (96). Yet most field studies that measure SNA at altitude have primarily focused on the circulating catecholamine technique. This is due to the simple nature of the technique for data collection over multiple time points in the field (52, 111, 115, 116, 143). However, the respective sensitivity of measures is of concern due to high inter/intra variability in participants, resulting in conflicting findings of noradrenaline and adrenaline release under acute hypoxia exposure. Rostrup (143) previously reviewed earlier studies measuring catecholamine plasma levls under both acute and chronic hypoxia. He only noted an increase of noradrenaline spillover in one of the 6 studies during short-term altitude (3658-4559m) exposure (4 hours to 3 days). Yet in twelve studies that measured noradrenaline release following a week or more at altitude (ranging from 3500-6000m); he further reported 11 studies that demonstrated an increase in noradrenaline spillover. This discrepancy between acute and chronic hypoxic conditions may be explained in part due to differences in protocol, time points of measurement, and maximum elevation achieved (147), which all have effects on overall catecholamine spillover, reuptake, and excretion at altitude (67, 143). Despite this, there is a general trend towards greater plasma noradrenaline spillover at altitude appears to be duration dependent with heightened catecholamine spillover under chronic hypoxia (figure 3).

The measurement of MSNA allows for region specific measurements of SNA that are sensitive to acute hypoxia interventions (147). Assessment of MSNA is performed via microneurography, a technique used to measure action potentials that travel along efferent post-ganglionic c-efferent nerve fibers (Figure 4). The microneurography technique began in the mid 1960's, with Hagberth and Valbo first successfully demonstrating rhythmic neural discharges from the peroneal and radial nerves (65). Delius *et al.* were subsequently able to elaborate on this groundbreaking work by establishing the sympathetic characteristics associated neurons innervating the vasculature within skeletal muscle (35). The microneurography technique has since been extensively by researchers for measuring SNA in both healthy and clinical populations. The studies of both Saito *et al.* (145) and Rowell *et al.* (144) were amongst the first to demonstrate augmented MSNA under acute hypoxemia in healthy populations. Leuenberger *et al.* (96) subsequently demonstrated a strong correlation between MSNA and forearm plasma noradrenaline concentrations under acute hypoxemia conditions. Thus there is validity in using microneurography to measure SNA under hypoxia. However, the relative difficulty associated with the microneurography technique has been a common drawback for performance within field studies at altitude. MSNA measurements have only more recently been recorded at altitude in acclimatized Lowlanders (40, 69, 103). The following sections will discuss the microneurography technique (sub-section 3.5.2) while reviewing MSNA response to both acute and chronic hypoxia (Sections 2.4-2.5).

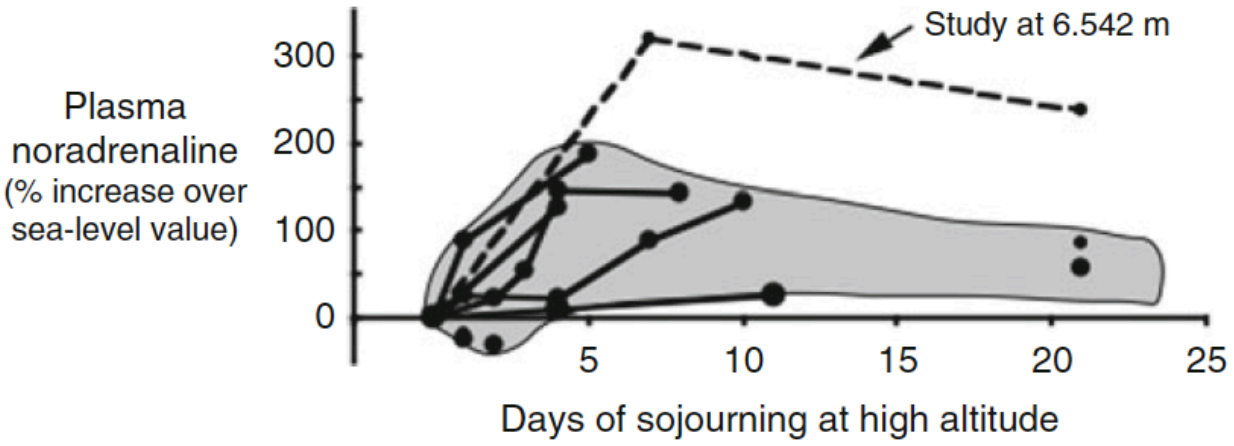

Figure 3. Summary of studies measuring plasma catecholamines at altitude. Majority of studies performed between 4200- 4559m, with one performed above 6500m as indicated by the dotted line. Studies indicate elevation of circulating plasma noradrenaline spillover while at altitude, with the degree of spillover varying between studies. Trend shows a progressive increase in plasma noradrenaline with longer duration. From Sander (147).

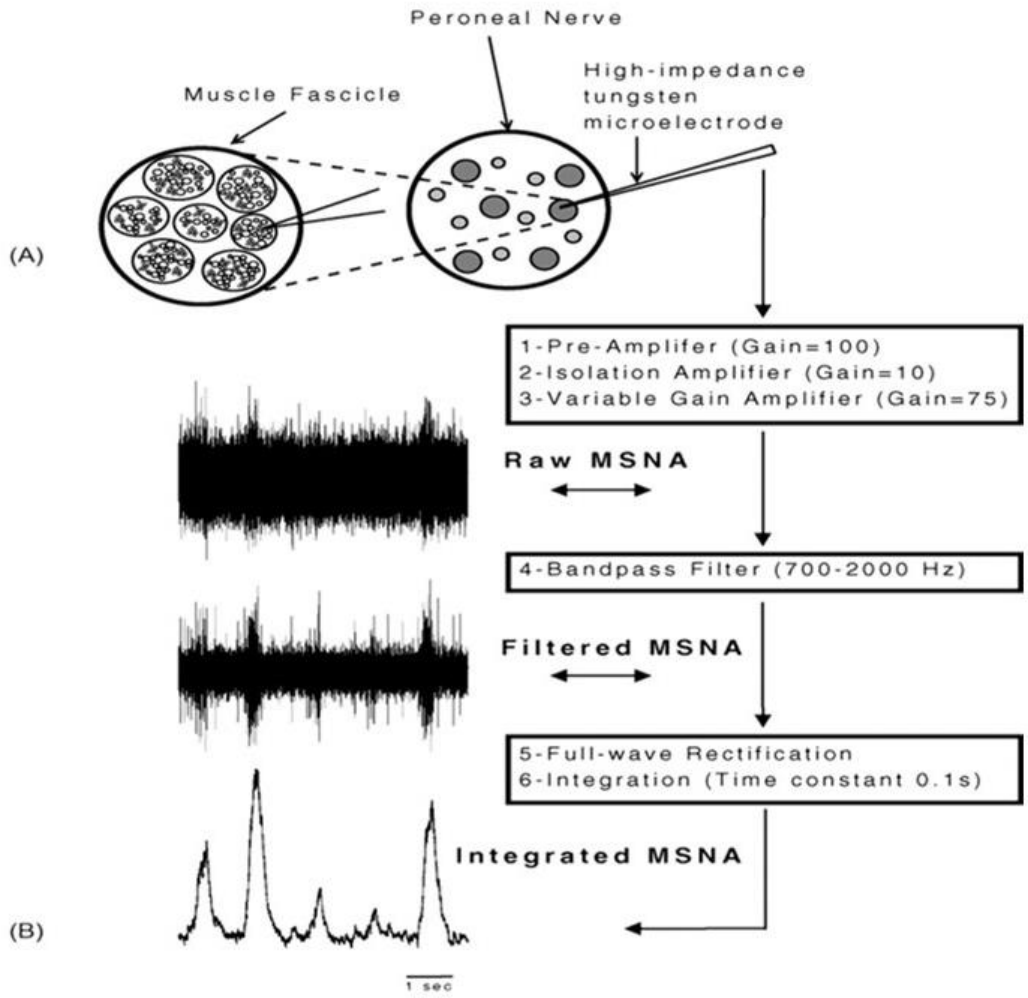

Figure 4. Representation of the microneurography and filtering of MSNA signal. (Panel A) Schematic representation of the microneurography technique. A recording microelectrode is inserted into a human peripheral nerve for measurement of post-ganglionic sympathetic nerve activity. The nerve fascicle contains bundles of sympathetic nerves that target the smooth muscle surrounding blood vessels. Increased axon discharge is recorded by the electrode and represented in the Raw MSNA signal following amplification. Following the filtering of the raw MSNA signal (Band pass Filter 700-2,000 Hz) the signal undergoes full wave rectification and time integration (constant 0.1s) (see panel B). From Salmanpour *et al.* (146)

2.5.1 The Pathways of Sympathetic Activation under Hypobaric Hypoxia

Hypoxia triggers a negative feedback response within the autonomic nervous system that attempts to correct for a fall in PaO_2. Reductions of PaO_2 excite oxygen-sensing bodies (chemoreceptors) within the carotid bifurcation (114). Greater chemoreceptor discharge sends excitatory signals towards ventilatory and cardiovascular control centers within the brainstem (147). Depending on the degree of central nervous system (CNS) activation, there is a corresponding increase in SNA towards various regions of the human body (38). These corrective (ventilatory) and compensatory (vasoconstrictive) responses to hypoxia improve tissue oxygenation while maintaining both vascular tone and blood flow to oxygen critical organs (114). The following sections will provide an overview of the sympathetic vasomotor response to hypoxia. This will include initial detection, relaying of information to-and-from CNS, and corresponding vasoconstrictive response within the vasculature to increased SNA.

2.5.1.1 Activation of Afferent Pathways to Hypobaric Hypoxia

The neural response to hypoxia begins with the initial detection of reduced PaO_2 through oxygen sensitive bodies (peripheral chemoreceptors) located in the carotid bifurcation. These peripheral chemoreceptors consist of grouped Type I glomus cells that monitor chemical changes in PaO_2 through a dense network of capillaries branching from the external carotid artery (62, 99) (Figure 5). These cells are sensitive to multiple stimuli that circulate within the blood, though they are most notably known for monitoring and responding to changes in PaO_2 (99). Under hypoxic stress, the peripheral chemoreceptors become activated through blocking of voltage-gated channels located on the glomus cell membrane. The closing of potassium ion (K^+) channels promote subsequent cell membrane depolarization (Figure 6). Although it is well established that glomus cell depolarization is through closing of K^+ channels; the mechanisms associated with the closing of K^+ channels are not fully understood, though several mechanisms have been proposed. The inhibition of nicotinamide adenine dinucleotide phosphate oxidase (NADPH) within mitochondria that inhibit intracellular reactive oxygen species (ROS) accumulation,

increased cyclic adenosine monophosphates, or heme-containing proteins , have all been suggested (99, 140). The depolarization of the glomus cell membrane opens calcium (Ca^{2+}) channels leads to increased $Ca2^{+}$ influx, and intracellular content, within the glomus cell (99). Greater cytosolic Ca^{2+} concentration promotes fusion of dopamine containing vesicles with the pre-synaptic cleft. Dopamine travels across the synaptic cleft to the receptors of the carotid sinus nerve (a branch of the cranial nerve IX). Greater carotid sinus nerve discharge occurs following dopamine binding, with the action potentials travelling along the glossopharyngeal nerve. These signals continue along the nucleus tractus solitarious (NTS) towards the ventrolateral medulla (VLM) within the brainstem (32, 62).

Chemoreceptor activation depends on the duration and degree of the hypoxic stimuli. Direct measurements within animal models show that the stimulus-response curve of carotid sinus discharge is exponential when CO_2 and pH is controlled, with significantly greater discharge occurring below a PaO_2 threshold of 60mmHg (79, 175) (Figure 7). Yet with longer hypoxia exposure periods there is progressive carotid body sensitization. This results in greater carotid body discharge at the same hypoxia stimuli (9, 38, 128). The proposed mechanisms to chemoreceptor sensitization will be discussed within further sections.

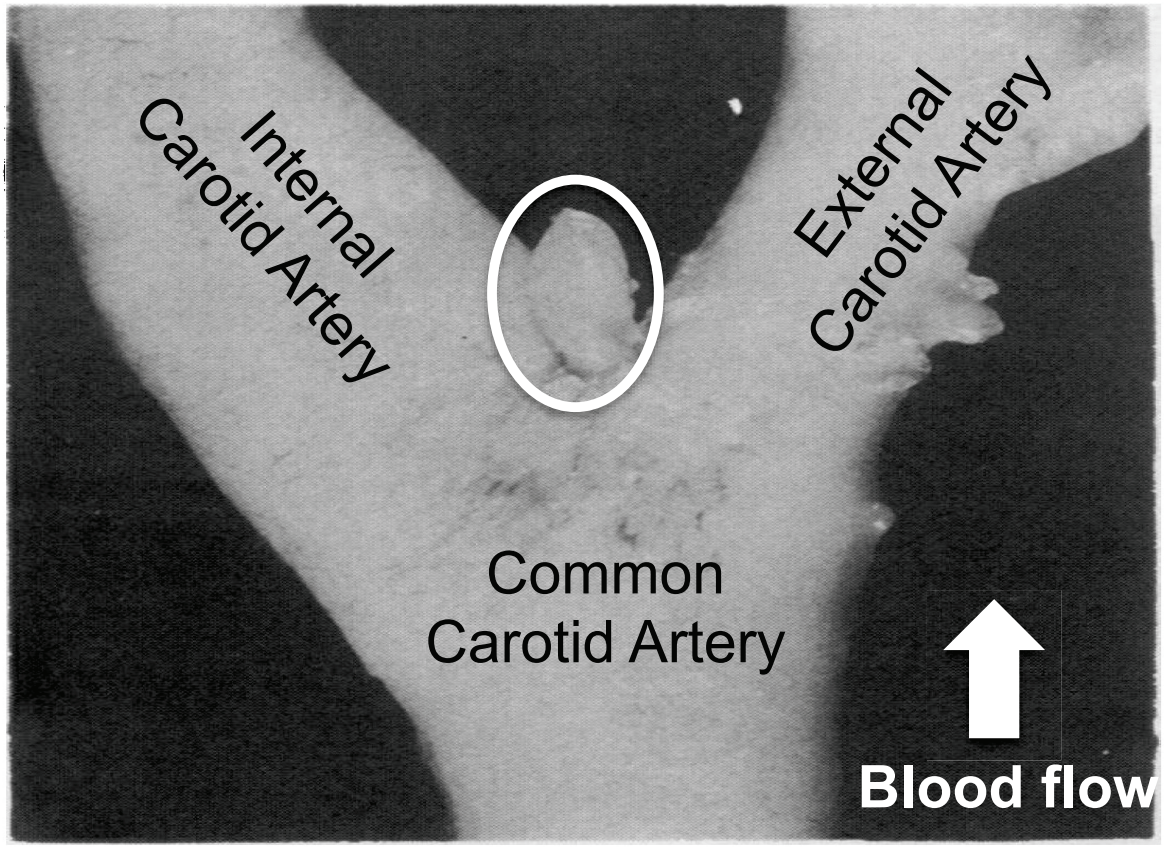

Figure 5. Gross anatomical carotid sinus body (circled in white) at the left carotid bifurcation. The carotid body receives blood flow through a dense capillary network that surrounds the type-1 glomus cells. Decreased PaO_2 is detected by the glomus cells through inhibition of voltage-gated channels on the cell membrane, initiating cell depolarization. Modified from Kahn *et al.*(91)

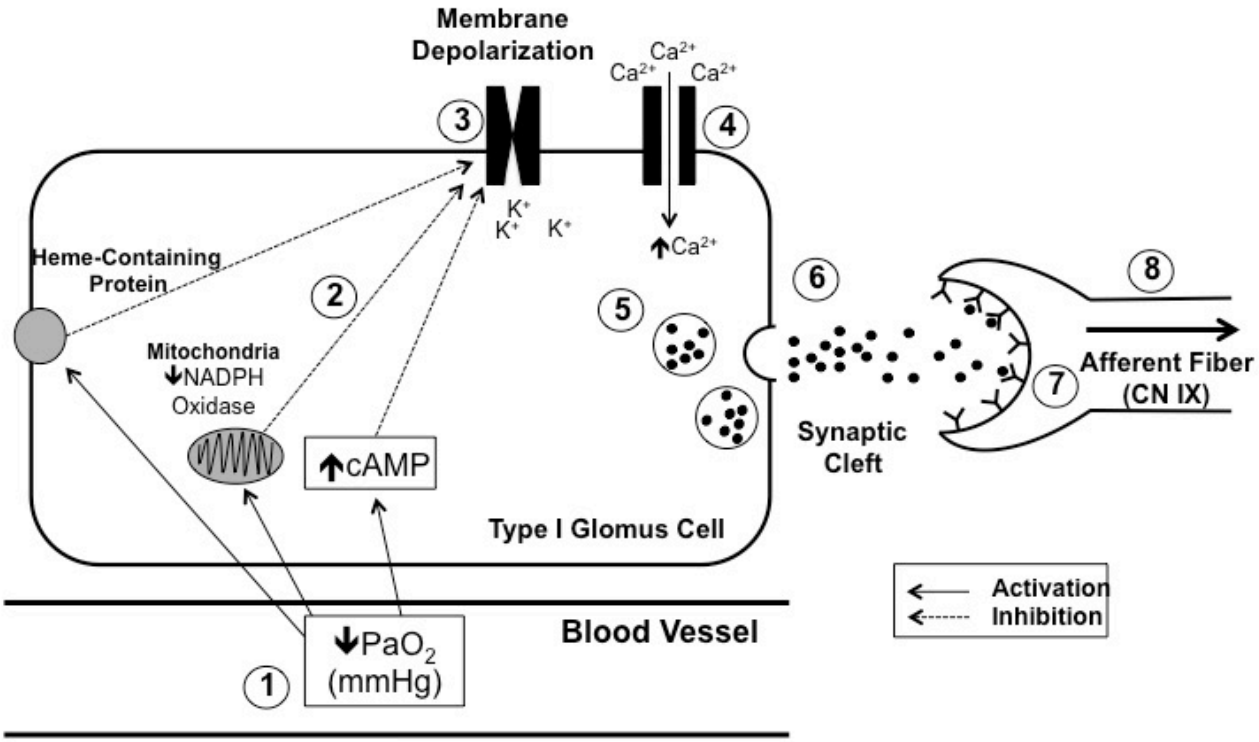

Figure 6. Model of Glomus cell detection of hypoxia and post-synapse activation. 1.) Initial reduction in PaO_2 is detected by type I glomus cells of carotid body, 2.) Mechanisms associated with cellular depolarization, though not completely understood, become active. Several proposed mechanisms to glomus membrane depolarization include activation of heme-containing proteins, inhibition of mitochondrial nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, and increase cyclic adenosine monophosphate (cAMP) concentrations within cell, 3.) Closure of potassium (K^+) gated channels inhibits K^+ efflux from cell to promote cell depolarization, 4.) Depolarization opens calcium (Ca^{2+}) channels to increase Ca^{2+} influx and intracellular concentration, 5.) Ca^{2+} influx activates synaptic vesicles, which fuse with the pre-synaptic cleft to release dopamine, 6.) Dopamine travels across the synaptic cleft, 7.) Dopamine binds with afferent receptors on post-synaptic cleft, 8.) Activation of afferent carotid sinus nerve fibers sends excitatory signals through the cranial nerve branch IX towards the central nervous system (CNS).

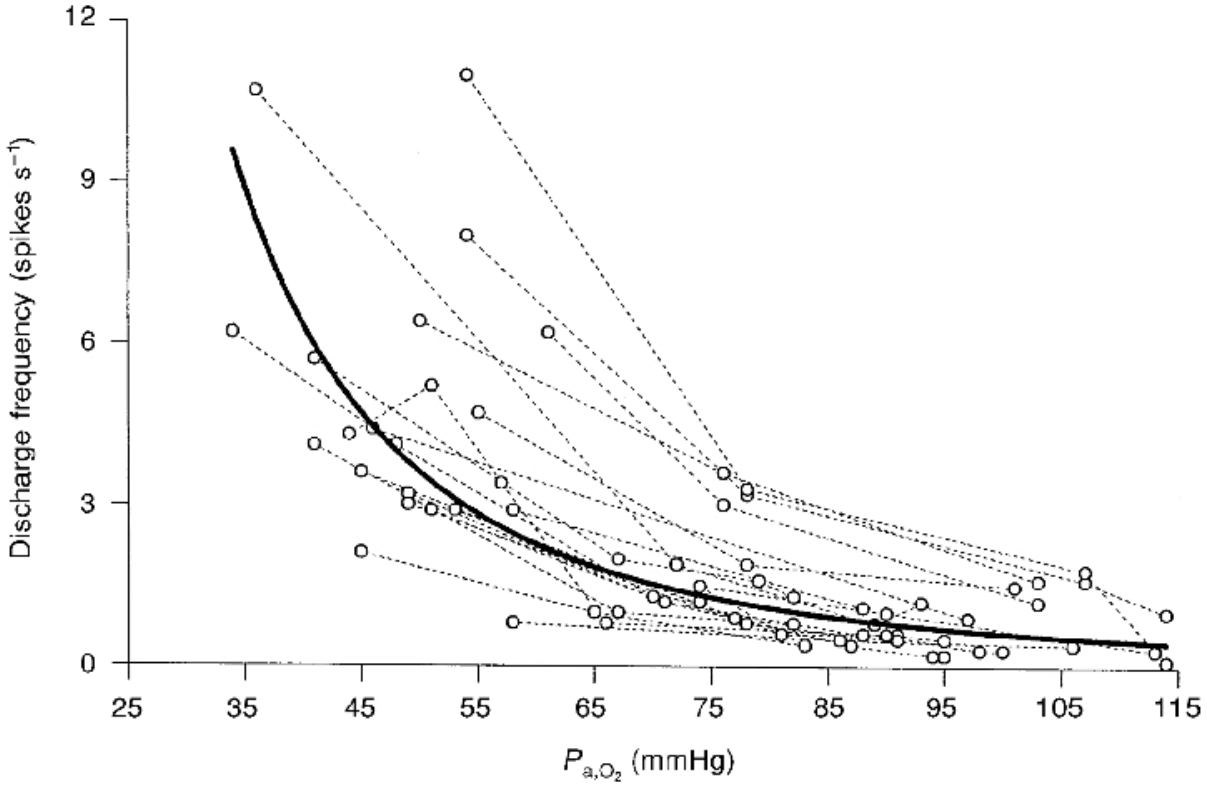

Figure 7. Carotid sinus single unit discharge at progressively reduced PaO_2. Initial carotid discharge occurs around 80mmHG. Discharge frequency from the carotid sinus exponentially increases below a PaO_2 threshold of 60mmHg. From *Vidruk* (175).

2.5.1.2 Activation of Central Neuronal Pools and Pre-Ganglionic Divisions

Hypoxia is detected by the peripheral chemoreceptors, which increase discharging along the glossopharyngeal nerve towards the CNS. The glossopharyngeal nerve merges with the CNS through the NTS. However, it is the central neuronal pools that ultimately regulate both phasic and tonic SNA outflow during hypoxia (31, 62). These pools are specifically located within the medulla oblongata (the ventrolateral medulla; VLM) and consist of several sub regions that control different cardiovascular and respiratory responses. Several regions of the VLM become highly active under hypoxia that concurrently increases ventilatory, cardiac, and vasomotor responses (62). The rostral ventrolateral medulla (RVLM) sub region within the VLM regulates sympathetic outflow. The degree of SNA outflow is based on converging stimuli from several reflex pathways that merge within the RVLM (Figure 8), including the chemoreceptors (31, 32). The specific pathways that connect the NTS and RVLM are not fully understood. However, it is believed that chemoreflex activation of the RVLM is facilitated either through a direct connection with the NTS (via series of monosynaptic inputs), or through a series of interneurons that span from the NTS across and merge with several different regions of the VLM (62). The activation of the RVLM appears to be facilitated by excitatory glutamatergic synapses. This was previously seen with the abolishment of SNA following glutamatergic blockade under chemoreflex stress in rats (63). Discharge frequency within the RVLM can be modified through additional excitatory (central chemoreflex, baroreflex), or inhibitory (pulmonary stretch reflex) stimuli (62). For example, the baroreflex (which monitors blood pressure through bodies that detect vessel wall distension) integrates with the chemoreflex through the NTS, and provides additional inhibitory (via the caudal ventrolateral medulla [CVLM]) stimuli to the RVLM when blood pressure is increased. This will reduce RVLM discharge and overall improved tonic regulation of blood pressure (32). With greater RVLM discharge, additional sympathetic activity travels from the efferent arm of the CNS towards the periphery via neural impulses. These neural impulses travel along nerves within the intermediolateral (IML) cell column towards numerous pre-ganglion synapses throughout the body.

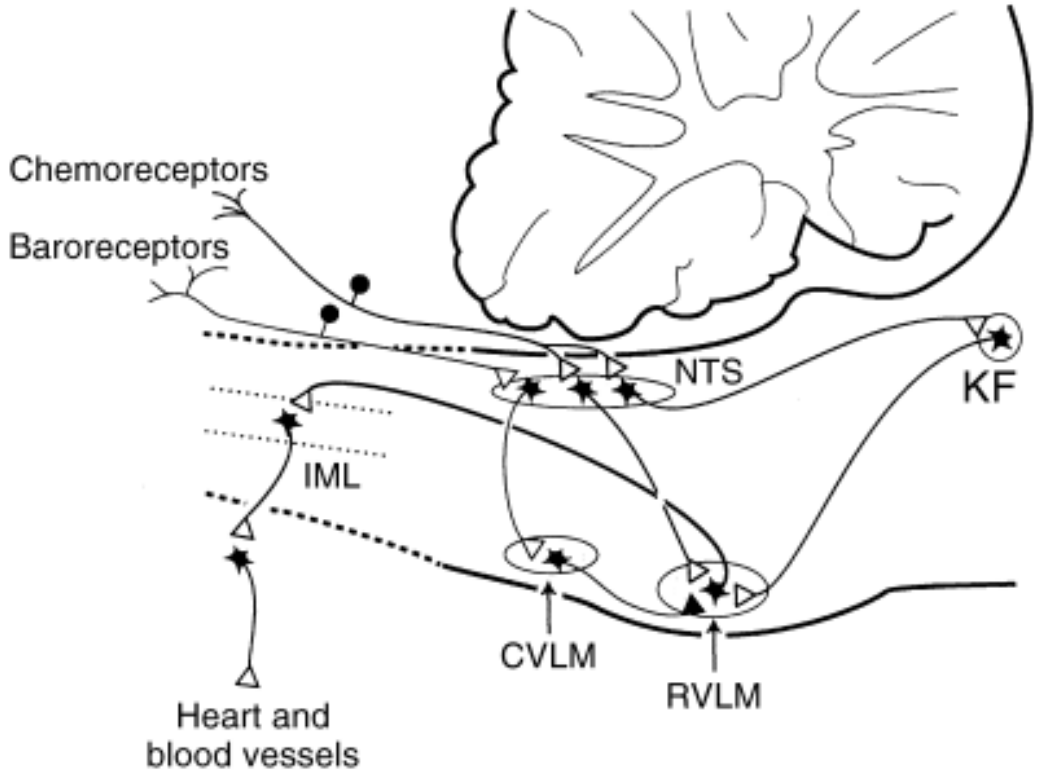

Figure 8. Sagittal view of central neuronal pools (the VLM) within the brainstem. The RVLM is a sub region that regulates sympathetic outflow. The frequency of discharges depends on the excitatory and inhibitory stimuli received from several reflexes throughout the body. The white triangles represent excitatory inputs, while the black triangles represent inhibitory inputs. Increased peripheral chemoreceptor discharge sends excitatory signals to the RVLM through the NTS. This excitatory stimulus is processed and integrated with several other stimuli. The CVLM provides inhibitory signals to the RVLM, as based on activation from the baroreflex. Following integration of excitatory and inhibitory signals, RVLM discharge becomes more frequent. These neural impulses travel through the efferent division of the nervous system (via the IML cell column), where they can innervate the heart, blood vessels, and other various organs throughout the body. *From* Dampney *et al*.(32)

2.5.1.3 Post-Ganglionic Neurovascular Co-Transmission within Smooth Muscle.

Greater RVLM activation within the SNS promotes a vasoconstrictive response within smooth muscle through neural impulses that travel from the RVLM to various regions of the body. The nerves within the IML cell column are pre-ganglionic, as the neural impulses travelling down the spinal cord soon arrive at ganglia following their respective branching site. These pre-ganglionic neural impulses promote binding of neurotransmitters (acetylcholine) to nicotinic receptors within the ganglia (62, 147). The increase in post-ganglion SNA innervates nerve varicosities that surround the respective smooth muscle (62). The type of neurotransmitter released from the pre-synaptic nerve terminal will vary depending on the effector organ. Neurotransmitters bind to either beta adrenergic (heart, airways of the lung) or alpha adrenergic (smooth muscle) receptors to promote a vasodilatory or vasoconstrictive response respectively (147). However, the predominant response within smooth muscle to heightened SNA under hypoxia (with exception to the brain and heart) is vasoconstriction.

