DOCTOR OF PHILOSOPHY

Sympathetic Nervous System Activity and Autonomic Control of Resting Blood Pressure at High Altitude, in Lowlanders and Highland Natives

Simpson, Lydia

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Sympathetic Nervous System Activity and Autonomic Control of Resting Blood Pressure at High Altitude, in Lowlanders and Highland Natives

Lydia Simpson BSc, MSc

Submitted in partial satisfaction of the requirements for the Degree of Doctor of Philosophy

School of Sport, Health and Exercise Sciences, Bangor University, UK, June 2020

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ABBREVIATIONS

Ach: *acetylcholine*

ANS: *autonomic nervous system*

ATP: *adenosine triphosphate*

au: *arbitrary units*

au·min^{-1}: *arbitrary units per min*

au·100HB^{-1}: *arbitrary units per 100 heartbeats*

AV node*: atrioventricular node*

BP: *blood pressure*

bpm: *beats per minute*

Bursts·min^{-1}: *bursts per minute*

Bursts·100HB^{-1}: *bursts per 100 heartbeats*

CaO_2: *arterial oxygen content*

CMS: *chronic mountain sickness*

CVLM: *caudal ventrolateral medulla*

DBP: *diastolic blood pressure*

ECG: *electrocardiogram*

EPO: *erythropoietin*

FiO_2: *fractional inspired oxygen*

[Hb]: *haemoglobin concentration*

Hct *haematocrit*

HPV: hypoxic pulmonary vasoconstriction

HR: *heart rate*

IML: *intermediolateral cell column*

iNO: *inhaled nitric oxide*

LBNP: *lower body negative pressure*

LBPP: *lower body positive pressure*

MAP: *mean arterial pressure*

MSNA: *muscle sympathetic nerve activity*

NA: *noradrenaline*

NO: *nitric oxide*

NTS: *nucleus tractus solitarius*

PO_2: *partial pressure of oxygen*

$PaCO_2$: *arterial oxygen partial pressure*

PaO_2: *arterial oxygen partial pressure*

PASP: *Pulmonary artery systolic pressure*

PE: *phenylephrine*

PVN: *paraventricular nucleus*

Qc: *cardiac output*

RBC: *red blood cell*

RRI: *R-R interval*

RVLM: *rostral ventrolateral medulla*

SA node*: sinoatrial node*

SaO_2: *arterial oxygen saturation*

SNS: *sympathetic nervous system*

SpO_2: *peripheral oxygen saturation*

SBP: *systolic blood pressure*

SNP: *sodium nitroprusside*

TPR: *total peripheral resistance*

α: *alph*a

β*: beta*

ABSTRACT

The main aim of this thesis is to characterise sympathetic neural activity and autonomic regulation of BP at high altitude, in Lowlanders during acclimatisation, and in highland native populations, who have adapted to high altitude hypoxia over generations. To address this aim, resting sympathetic neural activity to the skeletal muscle vasculature (MSNA) was assessed, via microneurography, and baroreflex control of MSNA (vascular sympathetic baroreflex) and baroreflex control of the heart (cardiovagal baroreflex) was assessed using the modified Oxford test. Experimental study 1 examines sympathetic neural activity and arterial baroreflex function in Lowlanders following 10–20 days at 5050 m and compares them to Nepalese Sherpa. Experimental study 2 examines sympathetic neural activity and arterial baroreflex function in Andean Quechua who have developed the maladaptation syndrome chronic mountain sickness (CMS), and compares them to healthy Andean Quechua. Experimental study 1 and 2 also examine the mechanistic contribution of the peripheral chemoreflex to sympathetic neural activity and arterial baroreflex function in both Lowlanders and highland native populations. Experimental study 3 aims to extend these findings by investigating the previously unexplored mechanistic role of pressure-sensitive receptors in the pulmonary arteries in Lowlanders at high altitude. The major findings of this thesis are 1) heightened sympathetic neural activity is a feature of high altitude exposure in both acclimatising Lowlanders and highland natives, compared to Lowlanders at low altitude. Nepalese Sherpa, however, appear to have adapted to favour lower basal sympathetic neural activity, compared to Lowlanders and Andean Quechua at high altitude. Thus, divergent pathways of physiological adaptation between highland populations extend to autonomic regulation of resting arterial pressure. Despite heightened sympathetic neural activity, the responsiveness of the sympathetic nervous system to acute fluctuations in BP (i.e. vascular sympathetic

baroreflex gain) is well preserved at high altitude. The vascular sympathetic baroreflex is reset to a higher MSNA set-point in all populations, compared to Lowlanders at low altitude. Such resetting permits elevated basal sympathetic vasomotor activity that allows normal resting arterial pressure to be maintained in the face of hypoxic local vasodilation, and potentially altered vascular sensitivity. Despite maintained vascular sympathetic baroreflex gain, the responsiveness of the cardiovagal baroreflex is depressed in both Lowlanders and healthy highlanders at high altitude. This thesis also demonstrates that the peripheral chemoreflex does not play a major role in sympathoexcitation and vascular sympathetic baroreflex resetting at high altitude in either acclimatising Lowlanders or highland natives. Moreover, this thesis demonstrates, for the first time, that hypoxia induced elevations in pulmonary arterial pressure contribute to the sympathoexcitation and baroreflex resetting during acclimatisation to high altitude in Lowlanders.

CHAPTER 1. INTRODUCTION

INTRODUCTION

It is estimated that 140 million people reside permanently at high altitude (>3000 m) with more than 100 million individuals travelling to high altitude each year (Burtscher, 1999; Basnyat, 2014). These figures are only expected to rise due to the increasing accessibility and economic growth of these mountainous regions. Ascent to high altitude presents many challenges to the human body; however, the greatest physiological challenge is ambient hypoxia (West, 2007). Atmospheric pressure progressively falls with ascent to altitude, which lowers the partial pressure of oxygen (Bert, 1878). Less oxygen in the inspired air reduces the amount of oxygen diffusing into the blood stream, and thus reduces the amount of oxygen available to the cells (Hurtado, 1964). A constant supply of oxygen is required by the cells for energy producing metabolism in the mitochondria and is required to maintain cellular function.

Despite the significant challenge to oxygen homeostasis, humans native to low altitude demonstrate a remarkable ability to cope with, and survive exposure to, extremely high altitudes, for example to the summit of Mount Everest without supplemental oxygen. This is due to the process of acclimatisation, which involves numerous respiratory, cardiovascular and hematological adjustments (West, 2007), which collectively attempt to maintain oxygen delivery. These physiological adjustments include profound changes in autonomic nervous system activation (ANS) (Hainsworth et al., 2007).

At high altitude, there is a decrease in parasympathetic outflow to the heart and an increase in sympathetic outflow to the vasculature, altering autonomic regulation of the cardiovascular system (Hansen & Sander, 2003; Siebenmann, et al., 2017). Autonomic outflow to the heart and vasculature influences heart rate (HR), myocardial contractility and vascular resistance; thus, is a primary regulator of blood pressure (BP). The arterial baroreflex is one

of the body's major BP control mechanisms, regulating arterial BP around a set-point via its modulation of efferent autonomic activity on a beat-by-beat basis. Appropriate BP homeostasis is required to ensure adequate organ perfusion and oxygen delivery, whilst simultaneously preventing over perfusion and end organ damage (Fagard & Cornelissen, 2007). Nevertheless, autonomic regulation of BP during high altitude acclimatisation is poorly characterised.

Indigenous populations of the Andean Altiplano of South America, the Tibetan plateau of Asia, and the Ethiopian highlands of East Africa have been exposed to the stress of chronic hypoxia for millennia (Aldenderfer, 2003). Thus, these populations have been exposed to the opportunity for natural selection, which has given rise to distinct cardiorespiratory, haematological and metabolic adaptations (Gilbert-Kawai et al., 2014; Bigham, 2016; Horscroft et al., 2017; Moore, 2017) to try and offset ambient hypoxia. Collectively, such physiological adaptations have allowed these populations to survive and thrive at high altitude, and demonstrate remarkable hypoxia tolerance. However, despite exposure to the same stress of chronic hypoxia, these geographically distinct populations have developed different physiological adaptations to achieve successful oxygen delivery (Beall, 2006, 2007; Moore, 2017). It is unknown whether differences in adaptation patterns extend to autonomic regulation of the cardiovascular system.

Whilst most highlanders demonstrate excellent adaptation to their environment, a small percentage of individuals (5-10%), particularly those native to the Andean plateau, cannot adapt to high altitude (León-Velarde et al., 2005; Villafuerte & Corante, 2016). These individuals develop maladaptation syndrome chronic mountain sickness (CMS), which is characterised by an excessive haematological response to ambient hypoxia (i.e. excessive

erythrocytosis) and often accompanied by accentuated arterial hypoxemia. CMS places significant strain on the cardiovascular system, and increases cardiovascular disease risk (Corante et al., 2018). Nevertheless, whether CMS is accompanied by dysregulated autonomic control of the cardiovascular system is yet to be determined.