There are three mediators of sympathetic vasoconstriction within smooth muscle (figure 9). The most commonly reported is norepinephrine. Following release from the sympathetic nerve terminal, norepinephrine binds with α1-adrenergic receptors on the post-synaptic membrane. Increased α1-adrenergic receptor binding subsequently activates G-alpha protein receptors ($G\alpha_q$) within the endoplasmic reticulum (104) that breaks down phosphatidylinositol bisphosphate (PIP2) to form inositol triphosphate (IP_3). IP_3 binds with receptors on the sarcoplasmic reticulum (SR) that allow the efflux of $Ca2^+$ from the SR to promote smooth muscle vasoconstriction (104). Norepinephrine activates α2 receptors, which can inhibit the release of norepinephrine and additional co-transmitters (104). The additional co-transmitters, Neuropeptide-Y (NPY) and ATP, aid in greater control over vasoconstriction within smooth muscle. NPY is co-localized with norepinephrine in large vesicles, while ATP is believed to be stored in smaller vesicles (with norepinephrine) prior to release from the sympathetic nerve terminal. Upon release NPY binds with post-synaptic Y_1 while ATP binds with P_{2x} receptors, both of which have a direct vasoconstrictive effect on smooth muscle (104). However, both NPY and ATP can

additionally bind to Y_2 and P_{2y} receptors on the pre-synaptic terminal to inhibit release of all three neurotransmitters (60). The sympathetic stimulation required for co-activation and release of neurotransmitters from the varicosity can vary. It is speculated that lower SNA stimulation will recruit smaller vesicles (norepinephrine and ATP), higher SNA frequencies recruiting larger vesicles (101). In addition, NPY has previously been shown to exert a greater vasoconstrictive effect relative to norepinephrine (102). Although they are often co-released under increased SNA, there may exist a reserve available for greater vasoconstrictive response under heightened SNA. This allows for a greater vasoconstrictive response to counteract the vasodilatory response during hypoxia.

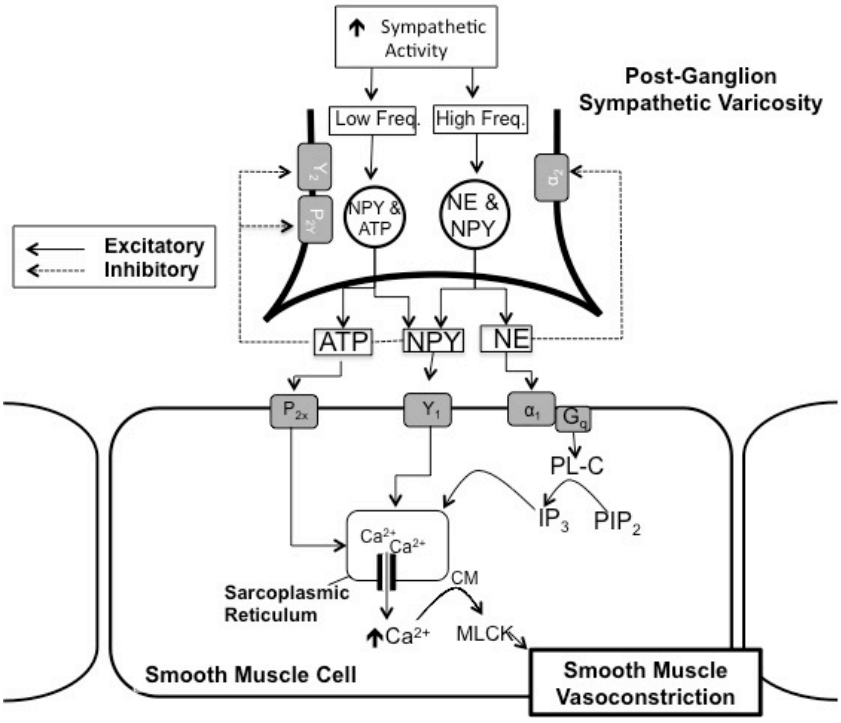

Figure 9. Representation of smooth muscle vasoconstriction through increased post-ganglionic sympathetic outflow. Co-transmission of low frequency SNA releases vesicles containing adenosine triphosphate (ATP) and neuropeptide Y (NPY) from the sympathetic varicosity, while high frequency releases vesicles containing norepinephrine (NE) and NPY. The release of neurotransmitters (NE, NPY, ATP) will see successive binding with their respective g-protein receptor on the smooth muscle (α_1, Y_1, and P_{2x}). NE binds with α_1 receptors and subsequent coupled g-protein (G_q), triggering the activation of phospholipase C (PL-C). PL-C produces inositol triphosphate (IP_3) through the breakdown of phosphatidylinositol bisphosphate (PIP2), which goes on to stimulate calcium (Ca^{2+}) release from the sarcoplasmic reticulum. NPY and ATP binding to their respective Y_1 and P_{2x} protein receptors have a direct effect on the sarcoplasmic through influx of intracellular Ca^{2+} to the smooth muscle cell. Ca^{2+} binds with calmodulin (CM) to activate myosin light chain kinase (MLCK), and signal vasoconstriction of the smooth muscle. Termination of vesicle release from the varicosity is through neurotransmitter (NE, NPY, and ATP) binding with inhibitory g-protein receptors on the varicosity (α_2, Y_2, and P_{2y}, respectively). Adapted from Macarthur *et al.* (104)

2.5.2 Sympathetic Response to Acute Hypoxia

Our current understanding of the SNA response to altitude is primarily based off lab studies examining acute FiO_2 manipulation under normobaric conditions. Although there is considerable variability between findings, studies show that SNA (including MSNA) increases under acute hypoxia (Figure 10). This large degree of variability may be explained through the large range of FiO_2 between studies (15-10% FiO_2), and duration of normobaric hypoxia exposure (approx. 5-30 minutes with most studies) (34, 70, 87, 115, 144, 145, 147, 166). For example, MSNA has been shown to increase in as little as 30 seconds following exposure within healthy Lowlanders (156), while the MSNA response is more pronounced under rapid changes of FiO_2 (145). In addition differences between studies may also be attributed to age (33), gender (87), or participants respective place of residence (33, 144). As previously stated, progressively larger hypoxic stimulus results in exponentially greater chemoreflex activation (145, 159). However, MSNA activity only appears to significantly increase once arterial saturation of oxygen (SaO_2) reaches a "threshold" of around 80% in humans (156, 159). Somers *et al.* (159) demonstrated this when they saw a significant increase in MSNA following 10%FiO_2, but not 14% (82% vs. 91% SaO_2 respectively). Saito *et al.* (145) also reported a more pronounced increase in burst frequency at a simulated altitude of 6000m (54% increase) compared with 4000m (34% increase). The maximal SNA response to hypoxia in unknown, though two studies have reported a "plateauing" of MSNA during acute hypoxia. Morgan *et al.* (121) reported a similar MSNA response during 5 and 20 minutes of acute hypoxia (SaO_2 80%), while Querdio *et al.* (134) reported a similar plateauing in MSNA response during 20 minute of hypoxia exposure with no further increase beyond 12 minutes (20 bursts/min to peak 28 burst/min). However, these two findings contrast field studies that clearly demonstrate further MSNA augmentation following acclimatization at altitude respective of acute lab exposure (103). This suggests considerable MSNA augmentation under longer hypoxia periods when compared to acute exposure, though it is unclear whether this affects sympathetic reserve.

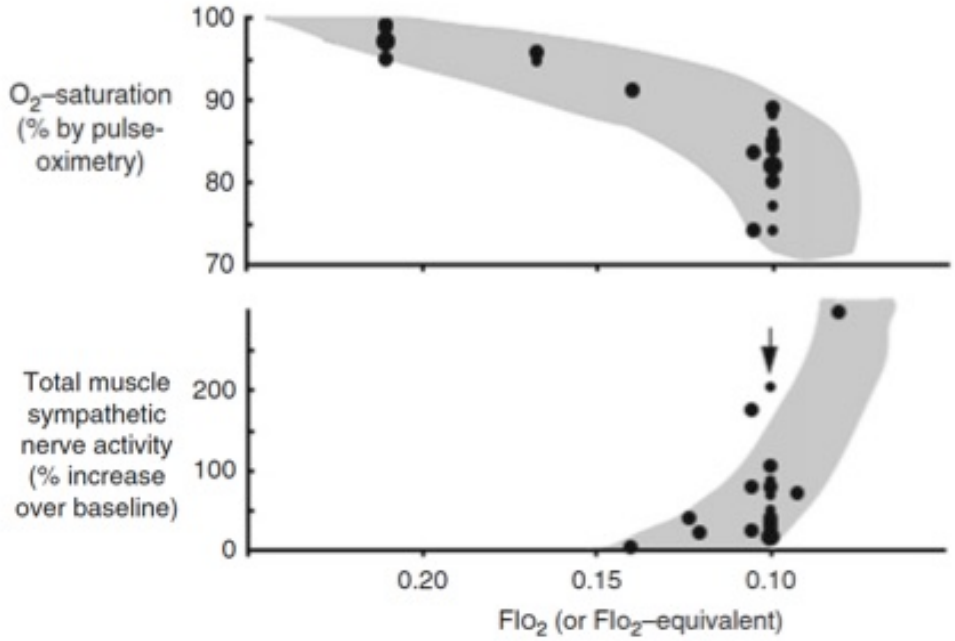

Figure 10. Summary of studies measuring the percent change in total MSNA and O_2 saturation during acute hypoxia exposure. These studies were performed either through manipulation of FiO_2 or within chamber studies. The degree of sympathetic activation appears to be governed by a "threshold" response, with higher sympathetic activity following a drop in SpO_2 (85-80%). Modified from *Sander* (147)

2.5.3 Sympathetic Acclimatization to Chronic Hypoxia

As of this review, there have only been four publications demonstrating augmented MSNA at altitude. The classic study performed by Hansen and Sander (69) showed a tripling in burst firing (from 15 to 48 bursts/min) following 4 weeks residency at 5260m in 8 lowlander residents (Figure 11). More recently, Lundby *et al.* (103) demonstrated an increase in burst frequency from sea level to days 10 and 50 at 4300m (15 vs. 42 and 42 bursts/ min respectively) in Lowlanders. These two studies are the only findings that show MSNA longitudinally, with the latter suggesting no further increases in MSNA following the acclimatization period at altitude. Fisher *et al.* (50) recently were able to indirectly measure further augmentation of MSNA, via hypoxia step test, in acclimatizing Lowlanders following several days at 3,454 m. Duplain *et al.* (40) saw an increase of 14 bursts/min (37 versus 23 bursts/min, respectively) during 24 hours post -rapid ascent to 4559 m in healthy HAPE resistant mountaineers. This study was novel for demonstrating the relationship between autonomic dysregulation and altitude illness, with HAPE susceptible individuals exhibiting higher burst frequency than their HAPE resistant counterparts. However, no published studies have followed up to explore the potential link between pulmonary hypertension and sympathetic hyperactivity at altitude.

The specific mechanisms behind MSNA augmentation at altitude are unknown. One often proposed mechanism is progressive peripheral chemoreceptor sensitization (38, 50, 69). This argues that the chemoreceptors become more responsive (through greater discharge frequency) to the same hypoxic stimulus under longer exposure periods. The respective time course for chemoreceptor sensitization may be early on following hypoxia exposure. Early animal studies show progressively greater discharge frequency within the carotid bodies over several hours following exposure (128, 176). Indirect markers of chemoreceptor sensitization were first reported in human models by Forster *et al.* (51),who saw ventilatory acclimatization (higher respective of low altitude) following 3-4 weeks at 3100m that was independent of changes in cerebral spinal fluid pH. Lundby *et al.* (103) recently demonstrated higher basal MSNA after 10 days at 4300m when compared to 15 minutes of acute normobaric hypoxia exposure

at a comparable FiO_2 in Lowlanders. Furthermore, findings of type I glomus cell proliferation within rat models following 3 days of moderate hypoxia exposure (Nitrogen titration for 12% FiO_2) indicate morphological adaptations of the carotid bodies to chronic hypoxia (179). These studies argue in favour of chemoreceptor sensitization as an important mechanism for sympathetic augmentation. However, chemoreceptor sensitization has more recently been questioned by studies that demonstrate only minor reductions in MSNA following chemoreceptor blockades via supplemental oxygen (69) and low-dose intravenous dopamine infusion (50) at altitude. As such, chemoreceptor sensitization at altitude may only partially contribute to the sympathetic hyperactivity following acclimatization, with further research required to evaluate alternative mechanisms.

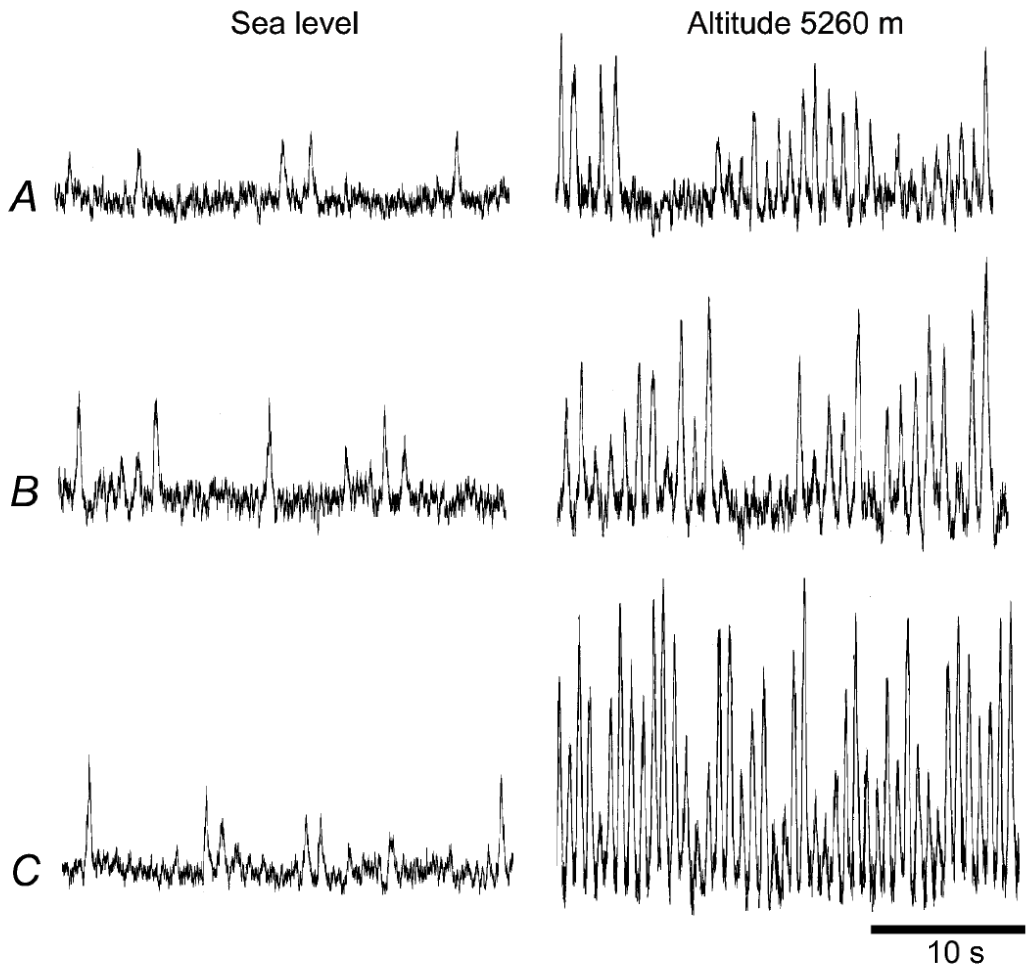

Figure 11. Integrated neurogram trace of Lowlander at sea level and following 4 weeks at 5260m. Traces taken from separate individuals that demonstrate low (Panel A), average (Panel B), and high (Panel C) MSNA at low and high altitude. Though the degree of SNA differs between Lowlanders, there is considerable augmentation of MSNA at high altitude. Modified from Hansen and Sander (69)

2.6 Introduction to High Altitude Dwellers

The cardiovascular adjustments in acclimatized Lowlanders, who have evolved to tolerate greater oxygen availability (thus increased PiO_2) at lower altitudes, allow for small increases in P_AO_2 and PaO_2. These adaptations improve functional capacity at altitude through increased oxygen delivery to the local tissue. However, there is an estimated 140 million individuals around the world that permanently reside at high altitude (>2500m) (66). Of these individuals, several ethnic groups permanently reside above 4000m, while also showing improved cardiovascular function when compared with acclimatized Lowlanders (55, 67, 168, 191, 194). Three main regions where these high altitude residents exist are the Himalayas of south-central Asia, the Andes in South America, and the Ethiopian highlands of eastern Africa. Yet the extent of adaptation within the groups varies between these regions due to different durations and degree of chronic hypoxia exposure at altitude between groups (194). One particular group that have received considerable attention are the Sherpa. This group are native to the Himalayan mountain ranges of Nepal, with their ancestry being traced to the nomadic tribes that resided on the Tibetan Plateau for thousands of years (55). This ethnic group currently resides within the Khumbu and Solo-Khumbu regions near Mt. Everest. Their high altitude capabilities are demonstrated through a wealth of personal accounts that acknowledge the Sherpas' extraordinary ability to function at extreme altitude (>5500m). It is for this reason that the local Sherpa population are often recruited as guides and porters on mountaineering expeditions to the worlds' highest peaks.

The altitude adaptations within Sherpa have been a unique area of research within the environmental physiology field. Numerous studies and reviews have demonstrated that Sherpa exhibit a combination of altitude-induced adaptations that improve cardiovascular function over their Lowlander counterparts (55, 67, 168, 191, 194). What remains less well understood is autonomic function within Sherpa. Therefore, the following sections will go over our current understanding in relation to physiological differences native Sherpa/ Tibetans and Lowlanders, as well as our current understanding of

autonomic regulation in Sherpa. These will be specifically focused on studies that suggest differential regulation of sympathetic activity within native Sherpa at altitude.

2.6.1 Physiological Adaptations in Sherpa at Altitude

Both phenotypic and genotypic adaptations within Sherpa are believed to be through an evolutionary response to permanent residency at altitude. These adaptions do not appear to affect either basal SaO_2 or the ventilatory response to hypoxia, which are both similar to that of acclimatized Lowlanders (11, 12, 55, 191). The improved hypoxic tolerance in Sherpa is not the product of one sole adaptive response, but rather a series of inter-related cardiovascular and biomechanical factors that improve circulating O_2 distribution and metabolism. These adaptations are meant to improve cardiovascular efficiency at altitude via improved blood flow redistribution. This is seen through greater capillary density within skeletal muscle (90) and improved ability to increase microcirculatory blood flow within Sherpa at altitude (54). Circulating nitric oxide (NO) expression and metabolism, which is considered an essential regulating factor of local vascular blood flow (via attenuation of endothelial resistance) (97), is also greater within Sherpa at altitude (45, 174). NO metabolism was previously seen within Sherpa that exhibit a greater ability to increase leg blood flow following 2 minutes of circulatory occlusion (150). Ezurum *et al.* (45) subsequently demonstrated that Tibetans have higher resting forearm blood flow and circulating NO by-products. Pulmonary artery pressure is also considered to be lower, as observed through greater exhaled NO within native Tibetans relative to acclimatized Lowlanders (78). These all suggest an improved ability to deliver oxygenated blood towards working tissues. Cerebral auto regulation has been investigated to a lesser degree within Sherpa. The few published studies suggest greater blood flow in Sherpa, which would be beneficial for improving oxygenation of the hypoxic brain. Jansen *et al.* (84, 85) demonstrated that cerebral blood flow is better maintained within Sherpa below 3700m when compared to Lowlanders. The internal carotid artery (ICA) also shows greater blood velocity within Sherpa (81), though vessel diameter was not successfully measured to determine flow. These findings suggest an overall reduced vascular resistance within Sherpa that improves systemic and

peripheral blood flow. Whether this lower vascular resistance is sympathetic mediated remains to be studies.

Some of the phenotypic differences within Sherpa initially appear to be detrimental towards performance at altitude, though they may be compensatory in order to improve cardiovascular efficiency. For example, Sherpa exhibit both a reduced hemoglobin (Hb) and hematocrit (Hc) content relative of Lowlanders (187, 188). In addition, Sherpa exhibit a blunted erythropoietic response at higher altitudes when compared with acclimatized Han Lowlanders (192). It has been hypothesized that a reduced Hb and Hc content is a trade off for improved circulatory efficiency through reduced blood viscosity. Another example is with hypoxic pulmonary vasoconstriction (HPV), which is proposed to facilitate greater ventilation perfusion matching in the lung during chronic hypoxia (55). Though not yet measured within Sherpa, Tibetans appear show a blunted HPV response at altitude with respects to Lowlanders (58). Histologically, the smooth muscles that surround pulmonary arteries within Tibetans are smaller than in Lowlanders (61), further suggesting a reduced vasoconstrictive ability within the lungs. Though this may hinder a more robust O_2 diffusion between the alveoli and pulmonary capillary, this trade off favours improved right ventricular efficiency through reduced pulmonary resistance and cardiac afterload. Both of these factors may also explain potential improvements in hemodynamic efficiency. Sherpa demonstrate an overall reduced ventricular mass (68) and ability to further increase cardiac output (120) at altitude. Somewhat interestingly though is that elevated Hc content and excessive pulmonary hypertension are commonly reported symptoms of chronic mountain sickness within high altitude populations (119, 187), further demonstrating these responses within Sherpa as beneficial towards residency at altitude. Therefore, these particular phenotypic adaptations within Sherpa may counter intuitively aid towards facilitating greater hypoxic tolerance through improved efficiency in redistributing blood towards essential organs.

2.6.2 Sympathetic Activity between Lowlanders and Sherpa

The previously discussed cardiovascular responses in Sherpa may be through differential regulation of SNA. Though very little evidence exists that pertains to autonomic function in Sherpa; field studies have potentially shown indirect markers that demonstrate a blunted SNA response (55, 66). Previous findings in high altitude natives of the Indian Himalayan range had lower urinary catecholamine relative to lowland Indians at 3500m (111). Several studies that have indirectly demonstrated a potentially altered sympathetic response that include both increased vascular responsiveness via improved blood flow (20, 45, 54) and diminished heart rate variability (195, 197) in native Tibetans when compare to Lowlanders. Additionally, healthy Andean populations have previously been shown to have a significantly lower plasma norepinephrine concentration than those suffering from chronic mountain sickness (52), indicating some degree of sympathetic maladaptation. However this finding in Andeans may not accurately translate to Sherpa due to differential genetic expression(194) or phenotypic differences (12, 13) that exist between high altitude populations.

These differences in cardiovascular regulation between Lowlanders and Sherpa may be explained through altered chemoreflex sensitivity to hypoxia. Several studies have reported a blunted chemoreflex response within Sherpa. Lahiri and Milledge (93) showed a blunted hypoxic ventilatory response in Sherpa's compared to lowlanders at 4800m. Dempsey reported similar findings within Sherpa at 3100m despite being able to maintain similar resting PaO_2 and SaO_2 as Lowlanders (37, 51). However, current evidence with regards to chemoreflex sensitivity in Sherpa is mixed with more recent studies suggest that Sherpa have chemoreflex sensitivity similar to that of acclimatized Lowlanders (12, 30, 64, 196). Therefore, further investigation is needed for determining whether high altitude natives exhibit an altered SNA response when compared with Lowlanders.

2.7 Introduction to Apnea

Measurements of MSNA following acclimatization at altitude have solely been performed during periods of supine rest. Therefore, it is unknown whether MSNA is further augmented under additional stress (sympathetic reactivity). We have chosen to use a voluntary breath hold for assessing sympathetic reactivity as it evokes both a quick and large MSNA response. In addition, breath holds require minimal movement, thus are used commonly within microneurography studies for determining a signal (19, 39, 76, 106, 112, 167). The physiology behind SNA augmentation and breath holding is complex. There are several reflexes that can potentially affect overall breath hold duration and sympathetic outflow prior to volitional breakpoint. In addition, breath hold duration involves a significant mental component to counteract the urge to breathe. Breath hold duration is affected by previous repetitive practice (132), trained versus untrained individuals (19, 76), mental distractions (6), and whether the individual is submerged underwater (greater activation of the mammalian diving reflex) (132). A brief review will be used to examine the mechanisms that drive sympathetic activation during breath holding. Particular focus will be paid on untrained individuals breath holding without facial submersion or being underwater.

Apnea is the absence of ventilation, resulting in non-existent airflow between the lungs and the environment (3, 125). Involuntary apneic periods are often seen under clinical (125, 126, 157, 159) or environmental (cheyne-strokes respiration at altitude) (3, 21) contexts, where the continuous bouts of apnea are immediately followed by an increased rate of breathing (hyperpnea). In contrast, volitional breath holds are both consciously intitated and terminated by the individual. Volitional breath holding is an integral aspect of aquatic activities, with its origins stemming from the hunting of fish (Japan and Korea) and collection of pearls (Persian gulf) underwater (1, 47). The sport of breath hold diving has evolved with continuously increasing dive depths and durations being achieved by elite level sport divers. An individuals respective breath hold duration varies as tolerance during the latter “struggle phase” dictates when they reach their volitional “breakpoint”. An individuals volitional breakpoint is often

shorter than their respective physiological breakpoint, where considerable hypercapnic and hypoxic stress evokes syncope, forcing the return to normal breathing (132, 155).

2.7.1 Mechanisms Governing Volitional Breath Holding

Breath holding is a complex physiological process that involves the integration of excitatory (chemoreflex, cortical descending drive) and inhibitory (pulmonary stretch reflex, baroreflex) stimuli. The integration of these stimuli provides a robust ventilatory drive to breathe and sympathetic response (Figure 12). Yet as of this review, the respective contribution of each mechanism towards the sympathetic augmentation prior to breakpoint remains relatively unknown. These mechanisms, and their respective contribution to sympathetic activation during breath holding, will be discussed further in the following sub-sections.

2.7.1.1 Central Chemoreceptor Activation of Sympathetic Function

Significant changes in both hypoxic and hypercapnic stress during long duration breath holds activate the peripheral and central chemoreceptors (39). However, shorter duration breath holds (ie. within untrained individuals) do not demonstrate a significant degree of hypoxic stress to cause a strong peripheral chemoreflex response (7, 76). Arterial CO_2 ($PaCO_2$) is tightly regulated within the body through several different regions within the brainstem, cerebellum, hypothalamus, and midbrain that tightly monitor both $PaCO_2$ (approx. 35-40 mmHg) and cerebral spinal fluid changes in blood pH *(7.30 au.)* (127). Hypercapnic stress increases interstitial fluid propagation of action potentials within these regions to the cardiovascular centers (VLM). The increase in sympathetic outflow from the RVLM travels towards to their respective regions similar to that seen with hypoxic stress (186).

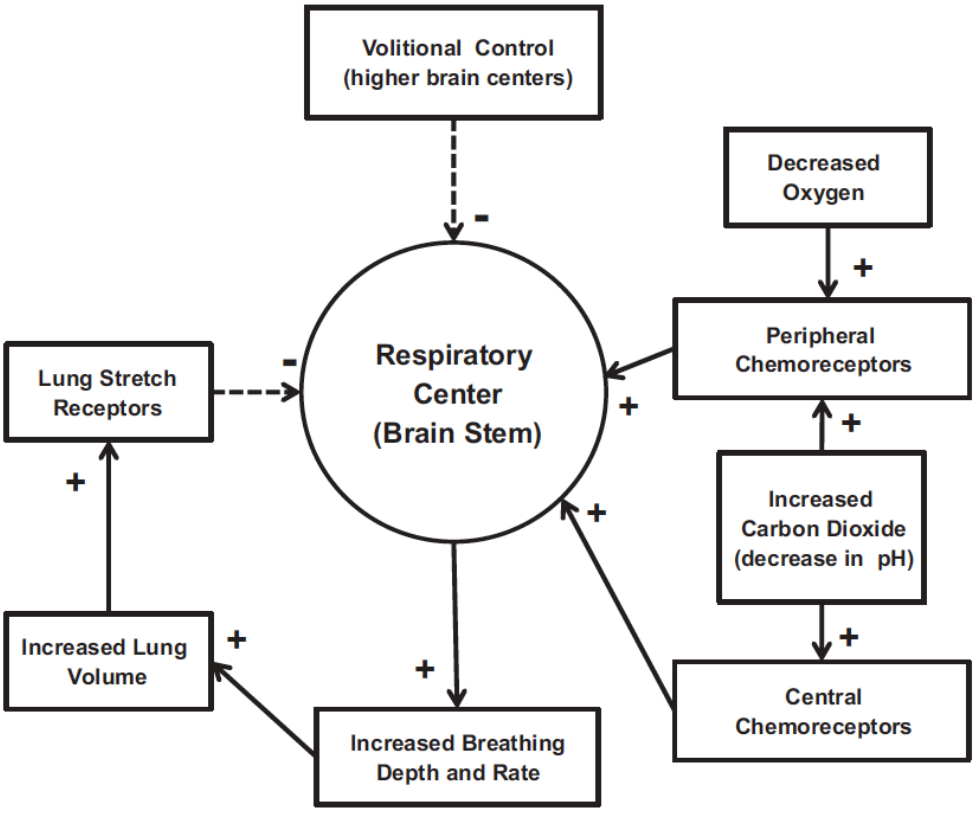

Figure 12. Schematic of excitatory and inhibitory pathways that control sympathetic outflow and tolerance during breath holding. Certain reflexes (chemoreflex, baroreflex) provide excitatory signals while others (pulmonary stretch reflex) send inhibitory feedback to the cardiovascular and respiratory control centers. Through integration of these excitatory and inhibitory signals, increased sympathetic, vagal, and ventilatory drive is sent to various regions of the body. However, during breatholding volitional control ultimately dictates when an individual reaches their volitional breakpoint, which is often well before mechanical breakpoint. *From Skow et al.* (155).

2.7.1.2 Pulmonary Stretch Inhibition of Sympathetic Function

Slow adapting pulmonary stretch receptors (SAR) monitor lung wall tension and aid in preventing lung over-inflation(149). SAR are distributed throughout the trachea, bronchial branches, and continuing down to the alveolar sacs (43). Heuring and Breuer (74, 75) first established their role in regulating airway tension within rat models that showed premature termination of inspiratory feedback following inflation within the lungs (known as the "Heuring-Breuer" reflex). SAR discharge follows a cyclical pattern during normal ventilation, with increased firing during the inspiratory phase followed by cessation during expiration (74). Sympathetic outflow follows a similar pattern of respiratory modulation with increased pulmonary stretch feedback during late inspiration suppressing the SNA response under normal ventilation (41, 65, 106, 152) and during artificial ventilation within lung-denervated patients (153). Muxworthy (122) originally showed a linear response in breath-hold duration and lung inflation, while Mithoefer (118) saw prolonged breath hold duration under both hypoxia and hypercapnic stress through increased lung volume. This translates over to sympathetic outflow during breath holding, with larger inspiratory volumes appearing to inhibit initial sympathetic outflow during the initial phase (105, 106).

2.7.2 Sympathetic Activation during Volitional Breath Holding

Prior to volitional breakpoint there is a gradual increase in SNA. This translates to considerable increases in MSNA that exists with more prolonged breath holds (Figure 13). The genesis of SNA can be considered biphasic, with each phase receiving stimuli from several different reflex mechanisms. SNA is governed during the initial breath hold phase through an interaction between transthoracic pressure and baroreflex activation (76, 105, 109) rather than chemoreflex strain. This is characterized with an initial increase in SNA that counteracts the reduction in MAP (19, 76, 106). Cardiac function is altered by increased intrathoracic pressure during breath holding. This is apparent with reductions in cardiac output, left ventricular filling, and SV with larger inspiratory volumes (19, 48). A reduction in venous return unloads the aortic and carotid stretch receptors, thus increasing baroreflex activation (8, 19, 109).

Heusser *et al.* (76) have previously reported an initial surge in sympathetic drive concurrent with overall MAP reduction following 15-20 seconds of maximal breath holding at total lung capacity. This initial increase in SNA does appear at lower lung volumes through withdrawal of pulmonary stretch feedback, though it is less pronounced (106).

Sympathetic activity progressively increases after the initial phase of breath holding through greater hypercapnic and hypoxic stress. As seen previously, the degree of SNA augmentation prior to volitional breakpoint will vary depending on the accumulation of chemoreflex stress (19, 76, 105, 109, 161). Direct measurements of carotid sinus nerve discharge within animal models show that the stimulus-response curve of carotid sinus discharge is exponential when CO_2 and pH is controlled, with an exponential increase in discharging until following a drop in arterial oxygen content below the threshold of 60mmHg (79, 175). Conversely, changes in blood brain pH under hypercapnia are more sensitive and provide a more rapid response (130). This stimulus though appears to be more responsive to hypercapnic stress initially, with greater contribution from hypoxia afterwards. There is greater SNA observed under normoxic hypercapnia than isocapnic hypoxia(158). However, this contrasts previous findings of elevated burst frequency and total SNA under isocapnic hypoxia and not hypercapnia(166). Under longer duration breath-holding manoeuvres it would appear that the synergistic effect of greater hypoxic and hypercapnic drive contribute to maximizing the sympathetic response prior to volitional breakpoint(158, 167) (Figure 14). Shorter duration breath holds (ie. end expiratory) do not rely on peripheral chemoreflex activation, but rather a combination of baroreflex-mediated activation, hypercapnic stress, and inhibitory pulmonary stretch feedback.

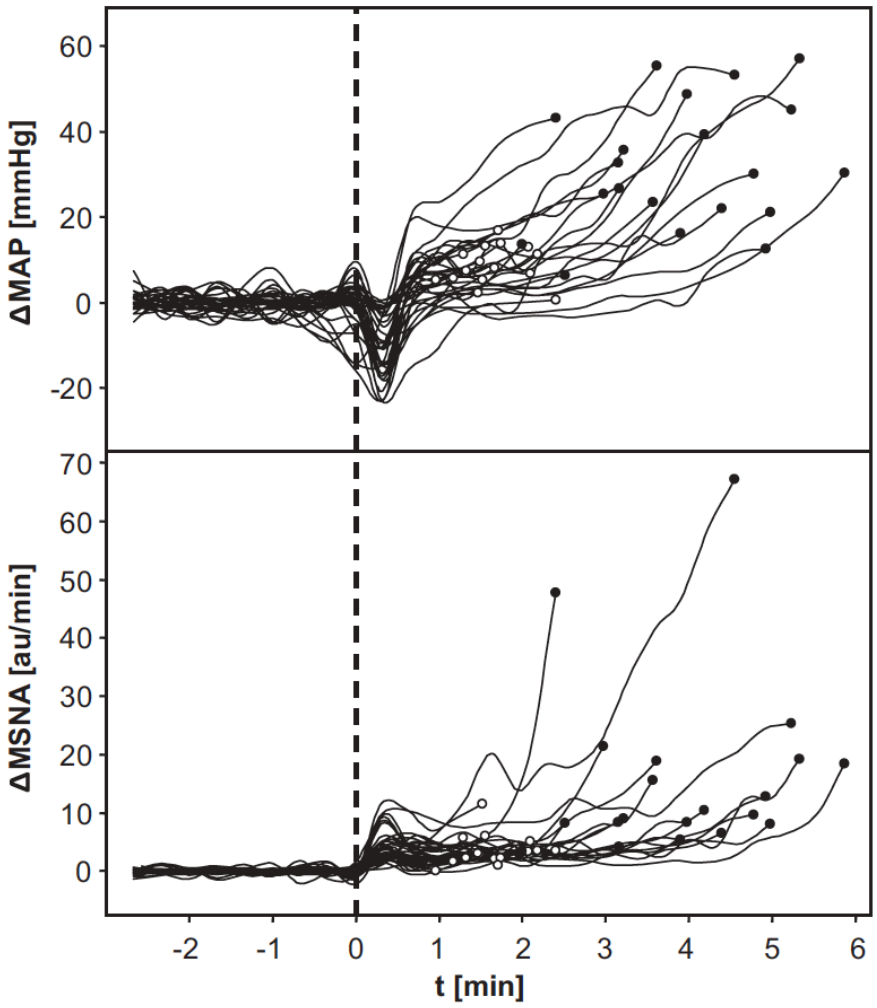

Figure 13. Time course of mean arterial pressure and MSNA burst frequency during breath holding. Initial reductions in MAP indicate reduced venous return due to increased intrathoracic pressure. An increased baroreflex discharge causes early on spike in MSNA innervates vascular smooth muscle to vasoconstrict. The gradual increase in MSNA overtime can be contributed to gradually reduce inhibitory stimuli (due to reduced pulmonary stretch from shrinking lung volume) and increased excitatory stimuli (increased hypoxic and hypercapnic stress). White circles represent control subjects while black circles represent trained divers, who are able to tolerate greater hypoxic and hypercapnic strain, thus see larger degree of sympathetic augmentation. From Heusser *et al.*(76)

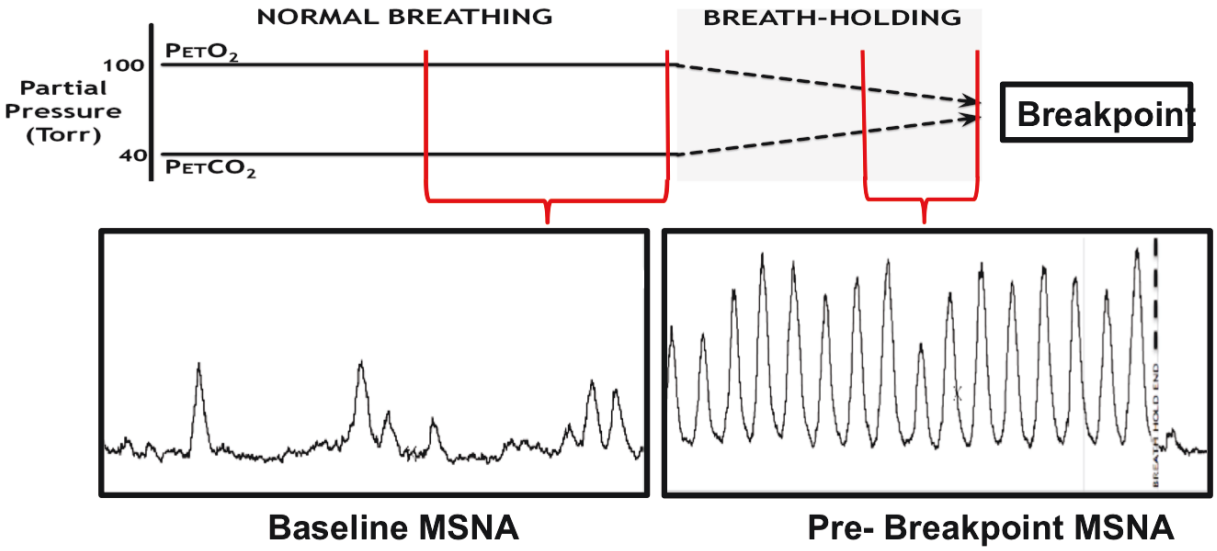

Figure 14. Integrated MSNA neurogram trace during baseline and prior to volitional breakpoint. There is a progressive increases in sympathetic activation during breath holding until volitional point. Return to eupneic breathing quickly withdraws sympathetic outflow. This trend is similar across different breath hold techniques, with the time to volitional breakpoint varying.

2.8 Summary

Augmentation of SNA under hypoxia allows for sympathetic-mediated recruitment of the cardiovascular system. Greater MSNA aims to maintain arterial pressure and perfusion of O_2 towards critical organs. The degree of SNA under hypoxic stress appears to be dependent on both the duration and severity of hypoxia. This becomes important within Lowlanders that are acclimatizing to high altitude, who show even greater SNA augmentation under chronic hypoxia exposure. The further increased SNA may be explained through several different mechanisms, with one of them being peripheral chemoreceptor sensitization. However, this autonomic response has not previously been assessed within native Sherpa that have adapted over thousands of years to life at high altitude. This becomes apparent when comparing physiological differences between Sherpa and acclimatized Lowlanders. Lowlanders exhibiting different vascular characteristics that suggest a higher degree of SNA at altitude than Sherpa.

The majority of studies measuring MSNA at altitude have only demonstrated heightened basal activity. This leaves a large gap within the current knowledge about SNA at altitude. More specifically, it is unknown if sympathetic reactivity to additional stress is also affected at altitude. Through the use of voluntary breath holding, which is a strong sympathetic stimulus; sympathetic reactivity can be quantified at altitude. This will also allow for comparison of sympathetic reactivity between acclimatized Lowlanders and Sherpa at altitude.

CHAPTER 3- TECHNIQUES AND INSTRUMENTATION

The following chapter provides a more detailed account of the techniques, instrumentation, and data analysis that will be discussed within Chapter 4. All measurements described within the following chapter were either performed during the research expedition to the Nepal, or were used for quantifying SNA during each respective protocol. The microneurography technique will be discussed with regards to practice, validity and reliability, and quantification during baseline and reactivity conditions.

3.1 Research Design

The research questions and hypotheses pertaining to this thesis are listed previously (see section 1.3). This study is considered exploratory, as there are no previous findings that have measured MSNA in Sherpa at altitude. As of this thesis, only one study has indirectly addressed sympathetic reactivity in Lowlanders at altitude (50). The design was based off of a quantitative research methodology and quasi-experimental approach. These were chosen due to the sampling protocol being used and lack of random assignment for subjects to particular groups. Lowlander participants served as their own controls at both low and high altitude. The study involves the analysis of MSNA under resting (frequency, incidence, burst amplitude) and apnea (burst integral area, total normalized SNA, incidence of bursts) conditions between low and high altitude. These methods of MSNA quantification will be discussed within the following sections.

3.2 Instrumentation

All instrumentation and testing had participants positioned in a resting, supine position. Cardiovascular function was measured through several devices, while regional MSNA was assessed through microneurography (see section 3.2.2). Participants remained fully instrumented during the entire protocol.

3.2.1 Cardiovascular Measurements

Cardiovascular measures were performed in addition to SNA collection at low and high altitude. Heart rate was calculated via ECG (Lead II) while arterial blood pressure (finger photoplethysmography; Finometer Pro, Finapres Medical Systems, Netherlands) was collected continuously at 1 KHz (ADInstruments, Chart Pro v8.3.1). Mean (MAP), systolic (SBP) and diastolic (DBP) pressures were calculated on a beat-by-beat basis from the arterial pressure waveform via photoplethysmography (Finapres Medical Systems; Netherlands). These were used to complement the MSNA measures for comparing neurovascular characteristics between Lowlanders and Sherpa. Beat-by-beat cardiac output (Q) was calculated using the Model Flow algorithm and used to calculate total peripheral resistance (TPR= MAP/Q). Peripheral oxygen capillary saturation (SpO_2) was collected via pulse oximetry (Nellcor, Medtronics, USA) from the index finger. Cardiovascular and sympathetic measures were collected at all points of the study with exception to SpO_2, which was not obtained in Lowlanders during the apnea protocol at low altitude.

3.2.2 Muscle Sympathetic Nerve Activity

Microneurography was used to measure MSNA, which has been used previously to assess regional SNA during hypoxia (162, 163, 167). All microneurography pokes were performed by trained microneurogrphers involved in the expedition. Microneurography instrumentation involves the placement of two electrodes (a reference and recording electrode) into a nerve site of the microneurographers choice. The common peroneal (fibular) nerve, which provides sensation and motor function to the lower leg, was chosen as the recording site. Anatomically, the peroneal nerve branches off the tibial nerve, travelling alongside the lateral portion of the popliteal fossa towards the head of the fibula. The common peroneal branches from the fibular neck into the superficial and deep peroneal nerve. Location of the peroneal

nerve can be performed through touch, and can be felt as it travels closely around the fibular head. The peroneal nerve is commonly used in microneurography studies due to ease of accessibility and minimal resistance for the electrode to overcome (112). In addition, the peroneal nerve has been used by several other studies that measured MSNA at altitude (40, 69, 103), thus allowing for an appropriate site comparison.

Once the microneurographer had identified/ landmarked a recording site along the nerve (Figure 15); a reference electrode was inserted, followed by the recording electrode. The reference electrode was positioned approximately 1-3cm away from the recording site in order to filter out background noise near the site. The recording electrode (200μm diameter, 35 mm long, tapered to a 1-5 μm uninsulated tip) was then inserted percutanesouly to the peroneal nerve. Once inserted, the recording electrode was manually manipulated until a pulse-synchronous bursts pattern was observed in the raw neurogram trace (36) (Figure 16). A MSNA signal was confirmed once the burst pattern exhibited i.) augmentation based on apnea and not audible (a strong sympathetic stressor) ii.) exhibited pulse synchronization with the cardiac cycle, and iii.) did not respond to light touching or exhibit skin parasthesis (112, 185). The raw MSNA signal was pre-amplified 1000x then further-amplified 100x through a variable gain isolated amplifier. The raw signal was 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). Both raw and integrated signals were sampled at 10 KHz (ADInstruments, Chart Pro v8.3.1).

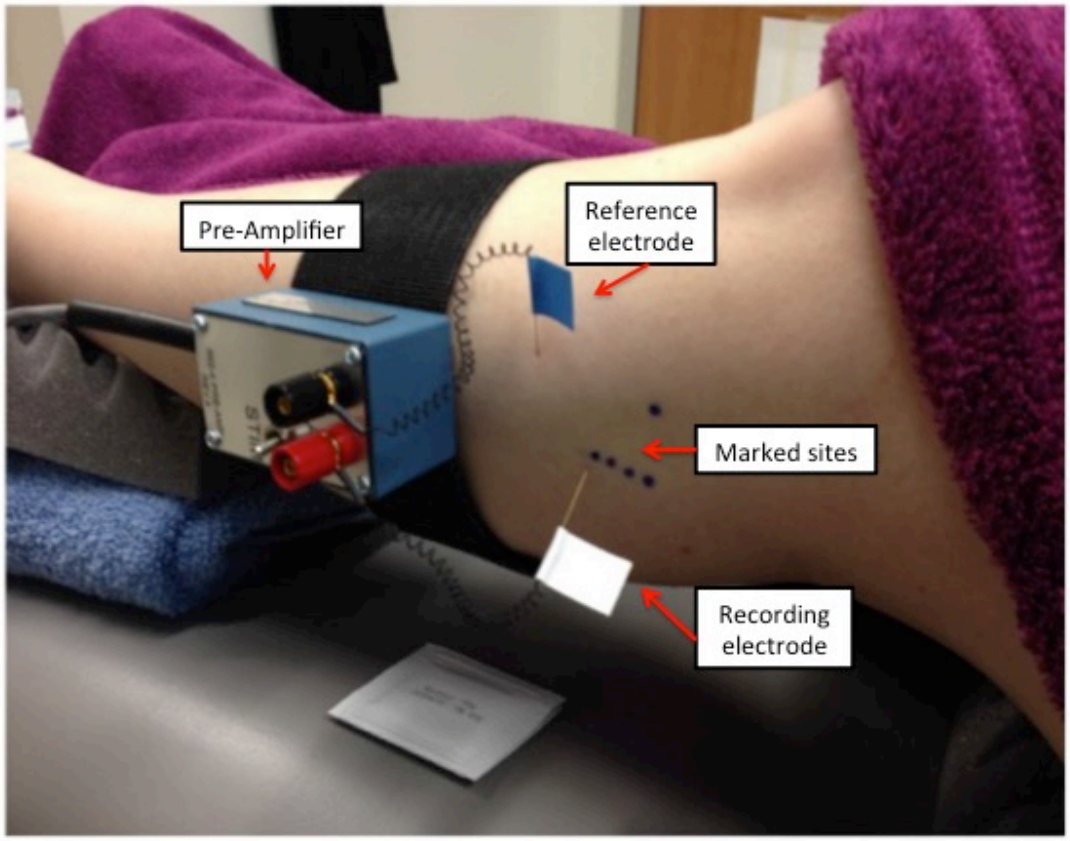

Figure 15. Example microneurography set up for the peroneal nerve. Once the peroneal nerve is located through manual manipulation, four potential insertion sites are landmarked with a black felt pen. The reference electrode (blue flag) is inserted approximately 1-3cm away from the potential recording sites. The recording electrode (white flag) is then inserted slightly inferior to the marked sites. The recording electrode is manually manipulated until an MSNA signal was confirmed. The raw MSNA signal is pre-amplified 1000x before additional 100x amplification was done through a variable gain isolated amplifier (model 662C-3; Iowa University Bioengineering).

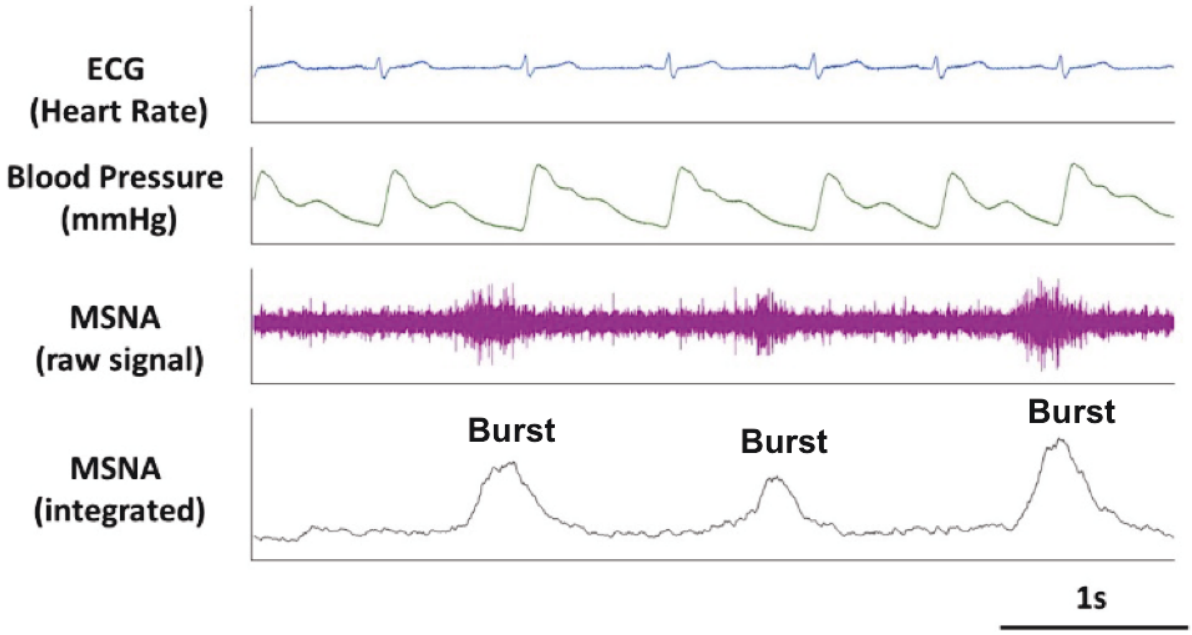

Figure 16. Burst identification from raw and integrated MSNA neurogram. The Raw MSNA neurogram represents the synchronized discharge of action potentials along the peroneal nerve that can be quantified as "bursts". A MSNA signal was confirmed once bursts exhibited i.) augmentation based on apnea, but not audible stimuli ii.) an exhibited pulse-synchronization with the cardiac cycle, and iii.) did not respond to light touching or exhibit skin parasthesis. Bursts were identified based on their pulse synchronization with the cardiac cycle as they can only occur once during a cardiac cycle (during to the QRS complex and nadir of diastole / termination during systolic upswing). The integrated neurogram is quantified through the rectification and time integration (leaky integrator with time constant 0.1 seconds) of the raw signal. MSNA data analysis is performed through bursts within the integrated neurogram channel.

3.2.2.1 Validity and Reliability of Microneurography for Measurement of MSNA

The use of microneurography for measuring SNA is relatively new, with the first established recordings being reported by Hagberth and Valbo in 1968 (65). The microneurography technique has since been widely adopted within many scientific fields, including physiology, for its advantages of real-time measurement within peripheral sympathetic nerves (112, 185). This is due to several reasons that demonstrate high validity and reliability of the technique. Regional SNA has previously been shown to accurately correlate with whole body norepinephrine spillover under acute hypoxia (178). The peroneal nerve is the most commonly used site in North America due to the relative ease of accessibility for researchers and reduced discomfort in subjects (112). Finally, the microneurography technique has been successfully used in both lab (33, 71, 121, 144, 163, 193) and field (40, 69, 103) studies for measuring hypoxia-induced sympathoexcitation.

The reliability of the microneurography technique with regards to quantification and analysis of MSNA (burst frequency, amplitude, incidence, and total MSNA) must be considered when measuring MSNA. The degree of accuracy is reliant on the recording electrodes proximity to sympathetic fibers within the nerve. Although Wallin *et al*. (178) have previously shown MSNA discharge to be equally represented between the radial and peroneal nerve under the same stimuli; action potentials generated along the axon will only be picked up if they are proximal to recording electrode. Therefore, the absolute MSNA signal strength will vary depending on the distribution of sympathetic fibers within that respective region of the nerve fascicle (178). Tompkins *et al*. (171) previously demonstrated differences in distribution of sympathetic nerve fascicles fibers along the nerves in dyed rat nerves / human cadavers (Figure 17). The MSNA signal strength therefore varies both between and within subjects due to the site chosen for measurement, bringing burst amplitude reliability into question (112, 185). Furthermore, there has been large variability reported in amplitude between studies, despite similar degrees of hypoxic stress (67, 147).

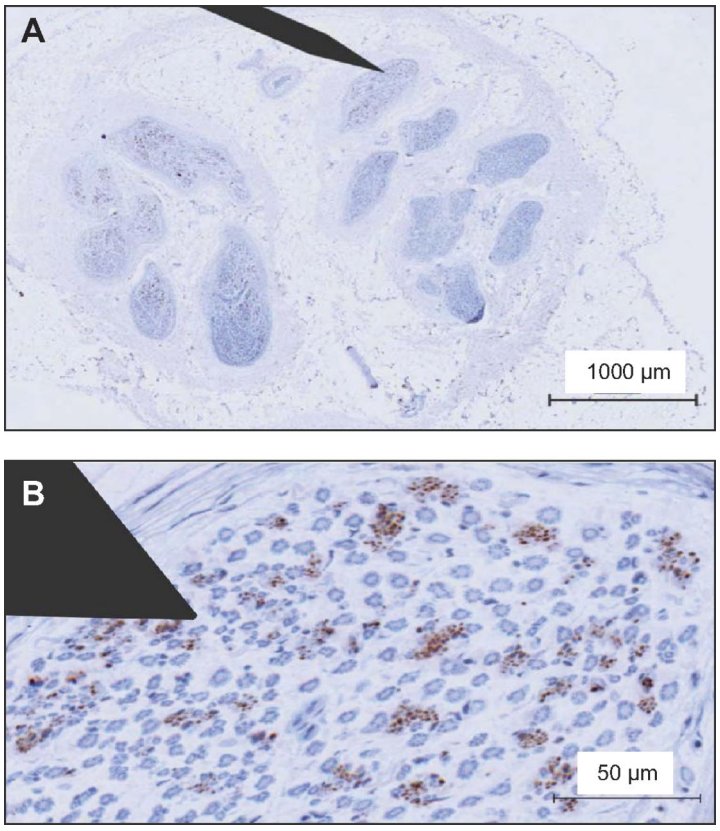

Figure 17. Dyed stain technique showing fascicle distribution within the peroneal nerve. *Panel A* represents cross section of the fascicles (darker stained fascicles represent greater concentration of sympathetic fibers) inside the nerve. *Panel B* represents zoomed in cross section of fascicle, where sympathetic fibers (positively stained for tyrosine hydroxylase) distribution varies considerably between fascicles. The insulated tip of the recording electrode can be seen in dark black at the top of both panels. The strength of an MSNA signal will vary depending on the number of sympathetic fibers that are proximal to the recording electrode Adapted from Tompkins *et al.*(171)

3.2.2.2 Safety of Microneurography for Measuring MSNA

The microneurography technique itself is considered an invasive procedure due to the breach of the recording electrode within nerve fascicles. The potential risk for adverse health affects with microneurography include infection, pain and tenderness, numbness, and more severe cases involving partial paresis (foot drop) (112). However, the prevalence of conditions caused through microneurography is considered to be unlikely. Eckberg *et al.* (42) reported an incidence of temporary peripheral nerve dysfunction (neuropathy) as extremely low (0.3% of reported cases that resolved within 6 months) while minor symptoms (tingling, slight numbness) only occurring less than two weeks (9% of reported cases) via follow up questionnaire in former microneurography subjects. Two trained microneurogrphers were implemented for the study (CDS and JPM) to ensure proper technique was enforced, including i.) proper land-marking and slow movements once skin was breached, ii.) minimal time within the nerve (maximum time allotment of 10 minutes of searching per site; total search time 45 minutes) and, iii.) a ratio of 1:1 for being used for electrode to subject (112). In order to minimize discomfort, constant communication of sensations was reported by participants to microneurographers. This was also used for identification of the electrodes location within a nerve site. Sherpa participants were provided with an individual fluent in Nepali during instrumentation to describe and translate any sensations and participant discomfort. Finally, proper sterilization and preparation techniques were implemented with each pair of electrodes. This included that both electrodes were autoclaved and sealed, with the recording and reference electrodes being discarded following completion of the protocol.