Therefore, the overall aim of this thesis is to comprehensively assess sympathetic neural system activity and arterial baroreflex control of the heart and vasculature at high altitude, in acclimatising Lowlanders and highland natives. To address this aim, three experimental studies were completed as part of two high altitude research expeditions, the first to the Ev-K2-CNR Research Facility (5050 m; Khumbu Valley, Nepal) in October 2016, and the second to the Universidad Peruana Cayetano Heredia's Instituto de Investigacions de Altura (4380 m; Cerro de Pasco, Peru) in July 2018. Experimental study 1 (Chapter 4) aims to examine and compare sympathetic neural activity and arterial baroreflex control of BP in Lowlanders during acclimatisation to high altitude to Lowlanders at low altitude, and compare acclimatising Lowlanders to highland native Nepalese Sherpa. Experimental study 2 (Chapter 5) aims to examine and compare sympathetic neural activity and arterial baroreflex control of BP in Andean natives with CMS to healthy Andean highlanders. Experimental studies 1, 2, and 3 (Chapter 4, 5 & 6) aim to examine the neural mechanisms involved in autonomic cardiovascular control at high altitude.

This thesis is structured as follows:

- Chapter 2 – Literature review
- Chapter 3 – General methodology
- Chapter 4 – Experimental study 1: Baroreflex control of sympathetic vasomotor activity and resting arterial pressure at high altitude: insight from Lowlanders and Sherpa

- Chapter 5 – Experimental study 2: Andean highlanders, chronic mountain sickness and the integrative regulation of resting blood pressure
- Chapter 6 – Experimental study 3: Evidence for a physiological role of pulmonary arterial baroreceptors in sympathetic neural activation in healthy Lowlanders at high altitude
- Chapter 7 – General discussion

All published abstracts (n=4) specifically relating to this thesis are included in appendix III.

CHAPTER 2: LITERATURE REVIEW

LITERATURE REVIEW

2.1 Arterial blood pressure

Mean arterial pressure (MAP) is the major regulated variable of the cardiovascular system and is tightly controlled to maintain appropriate perfusion and adequate oxygen delivery to vital organs. Impaired regulation of blood pressure (BP) can, therefore, have important pathophysiological consequences. Low BP can cause inadequate blood flow and oxygen delivery to peripheral tissues and potentially compromise cellular and organ system function (Wehrwein & Joyner, 2013). Conversely, an increase in BP or BP variability may cause end-organ damage (Kannel et al., 1998; Bussy et al., 2000; Verdecchia et al., 2003; Fagard & Cornelissen, 2007; Cuspidi et al., 2015a; Cuspidi et al., 2015b) that increases cardiovascular disease risk, including stroke, myocardial infarction and coronary and hypertensive heart disease. MAP is determined by both cardiac output (Qc) and total peripheral resistance (TPR), and is the distension pressure exerted on the systemic arteries. Qc is determined by myocardial contractility, end diastolic volume (i.e. stroke volume) and heart rate (HR), with end diastolic volume determined by venous blood volume and venous smooth muscle tone (i.e. venous pressure) (Guyenet, 2006). On the other hand, TPR is largely determined by arterial vessel diameter, and to a lesser extent changes in blood viscosity, as outlined by Poiseuille's Law. Arterial diameter is dependent on the balance of vasodilator and vasoconstrictor factors acting on the vascular smooth muscle. The efferent activity of the sympathetic and parasympathetic branches of the autonomic nervous system (ANS) modulate HR, myocardial contractility, TPR and thus arterial pressure (Guyenet, 2006).

2.1 Autonomic regulation of arterial blood pressure

Parasympathetic outflow to the heart originates from the parasympathetic preganglion neurons located in the nucleus ambiguous and the dorsal motor nucleus within the medulla oblongata. The preganglionic neurons project, via the vagus nerve, to the peripherally located parasympathetic ganglia of the heart. The preganglionic neurons release acetylcholine (ACh), which binds to nicotinic receptors on the postganglionic neurons. Increased parasympathetic activity causes the release of acetylcholine from the nerve terminals of postganglionic parasympathetic fibers, which acts on muscarinic receptors located on the 'pacemaker' cells of the sino-atrial (SA) and atrioventricular (AV) node of the heart (Brodde et al., 2001). Increased parasympathetic activity serves to reduce the spontaneous rate of SA and AV node depolarisation, reducing depolarisation rate of cardiac myocytes and consequently lengthening RR interval (RRI) (Brodde et al., 2001). Conversely, withdrawal of parasympathetic activity increases the rate of SA and AV node depolarisation, shortening RRI (Drew & Sinoway, 2012). The parasympathetic branch of the ANS influences RRI, and therefore HR, and is essential in the regulation of Qc.

Sympathetic outflow originates from the sympathetic preganglion neurons in the rostral ventrolateral medulla (RVLM) and project via the intermediolateral cell column (IML) of the spinal cord. Pre-ganglionic neurons synapse in the paravertebral and prevertebral ganglia and release neurotransmitter Ach, which binds to nicotinic receptors on post-ganglionic neurons that project through the peripheral nerves to innervate target organs. In response to increased sympathetic nerve activity (SNA), postganglionic sympathetic fibres release noradrenaline (NA) as the primary neurotransmitter, which acts on α1- and α2-adrenergic (e.g. in blood vessels) or β1- and β2- adrenergic receptors (e.g. on the heart) (Herring & Paterson, 2018). Sympathetic nervous system (SNS) innervation of the heart

includes innervation of both the SA and AV node and the myocardium, which modulates RRI and myocardial contractility, and thus influences Qc (Brodde et al., 2001). In addition, the SNS also innervates the arterial and venous vasculature. SNS innervation of the arterial vasculature is a major contributor to TPR and, venous SNS innervation modulates end diastolic volume, and thus stroke volume (Herring & Paterson, 2018).

Vascular sympathetic nerves release NA, which binds to post junctional α and β-adrenergic receptors located on vascular smooth muscle cells. NA binding to α-adrenergic receptors results in vasoconstriction, and NA binding to β-adrenergic receptors results in vasodilation (Macarthur et al., 2011). The greater sensitivity of α-adrenergic receptors to NA means that its release largely stimulates contraction of smooth muscle and vasoconstriction. In addition to NA, co-transmitters neuropeptide Y and adenosine tri phosphate (ATP) are released by vascular sympathetic nerves, which independently elicit vasoconstriction, but also act pre-junctionally to modulate the release of NA and/or potentiate its vasoconstrictor effect (Macarthur et al., 2011). Greater sympathetic outflow leads to greater release of NA from the sympathetic nerve fibres; however, the background level of sympathetic nerve activity also determines the release of co-transmitters. ATP is co-released with NA in response to lower levels of sympathetic nerve activity, and NYP is released with NA under conditions of high sympathetic outflow (Burnstock, 2008, 2011). Importantly, the sympathetic nerves innervating the vasculature display tonic activity, which sets a background level of vasoconstriction and vascular tone; thus, reducing sympathetic vasomotor outflow causes vasodilation. Overall, both the parasympathetic and sympathetic branches of the ANS are important in the regulation of Qc, via regulation of HR and stroke volume, and the sympathetic branch is fundamental to the regulation of TPR. Together, these neural pathways maintain MAP around an appropriate level on a beat-by-beat basis.

2.2 Assessment of the autonomic nervous system in humans

Functional assessment of autonomic control of the heart and peripheral vasculature is critical for understanding arterial BP control in humans. The following section outlines the primary methods employed to assess the ANS in humans.

2.2.1 Measurement of circulating noradrenaline concentrations

Analysis of circulating plasma NA concentrations is an indirect method commonly employed to estimate global SNS activity. In healthy adults, 20% of the NA released from sympathetic nerve fibres "spills over" into the plasma and a proportional relationship between plasma NA concentrations and direct measures of sympathetic nerve activity is observed at rest (Wallin et al., 1981). Plasma NA concentrations are, however, the net effect of not only NA release but also clearance, via re-uptake, metabolism and excretion (Esler et al., 1988). Blood flow through the tissue from which plasma is sampled and total plasma volume also influences NA concentrations (Esler et al., 1988). Notably, these factors complicate the interpretation of plasma NA concentrations during altitude exposure due to increased NA clearance and altered regional blood flow at high altitude (Leuenberger et al., 1991). Moreover, if applied at high altitude, the well-described reduction in plasma volume (Siebenmann et al., 2013; Ryan et al., 2014) would serve to increase plasma NA concentrations, independent of its release. In addition, plasma NA concentrations provide no information regarding the control of sympathetic outflow to different organs, and thus provide little information regarding autonomic control of the heart and vasculature independently.

2.2.2 Measurement of noradrenaline spillover

To overcome some of the limitations associated with analysis of plasma NA concentrations, Murray Esler and colleagues (1984) pioneered the NA spillover technique, which allows for quantification of the rate by which NA is released into the plasma. This is performed by infusing titrated radiolabelled NA and measuring the arteriovenous NA difference across the organ (Esler et al., 1984). This technique allows the assessment of both whole-body NA spillover and regional NA spillover from visceral nerves of specific organs. Therefore, the NA spillover technique provides information of sympathetic nerve activity to both the heart and vasculature. Despite the advantages, NA spillover is an invasive technique requiring central venous catheterisation and clinical expertise. Furthermore, this technique lacks significant temporal resolution and, thus, cannot be used to investigate autonomic reflex control on a beat-by-beat basis, including arterial baroreflex control of BP.