3.3 Data Collection and Quantification

Data collected during the study was stored offline (ADInstruments, Chart Pro v8.3.1) and analyzed upon return to Canada. Cardiovascular data (HR, MAP, SBP, DBP, Q, TPR) were analyzed on a beat-by-beat basis during the selected portions of baseline and breath holding across all conditions. SNA was quantified through bursts observed within the integrated neurogram traces. MSNA bursts were

identified using a semi-automated detection algorithm (Chart Pro 8.3.1) and confirmed by a trained observer (SAB). Criteria of observers for identifying bursts were that the bursts exhibited i.) uniform sharp peaks within the neurogram, ii.) pulse synchronicity with the cardiac cycle (one per cardiac cycle termination with systolic upswing), and iii.) a signal to noise ratio that was greater than 3:1, as is the recommended ratio from White *et al.* (185).

3.3.1 Basal Sympathetic and Cardiovascular Function at Low and High Altitude

Baseline data was collected for 10 minutes at both low and high altitude in order to collect resting cardiovascular (SpO_2, HR, SBP, DBP, MAP, Q, TPR) and MSNA data. MSNA was quantified as burst frequency (burst/ min), burst incidence (bursts/100 heart beats [hb]) and normalized burst amplitude (% relative to maximal sized burst) based on the synchronous bursting pattern formed within a raw neurogram trace (Figure 18). These three measures are commonly used in studies examining MSNA under acute (34, 70, 87, 115, 144, 145, 147, 166) and chronic (40, 69, 103) hypoxic interventions. Quantification of burst amplitude followed previous recommendations for standardization by White *et al.* (185), which involves the normalization of burst amplitude (%). The normalization of burst amplitude was performed through all integrated neurogram channels in Lowlanders and Sherpa. Integrated MSNA files were checked to ensure a stable (represented as 0 volts) without fluctuation in baseline voltage (due to movement of electrode in the nerve). The highest integrated absolute burst (measured in volts) during baseline, without artifacts or noise, was identified as "100%". Other bursts within the 10 minutes of baseline were then calculated as a % relative to the maximal normalized burst (100%).

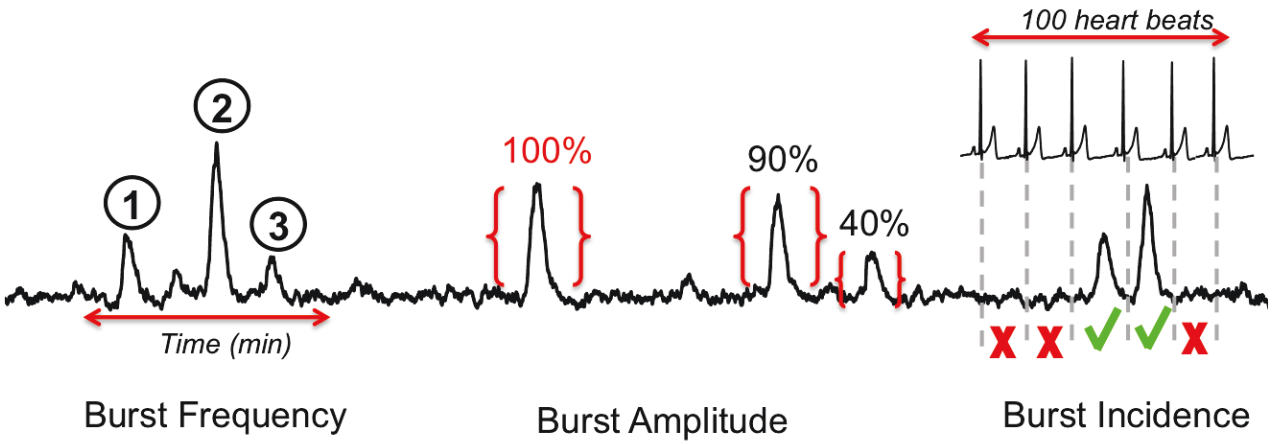

Figure 18. Baseline quantification of integrated MSNA bursts. MSNA was quantified as burst frequency (burst/ min), burst incidence (bursts/100 hb) and burst amplitude (% relative to maximal sized burst). The highest integrated absolute burst (measured in volts) during baseline, without artifacts or noise, was identified as "100%". Other bursts within the 10 minutes of baseline were then calculated as a % relative to the maximal normalized burst (100%).

3.3.2 Sympathetic Reactivity at Low and High Altitude

Sympathetic and cardiovascular reactivity data was collected on a beat-by beat basis during the last 10 cardiac cycles of breath holding. This was performed in both groups at both low and high altitude. Cardiovascular parameters were additionally calculated post-apnea for their nadir (S_pO_2, HR, MAP) and peak (SBP, DBP, MAP, and TPR) responses. These respective nadir and peak responses occurred within 10-15 seconds post-volitional breakpoint. MSNA was quantified as the burst integral area (under the curve; au) and incidence of bursts (% of individuals who exhibited a burst during that respective cardiac cycle) during the last 10 cardiac cycles prior to volitional breakpoint (Figure 19, 21). This method of calculating burst amplitude was chosen due to the development of prolonged "fusing" of bursts during apnea in Lowlanders at altitude (See Figure 20) that did not exhibit normal peak characteristics (36, 65, 112). Therefore, the conventional quantification of MSNA (burst amplitude) would not have accurately measured the size of each of this burst. In addition, burst prevalence indicated near 100% during the last 5 cardiac cycles in all groups for both low and high altitude. This would have resulted in burst incidence and frequency showing no difference. The average burst area was calculated from the mean burst amplitude during baseline for comparison to baseline. Finally, the calculation of burst area for quantifying abnormal bursts of MSNA has been used previously under periods of drastic baroreflex unloading (via Lower body negative pressure) (28). The burst integral area was normalized to the cardiac cycle through the calculation of total normalized sympathetic activity (au/min) during reactivity protocols (Figure 21). This was calculated by the sum of the absolute integral areas (au) being divided by the sum of the R-R Intervals (seconds) during the last 10 cardiac cycles. This value was then multiplied by *60 to give an indication of MSNA in au/min. Normalized total SNA during baseline was calculated in a similar manner, with exception that baseline values were calculated from 15 cardiac cycles obtained within 1 minute before beginning the breath holding protocol. The absolute change in normalized total SNA was calculated from the difference between baseline and reactivity values at each altitude. Raw data of RR-Interval, absolute burst integral area, and quantified normalized total SNA can be seen in appendix VI (Tables 1-9).

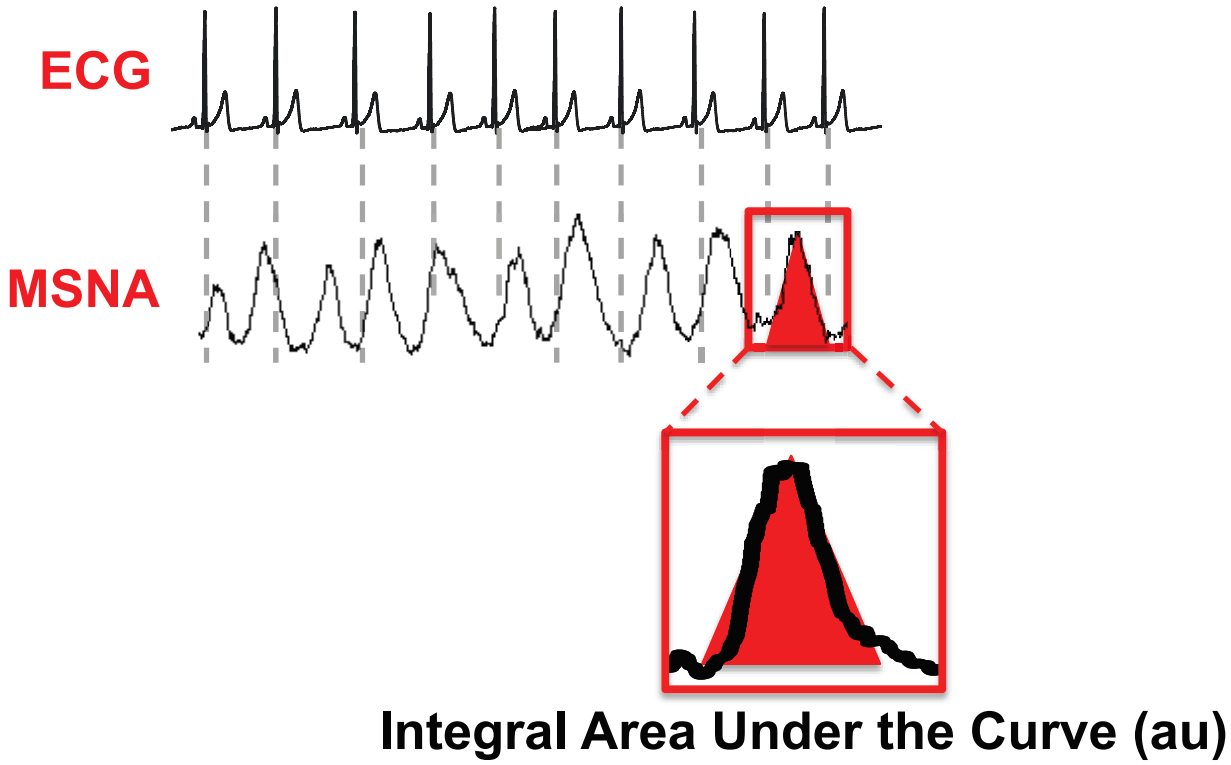

Figure 19. Quantification of burst integral area. Bursts were quantified as the integral area under the curve (au) for the reactivity protocol. Integral area was calculated relative to mean baseline (established as "0mv"). Area was visually confirmed to lie between RR intervals. Difference between baseline activity and reactivity protocol was performed through determining maximum normalized burst amplitude (100%) during baseline.

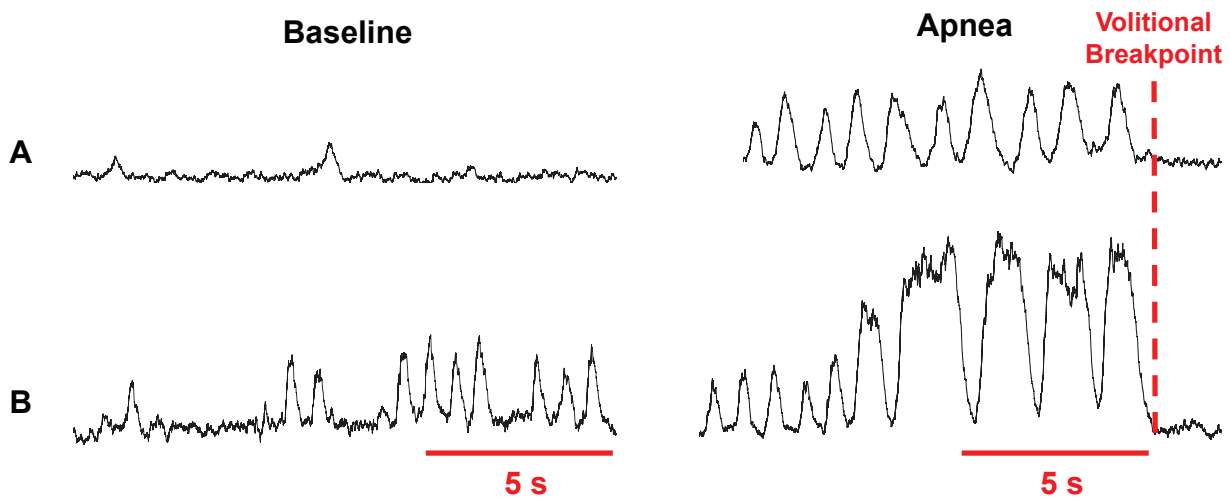

Figure 20. Baseline and breath holding neurogram traces obtained from one Lowlander at 344m (A) And 5050m (B). Apnea at altitude caused prolonged burst periods and loss of characteristic burst "peaks" prior to volitional breakpoint in 8 of the 14 Lowlanders.

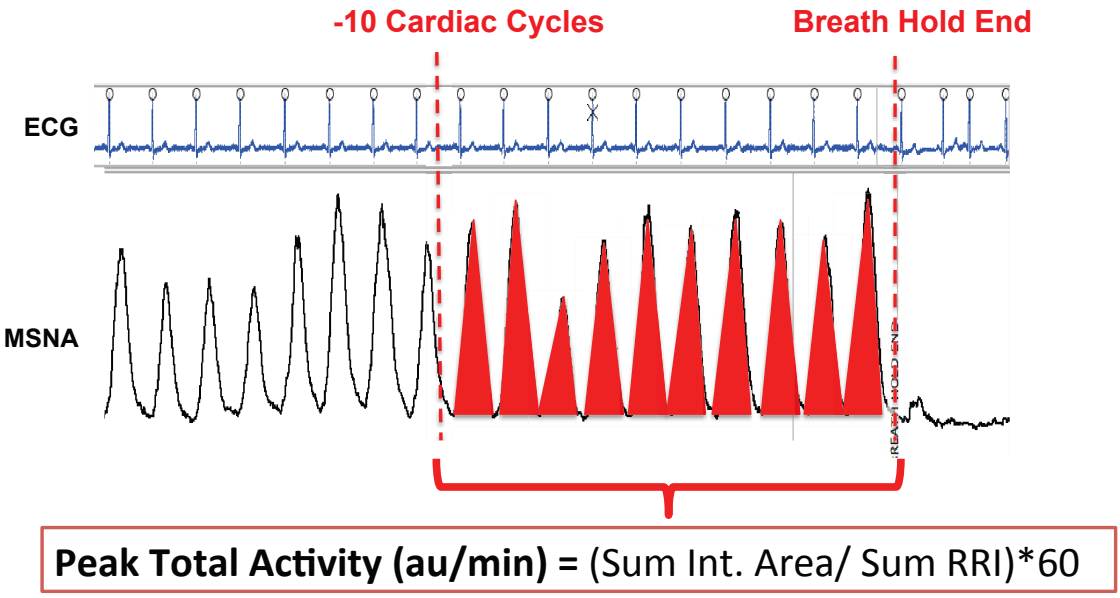

Figure 21. Quantification of total normalized sympathetic activity (au/min) during breath holding. Burst Integral Area (au) was calculated up to 10 cardiac cycles prior to volitional breakpoint for Lowlanders and Sherpa. The sum of these 10 integral areas was divided by the sum of the RR Intervals (seconds), and then multiplied to the minute (au/min) to calculate total normalized SNA. This was compared to the total normalized SNA at baseline (taken from 15 cardiac cycles during the last minute prior to breath holding).

CHAPTER 4 - STUDY SUMMARY

4.1 Abstract

We examined the hypothesis that Sherpa would exhibit lower basal sympathetic activity, as well as a blunted sympathetic reactivity to stress, compared with acclimatized Lowlanders at altitude. Muscle sympathetic activity (MSNA; microneurography) was measured in Lowlanders (n=14; age=27±6yrs) at 344m and 5050m, while Sherpa (age=32±11yrs) were tested at 1400m (n=4) and 5050m (n=8). MSNA burst frequency (bursts/ min), burst incidence (bursts/100 hb), and burst amplitude (% of max burst) was assessed during rest. Sympathetic reactivity was measured during an end-expiratory breath hold. Burst integral area (au), incidence of bursts (%), and total normalized SNA (i.e. au/min) was calculated during baseline and prior to volitional breakpoint. Lowlanders saw elevated burst frequency (11±5 bursts/ min to 30±6 bursts/ min; Mean±SD; $p<0.001$) and burst incidence (25±13 bursts/ 100hb to 50±15 bursts/ 100hb; $p<0.001$) at 5050m, while Sherpas had lower burst frequency (23±11 bursts/ min; $p<0.05$) and incidence (30±13 bursts/ 100hb; $p<0.05$) at 5050m. Breath holding increased burst area prior to volitional breakpoint across all conditions. However, both individual bursts (within 5 cardiac cycles prior to volitional breakpoint) and total normalized SNA were significantly lower in Sherpa ($P<0.05$). Our findings indicate for the first time that sympathetic basal activity and reactivity is lower in Sherpa when compared to acclimatized Lowlanders, despite the pressor responses to exercise being similar.

4.2 Background

Reductions in arterial oxygen tension (hypoxemia) activate the peripheral chemoreceptors to drive robust corrective and compensatory (e.g. ventilatory and cardiovascular) responses (56, 82, 135, 164, 181). This response to hypoxemia also includes an increase in chemoreflex-mediated sympathetic vasomotor activity (40, 114, 145, 163, 190) which facilitates cardiovascular redistribution of blood and oxygen to critical tissues (16, 25, 131, 137) under local hypoxic vasodilation (113). Chronically

heightened peripheral chemoreflex sensitization (9, 38) in low altitude dwellers has been associated with increased basal sympathetic activity during chronic exposure to altitude related hypoxia (40, 69, 103).

These neurovascular responses to chronic hypoxia in acclimatization Lowlanders may differ in high altitude natives, including Nepalese Sherpa, who demonstrate a multitude of beneficial cardiovascular adaptations (55, 66, 80, 86, 154, 168) that have improved their tolerance of chronic hypoxic stress. Several studies have indirectly suggested differential autonomic control between Tibetans and lowlanders that include both higher vascular responsiveness via improved blood flow, lower pulmonary artery pressures (20, 45, 54) and lower heart rate variability (195, 197). These differences in vascular control and cardiac regulation may in part be through differential regulation of sympathetic activity within high altitude natives, though these findings are indirect and do not directly demonstrate differences for sympathetic augmentation. A large gap exists in the current knowledge relating to neurovascular regulation within high altitude populations, with direct basal post-ganglionic sympathetic vasomotor activity only being reported once in native Bolivians (103). Therefore, we sought to examine whether baseline sympathetic vasomotor activity is differentially regulated between Lowland dwellers and native Sherpa populations at low and high altitude. We additionally determined whether sympathetic activity was further augmented during periods of additional stress at altitude, and if breath holding resulted in an altered vascular response. We hypothesized that Sherpas would exhibit both a reduced basal SNA and neurovascular reactivity to additional stress under chronic hypoxia.

4.3 Methods

The current study was a standalone experiment from a larger high altitude expedition to the Ev-K2-CNR Research Facility, in Nepal (5050m). Although participants took part in a number of independent investigations, care was taken to ensure no overlap of research questions and each study addressed distinct *a* priori research questions. Baseline demographics, cardiovascular characteristics (173) and heart rate responses (24) to apnea have been previously reported from Lowlanders and Sherpa at altitude. This

study focuses on independent questions and novel data related to sympathetic (re) activity in high altitude natives contrasted with acclimatized Lowlanders.

4.3.1 Study Participants

The populations of interest consisted of Lowlanders and Sherpa at low and high altitude. Both groups were administered a healthy history questionnaire that determined pre-existing neurological or cardiovascular dysfunction prior to testing (see appendix III-IV). This was administered to reduce possible confounding factors and underlying co-morbidities that may alter sympathetic and cardiovascular responses at low and high altitude. Lowlanders were composed of research members associated with the expedition. The inclusion criteria for Lowlanders were that they had resided near or at sea level and had not been at high altitude (<2500m) for at least three months prior to testing. Lowlander nationality varied with the majority of participants residing in Canada, while several others resided from the United States, United Kingdom, and New Zealand. High altitude natives were Nepalese Sherpas that were born, and had permanently resided, within the Khumbu region of Nepal (>3400m). The sample size consisted of 14 Lowlanders (27±6yrs; 2 female) and 8 Sherpa (32±11yrs). Participants exhibited no differences in age, height, weight, and BMI (Table 1). However, one Sherpa was 60 years old, while another four of the eight Sherpa were current smokers (0.42± 0.07 pack years). At high altitude, one Lowlander subject scored a 3 on the Lake Louise acute mountain sickness (AMS) scoring system (142), which classified them as having mild AMS prior to testing. Two Lowlander participants were taking oral medication (a single dose of acetazolamide/ day) during ascent while one of the two had an additional intramuscular corticosteroid injection (dexamethasone) administered at 5050m for relief of altitude related discomfort. However, both Lowlanders were tested following a 48-hour washout period at 5050m. As these individuals sympathetic function was not considered outliers, they were included within the analysis. All written consent was explained in Nepalese and English as needed, and were approved by the University of Alberta Biomedical Research Ethics Board, the University of British Columbia Clinical Research Ethics Board, and the Nepal Health Research Council under the Declaration of Helsinki (See Appendix I and II).

4.3.2 Testing Locations

The following sections outline the testing location of Lowlanders and Sherpa at low and high altitude. The ascent profile used by Lowlanders and Sherpa to high altitude is discussed as well.

4.3.2.1 Low Altitude

Testing location and date between Lowlanders and Sherpas for low and high altitude are listed below (Figure 22). Low altitude testing for Lowlanders occurred at the Center for Heart, Lung, and Vascular Health (University of British Columbia Okanagan, Kelowna, Canada; 344m) from August 21th until September 8th, 2016. Sherpa had arrived in Kathmandu (1400m), and were subsequently tested between days 1-4 after arrival. Before that they had been residing at high altitude (>2500m). The entire study group flew to Lukla (2860 m) and commenced ascent following both groups residing in Kathmandu.

4.3.2.2 Ascent Profile

In order to reduce the risk of altitude associated illness in Lowlanders; a conservative nine-day ascent profile was used (figure 23). Both Lowlanders and Sherpa resided together in Kathmandu for one week prior to flying to Lukla (2860m). Trekking days were restricted (<600m/day in altitude when possible). Acclimatization days were performed in Namche Bazaar (day 10-12) and Pheriche (day 14-15) to account for days when ascent exceeded 600 vertical meters/day.

4.3.2.3 High Altitude

Both Lowlanders and Sherpa were tested at the EV-K2-CNR Research Facility (Khumbu Valley, Nepal, 5050m) following a nine-day ascent profile (Figure 22; 23). Sherpa were tested between days 1-4 after arrival at 5050m (October 11-15th, 2016) while Lowlanders were tested on days 5-10 (October 16-20th, 2016). One lowlander was tested on day 1 after arrival at 5050m. Participants split into two groups at 4370m. The first team had one acclimation before ascending to 5050m to setup equipment, while the subsequent team had one additional acclimation day. The first group arrived one day earlier at 5050m than the second group. One Lowlander arrived at 5050m after residing at 3840m for 9 days and, who was considered to have been tested on the first day at 5050m. The day that participants were tested at altitude was corrected for differences in participants arriving at 5050m, with the first day of being at 5050m being considered "Day 1" (Figure 22). Two Lowlanders were administered acetazolamide or dexamethasone following 4 days arrival at 5050m but were tested after a 48-hour washout period. Sherpa did not require any medication and were tested on days 1-3 following arrival at 5050m, while Lowlanders were tested between days 1-10.

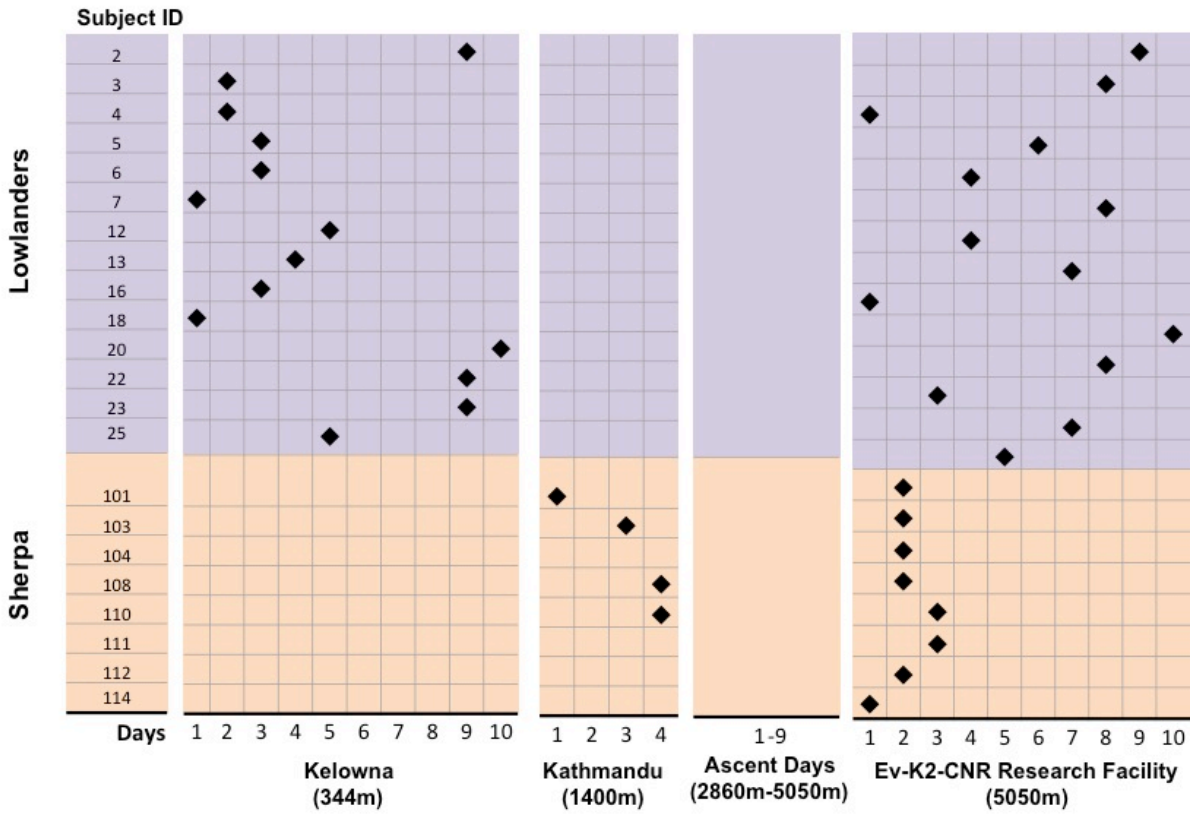

Figure 22. Testing dates for each participant at low and high altitude. Participants were not tested during ascent days from low to high altitude. Subsets of Sherpa (n.4) were successfully tested at 1400m in order to compare Sherpa re-acclimatization during 1-4 days after arrival in Kathmandu. Testing days at high altitude were corrected for when individuals arrived at 5050m (See figure 23 for ascent profile of Lowlanders and Sherpa). The corresponding testing date is in relation to the number of days they had already been at 5050m. Sherpa were tested on days 1-3 following arrival at 5050m while Lowlanders were tested between days 1-10.

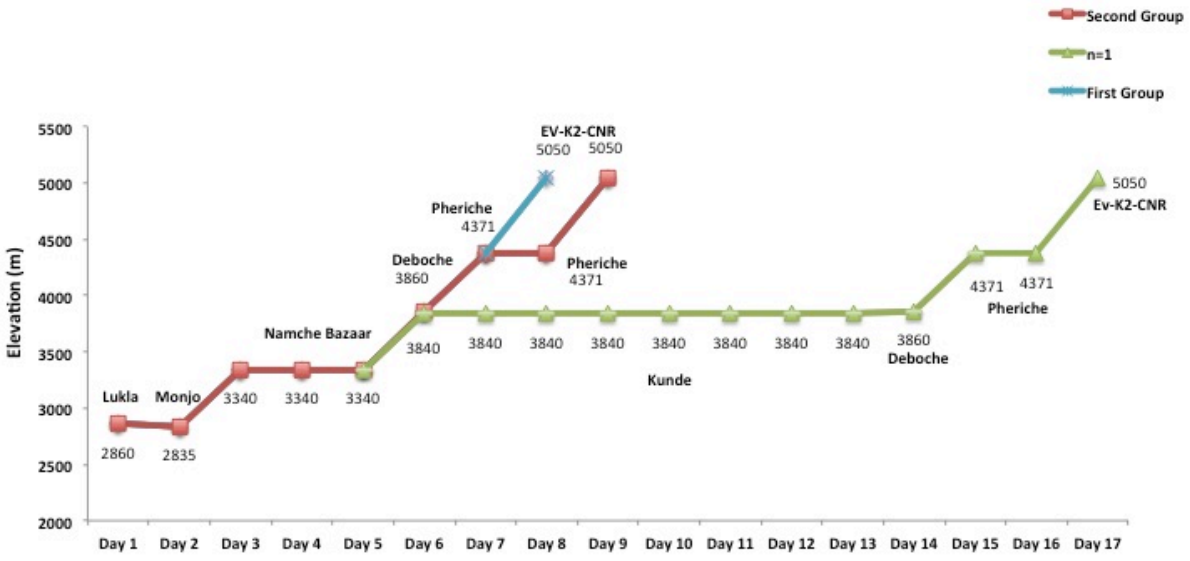

Figure 23. Schematic of ascent profile used from 2860m to 5050m. Ascent to Ev-K2-CNR Research Facility (5050m) was performed over a nine-day period through gradual ascent to higher (<600 vertical meters/day) elevations. Multiple successive days at the same altitude were established following days where vertical ascent achieved >600 vertical meters. These "rest days" were at 3340m (Namche Bazaar) for 3 days and 4371m (Pheriche) for two days. At 4371m participants split into two groups, with a lead team advancing to 5050m to set up the labs while the second team had one additional acclimation day before ascending to 5050m. One Lowlander arrived at 5050m after residing at 3840m for 9 days. They were considered to have been tested on "day 1", as they were tested on the same day as arriving at 5050m.