2.2.3 Microneurography

The development of the microneurographic technique by Hagbarth and Vallbo in the late 1960's, allowed for direct, beat-by-beat measurement of sympathetic nerve activity directed towards the cutaneous vasculature (SSNA) and skeletal muscle vasculature (MSNA) (Hagbarth & Vallbo, 1968). In this review, the focus will be on MSNA, as the skeletal muscle represents the body's largest vascular bed, and therefore plays a pivotal role in BP regulation, and haemodynamic stability, due to its influence on TPR (Wallin & Charkoudian, 2007).

Microneurography involves the percutaneous insertion of a tungsten microelectrode into a nerve bundle in a superficial nerve (most commonly the peroneal, median, or radial nerves). The tungsten microelectrode allows action potentials within efferent post-ganglion sympathetic neurons, which innervate the vascular smooth muscle within skeletal muscle, to

be recorded. These recordings can be made from individual sympathetic neurons, referred to as single-unit MSNA; however are more commonly recorded from multiple sympathetic post-ganglion neurons simultaneously, referred to as multi-unit MSNA. This thesis exclusively focuses on the measurement of multi-unit MSNA, shown in *Figure 1*.

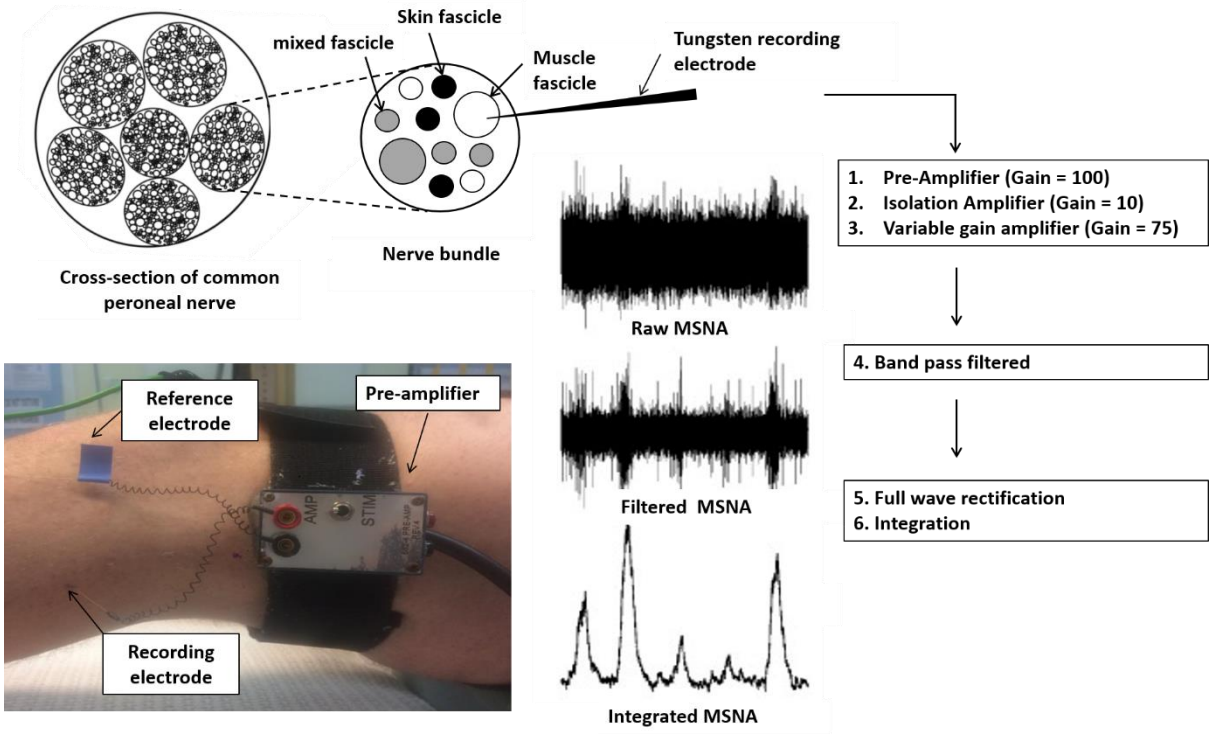

Figure 1. Assessment of muscle sympathetic nerve activity via microneurography.

A high impedance tungsten recording electrode (200 µm diameter) is inserted through the skin into a muscle fascicle of a peripheral nerve (peroneal nerve shown here). A reference electrode, which filters out background noise, is also inserted beneath the skin, 1-3cm from the recording site. The obtained raw nerve signal is amplified, band pass filtered, rectified, and integrated to produce 'bursts' of sympathetic nerve activity, which can be quantified, according to both their occurrence and amplitude.

Each 'spike,' in the raw MSNA trace represents an action potential within an efferent post-ganglionic sympathetic neuron of the peripheral nerve. However, due to the inherently low signal-to-noise ratio, the raw nerve signal is typically integrated, to produce 'bursts' of sympathetic nerve activity, which allow MSNA to be easily quantified. Each burst represents

a synchronized discharge of action potentials from a large number of efferent post-ganglionic sympathetic neurons, which results in the release of NA, in addition to other co-transmitters, from sympathetic nerve fibre terminals, leading to vasoconstriction of the vessel (Burnstock, 2008, 2011). An increase in sympathetic nerve firing increases the occurrence and/or the amplitude of bursts. Assessment of MSNA via microneurography demonstrates a high intra-individual reproducibility, where a similar resting MSNA is observed within individuals when assessed three weeks to 21 months apart (Fagius & Wallin, 1993).

Integrated MSNA burst quantification

MSNA can be quantified as four indices, i) burst frequency (bursts·min^{-1}), ii) burst incidence (bursts·100HB^{-1}), iii) burst amplitude (a.u), iv) total activity (a.u·min^{-1}) and v) total MSNA (a.u·100HB^{-1}) as described in *Figure 2*.

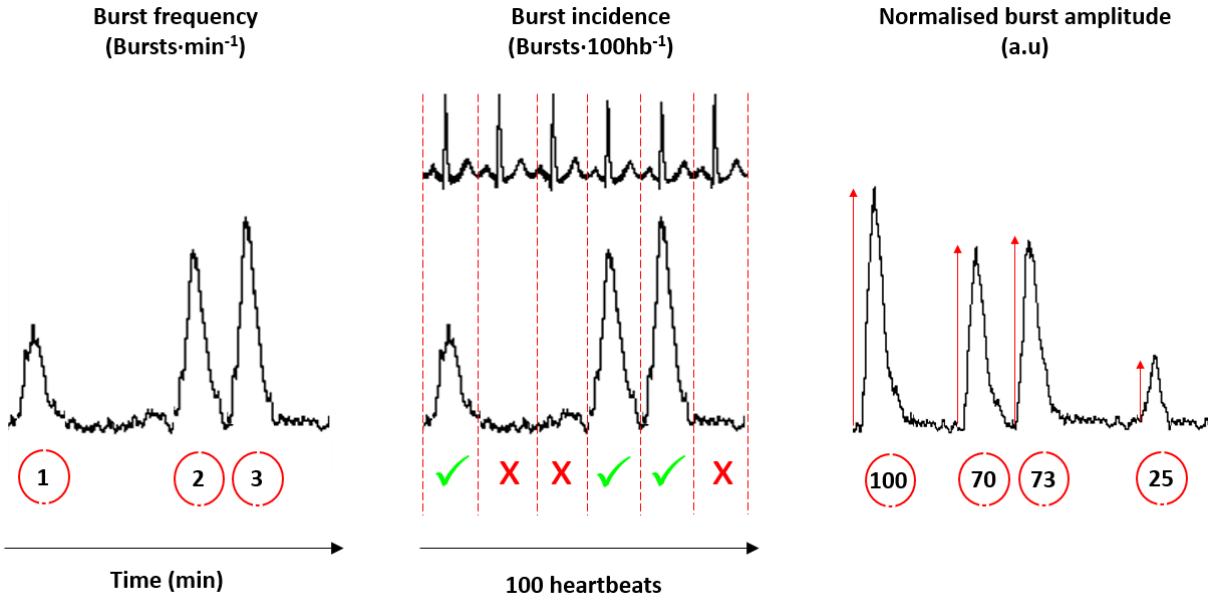

Figure 2. Quantification of muscle sympathetic nerve activity.

MSNA bursts can be expressed as the number of bursts occurring over a minute i.e. MSNA burst frequency (Bursts·min^{-1}), and expressed independent of time, as the number of bursts per hundred heartbeats i.e. MSNA burst incidence (Bursts·100HB^{-1}). MSNA bursts can also be expressed relative to their amplitude. Absolute burst amplitude (mv) is an indication of both the number and size of sympathetic neurons that are firing action potentials. However, burst amplitude is also dependent on the proximity of the microelectrode to the post ganglionic sympathetic neurons it is recording from (Steinback et al., 2010). Therefore, burst amplitude data is normalized to the largest burst observed during baseline (Hart et al., 2017; White et al., 2015). Normalized burst amplitude (a.u) can then be used to calculate total activity (average burst amplitude x burst frequency [a.u·min^{-1}]) and total MSNA (average burst amplitude x burst incidence [a.u·100HB^{-1}]).

The method of MSNA burst quantification provides different neurophysiological information into regulation of MSNA. Burst frequency (bursts·min^{-1}), normalized burst amplitude (a.u) and, total activity (a.u·min^{-1}) are reflective of the neurotransmitter release, and thus the sympathetic vasoconstrictor drive that the vasculature is exposed to in a given time (Wallin et al., 1992; Notarius et al., 2015). Therefore, burst frequency and total activity are an index of sympathetic vasomotor outflow, which determines vascular tone. In contrast, burst incidence represents the probability of a burst occurring per cardiac cycle, independent of time and HR. Therefore, MSNA burst incidence and total MSNA are reflective of central reflex control of sympathetic outflow and is an index of the baroreflex gating of sympathetic bursts.

2.2.4 Techniques to assess autonomic control of the heart

Microneurography allows direct assessment of sympathetic nerve activity to the skeletal muscle vasculature; however, sympathetic and parasympathetic nerve activity to the heart cannot be easily directly measured due to the inaccessibility of the cardiac autonomic efferents. Whilst recent advancements suggest this might be possible in the future (Ottaviani et al., 2020), other methods are commonly employed to assess autonomic control of the heart; including pharmacological autonomic blockade (Katona et al., 1977), assessment of heart rate variability (HRV) (Malik, 1996) and reflex mediated changes in RRI to determine cardiovagal responsiveness (Smyth et al., 1969). These methods will be briefly discussed here, but have been extensively reviewed elsewhere (Chapleau & Sabharwal, 2011).

Pharmacological autonomic blockade of the parasympathetic control of the heart (using muscarinic receptor antagonist atropine), alone or in combination with blockade of sympathetic control of the heart (using non selective β adrenergic receptor antagonist

propranolol), has provided valuable information regarding the contributions of the two branches of the autonomic nervous system to HR, both at rest and during physiological stressors including hypoxic exposure (Koller et al., 1988; Boushel et al., 2001; Siebenmann, et al., 2017). However, similarly to the assessment of plasma NA concentrations and NA spillover, autonomic blockade does not provide significant temporal resolution of autonomic outflow and, therefore, cannot be used to investigate autonomic reflex control of BP on a beat-by-beat basis.

HRV is also often used as an indirect and non-invasive measure of cardiac autonomic control, via power spectral analysis of the beat-by-beat variability in RRI. Briefly, this technique separates a series of continuous heart beats into its frequency components, typically identifying RRIs within the low frequency range (0.04–0.15 Hz) and high frequency range (0.15–0.4Hz). It is suggested that low frequency (LF) power represents cardiac sympathetic activity, high frequency (HF) power represents cardiac parasympathetic activity and the ratio of LF to HF power represents "sympathovagal balance" (Pagani et al., 1986). However, the validity of indices of HRV as markers of cardiac autonomic control has been widely questioned (Eckberg, 1997; Houle & Billman, 1999; Billman, 2013). Indeed, HRV is based on several assumptions that can oversimplify autonomic control of the heart, including that reciprocal changes in cardiac parasympathetic and sympathetic nerve activity occur. Furthermore, from autonomic blockade studies, LF and HF power cannot be solely attributed to changes in cardiac sympathetic and parasympathetic outflow respectively (Houle & Billman, 1999; Taylor et al., 2001). In addition, studies have argued that HRV is primarily dependent on prevailing HR rather than representing cardiac autonomic regulation (Monfredi et al., 2010).

The focus of this thesis, regarding assessment of autonomic control of the heart, is the RRI response to fluctuations in BP. The RRI response to alterations in BP predominantly reflects parasympathetic modulation of the heart by the arterial baroreflex (cardiovagal baroreflex) (Pickering et al., 1971; Scher et al., 1972; Sagawa, 2011) and can be evaluated from the relationship between beat-by-beat systolic blood pressure (SBP) and RRI across a range of pressures. A greater RRI responsiveness to a given change in BP indicates greater autonomic control of the heart.

2.3 Central regulation of autonomic outflow

The integration of converging inhibitory and excitatory inputs from peripheral reflex pathways, descending neural inputs, central modulators, and circulating factors determine efferent autonomic outflow to the heart and peripheral vasculature, which serve to regulate BP (*Figure 3*).

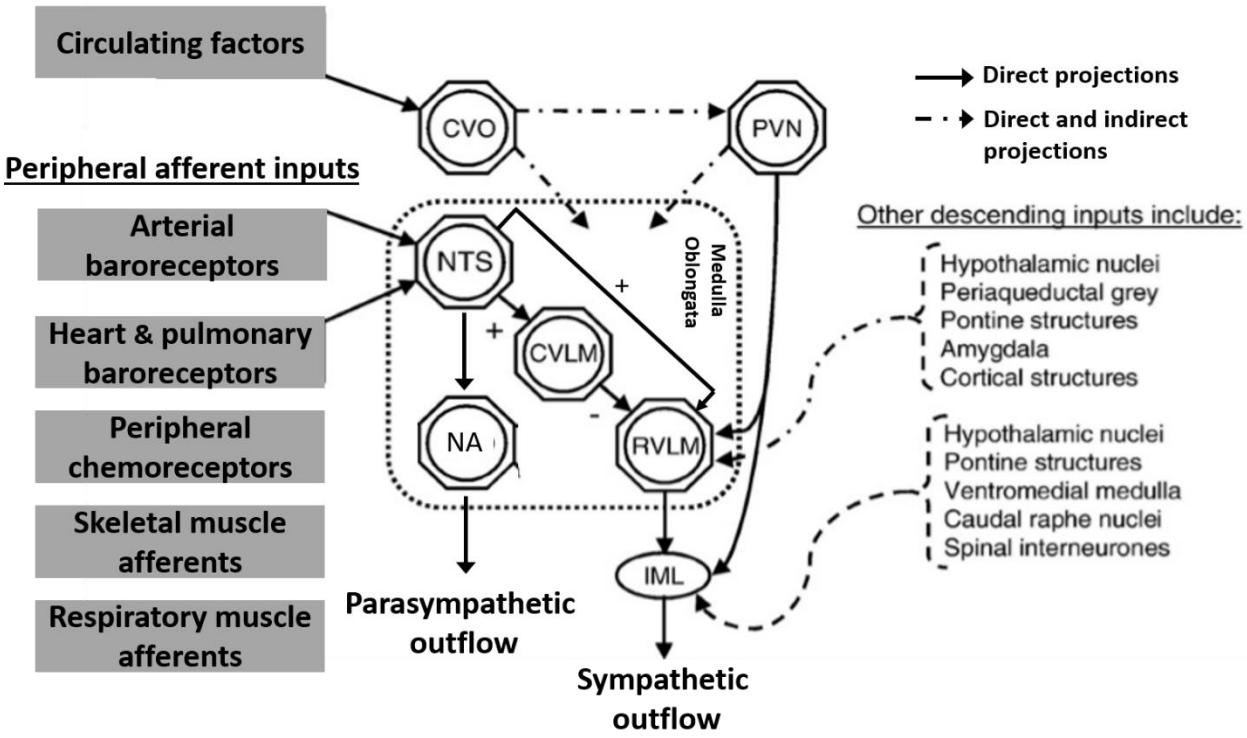

Figure 3. *Central regulation of autonomic outflow*.

Autonomic outflow is the product of central integration of numerous inputs to the cardiovascular control centres in the medulla oblongata. These include inputs from peripheral afferents that terminate in the NTS and project to the rostral ventrolateral medulla (RVLM), either directly or via the caudal ventrolateral medulla (CVLM), and project to the nucleus ambiguous (NA). The RVLM provides a direct excitatory drive to the sympathetic preganglionic neurons in the IML, determining sympathetic outflow. Conversely, the NA provides the excitatory drive to the parasympathetic preganglionic neurons, determining parasympathetic outflow. Descending neural inputs from higher brain structures (pons, hypothalamus, amygdala) and the paraventricular hypothalamic nucleus (PVN), and activity of central modulators (Ang II, central nitric oxide, reactive oxygen species), also influence sympathetic outflow by providing direct excitatory drive to the RVLM and IML. The circumventricular organs (CVO), which lack a blood brain barrier, also provide an additional means by which circulating factors, including Ang II, can influence autonomic outflow. Collectively, the integration of these components determines efferent sympathetic and

parasympathetic outflow to the heart and peripheral vasculature, which regulate arterial pressure as part of the arterial baroreflex circuitry. There are separate descending outputs from the RVLM, which regulates the sympathetic outflow to the heart and blood vessels, and blood vessels in different regions. This allows for a differentiated control of the sympathetic outflow. Adapted from (Fisher et al., 2009). + indicates an excitatory input and – indicates an inhibitory input.