4.3.3 Instrumentation

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). Heart rate (HR) was calculated from the ECG R-R interval. Beat-by-beat cardiac output (Q) was calculated using the Model Flow algorithm and used to calculate total peripheral resistance (TPR = MAP/Q). Beat-by-beat mean (MAP), systolic (SBP) and diastolic (DBP) pressures were calculated from the arterial pressure waveform that was calibrated against manual sphygmomanometry. Microneurography was used to measure muscle sympathetic nerve activity (MSNA) (65, 162, 163). 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 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 identified which was augmented in response to apnea but not a loud noise (35). 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). Both raw and integrated signals were sampled at 10 KHz (ADInstruments, Chart Pro v8.3.1).

4.3.4 Resting Baseline and Reactivity Protocol

Following instrumentation, basal sympathetic activity was measured during 10 minutes of quiet rest (see appendix V for description of protocol). Sympathetic reactivity was subsequently assessed during a maximal voluntary end-expiratory apnea (at functional residual capacity). An investigator paced the participants' breathing prior to the apnea (2-3 breaths) to maintain rate and depth and prevent hyperventilation. Participants were then instructed and encouraged to "hold their breath for as long as possible". Instructions were also provided in Nepali when needed.

4.3.5 Data and Statistical Analysis

Baseline sympathetic and cardiovascular data were averaged over ~10 minutes during resting tidal volume breathing, and during the last 10 cardiac cycles prior to volitional breakpoint. Cardiovascular parameters were additionally calculated post-apnea for their nadir (S_pO_2, HR, MAP) and peak (SBP, DBP, MAP, and TPR) responses. These respective nadir and peak responses occurred within 10-15 seconds post-volitional breakpoint. MSNA bursts were identified using a semi-automated detection algorithm (Chart Pro 8.3.1) and confirmed by a trained observer (SAB). MSNA was quantified as burst frequency (bursts/min), incidence (bursts/100 hb), and normalized burst amplitude (% of maximal burst size at baseline) during baseline. The data from the final 10 cardiac cycles prior to volitional breakpoint for each breath hold were analyzed to account for variation in apnea duration from low to high altitude. Bursts were quantified during the reactivity protocol through the calculation of burst area (area under the curve, [au]) and incidence of bursts (%). An incidence of burst that was100% would indicate that all individuals from were exhibiting a burst during that respective cardiac cycle. The burst area from each cardiac cycle was compared to the mean baseline burst area and represented as the % increase. Total normalized SNA (au/min) was also calculated during baseline and apnea to account for the greater bradycardic response seen with breath holding at altitude (24). Values calculated from the total normalized area are represented as (Δ absolute total normalized SNA [au/min]).

Results are reported as mean ± standard deviation (SD) with exception to baseline burst amplitude, which is calculated as median ± interquartile range (IQR). In addition, burst amplitude is represented as a distribution of normalized bursts obtained during that 10-minute period. Multiple comparisons were assessed for all measurments 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) (24, 77, 165):

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

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

For normalized bursts amplitude, a two ways repeated measures ANOVA compared the main and interaction effects within 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 within 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).

4.4 Results

All 14 lowlanders were successfully tested at both 344m and 5050m. Two Lowlander participants reported having mild acute mountain sickness (AMS) (Lake Louise scores of 3). However, the data from these two subjects were comparable to the averaged responses and hence included in the main analyses. Sherpa values from Kathmandu were not obtained in 4 of the 8 participants due to technical limitations. The 4 successfully measured Sherpa are included in the discussion for descriptive purposes. Baseline cardiovascular and autonomic characteristics for both Sherpa and Lowlanders are listed in Table 1. We have previously reported that Sherpa exhibited similar resting cardiovascular values to Lowlanders with no difference in SBP, DBP, MAP, Q, and TPR at 5050m (24). In Lowlanders, the SpO_2 during baseline was 98±1 % at low altitude. The resting SpO_2 of Sherpa (83±4 %) and Lowlanders (83±3%) were comparable at 5050m.

4.4.1 Baseline Sympathetic Characteristics in Sherpa and Lowlanders

Resting MSNA values for Sherpa and Lowlanders are exhibited in Table 1, and Figures 24 - 25. Lowlander burst frequency tripled (11±5 bursts/ min to 30±6 bursts/ min; $p<0.001$), while burst incidence doubled (25±13 bursts/ 100hb to 50±15 bursts/ 100hb; $p<0.001$) upon ascent to 5050m. At 5050m, Sherpa had a lower burst frequency (23±11 bursts/min; $p < 0.05$) and incidence (30±11 bursts/100hb; $p<0.05$) compared to Lowlanders at 5050m. The distribution of normalized burst area was also shifted towards larger sized bursts in Lowlanders at 5050m (42± 22 vs. 46±23 [Median± IQR]; $P<0.05$). There were no observed interaction effects between burst amplitude in Lowlanders from low to high altitude. The burst amplitude distributions in the Sherpa were similar to that of Lowlanders at 5050m (46± 19 vs. 46±23, respectively).

Table 1: Demographic, cardiovascular, and sympathetic function in lowlanders and Sherpa at low and high altitudes.

	LOWLANDERS		**SHERPA**
	334m (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)	11 ± 5	30 ± 7*	23 ± 11†
Burst Incidence (burst /100hb)	25 ± 13	50 ± 15*	30 ± 13†
Burst Amplitude (au) ●	42.0± 22.2	46.7±7.9	46.3± 19.1

♦ Model Flow.

●Burst amplitude calculated as median % relative to maximum burst during selection period. ± Indicate Interquartile Range

* Significantly different from Lowlanders tested at low altitude (334m); $p < 0.05$.

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

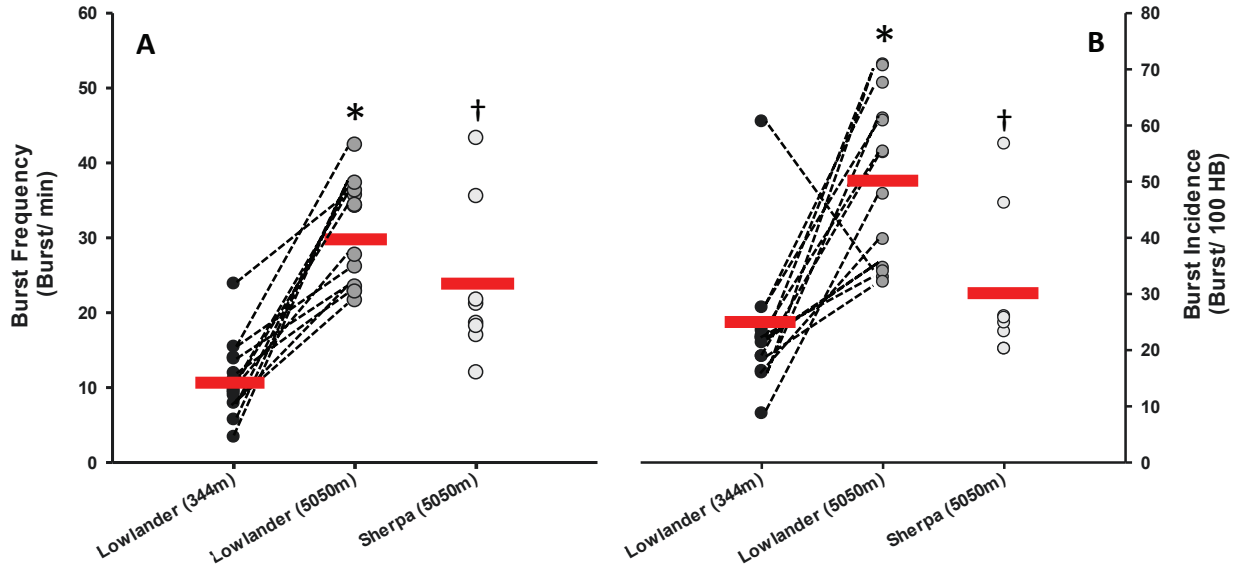

FIGURE 24. Baseline burst frequency and incidence in Lowlanders and Sherpa at 344m and 5050m**.** *Panel A,* baseline burst frequency (bursts/min) in Lowlanders at 344m (n=14), 5050m (n=14), and Sherpa at 5050m (n=8). *Panel B*, baseline burst incidence (bursts/ 100hb) in Lowlanders at 344m (n= 14), 5050m (n=14), and Sherpa at 5050m (n=8). Both burst frequency and incidence was significantly increased ($P<0.001$) at 5050m in Lowlanders. Both burst frequency and incidence was lower in Sherpa than Lowlanders at 5050m ($P<0.05$ and $P<0.05$ respectively). * Significant difference between Lowlanders at low altitude, $P<0.05$; † Significant difference between Lowlanders at high altitude, $P<0.05$.

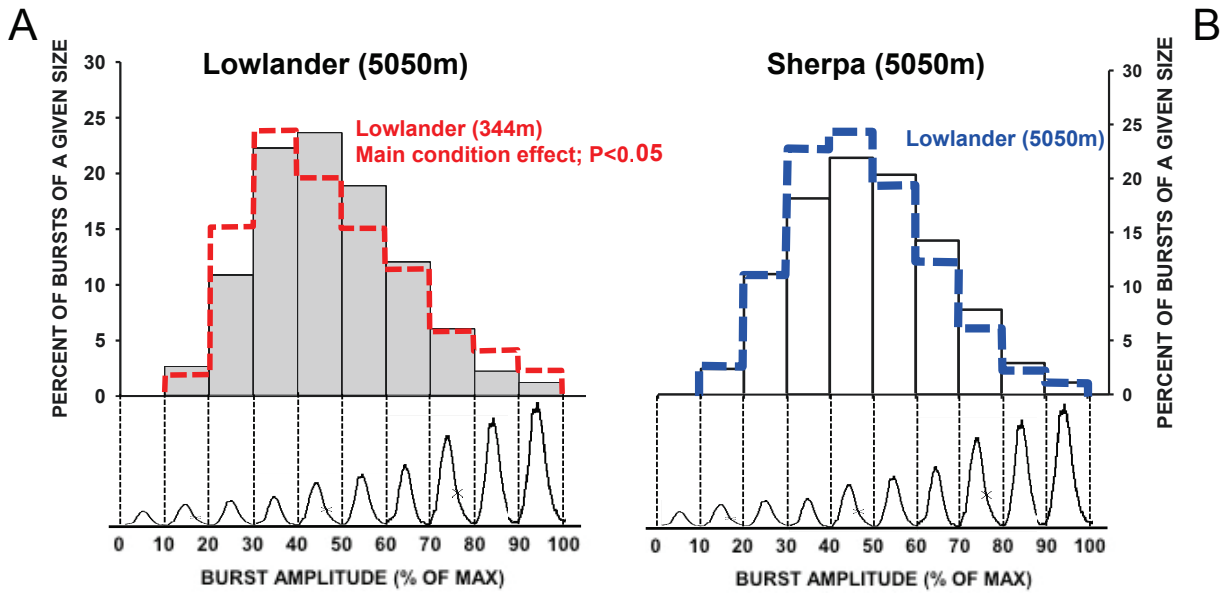

FIGURE 25: Burst amplitude (% of max burst) in Lowlanders And Sherpa at 344m and 5050m. Histograms represent normalized burst amplitude frequencies obtained during baseline. *Panel A,* Lowlander (n=14) distributions at low altitude (344m, red dotted line) plotted against high altitude (5050m, solid grey bars). *Panel B,* Lowlander (n=14) distribution at high (5050m, blue dotted line) altitude plotted against Sherpa (n=8) at 5050m (solid white bars). Burst amplitude showed a main effect ($P<0.05$) shift in Lowlanders towards larger sized bursts at altitude. However, there was no noted interaction effect observed between bursts. Sherpas at altitude demonstrated a similar distribution to Lowlanders at altitude.

4.4.2 Sympathetic Reactivity to breath holding in Lowlanders and Sherpa

At low altitude, Lowlanders had breath hold duration of 30 ±11s (range 15-74s), which was reduced to 15 ±5s (range 9-27s) ($P<0.001$) at 5050m. Lowlander SpO_2 nadir post-breath hold was 78±7%. Sherpa breath hold duration (15±2s; Range 12-19s) and desaturation (75±5%) post-breath hold were not different to that of Lowlanders. Absolute normalized total SNA was not obtained in one Lowlander participant during baseline due a considerable amount of noise within the MSNA neurogram. Therefore, reactivity for breatholding at low altitude is an N of 13 in Lowlanders.

Breath holding across all groups and conditions produced a robust increase in sympathetic activity (Figures 26 - 27). Individual burst area was increased respective of baseline during the last 5 cardiac cycles prior to volitional breakpoint in both Lowlanders and Sherpa across all conditions ($P<0.05$). The response of these burst areas were similar in Lowlanders from low to high altitude, though incidence of bursts was higher in Lowlanders during the last 5 cardiac cycles at altitude. However, Sherpa increase in burst area was significantly lower during the last 5 cardiac cycles compared to Lowlanders at 5050m. Low altitude breath holding was associated with an increase in normalized total SNA in Lowlanders (+144±126 au/min versus baseline; $P<0.01$) that was similar at high altitude (+133±71 au/min versus baseline). Interestingly, despite a similar magnitude of sympathetic response between low and high altitude, 5 out of the 14 Lowlanders had elongation of sympathetic bursts at high altitude, which did not exhibit normal burst firing characteristics seen at either low altitude, or within Sherpa at 5050m (Figure 28). In contrast, Sherpa sympathetic responses (+56.8± 49.6 au/min) were significantly lower ($P<0.01$) than Lowlanders at 5050m. Sherpa also exhibited both a smaller pressor response post-breath hold (+23± 20 mmHg; $P<0.05$) compared to Lowlanders (+35±20 mmHg) at high altitude. A measurement of transduction showed that Sherpa exhibited a larger response to sympathetic stimulation (0.38± 0.27 vs. 0.09±0.08 ΔmmHg/Δau in Lowlanders at 5050m). Finally, there was no relationship between days after arrival at 5050m with either baseline sympathetic function and neurovascular reactivity in either group.

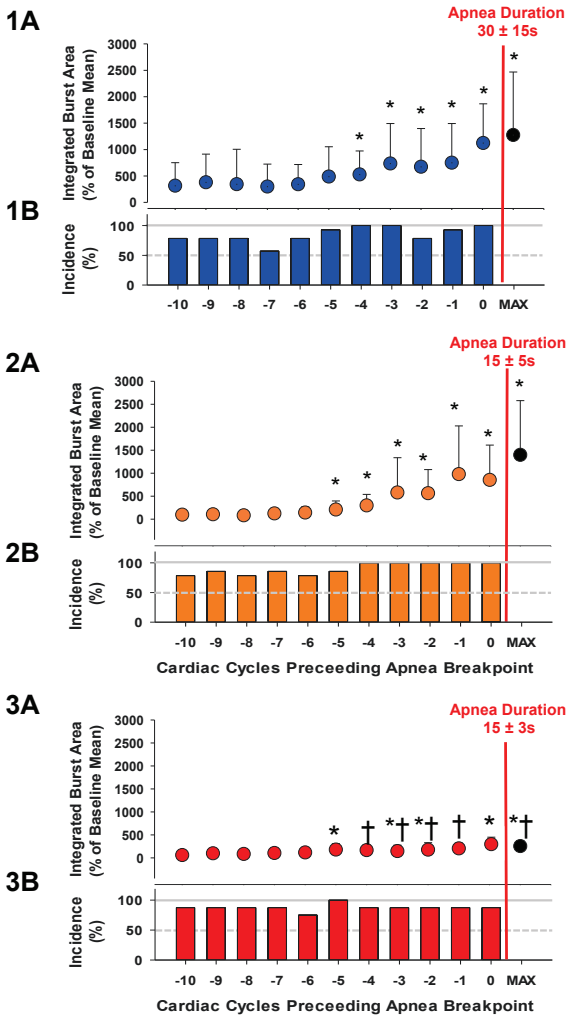

FIGURE 26. Percent change in burst integral area and percent incidence of bursts for the last 10 cardiac cycles in Lowlanders and Sherpa during breath holds. Integral burst area (% increase relative to baseline; mean ± SD; denoted as "#A") and incidence of bursts (% of individuals who exhibited a burst during the respective cardiac cycle; denoted as "#B"). Values represent each respective cardiac cycle up to 10 cycles prior to volitional breakpoint. *Panel 1,2*, Lowlanders at 344m (blue) and 5050m (orange). *Panel 3,* Sherpa at 5050m (red). Lowlanders showed an increase in sympathetic activity prior to volitional breakpoint at both low and high altitude ($P<0.05$). Sherpa exhibited a smaller 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 $P<0.05$; † Significant difference between Lowlanders at high altitude, $P<0.05$.

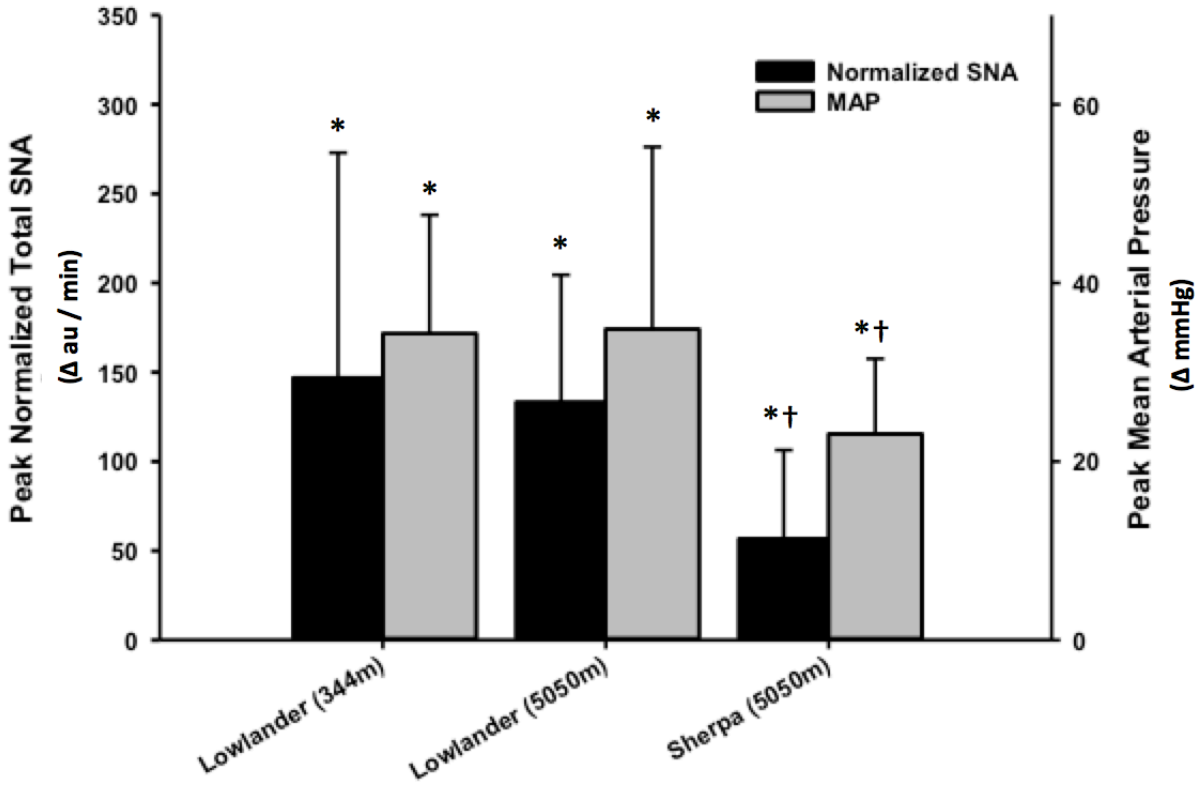

FIGURE 27. Absolute increase in total normalized SNA and mean arterial pressure in Lowlanders and Sherpa during breath holds. The normalized SNA during breath holding was compared to baseline, which increased across irrespective of condition. Lowlanders and Sherpa exhibited higher total SNA response that corresponded with a concurrent increase in MAP during apnea. The total sympathetic response was also similar across all conditions within Lowlanders. Sherpa had reduced sympathetic and blood pressure responses respective of Lowlanders at low and high altitude. * Significant difference versus respective baseline, $P<0.05$; † Significant difference between Lowlanders at high altitude, $P<0.05$

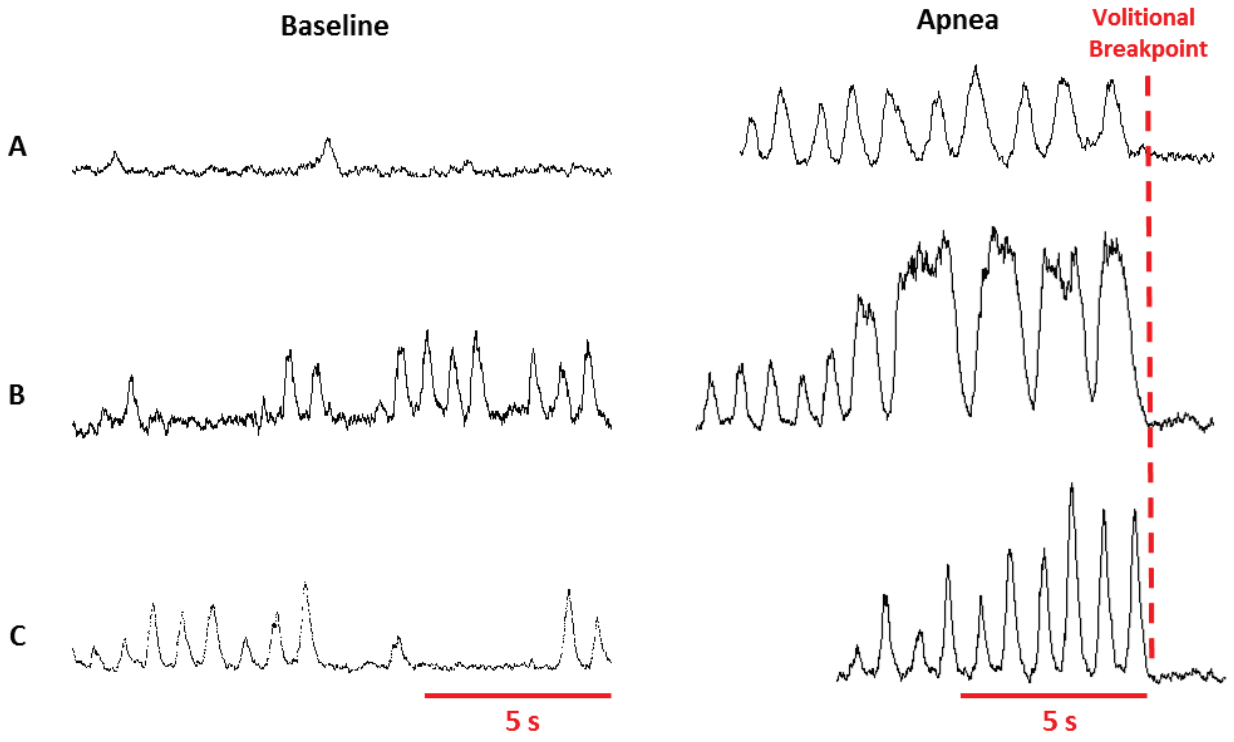

FIGURE 28: Integrated MSNA neurogram of Lowlanders and Sherpa at 344m and 5050m. Apnea shows last 10 cardiac cycles obtained prior to volitional breakpoint. *Panel A, B are* examples of the same male at 355m (A) and 5050m (B) against Sherpa at 5050m (C). Apnea at altitude caused prolonged burst periods and loss of characteristic burst "peak" in Lowlanders. However, these prolonged bursts were limited to between cardiac cycles. Whereas Sherpa did not develop prolonged burst firing patterns at 5050m.

4.5 Discussion

Within the current study, we report and compare direct recordings of post-ganglionic sympathetic activity between and Sherpa and acclimatized Lowlanders at altitude. With regards to research question 1; our data agrees with the hypothesis that stated Sherpas would exhibit lower basal sympathetic activity at altitude than Lowlanders. This is response is lower within Sherpa is present, despite no differences in blood pressure or arterial oxygen saturation between groups at altitude. We have shown the breath-holding model can promote a strong SNA response at altitude. However, Lowlanders do not exhibit further augmentation of MSNA to additional stress at altitude, which disagrees with our hypothesis stated in research question 2. Our stated hypothesis for research question 3 was also correct; with Sherpa demonstrating a lower sympathetic and blood pressure response at altitude relative of acclimatized Lowlanders. Therefore, we have demonstrated that sympathetic and vasomotor (re) activity at high altitude is lower in Sherpa compared to Lowlanders.

4.5.1 Sympathetic Regulation in Sherpa at Altitude

There have been a number of studies that attempted to indirectly assess SNA in high altitude natives. Healthy Andeans have previously been shown to exhibit significantly lower plasma norepinephrine concentrations than those suffering from chronic mountain sickness (52). These findings agree with earlier findings in HAPE susceptible Lowlanders (40), suggesting a link between sympathetic hyperactivity and acute/chronic maladaptation to altitude. Lundby *et al.* (103) more recently used MSNA to demonstrate that burst frequency was similar between native Bolivian Aymarans and acclimatized Lowlanders following 50 days at 4100m. High altitude natives of the Indian Himalayan range had lower urinary catecholamine relative to lowland Indians at 3500m (111). However, these finding in Andeans, Indians, and Bolivians may not accurately translate to Sherpa due to differential genetic expression (194) or phenotypic differences (12, 13) that exist between high altitude populations. In addition, the generational residency of Sherpa at altitude may confound the differences between high altitude groups. Native Tibetans and Sherpa residing at altitude are considered to have existed at high

altitude for a longer duration than either high altitude Andeans and Ethiopians (194). As such, the only previous findings of altered autonomic function within native Sherpa/ Tibetans have been through measurements of heart rate variability (HRV) that demonstrate greater parasympathetic dominance in high altitude natives (133, 197). Though these HRV findings suggest lower SNA in native Sherpa; HRV analysis is not currently capable of accurately representing differences in SNA between low and high altitude groups, nor a valid representation of whole body sympathetic activation. These are the first reported findings that directly demonstrate lower sympathetic function at altitude in native Sherpa. However, the specific mechanism related to the observed difference between SNA in Lowlander and Sherpa has not yet been determined. Sympathetic activation is primarily regulated through baroreflex or chemoreflex pathways. The gradual increase in carotid body discharge in Lowlanders under long-term hypoxia exposure (38) suggests the chemoreflex as a driver of sympathetic hyperactivity at altitude. The earliest findings of a lower hypoxic ventilatory response (an indicator of chemoreflex activation) within Sherpa (51, 93) supported that a difference in chemoreflex sensitivity to altitude may be present in Sherpa. However, the current consensus (including more recent publishings from this expedition) indicate a similar HVR between acclimatized Lowlanders and Sherpa (11, 12, 24, 55, 64). Therefore, the differences in SNA between Lowlanders and Sherpa may be a combination of other reflexes. This warrants further investigation in order to delineate the mechanisms underpinning autonomic and neurovascular control at high altitude.