2.3.1 The arterial baroreflex

The arterial baroreflex is the primary beat-by-beat controller of resting arterial pressure. The arterial baroreflex is a negative feedback mechanism that regulates arterial pressure around a set-point, via regulation of autonomic outflow to the heart (i.e. cardiovagal limb of the baroreflex) and vasculature (i.e. vascular sympathetic limb of the baroreflex). Baroreceptors located in the carotid sinus and aortic arch sense mechanical distension of the arterial wall, via mechanically activated ion channels PIEZO1 and PIEZO2 (Zeng et al., 2018). An increase in vessel wall distension increases baroreceptor afferent firing, which is relayed to the cardiovascular control centre in the nucleus tractus solitarius (NTS) of the medulla oblongata, via the glossopharyngeal and vagus nerves (cranial nerves IX and X) (Dampney et al., 2003; Dampney, 2016) (*Figure 4*). Increased baroreceptor afferent input to the NTS reflexively increases parasympathetic outflow to the heart and inhibits sympathetic outflow to the heart, resulting in a lengthening of the RRI and a decrease in myocardial contractility, and thus reduces Qc. Increased baroreceptor afferent input also reflexively inhibits sympathetic outflow to the arterial and venous vasculature. Decreased sympathetic outflow to the arterioles elicits vasodilation, reducing TPR, whereas decreased sympathetic outflow to the veins reduces venous return, end diastolic volume, and thus stroke volume (Thomas, 2011). Conversely, during short-term decreases in arterial pressure parasympathetic outflow to the heart decreases and sympathetic inhibition is removed. The resulting alterations in Qc and TPR help to return arterial pressure back to the arterial baroreflex set-point. The neural pathways involved in the central integration of baroreceptor signals are illustrated in *Figure 4*.

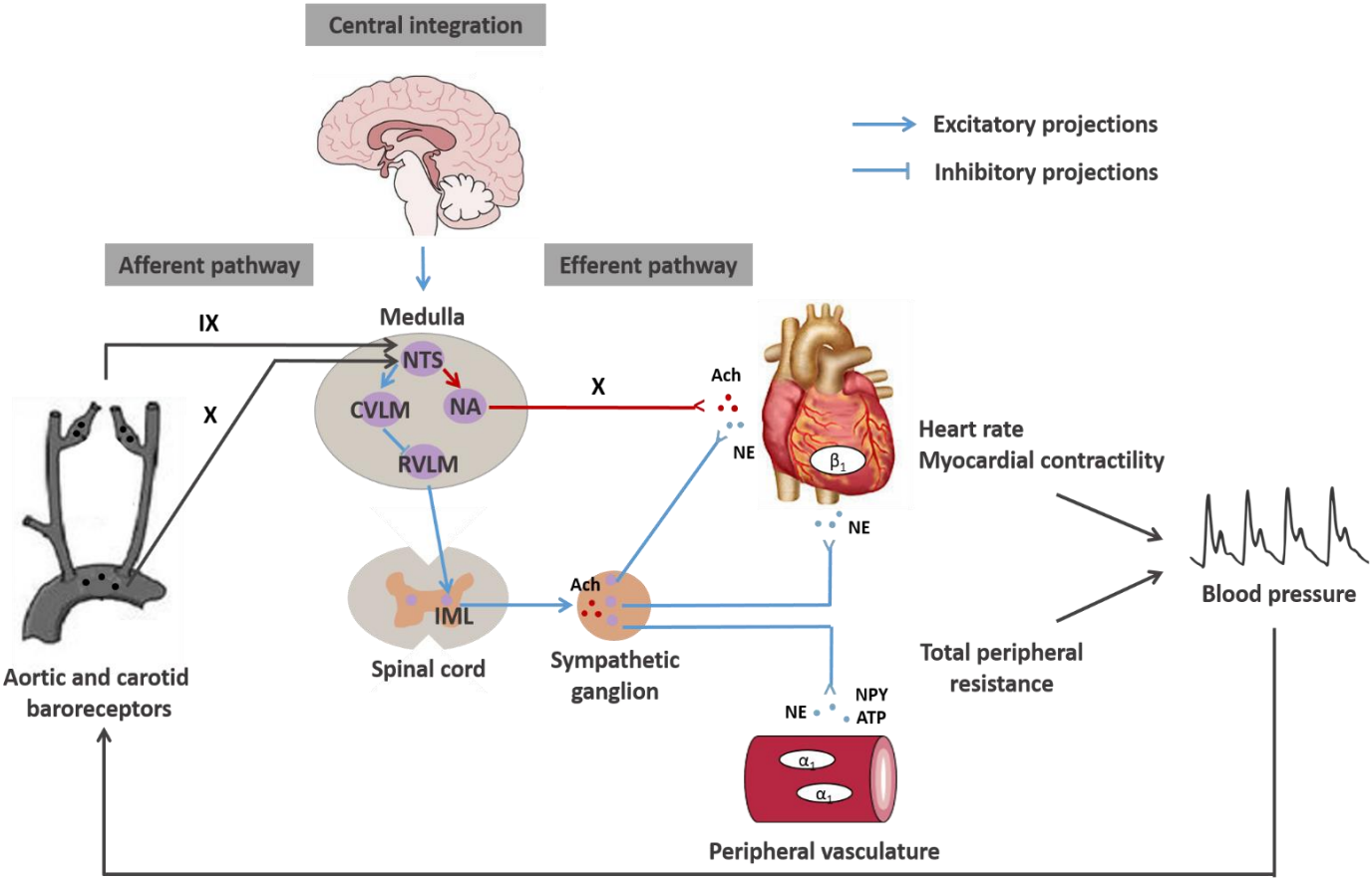

Figure 4. Arterial baroreflex regulation of arterial blood pressure.

Excitatory baroreceptor afferent signals are relayed to the cardiovascular control centre, the nucleus tractus solitarius (NTS), via glossopharyngeal (IX) and vagus nerves (X). Excitatory projections from the NTS synapse directly to the parasympathetic pre-ganglionic neurons within the nucleus ambiguous (NA) and increase parasympathetic outflow to the heart. In addition, neurons from the NTS synapse with inter-neurons of the caudal ventrolateral medulla (CVLM), which then project to, and inhibit, neurons of the rostral ventrolateral medulla (RVLM). The excitatory output of the RVLM then projects to the sympathetic preganglionic neurons in the interomediolateral (IML) cell column in the spinal cord and determines sympathetic outflow to the target organs (heart and blood vessels). Much of the work regarding central integration of baroreceptor afferent input comes from animal studies (Dampney *et al*., 2003). However, Macefield and Henderson (2010) demonstrated that the NTS, CVLM and RVLM were all activated during spontaneous increases in BP in humans, when assessed via functional magnetic resonance imaging.

The sympathetic vasomotor outflows to different vascular beds are influenced to different degrees by baroreceptor afferent inputs. Sympathetic outflow to the skeletal muscle, splanchnic, and renal vascular beds are under strong baroreflex control, whereas sympathetic nerve activity to the skin is little affected (Wallin & Charkoudian, 2007). The skeletal muscle represents the body's largest vascular bed, therefore, MSNA plays a pivotal role in determining TPR, and thus BP (Dornhorst, 1963).

Baroreflex control of arterial pressure occurs predominantly via alterations in sympathetic vasoconstrictor drive, and TPR rather than through changes in Qc (Ogoh et al., 2003). However, both the cardiovagal and vascular sympathetic limbs of the baroreflex are important to the regulation of sympathetic vasomotor outflow. The baroreflex influences the probability of a sympathetic burst occurring per cardiac cycle, through the vascular sympathetic limb, and also influences the number of opportunities for a burst to occur (i.e. HR), through the cardiovagal limb. Thus, together, these determine burst frequency (bursts·min^{-1}), which is indicative of sympathetic vasoconstrictor drive to the vasculature.

Kienbaum and colleagues (2001) proposed two central sites for baroreflex regulation of MSNA, one modulating the occurrence of sympathetic bursts (i.e. burst incidence) and the other modulating the strength of those bursts (i.e. burst amplitude). The baroreflex primarily influences burst occurrence (i.e. whether a burst will occur), via a gating mechanism. When BP increases, there is an increase in baroreceptor afferent firing. When baroreceptor afferent input reaches a 'threshold', a theoretical gate is closed causing inhibition of sympathetic outflow (i.e. MSNA bursts do not occur). Conversely, when BP falls, there is a decrease in baroreceptor afferent firing. When baroreceptor afferent input falls below the 'threshold' the theoretical gate is opened and inhibition of sympathetic outflow is removed (i.e. an MSNA

burst occurs). The baroreflex exerts a weaker effect over the amplitude of these bursts, which is believed to be largely determined by other central (e.g. central command, temperature, etc.) and peripheral (e.g. peripheral chemoreflex, skeletal muscle afferents etc.) inputs.