4.5.2 Sympathetic Regulation during Acclimatization in Lowlanders

Previous studies clearly demonstrate modest increases in sympathetic activity in Lowlanders during acute hypoxia that depends on both the duration (minutes – hours) and degree of exposure (70, 87, 115, 144, 145, 147, 166, 193). However, few field studies have demonstrated the effects of acclimatization to chronic hypoxia (days – weeks) on sympathetic function. Duplain *et al.* (40) first showed increased basal sympathetic activity 24hr following ascent to 4559. Hansen and Sander further demonstrated a tripling in burst firing (from 16 ± 3 bursts/ min to 48 ±5 bursts/ min) following 4 weeks

residency at 5260m (69). However, it was Lundby *et al.* (103) that recently demonstrated further SNA augmentation following ten days at 4100m (42± 5 bursts/ min versus 15± 2 bursts/min at 25m above sea level) relative to acute normobaric hypoxia exposure (16± 2 bursts/min with FiO_2 0.126). These earlier studies demonstrate that SNA becomes further augmented at altitude. These previous results are also consistent with our current findings. Our acclimatized Lowlanders showing a tripling in burst frequency, doubling of burst incidence, as well as shift towards larger bursts within 10 days of arrival at 5050m. Lundby *et al.* (103) also saw that the tripling of MSNA within 10 days at altitude was sustained following 50 days (42± 5 bursts/min) at the same altitude. In other words, MSNA does not appear to increase further after a certain period time point at altitude. The degree of sympathetic activation may be dose dependant with regards to the hypoxic stimulus. Previous studies have demonstrated increases in MSNA with progressively greater hypoxic stimulus during hypobaric chamber and normobaric hypoxic exposure (145, 159). Whether this dose response affects the duration and degree of progressive sympathetic hyperactivity at altitude requires further investigation.

The mechanisms contributing to sympathetic hyperactivity in acclimatized Lowlanders at altitude remain unknown. The peripheral chemoreceptors have traditionally been proposed as the mechanism that influences sympathetic activation under hypoxia. This is shown with greater carotid body discharge under progressively increased acute hypoxic stimuli (114, 175). Furthermore, an augmented ventilatory response within acclimatizing Lowlanders at altitude (51) suggests that chemoreceptor sensitization may also play a further role in sympathetic hyperactivity at altitude. However, the role of the chemoreceptors has more recently been challenged two studies at altitude. Hansen and Sander (69) only saw modest reductions of total SNA were reported following 15 minutes of either 100% supplemental O_2 administration or volume loading (via 1000 ml intravenous saline infusion) at 5260m (69). It can be argued though that O_2 administration would suppress the ventilatory response while lowering the ventilatory-induced hypocapnia present at altitude, thus affecting sympathetic outflow. Fisher *et al.* (50) more recently saw no reduction in burst frequency following low-dose dopamine infusion under either

basal activity and an incremental hypoxia step protocol following 2 weeks at 3454m. Other mechanisms have been proposed, including long-term potentiation of central regulatory mechanisms (193), central resetting, or baroreflex-mediated changes. Though outside of the scope of this thesis; the differences in basal sympathetic function at altitude may be due to interactive effects between several mechanisms and/or central augmentation.

4.5.3 Sympathetic Reactivity in Lowlanders and Sherpa

We used voluntary apnea at altitude to demonstrate reduced sympathetic reactivity to stress in Sherpa compared to Lowlanders. This was seen despite similar breath holding duration and degree of desaturation between groups. Lowlanders exhibited the same reactivity when tested at low and high altitude. This is interesting considering previous findings that demonstrate no difference in total SNA during apnea in novice and trained breath-hold divers, the latter having developed acute tolerance to this form of stress (19). In the context of significant increases in basal activity, reduced apnea duration (indicative of an increase chemosensitivity), and a significantly reduced SpO_2 at rest and during apnea itself at altitude argue that breath holding at altitude produces a larger sympathetic stressor. Yet these results suggest a "sympathetic plateau" during maximal apnea in Lowlanders. Regulation of sympathetic vasomotor outflow is tightly regulated by the cardiac cycle. Thus, a finite degree of postganglionic activity/recruitment can occur within each R-R interval (107, 108). Yet despite our previous findings demonstrating a greater bradycardic response during apnea at altitude compared to sea level (24), it appears that sympathetic activity does not increase further. It may be that this plateau is lower in Sherpa due to central regulation of sympathetic outflow during stress. Yet after the increase in SNA during apnea was normalized to the last 10 cardiac cycles, Lowlanders showed a similar increase in SNA at low and high altitude. Furthermore, Sherpa still had a smaller reactivity to breath holding stress after normalization at altitude. Whether this is due to their own respective plateau being reached, or that they have additional reserve for increasing MSNA, requires further investigation. We cannot confirm a plateau response within Sherpa due to not obtaining measures of sympathetic reactivity in Sherpa at low.

Although the exact mechanisms behind these adaptations remain unknown; it suggests that long-term adaptation includes a decreased sympathetic activity and reactivity. As the pressor response and sympathetic reactivity was lower within Sherpa, this may be a result of other adaptations that are beneficial towards residency at altitude.

4.5.4 Neurovascular Response between Lowlanders and Sherpa at Altitude

The increase in sympathetic activity during hypoxia helps to redistribute blood flow to crucial organs (66, 98, 166). This can be seen through the gradual rise in blood pressure under chronic hypoxia exposure (131, 133, 147). Sherpa exhibited a blunted increase in SNA at altitude compared to Lowlanders, despite similar blood pressure responses. This observation suggests that the lower sympathoexcitation seen in Sherpa may be due to greater vascular sensitivity to sympathetic stimulation. Although altitude increases sympathetic vasomotor activity (40, 69), hypoxia acts as a local vasodilator within the peripheral vasculature (71, 131, 138). This in turn sees a reduction of blood pressure and increase peripheral blood flow. Overtime ,heightened sympathetic activity attempts to correct for local vasodilation and return blood pressure to normoxic conditions. However, the relationship between sympathetic activity (and reactivity) to vascular resistance has not been previously addressed. The voluntary apnea model allows for us to examine both the SNA and vascular responses. We not only found that the sympathetic response was lower in Sherpa, but the vascular response (as measured by the change in blood pressure) was lower as well. This may due to multiple mechanisms including lower epinephrine release, reduced receptor density, or an augmented hypoxic dilatory response. However, these need to be investigated further. Since the increases in MSNA and blood pressure were similar between altitudes in Lowlanders; acute acclimatization does not appear to influence the effects of efferent SNA on the vascular response.

CHAPTER 5 - GENERAL CONCLUSION

5.1 Main Findings

In Summary, our findings indicate an overall difference in autonomic regulation between Lowlanders and Sherpa at altitude. Our findings have reported, for the first time, direct neural recordings that show overall sympathetic function is lower in Sherpa at altitude. The lower basal MSNA suggests a potentially greater sympathetic reserve at altitude, though our findings from Kathmandu (see section 5.2 for further detail) are underpowered to confirm this. This lower MSNA translated to lower sympathetic reactivity within Sherpa during breath holds. MAP was lower similar in Sherpa during breath holding. However, the vasculature was more sensitive to increases in MSNA, suggesting an improved sympathetic responsiveness to stress. These findings contrast earlier published work by Lundby *et al.* (103) that demonstrate a similar MSNA response between Aymarans and acclimatized Lowlanders. We believe that our observation may not necessarily translate to other high altitude populations. Yet these findings add further to the numerous physiological adaptations that are present within Sherpa.

Through the use of breath holding, sympathetic reactivity was shown to increase MSNA across all conditions. Lowlanders did not see any further augmentation of sympathetic reactivity when compared to low altitude, despite basal MSNA being higher at 5050m. Rather, total SNA was similar prior to volitional breakpoint at both altitudes. The plateauing of MSNA in lowlanders within our study, despite the relatively short breath hold duration is a novel finding. This plateau maybe due to a potential sympathetic reserve, where only a finite degree of sympathetic augmentation is possible. This provides further insight into not only general integrative physiology, but also in relation to altitude physiology. Considering that breath holding may produce an extremely high degree of stress, other methods of assessing sympathetic reactivity (ie. physical work, sleep) should be further examined further.

5.2 Considerations

This study demonstrates an overall lower sympathetic response in Sherpa at altitude. However, it is still inconclusive if Sherpa exhibit this same response after de-acclimatization at altitude. In an attempt to assess sympathetic de-acclimatization within Sherpa; MSNA was recorded on a subset of 4 (out of 8) Sherpa who had descended to Kathmandu (1400m) 1-4 days prior to Lowlanders arriving. It was observed that both burst frequency (14 ± 2 bursts/ min) and incidence (23 ± 5 bursts/ 100 hb) appeared similar to that of Lowlanders when tested at low altitude (11 ± 5 bursts /min and 25 ± 13 bursts/ 100 hb). Although acknowledging the descriptive nature of these data, this suggests that a) altitude still represents a significant stress in high altitude natives, and b) the adaptations observed may be specific to response under hypoxic conditions and do not persist in relative normoxia. The latter findings likely differ from Lowlanders since Hansen and Sander (69) reported that a persistently elevated burst frequency and incidence in lowlanders existed within 3 days after return to normobaric conditions after 4 weeks residency at 5260m. Therefore, our limited data from Sherpa after ~1 week at low altitude suggest this persistence may be short-lived. The mechanism(s) that underpin this observation remain to be established.

As of these findings, only one recently published study has indirectly measured and quantified sympathetic reactivity at altitude within Lowlanders. Fisher *et al.* (50) measured MSNA reactivity via incremental hypoxia test (nadir $PETO_2$ 45mmHg at final stage) within Lowlanders following 15-17 days at 3454 m. Though they noted an increase in baseline burst frequency at altitude, the gain response in burst frequency was similar between low and high altitude. The findings of this study are difficult to completely contrast with our own. Firstly, their lowest reported burst frequency during the incremental test was similar to what we observed during baseline at 5050m. This can also be seen in their final stage SpO_2, which was also similar to our baseline. Therefore, their respective altitude may have been too small to get a considerable MSNA response. Secondly, they attempted to control for hyperventilatory induced hypocapnia during the incremental protocol stage by maintaining sea level end tidal O_2 and CO_2.

This would alter the ventilatory response at altitude and ultimately affect central chemoreceptor (hypocapnia) and ventilatory inhibition of MSNA at altitude. Though we were unable to report end tidal O_2 and CO_2 at altitude; the short duration of our end- expiratory breath holds would have minor effects on either values. We believe that our breath hold findings are a more accurate representation of sympathetic reactivity at altitude. We also demonstrate the maximal ability of sympathetic activity to increase during breath holding, though further studies would be required to confirm this.

Prior to volitional breakpoint at altitude, 5 of the 14 Lowlanders developed prolonged, "fused" burst-firing patterns that were not present under normoxic conditions. They were characterized as burst that had abnormally long firing durations (facilitated through increased cardiac R-R Intervals) and the absence of a distinct single, sharp peak (see figure 28). These patterns were benign, with return to normal burst characteristics upon cessation of breath holds at altitude. One interesting point was that the normalized the total peak SNA response within these individuals did not differ between those without the abnormal patterns. In addition, the normalized peak total SNA between Lowlanders at low and high altitude was still similar. These prolonged bursts were not seen within Sherpa. The phenomenon of prolonged/fused bursts has been previously observed under considerable sympathetic strain via drastic lower body negative pressure (28) and head-up tilt tests (83) that promote baroreflex unloading through reduced venous return. These earlier findings suggest a baroreflex-induced response. Both studies evoked a large baroreflex-mediated sympathetic response to correct for reduced arterial pressure. However, this is the first time that these observations have been found without considerable baroreflex activation, as can be speculated with the increase in MAP during breath holding that should suppressed afferent baroreflex feedback. Furthermore, these abnormal patterns still remained tightly regulated by baroreflex gating within individual cardiac cycles and became silent with the systolic upswing prior to volitional breakpoint. Cardiovagal gain is also seen to become reduced at altitude (131). This may be through chronic hypoxia pulmonary vasoconstriction and constant stimulation of cardiopulmonary stretch receptors that is similar to obstructive sleep apnea patients (29). As the chemoreceptors can activate both

sympathetic and parasympathetic pathways within the brain (114); chemoreceptor sensitization at altitude may promote both augmentation of parasympathetic and sympathetic activity. The performance of a breath hold evokes augmentation of both sympathetic (increase in MAP) and vagal (bradycardia) driv. As such, we have previously reported (24) that breath holding produces considerable autonomic conflict at altitude for acclimatized Lowlanders. This was seen specifically within the heart in the form of significantly greater bradycardia responses and the development of cardiac arrhythmias. Similar to our MSNA findings, these arrhythmic events were eliminated once the individuals stopped holding their breaths. Fagius *et al.* (46) were able to develop prolonged bursts with removal of cardiac burst rhythmicity during anaesthetic bilateral blocks of bilateral vagus and glossopharyngeal nerves. We believe that the these fused bursts at altitude may be explained through concurrently elevated vagal and sympathetic drive during breath holding, where prolonged R-R intervals allow for greater SNA periods.

5.3 Limitations

There are two main limitations that need to be addressed within this study. The use of voluntary apnea is a simple model of assessing muscle autonomic reactivity as it evokes both a quick and large sympathetic response (19, 39, 76, 106, 112, 167). Breath-hold tolerance can be objectively difficult to assess as duration can be affected by several factors including differences in ventilatory drive, previous repetitive practice (132), mental distractions (6), and overall tolerance of the individual. This raises the question of whether Sherpa truly demonstrated a maximal apnea at altitude. Two main considerations make us believe that Sherpa performed apnoea 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 breath hold duration and drop in SpO_2 to that of Lowlanders.

During prolonged periods of hypoxia exposure there is an apparent time-dependant sensitization of the peripheral chemoreceptors that results in progressively heightened sympathetic activity (38). This is supported by higher sympathetic activity observed by Hansen and Sander (69), who saw a tripling of

burst frequency following 4-week residency at 5260m, to our observed doubling of MSNA following 10 days at 5050m. Therefore, the findings of this current study may be in part influenced by the respective date individuals were tested at 5050m. As previously stated we assessed duration as a potential covariate through Pearson's correlation and follow up ANCOVA analysis. When baseline sympathetic function, and both sympathetic and vascular reactivity was examined between groups, there was no observable 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.

5.4 Conclusion

In summary, this study has shown several novel findings. First, there is differential regulation of sympathetic vasomotor activity between Lowlanders and Sherpa. Secondly, the reactivity of sympathetic activity at altitude to further stress is blunted in Sherpa. However in Lowlanders apneas appeared to maximize the augmentation of sympathetic activity. This altered response to altitude may be a beneficial adaptation to residency at altitude as reduced sympathetic activity prevents chronic hypertensive states to increased physical demands at altitude. The findings of normalized sympathetic function in Sherpa at low altitude are a unique finding and should be explored further to validate this observation.

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APPENDICES

Appendix I: Ethics Approval for Human Subjects (University of Alberta)

Approval Form

Date:	December 7, 2016
Principal Investigator:	Craig Steinback
Study ID:	Pro00064195
Study Title:	Mechanisms regulating sympathetic neurovascular control during altitude acclimatization in lowlanders and highlanders
Approval Expiry Date:	December 6, 2017
Funding/Sponsor:	University of Alberta Human Performance Fund Scholarship
Funding/Sponsor:	NSERC - Natural Sciences And Engineering Research Council

Thank you for submitting this application. We acknowledge that this research has been completed by the local researcher, under the University of British Columbia Clinical Research Ethics Board approval. Active approval by the UBC CREB was in place at the time that the research was conducted. This letter serves to acknowledge that the University of Alberta accepts the UBC ethical approval of this study, under the Western Provinces Harmonization Agreement.

A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date (November 28, 2017) you will have to re-submit an ethics application.

The membership of the Health Research Ethics Board - Biomedical Panel complies with the membership requirements for research ethics boards as defined in Division 5 of the Food and Drug Regulations and the Tri Council Policy Statement. The HREB - Biomedical Panel carries out its functions in a manner consistent with Good Clinical Practices and the Canadian General Standards Board (CAN/CGSB-101.1-2013).

Approval by the Health Research Ethics Board does not encompass authorization to access the patients, staff or resources of Alberta Health Services or other local health care institutions for the purposes of the research. Enquiries regarding Alberta Health administrative approval, and operational approval for areas impacted by the research, should be directed to the Alberta Health Services Research Administration office, #507 College Plaza, email nactrc.contracts@albertahealthservices.ca.

Sincerely,

Associate Chair, Health Research Ethics Board – Biomedical Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).

Appendix II: Consent Forms for Participants (Lowlanders and Sherpa)

SUBJECT INFORMATION AND INFORMED CONSENT

Mechanisms of acute adaptation and evolution in the human physiological response to high-altitude: a scientific expedition to the Nepal Himalaya

Principal Investigator:

[3]Philip N Ainslie, PhD

Co-Investigators:

[1]Brad Monteleone, PhD., MD; [2]Myp Sekhon, MD; [3]Nia Lewis, PhD; [4]David Macleod, MD; [5]Mike Stembridge, PhD; [3]Lindsey Boulet, BSc; [3]Matt Rieger, BSc; [3]Anthony Bain MSc; [3]Daniella Flueck, PhD; [3]Ryan Hoiland, BHK; [3]Joshua Tremblay, MSc; [3]Mike Tymko, BSc; [3]Alex Hansen, BHK; [3]Eric Delorme; [6]Geoff Hartley, BSc; [3]Christopher K. Willie, PhD; [7]James Anholm, MD; [7]Prajan Subedhi, MD; [8]Jonathan Moore, PhD; [9]Craig Steinback, PhD; [9]Steve Busch, BHK; [3]Chris McNeil, PhD; [3]Luca Ruggiero, MSc; [10]Joseph Donnelly, MD; [11]Ali McManus, PhD.

Investigator affiliation:

[1] Faculty of Medicine, UBCO
[2] Faculty of Medicine, Department of Critical Care, UBC
[3] Centre for Heart Lung and Vascular Health, UBCO
[4] Duke Clinical Research Unit, Duke University School of Medicine
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[6] Brock University, Saint Catharine's, Ontario
[7] Loma Linda University, School of Medicine, California
[8] Bangor University, Bangor, Gwynedd, Wales
[9] University of Alberta, Edmonton, Canada
[10] Department of Clinical Neurosciences, Cambridge University, UK
[11] Institute for Healthy Living and Chronic Disease Prevention, UBCO

Investigation Sites:

The University of British Columbia, Okanagan Campus; Ev-K2-CNR Research Pyramid, Khumbu Valley, Nepal.

1. INVITATION

Please read the following information carefully before deciding to participate in the study. If you have any questions, please do not hesitate to ask. You are being invited to take part in this research study because you are a healthy volunteer free of any cardiovascular or pulmonary disorders, and are potentially joining the research expedition to the Ev-K2-CNR Research Pyramid Laboratory, located at 5050m above sea level in the Khumbu Valley, Nepal, this October of 2016.

2. YOUR PARTICIPATION IS VOLUNTARY

Your participation is entirely voluntary. Before you decide to volunteer, it is important for you to understand what this research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study, and the possible benefits, risks and discomforts associated with your participation. If you wish to participate, you will be asked to sign this form. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide. If you choose not to participate in this study, you will not be penalized in any way. You do not need to disclose why you have chosen not to participate, and you will still receive complete clinical care if your still want to join the research expedition.

3. WHO IS CONDUCTING THE STUDY

The research team includes investigators from the Centre for Heart, Lung and Vascular Health, at the University of British Columbia, University of Alberta, Okanagan, the Duke University Medical Center, Cardiff Metropolitan University, Brock University, and the Loma Linda University. The research is, in part, supported by the National Science and Engineering Research Council of Canada and Canada Research Chairs program.

4. BACKGROUND

Many respiratory and cardiovascular diseases involve exposure to low levels of oxygen (hypoxia), high blood pressure in the lung, and difficulty in breathing. Some examples of these diseases include cerebral stroke, sleep apnea, chronic obstructive pulmonary (lung) disease, and congestive heart failure. Ascent to high altitude provides an excellent means to examine physiological adaptation to acute and chronic hypoxia. Because of the time necessary to study any chronic adaptation (i.e. several weeks of exposure), the profound limitations on quality of life, and related expense, studying the effects of high altitude at sea level using hypobaric (low barometric pressure) or hypoxic chambers is not feasible. This research expedition entails 13 distinct studies that will be performed at sea level (at the Centre for Heart, Lung and Vascular Health) and during 14 days at the Ev-K2-CNR Research Pyramid Laboratory, located at 5050m above sea level in the Khumbu Valley, Nepal. The 12 studies are outlined in section five (below) and in more detail in section 8 of this consent form.

5. WHAT IS THE PURPOSE OF THE STUDY?

Very few studies have taken an integrative (i.e. whole body) research approach to investigating biological changes to acute and chronic hypoxia. These 12 main research studies and related purposes take an integrative approach to study chronic hypoxic physiology, with particular focus on the cerebrovascular functioning (i.e. brain blood flow functioning).

NOTE: YOU WILL BE PERFORMING SOME BUT NOT ALL OF THESE STUDIES. THE INCLUSION OF CERTAIN STUDIES WILL BE ASSIGNED BASED ON AVAILABILITY (WHEN YOU ARE NOT ACTING AS A RESEARCHER YOURSELF), FOR LOGISTIC PURPOSES. YOU MAY ALSO DECIDE ON WHAT STUDIES YOU MAY WANT OR NOT WANT TO VOLUNTEER FOR.

1. **Neuromuscular function and adaptation to high altitude.**

   Aim: To assess impairment in the nervous and muscular systems and their recovery during exposure to 2-weeks at 5050m and following return to sea level.

   Synopsis: Because the function of muscles appears to become impaired somewhat at high altitude, this study will assess the severity of this impairment and the time course of regaining normal function after return to sea level. values for cerebral blood flow at rest and during exercise in Sherpa children.

2. **Comparative effects on the lung vasculature of ascent to high altitude in lowlanders and high altitude natives**

   Aim: To characterize lung arterial and right heart function during ascent to high altitude, and how these parameters are helped by supplemental oxygen.

   Synopsis: The pressure within the arteries of the lung (and consequently the pressure against which the right side of the heart must work) increases at high altitude, an effect that can be temporarily cured by supplemental oxygen, but the time course and magnitude of amelioration of supplemental oxygen is still not known.

3. **The influence of altitude on postural changes in cerebral blood flow, intrapulmonary shunting and gas exchange**

   Aim: To assess the altered brain vascular, heart, and lung physiological responses to postural change during ascent and acclimatization to high altitude.

Synopsis: The cardiovascular response to changes in posture appears to be affected by high altitude but little is understood about the time course of these changes and their severity.

4. **A non-invasive approach to the pathophysiology of acute mountain sickness**

   Aim: To assess the predictive relationship between optic nerve sheath diameter and acute mountain sickness using known physiological ramifications of ascent to high altitude.

   Synopsis: The diameter of the optic nerve can be measured non-invasively using ultrasound. There is evidence that the diameter of this nerve changes when pressure within the cranium changes which is known to occur with exposure to hypoxia and especially with altitude illness. A non-invasive measure of intracranial pressure would therefore be very useful in the diagnosis of altitude related illnesses.

5. **Sympathetic function at high altitude: lowlanders versus high altitude natives**

   Aim: To examine the effect of acute and chronic hypoxia on sympathetic activity and neural transduction and to contrast the impact of hypoxia on lowlanders and high altitude natives.

   Synopsis: Sympathetic nervous system (often associated with the fight-or-flight response) is known to increase at altitude, but it is not known if this is the case in people native to high altitude.

6. **Oxidative stress and cerebral blood flow at high-altitude**

   Aim: To examine the role of oxidative stress on cerebrovascular function during acute and chronic hypoxia in humans.

   Synopsis: Antioxidants influence the response of blood vessels throughout the body to hypoxia, but whether this response extends to the cerebral circulation is unknown.

7. **The mechanisms governing oxygen content mediated regulation of cerebral blood flow during acute and chronic hypoxia**

   Aim: To determine the role of oxygen content (CaO_2) versus arterial oxygen tension (PaO_2) in regulating cerebral blood flow (CBF) in acute and chronic hypoxia.

Synopsis: A long-standing question in physiology is whether the partial pressure of oxygen or the total content of oxygen in arterial blood is the stimulus for the myriad physiological responses to hypoxia. This study will utilize the constant lower PaO_2 at altitude while safely manipulating CaO_2 in order to establish which is the crucial physiological stimulus.

8. **Prediction of cerebral blood flow and acute mountain sickness at high altitude: an integrative approach**

   Aim: To predict cerebral blood flow and acute mountain sickness susceptibility utilizing a comprehensive physiological exam.

   Synopsis: Cerebral blood flow increases with ascent to high altitude, but the time course of this increase still remains somewhat ambiguous due to methodological issues in past studies. Moreover, an accurate means of predicting an individual's cerebral blood flow at a given altitude would be very useful medically.

9. **Cerebral vascular flow mediated dilation: sympathetic regulation in hypoxia**

   Aim: To determine the role of flow mediated mechanisms of cerebrovascular regulation in normoxia, hypoxia and during changes in carbon dioxide and if sympathetic nervous transduction is involved in these regulatory processes.

   Synopsis: Cerebral blood flow increases in response to carbon dioxide and hypoxia, a function partly of small arteries within the brain dilating and partly due to localized effects of the blood interacting with the blood vessel wall. This study will partition these mechanisms and test whether the sympathetic nervous system plays and addition role.

10. **Shear stress and the endothelium during acute and chronic hypoxia in humans**

    Aim: To determine whether endothelial function is preserved or worsened by periods of imposed retrograde shear stress during acute and chronic hypoxia

    Synopsis: Blood vessels respond to changes in flow by the action of the thin lining of cells on the inside of the vessel. In certain pathophysiological situations flow can transiently reverse and it is unclear the effect this may have on the vessel's properties.

11. **Adrenergic control of the extracranial arteries in response to a cold presor test and lower-body negative pressure**

Aim: To determine the specific role of alpha- and beta-adrenergic receptors on extracranial blood flow regulation in response to increased sympathetic outflow induced by the cold pressor test and lower-body negative pressure.

Synopsis: Blood vessels respond to changes in sympathetic nervous outflow by activation of alpha- and beta-receptors. However, the contribution of each of these receptors on maintaining blood flow to the brain is currently unclear.

6. WHO CAN PARTICIPATE IN THE STUDY?

Healthy English speaking human volunteers, ages between 18 and 50 years (inclusive), who will be a part of the research team to the Ev-K2-CNR research pyramid, can participate in this study. In total we will have 20 research subjects. All subjects, however, will not complete each study.

7. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You will not be permitted to participate in this study if you are below 18 or above 50 years of age, obese (body mass index greater than 30), diabetic, are taking any medications, have a history of smoking or have a history of pulmonary or cardiovascular disease. The investigators will directly assess such exclusion criteria during your initial laboratory visit. A clinician will screen you for systemic hypertension, obstructive coronary artery disease, or structural heart disease, assessed with resting and exercise ECG and echocardiograms. If you suspect you may be pregnant, or are trying to become pregnant, you should not participate in this study. We will be issuing a pregnancy test to females.

Participants will be excluded from studies if taking any nitrate medications. All members will be carefully screened by an independent clinician for co-morbidities, including sleep disordered breathing, systemic hypertension, obstructive coronary artery disease, or structural heart disease, assessed with resting and exercise ECG, and echocardiograms. They will be excluded if they are obese (body mass index greater than 30 kg•m-2), have a history of smoking, or have poor pulmonary function based on spirometry measurements (i.e. FEV1/FVC ratio less than 0.75).

8. WHAT DOES THE STUDY INVOLVE?

The sea-level studies will be conducted in the Integrative Cardiovascular and Respiratory Laboratory at the University of British Columbia (Room 118, Health Science Centre; Okanagan Campus). The trip from UBC to the Pyramid Laboratory, Khumbu Valley, Nepal, at the base of Mt Everest; (barometric pressure 412 ± 1 mmHg) will be done in two stages. First, the group will fly to Kathmandu (1340 m), where they will stay for 6-7 days. They will then fly to Lukla, located at 2860 m, and trek to the Pyramid Laboratory over 8 days, including two compulsory acclimatization days at 3450 m (day 4) and at 4252 m (day 7). The research team will then spend 3 weeks at the research lab conducting the proposed experiments below.

Overview of the Study

Depending on the studies you volunteer for, your participation in this study will involve between two and six visits to the laboratory at sea level prior to departure to Nepal. The first visit will be to ensure you meet the necessary criteria for participation and will involve medical history, pulmonary function testing (spirometry), ultrasound measurements of your heart and arteries, a short breathing test to familiarize you to the breathing tests where oxygen and carbon dioxide levels in the air you breathe are altered, and an exercise stress test on a bike. This testing session will last 1-2 hours. The next visits will last between 2 and 8 hours, depending on the study. The specific details of each of the measurements that will be performed are detailed below under 'procedures of the study' below.

Procedures of each study

1. Neuromuscular function and adaptation to high altitude.

You will visit the laboratory on four separate occasions for the assessment of neuromuscular function (as described below) while 1) breathing room air; 2) while breathing a hypoxic gas mixture; 3) following acclimatization to 5050m at the Ev-K2-CNR Pyramid Laboratory, Nepal; and 4) while breathing room air and a hypoxic gas mixture two weeks after return to sea-level.