Arterial baroreflex function

The function of the arterial baroreflex is characterised by three indices i) operating pressure, ii) operating point and iii) baroreflex gain (Figure 5). The operating pressure, systolic BP (SBP) for the cardiovagal baroreflex and diastolic BP (DBP) for the vascular sympathetic baroreflex, is the pressure the arterial baroreflex is defending. The operating point is the corresponding RRI or MSNA burst incidence required to maintain that operating pressure. The operating pressure and operating point are collectively referred to as the baroreflex set-point (*Figure 5*. *Panel A*). Baroreflex gain is the RRI (cardiovagal baroreflex gain) and MSNA (vascular sympathetic baroreflex gain) responsiveness to changes in BP. A greater change in RRI and/or MSNA for a given change in BP is indicative of a greater reflex gain, a greater ability to buffer beat-by-beat changes in BP and thus a greater ability to defend the operating pressure (Charkoudian & Wallin, 2014) (*Figure 5. Panel C and D*). Baroreflex gain has important implications for cardiovascular control. Low baroreflex gain is associated with increased BP variability and, thus, end organ damage (Cowley et al., 1973) e.g. endothelial dysfunction, vascular and cardiac hypertrophy, which elevate cardiovascular risk (Bristow et al., 1969; La Rovere et al., 2008). Furthermore, low baroreflex gain is associated with orthostatic intolerance and an exaggerated BP response to exercise (Sharman et al., 2018), which is an independent risk factor for cardiovascular events and mortality (Schultz et al., 2013, 2017). Importantly, the baroreflex operating pressure, operating point, and baroreflex gain are not

fixed, and can be modulated by central and peripheral mechanisms (Chapleau et al., 2006; Dampney, 2017).

The arterial baroreflex regulates BP around a set operating pressure on a beat-by-beat basis. However, arterial pressure is not constant and exhibits substantial diurnal variation (Millar-Craig et al., 1978; Veerman et al., 1995) during changes in activity or arousal (e.g. sleep, mental stress and exercise) (Rowell & O'Leary, 1990; Somers et al., 1993). During such conditions, the arterial baroreflex is reset to defend a different operating pressure, so that the baroreflex remains functional (i.e. continues to be highly effective in regulating BP on a beat-by-beat basis), but at a level that is appropriate for the physiological condition (Dampney, 2017). The arterial baroreflex can also be reset to a different operating point (RRI and MSNA), to maintain operating pressure (*Figure 5, Panel B*). In addition, the gain (i.e. responsiveness) of the reflex can be altered (O'Leary et al., 2003; Keller et al., 2006)

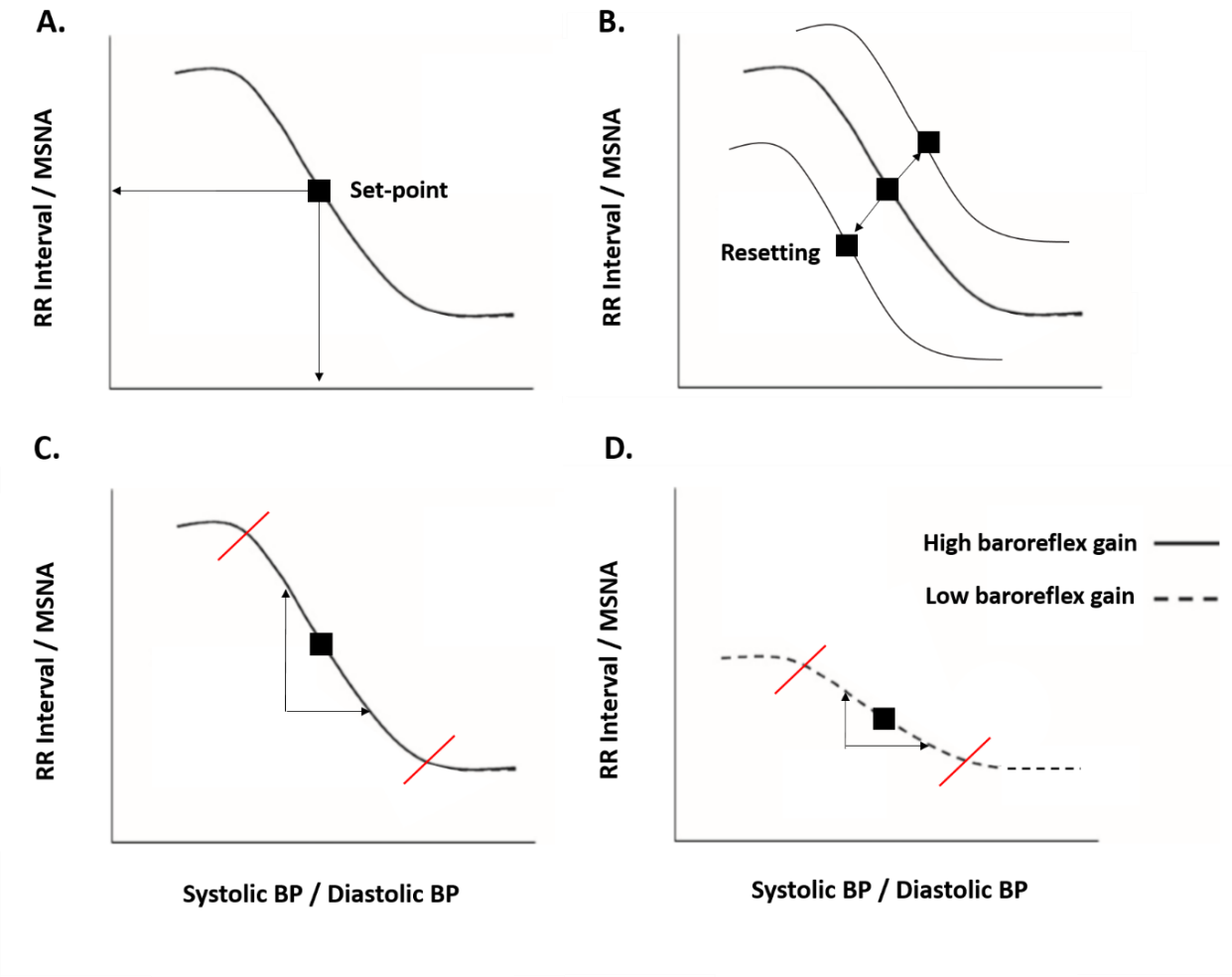

Figure 5. Overview of arterial baroreflex function.

Panel A indicates the arterial baroreflex set-point. Panel B illustrates arterial baroreflex resetting. Panel C and D demonstrate arterial baroreflex gain. Arterial baroreflex gain is quantified by plotting the stimulus-response relationship between BP (i.e. stimulus) and MSNA or RRI (i.e. response), across a range of blood pressures. This stimulus-response relationship is sigmoidal when the entire baroreflex curve is characterised. However, in humans, the threshold and saturation regions are not consistently observed; therefore, the linear portion of the relationship is typically isolated (as shown in panel C. and D). The slope of the regression line of the linear portion of the relationship is used as an index of reflex gain. Panel C illustrates a high baroreflex gain, where a steep slope is associated with a large change in heart rate or MSNA for a given change in pressure. Panel D illustrates low baroreflex gain, where a flatter slope is associated with a small change in heart rate or MSNA for a given change in pressure.

2.3.2 Heart and pulmonary baroreceptors

In addition to baroreceptors in the high-pressure arterial circulation, electrophysiological evidence in animal preparations has shown the existence of pressure sensitive receptors in the low-pressure cardiopulmonary circulation (Coleridge & Kidd, 1960; Coleridge et al., 1964; Coleridge et al., 1973; Hainsworth, 1995). These receptors are located primarily in the walls of the atria, ventricles, coronary arteries, and pulmonary arteries. Studies in anaesthetized animals, which allow a discrete physiological pressure stimulus to be applied to baroreceptive areas in cardiopulmonary circulation, have demonstrated that these receptors exhibit heterogeneous reflex responses.

Distension of atrial baroreceptors elicit increases in cardiac and renal sympathetic nerve activity, and thus increases HR and urine flow (Kappagoda et al., 1972, 1973; Karim et al., 1972). Atrial receptor activation, however, has little or no influence over vascular resistance (Carswell et al., 1970; Kappagoda et al., 1972, 1973), with no effect on sympathetic efferent activity in splenic and lumbar nerves, which is an analogue of human MSNA (Karim et al., 1972). Ventricular baroreceptor stimulation, via increases in ventricular filling pressure, elicits decreases in heart rate and vasodilation. However, ventricular baroreceptors play a minimal role in normal circulatory control (Drinkhill et al., 2001; Hainsworth, 2014), as non-physiological pressure stimuli are required to activate these receptors, and they instead primarily respond to chemical stimuli released in response to myocardial ischemia.

Coronary artery baroreceptors exert a negative feedback control over the peripheral vasculature, similar to that of the aortic and carotid (arterial) baroreceptors. Increased distending pressure to the coronary baroreceptors elicit decreases in efferent lumbar and renal sympathetic nerve activity , inducing vasodilation, with no effect on HR (Drinkhill et al.,

1996). In contrast to the negative feedback control originating from the coronary and arterial baroreceptors, pulmonary arterial baroreceptors exert positive feedback control over the circulation. Indeed, distension of these receptors, located at the pulmonary artery bifurcation and in the extrapulmonary artery branches, elicits reflex sympathoexcitation and systemic vasoconstriction in anesthetised dogs (Ledsome & Kan, 1977; McMahon et al., 2000a, 2000b; Moore et al., 2004a, 2004b, 2011). Moreover, an interaction exists between pulmonary arterial and carotid sinus baroreceptors, whereby increasing pressure within the pulmonary arteries acutely resets the vascular limb of the carotid sinus baroreflex to operate at higher systemic pressures with unaltered reflex gain (Moore et al., 2011). The positive feedback control of the pulmonary arterial baroreceptors does not compete with the negative feedback control of the arterial baroreceptors to modulate efferent sympathetic outflow. Instead, increased pulmonary arterial baroreceptor input serves to reset the arterial baroreflex to operate around a higher operating pressure and vascular resistance operating point, suitable for current physiological needs. This occurs whilst maintaining the ability of the arterial baroreflex to increase and decrease vascular resistance in response to acute fluctuations in BP, in a negative feedback fashion. Afferent input from pulmonary arterial baroreceptors, therefore, are likely important in control of autonomic outflow and BP control during physiological conditions where pulmonary arterial pressure is significantly elevated.

Due to the difficulty in isolating a pressure stimulus to intrathoracic receptors in a closed-loop system in humans, the reflex responses of these distinct baroreceptor populations have not been fully elucidated; although, a similar heterogeneity to that observed in animals is likely to exist in humans. Nevertheless, baroreceptors in the heart and pulmonary circulation tend to be grouped together in humans, and are widely considered to be a homogeneous group, which constitute a single ‘cardiopulmonary’ baroreflex. Roddie et

al., (1957) first reported reflex vasodilation in skeletal muscle vasculature during increases in central venous pressure, via passive leg raising. The authors proposed that this vasodilator response was mediated by activation of receptors in the cardiopulmonary circulation, as an increase in central venous pressure occurred in the absence of changes in MAP and pulse pressure. The lack of change in MAP and pulse pressure seemingly eliminatied any contribution from the arterial baroreceptors. This led to non-hypertensive lower body positive pressure (LBPP) and non-hypotensive lower body negative pressure (LBNP) being a commonly employed method to 'selectively' load and unload the baroreceptors in the heart and pulmonary circulation and to investigate the so-called 'cardiopulmonary 'baroreflex. Non-hypertensive LBPP elicits decreases in MSNA (Fu et al., 1998) and non-hypotensive LBNP elicits increases in MSNA and peripheral vasoconstriction (Victor and Leimbach, 1987, Oren et al., 1993). Together these findings led to the commonly held notion that, in humans, the so-called 'cardiopulmonary' baroreflex exerts negative feedback control over the circulation, similar to that of the arterial baroreflex. However, low levels of LBNP (-5 to -10 mmHg) also alter the loading of the carotid and aortic baroreceptors despite no change in MAP or pulse pressure (Lacolley et al*.,* 1992; Taylor et al*.*, 1995; Fu et al*.*, 2008). Thus, selective unloading of cardiopulmonary baroreceptors should not be assumed during non-hypotensive LBNP, with the negative feedback responses likely influenced by the arterial baroreflex.

Overall, in anesthetised animals, distinct populations of baroreceptors within the heart and pulmonary circulation exhibit distinct reflex responses (Hainsworth, 2014); therefore, grouping baroreceptors in the heart and pulmonary circulation together is unjustified. Despite this, the specific role of these distinct groups of baroreceptors to autonomic control of BP in humans remains unclear, due to the difficulty in isolating a pressure stimulus in a closed loop system. Nevertheless, baroreceptors in the heart, and particularly those in the pulmonary circulation, likely play an important, but poorly understood, role in autonomic regulation of BP at high altitude, where pressure within the pulmonary artery is greatly increased.

2.3.3 Peripheral chemoreflex

The peripheral chemoreflex attempts to maintain blood gases within normal range and strongly influences autonomic outflow. Peripheral chemoreceptors, located in the carotid body and in the arch of the aorta, primarily consist of grouped Type I glomus cells. These Type I glomus cells are polymodal and detect changes in arterial PCO_2 and pH, arterial concentrations of lactate, potassium, glucose, insulin, angiotensin, cytokines and PO_2, through a dense network of capillaries branching from the external carotid artery. Despite responding to a multitude of stimuli, the peripheral chemoreceptors respond mainly to a lowering in PaO_2 (aortic sensors respond mainly to CaO_2) (Biscoe et al., 1970; Lahiri et al., 1981), and play an important role in the physiological responses to hypoxia. Decreases in PaO_2 progressively activate peripheral chemoreceptors (Vidruk et al., 2001) via blocking of voltage-gated channels located on the glomus cell membrane, which prevent potassium ion (K^+) uptake and promote subsequent glomus cell membrane depolarization. Depolarization opens calcium (Ca^{2+}) channels, increasing intracellular Ca^{2+} concentration, which ultimately leads to the release of neurotransmitter dopamine. Binding of dopamine to post-synaptic receptors on the afferent fibres then serves to increase afferent firing (Kumar & Prabhakar, 2012).

Carotid and aortic chemoreceptor afferents run in the glossopharyngeal and vagal nerves, respectively, and synapse in a primary cardiovascular control center located within NTS. Excitatory afferent signals from the peripheral chemoreceptor are integrated in the respiratory centres of the brainstem and lead to increased efferent outflow to the respiratory muscles via the phrenic nerve (Koshiya & Guyenet, 1996; Guyenet, 2000, 2014), increasing respiratory rate and depth. Increased peripheral chemoreceptor afferent activity also elicits changes autonomic nervous system activity to the heart and vasculature. Peripheral chemoreceptor stimulation during hypoxia elicits reflex increases in sympathetic vasomotor

outflow to the skeletal muscle and visceral beds, increasing sympathetic vasoconstrictor drive. The effect of peripheral chemoreceptor activation on vascular resistance, however, is dependent on concurrent hypoxia-induced alterations in local vasodilator signaling (Marshall et al., 2004; Dinenno et al., 2016). Despite an increase in sympathetic activity to peripheral blood vessels, activation of peripheral chemoreceptors simultaneously increases parasympathetic activity to the heart, at least in isolated preparations and apneic animals. However, in spontaneously breathing humans, this response is modified by the secondary influence of alterations in ventilation (Kato et al., 1988) and, to a lesser degree, changes in $PaCO_2$. Augmented ventilation stimulates lung stretch receptors (during inspiration), which leads to an inhibition of cardiac parasympathetic activity and increases in HR (Kato et al., 1988). In summary, peripheral chemoreceptor activation in spontaneously breathing humans elicits an increase in sympathetic vasomotor activity and, indirectly, leads to parasympathetic withdrawal, which serve to increase MSNA and HR. The carotid baroreceptors appear to mediate the ventilatory responses to acute hypoxia, and aortic chemoreceptors mediate the cardiovascular response (De Burgh Daly & Ungar, 1966; Niewinski et al., 2014).

Peripheral chemoreflex function can be assessed by quantifying the ventilatory and cardiovascular responses to acute manipulations of peripheral chemoreceptor activity. Most commonly, brief hypoxic exposures are employed to progressively increase peripheral chemoreceptor activity. The peripheral chemoreflex ventilatory, HR and BP responsiveness to hypoxia can be determined from slope of the linear relationship between oxygen saturation and the investigated variable (Limberg et al., 2016). Alternatively, inhalation of 100% O_2 or intravenous infusion of low-dose dopamine have been employed to inhibit peripheral chemoreceptor activity. From this, the contribution of peripheral chemoreceptor activity to ventilation, HR, MSNA, and BP can be determined. Inhalation of hyperoxia or low dose

dopamine, has previously been employed to determine the peripheral chemoreceptor involvement in the cardiovascular responses to acute and chronic hypoxia and glucose ingestion (Niewinski et al., 2014; Mozer et al., 2016; Fisher et al., 2018; Smorschok et al., 2019).

2.4 Summary of autonomic control of arterial blood pressure

Appropriate regulation of BP is critical to ensure adequate perfusion and oxygen delivery to vital organs. The arterial baroreflex is the primary autonomic blood pressure control mechanism, which tightly regulates BP around a set-point by altering efferent parasympathetic and sympathetic outflow to the heart and blood vessels on a beat-by-beat basis. Arterial baroreflex function (i.e. operating pressure, MSNA/RRI operating point and arterial baroreflex gain) is modulated by input from central factors and other peripheral afferent inputs, including peripheral chemoreceptors and baroreceptors in the heart and pulmonary circulation.