Other than the location (i.e., Kelowna or Nepal) and the gas mixture you will breathe, all other procedures are identical for each laboratory visit, as detailed below:

1. *Quadriceps neuromuscular function*
    a) You will sit in a chair with one leg secured with velcro straps to an "arm" that allows the force you exert against it to be measured. An ultrasound machine will be used to measure the cross-sectional area of your quadriceps muscle. This is painless and safe, using the same technology as that used to visualize an unborn foetus.
    b) You will then extend your leg to your maximum effort and the force generated will be recorded.
    c) A device that measure blood oxygenation – the same technology as that used on your finger in the hospital – will be taped to your leg.
    d) Electrodes will be taped to your leg to measure the electrical activity of your quadriceps. These will be used to stimulate the nerve that controls your quadriceps. A small electrical current will be delivered through these electrodes to induce a contraction of your muscle and the resultant force measured. This current will than be applied constantly for about two minutes in order to fatigue the quadriceps muscle.

2. *Forearm neuromuscular function* – the identical protocol as for Quadriceps Neuromuscular function, above, will be repeated but in your forearm.

3. *Motor Unit Testing*

    a. A single, very brief (one thousandth of a second) magnetic pulse will be applied to the back of your head. This painless pulse causes a brief nerve impulse to your quadriceps muscle in order to measure the maximum force that muscle can generate. This will be repeated once, one minute later.

2. Comparative effects on the pulmonary vasculature of ascent to high altitude in lowlanders and high altitude natives

You will visit the laboratory once at sea level (UBCO campus) to have a series of measurements and a venous blood sample taken from an arm vein (2 ml). These measurements (but not the blood sample) will be repeated at Namche and Periche during the trek from Lukla to the research pyramid, and during the first two days at the research pyramid. During one of these days one additional venous blood sample (2 ml) will be taken from a vein in your arm.

Measurements:

1. While you are lying down heart rate and oxygen saturation will be measured using a pulse oximeter – a device that sits on one finger and shines light through your finger-tip. A cardiac ultrasound probe will be placed on your chest to measure the amount of blood your heart pumps per minute.
2. You will then breathe 100% oxygen through a mask for 30 minutes while heart rate and blood oxygen saturation are continuously measured.
3. Another cardiac ultrasound will be completed.

3. The influence of altitude on postural changes in cerebral blood flow, intrapulmonary shunting and gas exchange

This study will be carried out in three phases. The first phase will be low altitude testing at the University of British Columbia Okanagan (near sea level). The second phase will be performed at Namche (3440m) and the third phase at the Ev-K2-CNR Research Pyramid, Khumbu Valley, Nepal (5050m).
At UBCO you will breathe room air and an hypoxic gas mixture while the measurements described below are taken. In Namche and at the research pyramid you will breathe room air while the measurements described below are taken.
Measurements

1. An ultrasound probe will be placed on your head near your temple and held in place with a head frame that rests comfortably on your head like a hat.
2. You will breathe normally through a mask connected to a computer in order to measure your respiratory function.
3. Heart rate and blood oxygen saturation will be measured by a pulse oximeter that sits on your finger.
4. An ultrasound probe will be placed on your chest to measure your heart and lung function.

5. A thin plastic tube will be inserted into an arm vein using a needle (the needle does not remain in your vein) in order to take a small sample of blood. This tube will be connected to a syringe that contains saline (salt water) agitated with numerous tiny bubbles. A small amount of this saline will be injected into your vein and ultrasound used to see their path through your heart. This procedure does not cause any discomfort and is safe.

4. A non-invasive approach to the pathophysiology of acute mountain sickness

You will visit the laboratory once at sea-level and once per day for the first three days at the research pyramid. You will lay down for five minutes then an ultrasound probe will be placed over your eye to measure the diameter of your optic nerve. This is a painless and safe procedure that is regularly carried out clinically.

5. Sympathetic function at high altitude: lowlanders versus high altitude natives

You will visit the laboratory two times, once at UBCO and once at the pyramid research laboratory. A very thin tungsten needle will be inserted into a nerve on your leg, just below your knee. This allows for the measurement of nervous activity that is travelling to your blood vessels, as this activity changes at altitude. Insertion of this needle causes very slight pain and may sometimes cause a tingling or hot sensation in your foot. The needle will remain in place for the duration of the experiment (less than 2 hours) and be removed after the experiment is complete or at any time you wish should you feel discomfort of any kind.

During the study time you will breath gases mixtures through a mask with different concentrations of oxygen and carbon dioxide, while your blood pressure, heart rate, and cerebral blood flow are measured non-invasively with a cuff wrapped around your finger and an ultrasound probe placed on your neck. These procedures are safe and comfortable and will take approximately 2 hours for each visit.

6. Oxidative stress and cerebral blood flow at high-altitude

You will visit the laboratory on two occasions, once at UBCO and again at the pyramid research laboratory. Two hours prior to your visit you will take an antioxidant pill containing vitamin C, vitamin E, and lipoic acid – these are all found in conventional multi-vitamin supplements and are available at any pharmacy store. After these 2 hours you will lie on your back while a serious of tests are conducted as outlined below:

1. *Brain blood flow measures*: We will non-invasively assess the health and function of your blood vessels in your neck and brain. Non-invasive ultrasound probes will be placed on your neck and head to estimate blood flowing through arteries. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of your heart and blood vessels, and measurement of blood flow through arteries and veins.

2. *Breathing and Cardiovascular Measurements*: We will record breathing, heart rate, and blood pressure parameters. To do this you will breathe through a facemask, will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.

3. *Chemoreflex (breathing) testing*: During this test you will breathe through a facemask while the level of oxygen and carbon dioxide in your air is altered. You will initially breathe room air for 5 minutes after which the amount of carbon dioxide is maintained while the oxygen is decreased for a period of 10 minutes, or seconds, depending on the protocol. During these experiments you will find the need to breathe is increased, which is normal. You will be monitored during these procedures to ensure the level of oxygen and carbon dioxide is safe. We will measure your level of oxygen saturation with a cuff placed over your finger. At any time if you feel discomfort simply removing the facemask and breathing room air will normalize symptoms. In addition, you will breathe an increased level of carbon dioxide. Like breathing low oxygen, increasing the amount of inspired carbon dioxide will increase your desire to breathe deeper and faster, and your heart rate will be increased. This is normal, and symptoms will disappear immediately upon taking out the mouthpiece.

7. The mechanisms governing oxygen content mediated regulation of cerebral blood flow during acute and chronic hypoxia

You will visit the lab once at UBCO and once at the pyramid research laboratory.

Placement of catheters:

Following local anaesthetic (similar to what a dentist would use), we will place one flexible sterile catheter into an artery in your arm; another flexible sterile catheter will be carefully placed into a vein in your neck (only at UBCO, not at the Pyramid Laboratory). There may be a brief burning or stinging sensation on immediate insertion of the local anaesthetic; afterwards you should experience minimum discomfort. Highly experienced physicians (Drs David MacLeod or Myp Sekhon) who are specialists in these techniques will complete all procedures. Strict adherence to full sterile procedures will be followed at all times. The placement of these catheters allows us to sample a small amount of blood (2 ml - around half a teaspoon) at certain points throughout the experiment. You will not experience any pain, or be aware, when we do this. Blood samples will be ultimately analyzed to assess the level of oxygen and carbon dioxide in your blood and also for chemicals which will tell us why blood flow to your brain may be changing. Any remaining blood will be discarded using normal bio-safety procedures at the conclusion of the study.

Blood volume measurement:

You will be seated for 15 minutes before one blood sample is drawn from the forearm to assess baseline concentrations of carbon monoxide in the blood. You will then be

fitted with a nose clip and asked to breathe normally through your mouth for 2 minutes. After this you will exhale maximally and we will place a re-breathing mouthpiece in your mouth. You will then inhale maximally and then hold your breath for 10 seconds. Prior to the maximal inhalation, you will be switched on to the rebreathing apparatus, which will contain a calculated volume of carbon monoxide (based on body weight) and a small reservoir (~3-5L) of 100% oxygen. You will rebreathe from this system for 1 min and 50 seconds, after which the mouthpiece is removed and you will resume breathing room air. A second blood sample (2 ml) will be drawn from the forearm at minute 7 (from the start of the procedure). Your expired carbon monoxide levels (ppm) will also be measured by simply expiring onto an analyzer.

Cardiopulmonary testing

After placement of these catheters and measurement of blood volume is completed, all of the measurements described in *Study 6* above will be repeated.

Haemodilution

During your visit to the UBCO laboratory 20% of your total measured blood volume will be removed in three stages and briefly stored before being returned into the same vein from which it was withdrawn. During each stage an equivalent amount of saline (salt water) will be re-infused so that your blood volume never changes, but where the amount of red blood cells in your circulation decreases. At each stage of blood removal the measures described above will be repeated; in addition small samples (~1-2 mL) of blood will be withdrawn through the catheter in your arm and stored in a tube for subsequent analysis. During your visit to the Pyramid Laboratory no blood will be removed but saline will be infused into a vein in three stages. An intensive care physician will closely monitor you during the entire study and if at any time your heart rate or blood pressure varies too much your blood will be immediately re-infused and the study stopped. If at any time you feel uncomfortable or simply wish to stop the study your blood will be immediately re-infused and the study will be stopped. After the samples of your blood have been analyzed any remaining blood will be destroyed.

8. Prediction of cerebral blood flow and acute mountain sickness at high altitude: an integrative approach

You will visit the UBCO lab first and complete all tests described in Study 6. In addition, fitness will be assessed with an exercise test to exhaustion. This test will commence by 5-minutes of on a treadmill at a pace you select to warm up. You will then commence running at a pace you think you could maintain for one-hour, with the grade of the treadmill increased by 2% every two minutes until you cannot maintain that pace anymore; this test will likely take between 15-30 minutes.

During the ascent to the pyramid lab the *Breathing and cardiovascular measurements* described in Study 6 will replicated in Namche (3500m), Periche (4500m), and at the pyramid during the first 2 days, and during days 14-16.

9. Cerebral vascular flow mediated dilation: sympathetic regulation in hypoxia

You will visit the laboratory once at UBCO and once at the pyramid research laboratory. All tests described in *Study 6* will be carried out while the *Breathing and cardiovascular,* and *cerebrovascular* variables are measured. In addition, you will complete a brief (4 minute) test where you will breath increased levels of carbon dioxide while your cerebral blood flow is measured with ultrasound.

10. Shear stress and the endothelium during acute and chronic hypoxia in humans

You will visit the UBCO laboratory twice, and once at the pyramid research laboratory. The first two visits will comprise a flow mediated dilation test and rhythmic handgrip exercise test (see below) while you breathe either room air or hypoxic air through a max; you will not be told which gas you're breathing on each visit. Blood pressure and heart rate will also be measured during this visit. These tests will be completed following arrival to the pyramid lab while breathing room air.

Flow mediated dilation: This test is done to assess the function of your blood vessels. A blood pressure cuff will be placed around your left forearm, and pumped up to a level that will reduce blood flow for 5-minutes. After 5-minutes the cuff pressure will be released, and using an ultrasound machine, we will monitor the return of blood flow in the arm.

Rhythmic handgrip exercise flow-mediated vasodilation: This test is also done to assess the function of your blood vessels. You will hold a handgrip device and squeeze it in time to an audio and visual cue for six minutes while the blood flow in your arm is measured using an ultrasound machine.

11. Adrenergic control of the extracranial arteries in response to a cold pressor test and lower-body negative pressure

Your participation will involve one visit to the laboratory lasting six hours (please see below). These tests will require you to avoid caffeine, rigorous exercise, and alcohol for 24 hours prior to experimentation. Each participant will take part in protocols.

Respiratory and Cardiovascular Measurements: During all procedures we will record tidal volume (size of breath), respiratory frequency, minute ventilation (how many liters of air you breathe per minute), end-tidal O_2 and CO_2, heart rate, and blood pressure parameters. To do this you will breathe through a mouthpiece with nose clip, will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.

Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels within your brain, and within your neck. Non-invasive ultrasound probes will be placed on the side of your head and on your neck to estimate blood flowing through

arteries. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.

Experimental Protocol: You will breathe through a mouthpiece with nose clip so that we can measure your breathing. You will then rest quietly for 15 minutes to ensure stable baseline recordings of physiological variables. Following a 15-minute baseline period, each subject will undergo two protocols; and these protocols will by repeated within the same day separated by a one-hour rest period.

Cold Pressor Test: You will be instrumented and allowed to relax and breathe normally through a mouthpiece and noseclip for a 10-minute baseline period, while resting supine on a hospital bed.

End-tidal gas control: The partial pressures of oxygen and carbon dioxide in your lungs wil be controlled by manipulating the concentrations of those gases you inspire. You will breath through a mouth piece and instructed to avoid any irregular breathing. After a 45 minute break you will repeat this procedure.

Lower-body Negative Pressure Test: You will be instrumented as above and allowed to relax and breathe normally through a mouthpiece and nose clip. You will lie on your back with your lower body inside of a chamber, the pressure in which can be increased or decreased. After you lie still for 5 minutes for baseline measurements the pressure inside the chamber will be decreased by -10 mmHg every 2 minutes in a step-wise fashion for 10 minutes.

9. WHAT ARE THE POSSIBLE HARMS AND DISCOMFORTS?

A physician will be either on-sight (studies involving arterial or jugular catheters; Drs Macleod and/or Sekhon) or on-call during all experimental sessions should any complications arise. In the unlikely event of any complication, such as cardiac arrest or syncope (fainting), an emergency medical response will be immediately initiated. All investigators are certified to perform cardiopulmonary resuscitation and in the use of an automated external defibrillator and will follow standard emergency protocols. However, complications are very unlikely given the rigorous screening you will first undertake prior to admission to the study.

You are asked to report any unusual symptoms during each of the tests. You can stop any test at any time if you are feeling uncomfortable. Every effort will be made to conduct the tests in such a way to minimize discomfort and risk. Female participants in this study must avoid pregnancy. Failure to do so may result in potential harm to your fetus. You should discuss the issues surrounding this necessity (of not being or becoming pregnant during the course of the study) with your study doctors, and find an acceptable solution that will address this matter.

It must be noted that individual responses to the experimental procedures exist and you are encouraged to report any unusual sensations or symptoms to the investigator. You are permitted to end testing at any time for any reason. If you do experience undesirable symptoms during the experiment, the onsite physician(s) will provide immediate care. In the unlikely event you develop severe illness that cannot be adequately treated at the pyramid emergency evacuation by helicopter to Kathmandu will be immediately organized. All procedures used to collect physiological data will pose no risk to your continued health and well-being. These procedures have been performed around the world since the 1960's. However, the following risks should be considered:

Arterial and jugular catheters: A catheter is a thin plastic tube placed in a blood vessel. Qualified, highly experienced physicians who are specialists in such procedures will make all the arterial and venous catheterizations. These procedures are part of routine clinical care for patients in intensive care, but differ from those you would normally expect to receive in normal clinical care. There is typically brief pain on immediate insertion of the local anaesthetic. Once the needle is in place, the pain should subside. Blood draws through the needles should not be painful, and there should only be minor swelling at the site. At the end of the study, the needle will be withdrawn and a sterile dressing will be applied.

The main risks of the placing this catheter in your neck vein include hematoma (localized blood clotting) formation (6.1%) that is usually minor requiring no specific therapy other than temporary local pressure; infection (0.78%); and pneumothorax and tension pneumothorax (0.2%). These are very rare, and the risk of the latter two are generally considered negligible since the catheter is advanced cephalad, rather than towards the heart (apex of the lung). These risks will be further minimized by; 1) the use of ultrasound to image the jugular vein and enable the precise location of the catheter to be quantified; 2) having two experienced physicians overseeing the experimental procedures and catheterization at all time; 3) all lines will be inserted under strict aseptic techniques. This includes full surgical scrub, the wearing of hat, facemask and sterile gowns and the use of clinically-approved cleaning solutions and sterile towels; 4) risks are also minimized by lines that are shorter (8 cm) than previously used (15 cm) and being left in place for short periods of time (less than 7 hours); 5) as mentioned, oxygen saturation, ECG and blood pressure will be continuously monitored throughout each experimental procedure. Oxygen will be available at all times, as will resuscitation drugs and equipment; and 6) allowing 2-3 hours following completion of protocol and removal of catheterization to fully assess the participant. You will be instructed how to keep the area clean for the day or two following the study.

The most common complications of inserting a small needle into an artery is a small bruise and pain at the site of the needle location, which may last several days after removal of the catheter. Other complications of radial artery canalization include temporary occlusion/spasm, permanent ischemia, pseudoaneurysm, thrombosis, AV fistula, air embolism, compartment syndrome, carpal tunnel syndrome, and median nerve paralysis. These are very rare however, and these risks will be minimized by; 1) the use of ultrasound; 2) having an experienced physician (Dr. Sekhon), and; 3) adhering to strict aseptic techniques. The latter includes full surgical scrub, and the use of chlorohexidine or

iodine-based cleaning solutions and sterile towels. Having lines *in situ* for short periods of time (<5 h) further minimizes risks.

Approximately 20 mL (4 teaspoons) of blood will be withdrawn over the course of the experiment in order to measure arterial blood concentration of oxygen and carbon dioxide. Two mL of blood will be frozen for subsequent analysis of standard markers of metabolism; any remaining blood will be discarded immediately using normal bio-safety procedures. Experienced physicians who have appropriate resources available to manage most complications will supervise experiments. In the unlikely event of an emergency that cannot be adequately managed at the research pyramid, a helicopter evacuation to Kathmandu will be arranged immediately. However, as in our normal operating procedures, we will be largely self-contained: For example, we will have ample amounts of oxygen and advanced resuscitation drugs will be available. All experiments will be supervised by experienced ICU physician Dr Sekhon and Dr MacLeod (senior staff anesthetist). These experienced physicians have carried out >1000 of these procedures with no adverse-effects, including identical interventions at pyramid research laboratory in 2012

Acute Hypoxia at Sea-level: There are few risks associated with mild exposures to high altitude, a condition that will be simulated in this experiment. The level of low oxygen (hypoxia) that you will be exposed to is equal to approximately 3000 – 4600 m. This is approximately equal to being at the summit of Pike's Peak, Colorado. At this level of simulated high altitude you will breathe more quickly and more deeply. You may feel shortness of breath, dizzy or faint, and you may develop a temporary headache. These sensations will go away very quickly when you breathe room air. Your responses to the exposures to low levels of oxygen will be monitored during the test, and the test will be terminated if abnormal responses are observed (not anticipated). There is no risk of developing altitude illness. You may feel discomfort from lying in the same position for two to four hours. These discomforts will be alleviated once the testing is terminated and you are permitted to move around.

Hyper/hypo-capnia (high and low CO_2): There are no risks associated with the mild changes in carbon dioxide (CO_2). You will be asked to increase your breathing until a set (and lower) level of CO_2 has been reached, which may cause lightheadedness or dizziness in which case you will be instructed to breathe normally. You will be closely monitored throughout the protocol, however, in our experience of conducing greater than 2000 of these tests there have been no ill effects reported.

Ultrasound: Ultrasound is non-invasive, painless technique used for measuring blood flow in this study. It poses no risk.

Exercise in normoxia or hypoxia: The American Thoracic Society and the American College of Chest Physician statements associated with cardiopulmonary testing for patients with respiratory and cardiovascular disease (ATS/ACCP Statement on Cardiopulmonary Exercise Testing (2001), AJRCCM, pp. 213) state that the estimated risk of sudden cardiac death in testing in patients being assessed for a variety of medical reasons is stated to be 2

to 5 per 100,000 tests. No prospective study has ever been performed to assess the risk of exercise in any disease, however, this statement comes from a review of all studies that have provided estimates of sudden cardiac death from 100,000s of tests performed in medical centers across the World assessed in patients with high risk of cardiovascular complications to exercise. In general, exercise testing is considered extremely safe in healthy individuals, but risk of sudden cardiac death is higher in people with history of cardiovascular disease. As stated in our exclusion criteria, any participants with cardiovascular or cerebrovascular complications will not be eligible to participate in this study and as such the risk of an adverse event is considered extremely low.

Ascent to high altitude and altitude illness: The planned trek to the laboratory at 5050m is staged over 8 days, including two compulsory acclimatization days at 3450 m (day 4) and at 4252 m (day 7). This is an extremely conservative approach to trekking at high altitude. Members of this research team, on previous research expeditions, have made this ascent 3-5 times before and hence are highly experienced in identifying and treating altitude illness should it occur. Please note that faster ascents rates to basecamp Everest may result in a 30-40% risk of mild acute mountain sickness which is largely manifested in headache and some nausea (West, 2007). The risk of moderate to severe acute mountain sickness is less than 15% (again with faster ascents and no prior administration of acetazolamide to help speed acclimatization). The risk of High Altitude Pulmonary or Cerebral Edema is less than 0.05% (West, 2007). Normally high altitude medications (e.g., acetazolamide, dexamethasone, etc) and oxygen will be available at all times in case of an emergency. The Principal Investigator will also carry a satellite phone in case of the need for an emergency helicopter evacuation back to Kathmandu, the costs for which would be borne by the expedition if evacuation were necessary. Finally, as outlined, participants will have detailed physiological monitoring during their first 48 hours at high altitude; this monitoring will allow for any early detection of any serious AMS complications. Please note that should you need emergency evacuation down the mountain, depending on the severity of the injury, you will be accompanied by one of the team physicians or researchers. You will be flown directly via helicopter to the Kathmandu general hospital. We will make sure you have made full recovery before you travel home.

Blood volume measurement: There have been few, if any, complications for those participating in this test. A small amount of pain may be associated with routine blood sampling from a vein and skin puncture. It is possible that some individuals may experience lightheadedness, fainting, and/or nausea from the blood collections. You may experience localized bruising and swelling at or near the puncture site. You may also experience petechiae (small, non raised red spots on skin) where the tourniquet was applied. Occasionally, some individuals are allergic to the antiseptic used in skin preparation, the glue used in adhesive bandages, or latex. In this case, an alternate antiseptic, paper tape, and non-latex gloves will be used. Carbon monoxide is known to compete strongly for oxygen binding sites in the blood. This attribute is the basis on which the CO-rebreathe technique has been developed. This 'optimised' procedure has been further refined to reduce exposure in terms of the total quantity (~100ml) of carbon monoxide to which you will be exposed. The blood concentration will be minimal (5%), which is related to a small (3%) reduction in your maximal oxygen consumption. The CO

has a short half-life of 90 minutes. This study should not leave you with any long-term adverse effects. Qualified technicians will be present for all the testing sessions to reduce discomfort and monitor your condition.

Haemodilution: Blood volume removal has previously been well tolerated in similar studies in both healthy volunteers and patients. The risks associated with blood transfusion are usually related to receiving blood from someone else and if the blood has been stored for a long time. In this study you will only receive your own blood and the risks of a reaction are very rare. The risks associated with blood volume removal in this study are therefore only related to changes in heart rate and blood pressure. The blood pressure and heart rate will be continuously monitored by the research team and if any significant changes occur the study will be stopped, and you will be given oxygen by facemask and saline and/or your previously removed blood by IV immediately.

Remote ischemic precondition: There is mild initial discomfort associated with the automated occlusion cuffs that will be placed around the upper thigh and inflated to 220 mmHg; however, this discomfort normally disappears within 30 seconds. There is no carry over effect of this initial discomfort, and blood pressure, breathing and heart rate should quickly return to normal.

α_1-adrenoreceptor and β-adrenoreceptor blockers, Prazosin and Propranolol: With oral administration of the drug Prazosin (mini-press), participants may feel what is known as 'first dose response', in which they may feel faint. The prescribed clinical dosage of Prazosin is between 0.5 and 20 mg. For this study, a conservative dosage of 1mg/20kg will be administered. The prescribed clinical dosage of Propranolol is typically around 10-80mg daily. For this study, a conservative dosage of 1mg/kg will be administered. The most common side-effect symptoms of these two drugs include mild diuresis (increased urine production and therefore need to urinate), a mild decrease in blood pressure (hypotension), or feelings of dizziness and sleepiness. After administration of these drugs, participants will remain in supine position to reduce the risk of hypotension. In any case, a researcher will be available to assist the volunteer should begin to feel faint from the hypotensive effect of the drug intervention. The small and acute dose of drug we are using in this study means that any side effects are very short lasting; 2-3 hours. All participants will be continually and closely monitored.

Infusion of vasoactive drugs: With one-time infusion of sodium nitroprusside you may feel short-term light- headedness. A single dose of phenylephrine raises blood pressure to a level similar to that during exercise for ~10-15 minutes. ECG and blood pressure will be continuously monitored, and the test will be terminated if you feel light-headed, nauseated, or experience any other adverse sign or symptom. If a vasovagal reaction occurs, we will lift your legs and administer oxygen in order to facilitate recovery. The possibility of a local or systemic allergic reaction to the drugs is minimal, but exists. If a local reaction occurs (excessive or prolonged redness in the area of the infusion) the test will be stopped and the attending physician will determine the appropriate course of action. There is also a small risk of limb ischemia with phenylephrine infusion, but in healthy people without vascular disease this is extremely unlikely. A physician will be present during all tests, and the laboratory is also equipped with an automatic electronic defibrillator. All laboratory personnel are also certified by the Canadian Red Cross in emergency first aid CPR/AED (Level C).

Magnetic stimulation: There is an extremely small risk (~1 in 50,000) of producing an epileptic fit (abnormal electrical activity in the brain) with magnetic stimulation. There have been fewer than 20 events reported since the technique was developed in 1985 and most of these occurred in patients taking medications that themselves can induce epileptic fits, and some are believed to represent fainting rather than a seizure. All stimulation will comply with published safety guidelines developed in collaboration with the Safety of TMS Consensus Group, which includes clinicians and researchers from around the world. These guidelines set safe standards for the frequency, intensity and duration of stimuli. After large numbers of stimuli some people (fewer than 1 in 20) complain of a mild-headache lasting up to several hours due to scalp muscle contraction. This is unlikely to occur in this experiment because the number of stimuli is small. However, if headache is developed, it can be treated with standard non-prescription medications (e.g. acetaminophen).
Electrical stimulation is achieved with isolated and grounded electric stimulators designed specifically for humans. Some participants perceive these stimuli to be uncomfortable (e.g., a pain rating of 2-4 out of 10) but they are very brief and cause no injury.

Motor unit testing: Measurement of motor unit potentials through needle concentric electrodes has been widely used, and it is considered a safe and extremely valuable technique to study single motor units behavior.

Cold Pressor Test: During the CPT participants may feel slight discomfort and mild pain having their foot submerged in cold-ice water for 120 seconds. These symptoms will alleviate shortly after removal from ice bath

Lower-Body Negative Pressure: LBNP results in a reduction in central blood volume. During LBNP it is possible that participants feel light-headed and nauseas due to a transient decrease in blood pressure. All symptoms will dissipate immediately after LBNP is terminated. Blood pressure and heart rate will be continuously monitored during LBNP to ensure participant safety.

10. WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

You will not directly benefit from this study. However, you will gain information regarding your physiological makeup, including the structure and function of vessels that feed your brain, and your unique tolerance to heat stress.

11. WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of your information collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data will not be able to be withdrawn for example where the data is no longer identifiable (meaning it cannot be linked in any way back to your identity) or where the data has been merged with other data. If you would like to request the withdrawal of your data, please let your study doctor know.

12. AFTER THE STUDY IS FINISHED

The tests performed in this study are not intended to be diagnostic and are not performed under diagnostic conditions. However, if any medical issue (incidental finding) is presumed, you will be notified. You will be recommended to contact your medical doctor, and we will provide you with a written letter detailing our observations. If the information is thought to be serious by the research physician (Drs. Anholm, Subedhi, Sekhon or Dr. MacLeod), we will follow the emergency procedure (see below), which may involve contacting emergency service and transporting you to the emergency department at Kelowna General Hospital (for sea level testing) or Norvic International Hospital (for high altitude testing).

13. WHAT WILL THE STUDY COST ME?

You will not be paid for participation in this study. Transportation to the pyramid research laboratory from Kelowna will be covered.

14. WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator, Health Canada, UBC Clinical Research Ethics Board, and the Natural Sciences and Engineering Research Council of Canada (NSERC; the funding agency) for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a subject in this study. Only this number will be used on any research-related information collected about you during the course of this study, so that your identity [i.e. your name or any other information that could identify you such as your Personal Health number] as a subject in this study will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected and also give you the right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

A trained research assistant will be available on every occasion to explain the procedure and answer any questions.

Disclosure of Race/Ethnicity: Studies involving humans now routinely collect information on race and ethnic origin as well as other characteristics of individuals because these characteristics may influence how people respond to different medications. Providing information on your race or ethnic origin is voluntary.

15. WHAT HAPPENS IF SOMETHING GOES WRONG?

By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided on-site by one of the expedition intensivists (James Anholm, Myp Sekhon, Prajan Subedhi). If you require evacuation and further medical care the expedition and University of British Columbia Okanagan will pay for any costs associated with your medical treatment that are not covered by travel insurance.

In an event of a medical emergency, Drs. MacLeod, Subedhi, Sekhon, and Anholm will guide the research team.

In the event of emergency during sea level testing:

- The individual present with the highest level of medical training will guide the research team – this will very likely be one of the critical care physicians listed above, unless the medical emergency takes place outside of an experimental session.
- A member of the research team will dial 911 on the laboratory phone and contact emergency services for their help.

- A second member of the research team will dial campus security and summon a university-designated first aid to attendant the research laboratory. The first aid dispatch is ~1min walk to the research laboratory. These first aid attendants are equipped with a Level 2 kit including oxygen and an automated external defibrillator, and can assist with interim treatment while waiting for emergency services. They will also facilitate transport of emergency services to the building.
- The research laboratory is 15-20 minutes away from the emergency department at Kelowna General Hospital, and one of the research team will accompany the participant at all times

In the event of emergency during high altitude testing:

- The high altitude physicians will provide acute care (Drs Sekhon, Anhome, and/or Subedhi). All first aid amenities, including an automated external defibrillator, will be on site. The physicians will also be equipped with a standard emergency room crash cart (including epinephrine, oxygen etc.).

- If required a helicopter can reach the Ev-K2-CNR research pyramid within 20 minutes of contact. Because the research pyramid also acts as the air traffic control center for the Khumbu valley numerous forms of communication are always maintained (VHS radio, satellite phone, cellular phone, satellite internet). The patient would be immediately transported by air in the company of one of the high altitude physicians to Norvic International hospital in Kathmandu to receive treatment.

16. WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If you have any questions or desire further information about this study before or during participation, or if you experience any adverse effects, you can contact Dr. Phil Ainslie at 001-250-807-8089 or Dr. Kami Sherpa (+977 38 540053, +977 38 540113) In the event of a research related injury post the experimental testing, please speak to your doctor and contact the Dr. Phil Ainslie about the event on the above number.

17. WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?

If you have any concerns or complaints about your rights as a research subject and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598).

18. SUBJECT CONSENT TO PARTICIPATE

Mechanisms of acute adaptation and evolution in the human physiological response to high-altitude: a scientific expedition to the Nepal Himalaya

In signing this form you are consenting to participate in this research project. Furthermore, signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

- *I have read and understood the subject information and consent form.*
- *I have had sufficient time to consider the information provided and to ask for advice if necessary.*
- *I have had the opportunity to ask questions and have had satisfactory responses to my questions.*
- *I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.*
- *I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.*
- *I understand that I am not waiving any of my legal rights as a result of signing this consent form.*
- *I understand that there is no guarantee that this study will provide any benefits to me*
- *I have read this form and I freely consent to participate in this study.*
- *I have been told that I will receive a dated and signed copy of this form.*
- *I understand the procedure that will be followed in an event of a medical emergency*

SIGNATURES

_____

Printed name of subject Signature Date

_____

Printed name of witness Signature Date

_____

Printed name of principal investigator/
designated representative Signature Date

Appendix III: Sample "Day Of" Sheet and checklists for protocol

Participant ID:
Date:
Time:
Location:

SYMPAL Participant Information Form

Daily Pressure (mmHg) _____, Temperature: _____, Age -

_____,

Date of birth_____, Times since last meal_____,

Size of last meal_____, Gum Y / N

What day did you last start menstruation:_____, Altitude Dweller: High / Low

---

Y / N Have you abstained from caffeine for the past 12 hours, if not how long

_____

Y / N Have you abstained from alcohol for the past 12 hours, if not how long

_____

Y / N Have you abstained from strenous exercise for the past 12 hours, if not how long

_____

Forms Filled Out:

Y / N Health History Questionnaire

Y / N Consent Form

Y / N PSQI

Computer: _____

Ultrasound: _____

Drug Insertion: _____

SNA: _____

Tonometry: _____

Bloods/ Catheter:_____

PRE- BASELINE:

BL SpO_2: _____

MANUAL BP			

RTF:_____

_____

_____

BAROREFLEX TEST:

Sodium Nitroprusside dosage (µg) :_____ SNP Dosage/ Blood Volume: _____

Phenylephrine dosage (µg) :_____ PE Dosage/ Blood Volume:_____

Comments:

_____

_____

METABOREFLEX TEST:

MVC (N)_____ MVC 30%_____

Finger Poke Time: 1st _____ 2nd _____

Comments:

_____

_____

CHEMOREFLEX TEST:

High Altitude: Yes/ No

(IF HIGH ALTITUDE) ORDER OF PROTOCOL

Hyperoxia (~300 Torr) :_____ Normoxia (~160 Torr) :_____

	MEASUREMENT (CM)
Sternum – Finger	
Sternum – Brachial	

MSNA:

Nerve:

_____

Number of Sites:

_____

Total Search Time:

_____

Quality:

Sternum – Toe	
Sternum- Carotid	
Waist	
Hips	
Height	
Weight	

FINAL Comments:

Appendix IV: Health History Questionnaire used for Lowlanders

STUDY: **Neurovascular Regulation at Altitude: Low-Landers vs. High-Landers**
SUBJECT ID #:
RESEARCHER INITIALS: **DATE:**

Date of Birth: _____/_____/_____ Height: _____ Weight: _____

Ethnic Background: _____ **Family Physician: _____**

ALL PARTICIPANTS

Please check **any and all** that apply	**Personal History**	**Family History**
Stroke	□	□
Hypertension	□	□
Heart Attack	□	□
Heart Murmur	□	□
Blood clots	□	□
Other cardiovascular disorders (please specify)	□	□

_____2

	Personal History	**Family History**
Type I Diabetes	□	□
Type II Diabetes	□	□
Obesity	□	□
Other metabolic disorders (please specify)	□	□

_____

	Personal History	**Family History**
Asthma	□	□
Sleep Apnea	□	□
COPD	□	□
Other respiratory/breathing disorders (please specify)	□	□

_____

	Personal History	**Family History**
Alzheimers	□	□
Cognitive impairment	□	□
Parkinsons	□	□
ALS (Lou Gerhigs Disease)	□	□
Seizures	□	□
Other neurological disorders (please specify)	□	□

_____

	Yes	**No**
Any other major surgery, illness or injury not listed above? (If yes, please Specify)	□	□

_____

	Yes	**No**	**Unknown**
Were you born pre-mature (before 37wks)	□	□	□

	Yes	**No**
Do you smoke? (If yes, how many cigarettes per day?)	□	□

_____

(If you have quit, how long since your last cigarette?)

_____

	Yes	**No**
Have you ever fainted before? (If yes, under what circumstances?)	□	□

_____

	Yes	**No**
Are you currently taking any medications? (If yes, please list medications)	□	□

_____

_____

_____

STUDY: Neurovascular Regulation at Altitude: Low-Landers vs. High-Landers
SUBJECT ID #:
RESEARCHER INITIALS: **DATE:**

What have your **eating habits** been like in the past **month**? Check all that apply:

- □ One meal per day, specify when _____
- □ Two meals per day, specify when _____
- □ Three meals per day
- □ Snack(s) every day, specify when _____
- □ Special diet, please specify name _____
- □ Trying to follow Canada's Food Guide to Healthy Eating
- □ Other nutrition plan, please specify _____

What was your pattern of **physical activity** in the past **month**?

Type of Physical Activity	**Frequency**	Average **Duration** of your exercise sessions	**Intensity** (light, moderate or strenuous)	**Location** (home, outdoors, gym, etc.)
	_____ time(s) per week	_____ minutes		
	_____ time(s) per week	_____ minutes		
	_____ time(s) per week	_____ minutes		
	_____ time(s) per week	_____ minutes		

DEFINITIONS:

Light Intensity (minimal effort; e.g. yoga, easy walking, golf, bowling, stretching).

Moderate Intensity (not exhausting; e.g. fast walking, baseball, tennis, easy bicycling)

Strenuous Intensity (heart beats rapidly; e.g. running, jogging, vigorous swimming, vigorous long distance cycling).

During a typical **7-Day period** (a week) in the past **year**, in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

- ❍ often
- ❍ sometimes
- ❍ never/rarely

STUDY: **Neurovascular Regulation at Altitude: Low-Landers vs. High-Landers**
SUBJECT ID #:
RESEARCHER INITIALS: **DATE:**

WOMEN

Please check **any and all** that apply

	Yes	**No**
Are you post-menopausal?	□	□
(If not, how long since the first day of your last period?)		

_____

	Yes	**No**
Are you on hormone replacement therapy?	□	□

	Yes	**No**
Are you currently using oral contraceptives?	□	□
(If yes, what is the brand?)		

_____

	Yes	**No**
Are you pregnant?	□	□
(If yes, how many weeks?)		

_____

	Yes	**No**
Have you been pregnant previously?	□	□
(If yes, please indicate the number of previous pregnancies. Were there any complications, including pregnancy related hypertension, gestational diabetes, or pre-eclampsia?)		

_____

_____

Do you have any other health concerns you think we should be aware of?

_____

_____

Appendix V: Protocol Description with Computer Commands

(Used at Low and High Altitude for Both Groups)

Protocol	Time	Action	Comp. Comment
EQUIPMENT SETUP			
Equipment Setup	~5-10 min	-ECG, Photoplethysmography (Finapres) , Occlusion Cuff, zero handgrip transducer -Ensure forms filled during this period (PSQI, HH, Day-of), - Consent form signed	
CALIBRATION	~5-10 min	-CALIBRATE: (GASES, ULTRASOUND, 100%MVC, HYPOXIA MIX 37% O_2=355m) -Done in between equipment set up	☐ (MVC 100%) ☐ (GAS CAL ##%O2/##%CO2)
MSNA Search	~45-90 min	-Search for MSNA site -Remainder of setup	☐ (SNA START SEARCH) ☐ (SITE ###)
After MSNA	~5-10 min	-3 Manual Blood Pressures	☐ (MANUAL BP #: ###/###)
		- RTF cal (after 2nd manual BP)	☐ (MANUAL BP: ###/###) ☐ (RTF: ###/###)
PRE PROTOCOL			
Normal Baseline	~10 min	-Epiphan capturing of brachial during this point **(after 5 min normal bl)** - After recording of ultrasound begins allow for 2 min of recording	☐ (NORMAL BL) ☐ (RECORD START) ☐ (RECORD TIME ##:##:##) ☐ (RECORD END)
MAXIMAL END EXPIRATORY APNEA			
Baseline	~1 min		☐ (EE BH BASELINE)
End Expiratory Apnea	~	-Perform maximal apnea at end expiration (FRC) -**Marker starts once BH begins**	☐ (BH START)
Recover	~3 min	- Marker placed once persons resumes breathing	☐ (BH RECOVERY)
CONTINUE THROUGH REMAINDER OF PROTOCOLS within study…			

Appendix VI: Raw Data Tables from Sympathetic Reactivity Protocol in Sherpa and Lowlanders at Low and High Altitude

Table 1. Lowlander R-R Interval of each beat from the last 10 cardiac cycles during breath holding at low altitude. Values represented in seconds, with a sum of the calculated R-R Intervals in column 13.

Participant ID	Cardiac Cycles Prior To Volitional Breakpoint											SUM 10 cycles (sec.)
	Beat -10	Beat -9	Beat -8	Beat -7	Beat -6	Beat -5	Beat -4	Beat -3	Beat -2	Beat -1	Beat -0	
2	1.23	1.21	1.22	1.24	1.25	1.28	1.27	1.30	1.23	1.07	0.98	**13.28**
3	0.74	0.79	0.76	0.69	0.73	0.72	0.68	0.76	0.75	0.81	0.69	**8.12**
4	0.72	0.72	0.71	0.71	0.72	0.74	0.76	0.77	0.81	0.82	0.88	**8.35**
5	1.14	1.12	1.11	1.07	1.01	1.05	0.90	0.86	1.15	1.12	1.74	**12.27**
6	1.55	1.56	1.56	1.57	1.59	1.59	1.55	1.53	1.64	2.12	2.68	**18.93**
7	1.12	1.11	1.10	1.00	1.06	1.22	1.18	1.15	1.16	1.18	1.10	**12.39**
12	1.82	1.82	1.80	1.60	1.72	1.75	1.60	1.67	1.49	0.99	0.83	**17.09**
13	0.80	0.79	0.83	0.81	0.75	0.84	0.81	0.84	0.78	0.76	0.71	**8.71**
16	1.03	0.97	1.03	1.10	1.17	1.19	1.31	1.28	1.29	1.82	1.14	**13.34**
18	0.61	0.64	0.62	0.65	0.64	0.65	0.65	0.67	0.72	0.70	0.60	**7.15**
20	1.00	0.88	0.86	0.92	0.95	0.89	0.95	0.88	1.04	0.87	0.74	**9.99**
22	1.09	1.16	1.09	1.07	1.01	1.08	1.08	1.12	0.83	0.78	0.87	**11.18**
23	0.72	0.92	0.93	0.94	1.35	0.88	1.26	1.06	1.33	1.11	0.96	**11.46**
25	1.15	1.13	1.08	1.15	1.11	1.27	1.16	1.17	1.17	1.17	1.06	**12.62**

Table 2. Lowlander R-R Interval of each beat from the last 10 cardiac cycles during breath holding at high altitude. Values represented in seconds, with a sum of the calculated R-R Intervals in column 13.

Participant ID	Cardiac Cycles Prior To Volitional Breakpoint											SUM 10 cycles (sec.)
	Beat -10	Beat -9	Beat -8	Beat -7	Beat -6	Beat -5	Beat -4	Beat -3	Beat -2	Beat -1	Beat -0	
2	1.23	1.20	1.20	1.19	1.26	1.34	1.45	1.47	1.45	1.43	1.40	**14.63**
3	0.59	0.60	0.62	0.63	0.68	0.71	0.75	0.81	1.14	2.12	1.29	**9.93**
4	0.98	0.97	0.94	0.89	0.87	0.87	0.88	0.90	0.98	3.83	3.15	**15.26**
5	0.88	0.92	0.98	0.82	0.95	0.97	0.80	0.78	1.04	1.77	0.90	**10.82**
6	0.79	0.80	1.34	1.40	1.39	1.38	1.37	1.40	1.57	2.98	1.77	**16.20**
7	1.13	1.14	1.15	1.16	1.15	1.20	1.26	1.30	1.53	2.03	1.17	**14.21**
12	1.17	1.22	1.31	1.56	1.81	1.83	2.01	1.88	1.85	1.82	1.67	**18.13**
13	0.91	0.92	0.95	1.00	0.94	1.04	0.97	0.89	0.87	0.88	0.65	**10.02**
16	0.92	0.94	0.93	0.91	0.91	0.93	0.98	1.09	1.31	3.88	1.16	**13.97**
18	0.91	0.88	0.86	0.88	0.92	0.98	1.07	1.12	1.40	1.10	0.84	**10.96**
20	0.62	0.65	0.69	0.79	0.93	2.23	1.61	1.34	1.25	1.20	1.12	**12.43**
22	0.76	0.78	0.79	0.82	0.91	0.85	1.14	1.17	1.06	0.80	0.77	**9.85**
23	0.79	0.80	0.83	0.85	0.81	1.17	2.17	1.71	1.61	1.45	1.33	**13.52**
25	0.76	0.73	0.71	0.71	0.80	0.94	1.06	1.25	1.87	2.52	1.85	**13.20**

Table 3. Sherpa R-R Intervals of each beat from the last 10 cardiac cycles during breath holding at high altitude. Values represented in seconds, with a sum of the calculated R-R Intervals in column 13.

Participant ID	Cardiac Cycles Prior To Volitional Breakpoint											SUM 10 cycles (sec.)
	Beat -10	Beat -9	Beat -8	Beat -7	Beat -6	Beat -5	Beat -4	Beat -3	Beat -2	Beat -1	Beat -0	
101	0.96	0.93	0.92	0.89	0.85	0.83	0.86	0.85	0.85	0.84	0.85	**9.63**
103	0.90	0.90	0.90	0.90	0.90	0.87	0.84	0.85	0.88	0.83	0.76	**9.55**
104	0.86	0.86	0.85	0.85	0.87	0.86	0.87	0.84	0.78	0.74	0.74	**9.13**
108	0.87	0.90	1.01	0.95	0.98	1.03	1.10	1.14	1.18	1.19	0.71	**11.05**
110	0.83	0.86	0.86	0.90	0.97	0.98	1.06	0.89	0.95	0.87	1.02	**10.19**
111	0.89	0.88	0.87	0.88	0.88	0.88	0.88	0.86	0.85	0.83	0.82	**9.53**
112	0.69	0.71	0.70	0.71	0.68	0.67	0.67	0.66	0.67	0.64	0.64	**7.45**
114	0.99	0.99	0.98	0.97	0.96	0.95	0.93	0.91	0.90	0.88	0.83	**10.30**

Table 4. Lowlander burst integral area from each beat of the last 10 cardiac cycles during breath holding at low altitude. Values represented in arbitrary units (au), with a sum of the integral areas in column 13. Cycles with (0.00 au) represent no burst observed during that respective heartbeat.

Participant ID	Cardiac Cycles Prior To Volitional Breakpoint											SUM 10 cycles (au)
	Beat -10	Beat -9	Beat -8	Beat -7	Beat -6	Beat -5	Beat -4	Beat -3	Beat -2	Beat -1	Beat -0	
2	0.51	1.28	0.41	0.61	1.53	0.14	0.72	0.58	1.70	1.12	1.47	**10.06**
3	0.00	0.70	2.98	2.64	1.46	1.49	2.61	2.92	2.67	4.20	3.76	**25.42**
4	0.71	1.32	0.84	0.00	0.90	1.22	1.52	0.65	1.95	1.10	4.15	**14.35**
5	0.09	0.00	0.16	0.81	0.00	0.00	0.25	0.71	0.00	0.82	1.06	**3.90**
6	0.00	0.00	0.05	0.00	0.34	0.03	0.93	0.69	0.00	3.13	11.71	**16.87**
7	0.39	0.29	0.00	0.00	0.00	0.31	1.07	1.00	0.87	1.20	0.68	**5.81**
12	0.00	1.28	1.46	1.67	1.78	2.16	2.35	3.38	3.02	4.21	2.61	**23.92**
13	2.29	3.44	1.11	0.00	2.52	1.79	0.91	1.03	2.06	1.35	4.18	**20.69**
16	0.65	0.00	0.00	0.00	0.26	2.59	3.26	7.03	7.27	7.16	14.51	**42.74**
18	0.66	1.43	0.00	1.44	0.00	1.22	1.36	1.39	0.00	0.93	1.51	**9.93**
20	2.81	5.06	6.61	3.78	2.43	5.14	3.87	5.51	4.93	5.98	5.09	**51.20**
22	2.51	1.11	0.00	0.00	4.07	2.12	3.73	2.64	3.96	0.00	4.21	**24.35**
23	1.00	1.67	2.94	2.04	2.89	3.87	2.89	5.08	3.32	4.69	4.26	**34.64**
25	10.65	8.60	4.65	7.37	5.77	10.11	8.81	15.45	13.04	13.05	7.20	**104.72**

Table 5. Lowlander burst integral area from each beat of the last 10 cardiac cycles during breath holding at high altitude. Values represented in arbitrary units (au), with a sum of the integral areas in column 13. Cycles with (0.00 au) represent no burst observed during that respective heartbeat.

Participant ID	Cardiac Cycles Prior To Volitional Breakpoint											SUM 10 cycles (au)
	Beat -10	Beat -9	Beat -8	Beat -7	Beat -6	Beat -5	Beat -4	Beat -3	Beat -2	Beat -1	Beat -0	
2	0.82	0.97	0.79	0.75	0.76	1.10	1.36	1.88	3.06	6.39	7.96	**25.83**
3	0.00	0.90	0.00	0.80	0.92	1.71	1.40	2.00	2.72	4.74	7.29	**22.47**
4	0.26	0.70	0.35	0.21	0.63	0.58	0.85	1.13	2.22	20.82	5.14	**32.88**
5	0.16	0.25	0.00	0.00	0.17	1.13	0.40	0.67	0.73	1.81	5.74	**11.07**
6	0.00	0.00	1.28	2.77	0.00	3.17	2.19	2.50	4.84	9.44	17.36	**43.54**
7	1.78	1.20	1.33	3.73	3.04	1.75	4.59	5.26	7.63	11.80	13.24	**55.36**
12	0.68	2.19	1.80	2.75	4.70	7.66	9.21	6.74	3.26	12.43	3.45	**54.87**
13	8.99	4.23	4.24	4.93	6.42	6.81	7.56	6.99	6.09	6.33	6.33	**68.93**
16	0.00	1.08	0.00	0.90	0.00	0.00	1.30	3.27	4.30	8.50	29.71	**49.06**
18	1.31	0.79	0.77	0.57	1.11	0.75	1.54	1.94	2.73	3.48	0.23	**15.24**
20	0.20	0.67	0.61	0.86	1.17	2.15	3.34	11.92	8.39	*6.39*	*5.52*	**41.21**
22	0.96	0.68	0.41	1.13	1.20	1.40	1.77	2.07	3.69	2.90	0.46	**16.68**
23	0.90	0.92	1.24	1.38	1.13	1.85	5.83	20.50	15.18	13.00	*11.09*	**73.03**
25	1.40	0.00	1.14	0.00	0.00	0.00	0.96	2.83	3.86	6.05	2.86	**19.09**

Table 6. Sherpa burst integral area from each beat of the last 10 cardiac cycles during breath holding at high altitude. Values represented in arbitrary units (au), with a sum of the integral areas in column 13. Cycles with (0.00 au) represent no burst observed during that respective heartbeat.

Participant ID	Cardiac Cycles Prior To Volitional Breakpoint											SUM 10 cycles (au)
	Beat -10	Beat -9	Beat -8	Beat -7	Beat -6	Beat -5	Beat -4	Beat -3	Beat -2	Beat -1	Beat -0	
101	0.19	1.26	1.66	0.43	0.47	1.04	0.00	0.00	1.03	1.25	1.80	**9.13**
103	1.08	0.62	0.42	0.33	0.74	0.63	0.81	0.56	0.43	1.01	1.10	**7.73**
104	2.33	0.59	2.96	1.47	4.36	3.43	5.05	3.92	4.92	5.72	4.97	**39.72**
108	0.64	0.00	1.02	1.07	0.00	0.37	1.73	1.74	1.84	2.15	1.32	**11.88**
110	0.28	0.39	0.00	0.87	1.13	1.20	1.52	0.94	0.00	0.32	1.51	**8.17**
111	0.00	0.19	0.30	0.19	0.53	0.71	0.57	1.07	0.60	0.96	1.64	**6.76**
112	1.07	0.38	0.89	0.77	0.00	0.34	1.34	2.08	1.06	0.00	0.00	**7.93**
114	0.46	0.39	0.23	0.00	0.24	0.20	0.49	0.46	0.57	0.40	0.47	**3.92**

Table 7. Quantification of total normalized sympathetic activity (au/min) in Lowlanders at low altitude. Sum of RRI, sum of integral area, and total normalized SNA values calculated for baseline and reactivity protocols (see tables 1-6 for raw data of RR- intervals and integral area during apnea). Baseline integral area and RR interval sums analyzed from 15 cardiac cycles at approximately 1 minute prior to breath hold protocol. * Difference in total normalized SNA is between baseline and reactivity protocol. N/O** represent baseline integral area not obtained (n=1) due to high MSNA signal noise.

Participant ID	Baseline Sum Int. Area (15 cycles) (au)	Baseline Sum RRI (15 Cycles) (sec.)	Baseline Normalized Total SNA (au/min)	BH Sum Int. Area (10 cycles) (au)	BH Sum RRI (10 Cycles) (sec.)	Normalized BH Total SNA (au/min)	Abolute Difference* Normalized Total SNA (Δ au/min)	% Difference * Total SNA
2	4.94	15.03	19.7	10.06	13.28	45.4	25.7	230
3	3.33	11.05	18.1	25.42	8.12	187.8	169.7	1039
4	2.20	8.02	16.5	14.35	8.35	103.1	86.7	626
5	0.59	10.78	3.3	3.90	12.27	19.1	15.8	584
6	2.20	15.77	8.4	16.87	18.93	53.5	45.1	638
7	N/O**	N/O**	N/O**	N/O**	N/O**	N/O**	N/O**	N/O**
12	5.37	30.18	10.7	23.92	17.09	84.0	73.3	786
13	3.05	27.06	6.8	20.69	8.71	142.5	135.8	2107
16	2.41	18.27	7.9	42.74	13.34	192.3	184.3	2425
18	4.57	13.24	20.7	9.93	7.15	83.4	62.7	403
20	1.55	24.46	3.8	51.20	9.99	307.6	303.8	8081
22	4.95	21.21	14.0	24.35	11.18	130.7	116.7	934
23	2.47	29.69	5.0	34.64	11.46	181.3	176.3	3625
25	10.29	21.64	28.5	104.72	12.62	498.0	469.5	1745

Table 8. Quantification of total normalized sympathetic activity (au/min) in Lowlanders at high altitude. Sum of RRI, sum of integral area, and total normalized SNA values calculated for baseline and reactivity protocols (see tables 1-6 for raw data of RR- intervals and integral area during apnea). Baseline integral area and RR interval sums analyzed from 15 cardiac cycles at approximately 1 minute prior to breath hold protocol. * Difference in total normalized SNA is between baseline and reactivity protocol.

Participant ID	Baseline Sum Int. Area (15 cycles) (au)	Baseline Sum RRI (15 Cycles) (sec.)	Baseline Normalized Total SNA (au/min)	BH Sum Int. Area (10 cycles) (au)	BH Sum RRI (10 Cycles) (sec.)	Normalized BH Total SNA (au/min)	Abolute Difference* Normalized Total SNA (Δ au/min)	% Difference * Total SNA
2	7.44	16.45	27.1	25.8	14.63	105.9	78.8	390
3	2.77	8.03	20.7	22.5	9.93	135.8	115.1	656
4	4.34	25.89	10.1	32.9	15.26	129.3	119.2	1285
5	3.15	11.21	16.8	11.1	10.82	61.4	44.5	364
6	16.45	14.16	69.7	43.5	16.20	161.3	91.6	231
7	16.45	14.33	68.9	55.4	14.21	233.7	164.8	339
12	15.46	14.53	63.9	54.9	18.13	181.5	117.7	284
13	31.27	13.58	138.2	68.9	10.02	412.7	274.6	299
16	13.20	10.78	73.5	49.1	13.97	210.7	137.2	287
18	8.01	18.94	25.4	15.2	10.96	83.4	58.1	329
20	1.35	18.28	4.4	41.2	12.43	198.9	194.5	4500
22	5.11	16.33	18.8	16.7	9.85	101.6	82.8	541
23	14.07	17.57	48.1	73.0	13.52	324.2	276.1	674
25	5.45	21.35	15.3	19.1	13.20	86.8	71.4	566

Table 9. Quantification of total normalized sympathetic activity (au/min) in Sherpa at high altitude. Sum of RRI, sum of integral area, and total normalized SNA values calculated for baseline and reactivity protocols (see tables 1-6 for raw data of RR- intervals and integral area during apnea). Baseline integral area and RR interval sums analyzed from 15 cardiac cycles at approximately 1 minute prior to breath hold protocol. * Difference in total normalized SNA is between baseline and reactivity protocol.

Participant ID	Baseline Sum Int. Area (15 cycles) (au)	Baseline Sum RRI (15 Cycles) (sec.)	Baseline Normalized Total SNA (au/min)	BH Sum Int. Area (10 cycles) (au)	BH Sum RRI (10 Cycles) (sec.)	Normalized BH Total SNA (au/min)	Abolute Difference* Normalized Total SNA (Δ au/min)	% Difference * Total SNA
101	0.94	26.60	2.1	9.13	9.63	56.9	54.7	2681
103	4.09	29.40	8.3	7.73	9.55	48.6	40.2	582
104	33.02	23.43	84.6	39.72	9.13	261.1	176.5	309
108	8.20	29.13	16.9	11.88	11.05	64.5	47.6	382
110	3.65	22.60	9.7	8.17	10.19	48.1	38.4	496
111	4.01	25.80	9.3	6.76	9.53	42.5	33.2	456
112	5.44	18.19	17.9	7.93	7.45	63.9	45.9	356
114	1.71	20.06	5.1	3.92	10.30	22.8	17.7	446