2.5 High altitude

High altitude represents one of the most hostile environments on earth and poses a profound challenge to oxygen homeostasis. Barometric pressure decreases in a non-linear fashion during ascent to high altitude, which lowers inspired PO_2 (*Figure 6*) (Bert, 1878). In fact, on the summit of Mt Everest, inspired PO_2 is only one-third of that at sea level (West, 1983). A decreased inspired PO_2 lowers alveolar PO_2, reducing the driving pressure for gas exchange (alveolar-arterial pressure gradient) in the lungs and diffusion of oxygen into the arterial blood (PaO_2), which leads to arterial hypoxemia (Hurtado, 1964). The resulting reduction in systemic oxygen delivery poses a significant challenge to the human body, as cells require a continuous oxygen supply for normal cellular function. On exposure to high altitude there are numerous respiratory, cardiovascular, and hematological adjustments that occur, both acutely (minutes to hours) and during acclimatisation (days to weeks) (West, 2007). Collectively, these physiological adjustments attempt to offset the reduction in oxygen availability, restore oxygen delivery towards sea level values, and thus maintain normal cellular function. The physiological responses to hypoxia involve altered autonomic outflow to the heart and vasculature, which has important consequences for neural control and autonomic regulation of BP. The following section i) outlines the integrative physiological responses to acute hypoxic exposure, high altitude acclimatisation and adaptation and ii) discusses autonomic control of BP and arterial baroreflex regulation during acute hypoxic exposure, high altitude acclimatisation and adaptation.

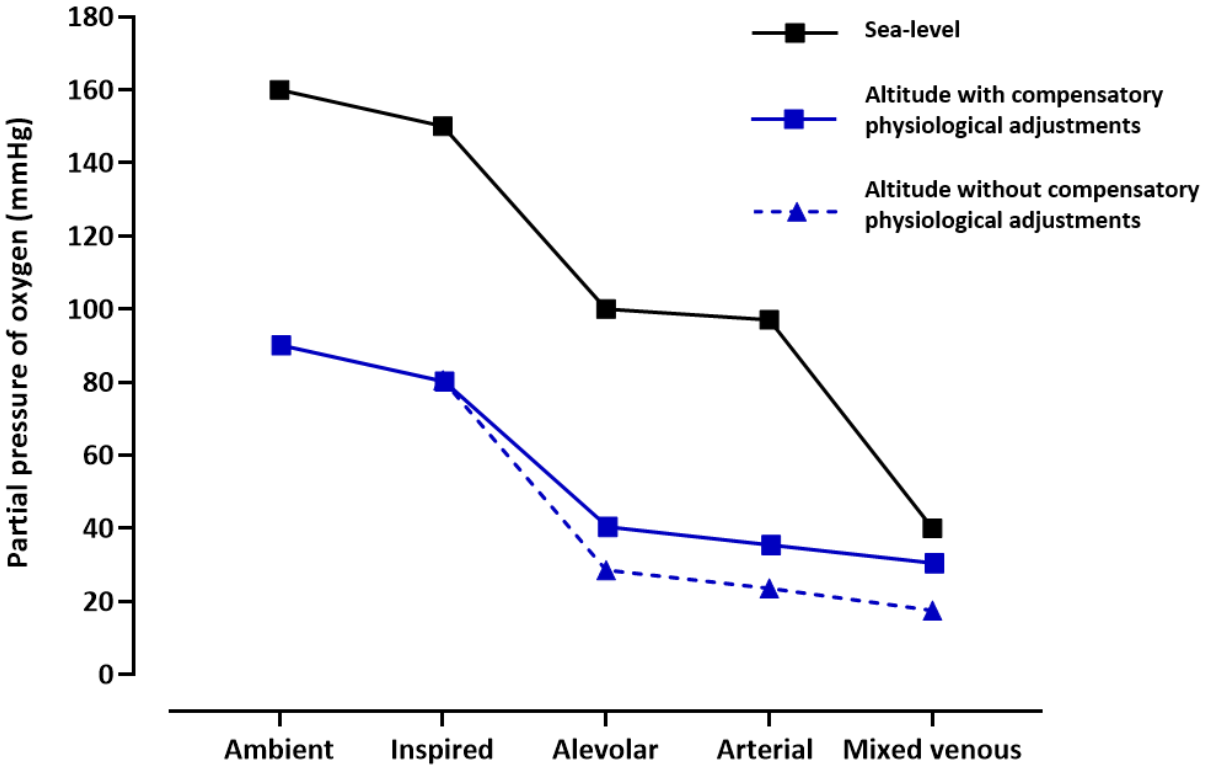

Figure 6. The oxygen cascade illustrating the stepwise decrease in PO_2 from the atmosphere to the mitochondria.

The black line illustrates the changes in PO_2 from ambient air to the mitochondria at sea level and the solid blue line illustrates the downward shift in the oxygen cascade that occurs at altitude. Numerous physiological adjustments occur at various levels of the cascade at altitude, which serve to compensate for the reduction in PO_2. Whilst such adjustments cannot return PO_2 to sea level values, a further reduction in PO_2 would occur if these adjustments did not occur. Figure redrawn from West (2012).

2.6 Acute hypoxic exposure

2.6.1 Integrative physiological responses to acute hypoxia

Acute hypoxia elicits profound changes in the ANS, which causes alterations in ventilation, cardiovascular function, and BP. Once PaO_2 falls below ~60mmHg (3000 m), hypoxic stimulation of peripheral chemoreceptors elicits a dose-response increase in minute ventilation, mediated by increases in tidal volume (Powell et al., 1998). This increase in ventilation raises alveolar PO_2 closer to inspired PO_2, which increases PaO_2 and serves as the first line of defence to hypoxia to try to offset the reduction in oxygen availability. In addition, exposure to acute hypoxia elicits increases in HR. Acute hypoxia induced tachycardia is mediated by an increase in sympathetic outflow and withdrawal of parasympathetic outflow to the heart, as combined inhibition of β-adrenergic and muscarinic receptors abolishes this response (Koller et al., 1988; Siebenmann et al., 2015). Hypoxia induced tachycardia increases Qc, which increases in a dose-dependent manner, and helps to compensate for the fall in CaO_2, and maintain O_2 delivery (DO_2) to the cells ($DO_2 = CaO_2 \times Qc$). The elevated Qc is primarily mediated by increases in HR, with no consistent change in stroke volume (Vogel & Harris, 1967).

2.6.2 Sympathetic vasomotor activity response to acute hypoxia

In addition to increased sympathetic outflow to the heart, sympathetic outflow to the vasculature is also significantly elevated during acute hypoxic exposure, as demonstrated by increases in MSNA. Saito et al., (1988) and Rowell et al., (1989) first demonstrated augmented MSNA in healthy participants exposed to acute hypoxia. Furthermore, MSNA increases in an intensity dependent manner, once arterial oxygen saturation (SaO_2) falls below 85% (Saito et al., 1988; Rowell et al., 1989; Somers et al., 1989a, 1989b; Duplain et al., 1999). Somers et al.,

(1989b) demonstrated a significant increase in MSNA following 10% FiO_2, but not 14% O_2 (82% vs. 91% SaO_2 respectively). Saito et al., (1988) also reported a more pronounced increase in MSNA burst frequency at a simulated altitude of 5000 m (50% increase) compared with 4000 m (34% increase). Hypoxic-induced increases in MSNA are mediated by increases in both MSNA burst frequency and MSNA burst amplitude (Saito et al., 1988; Steinback et al., 2009). Notable, despite significant increases in sympathetic vasoconstrictor drive, the vasoconstrictor effects of increased MSNA on vascular tone contend with vasodilatory effects of β-adrenergic activity, circulating vasodilators and local release of vasodilators (Weisbrod et al., 2001).

2.6.3 Hypoxia induced local vasodilation of the systemic vasculature

Reduced CaO_2 during systemic hypoxic exposure elicits an intensity dependent local vasodilation in the systemic resistance vessels, particularly in the coronary, splanchnic and skeletal muscle circulations (Heistad & Wheeler, 1970; Rowell & Blackmon, 1986; González-Alonso et al., 2002; Markwald et al., 2011; Vermeulen et al., 2018). Local vasodilation serves to reduce TPR and thus increases tissue perfusion and oxygen delivery to maintain tissue oxygen consumption. A number of vasodilator substances and pathways have been implicated in hypoxia-induced vasodilation, and have been reviewed elsewhere (Halliwill, 2005; Dinenno, 2016). Hypoxia-induced activation of nitric oxide (NO) pathways, combined with concurrent increases in prostaglandin release are considered to be the primary mechanism responsible for hypoxia mediated vasodilation, as combined NO and prostaglandin inhibition abolishes hypoxic vasodilation in resting human skeletal muscle (Markwald et al., 2011). Furthermore, the release of adenosine appears to be important in hypoxia induced vasodilation, as 60% of hypoxic vasodilatation in the forearm was inhibited

by the adenosine receptor antagonist aminophylline (Leuenberger et al., 1999). However, several other pathways have also been shown to play a minor role in promoting hypoxia induced vasodilation, including adrenaline mediated stimulation of β-adrenergic receptors (Weisbrod et al., 2001). Furthermore, ATP released from red blood cells when O_2 is off-loaded from haemoglobin can induce vasodilation through binding to P_{2y}-purinergic receptors located on the vascular endothelial cells (González-Alonso et al., 2002).

2.6.4 Systemic blood pressure during acute hypoxia

Increases in local vasodilatory signalling outweigh any increase in sympathetic vasoconstrictor drive and thus there is a net decrease in TPR during acute hypoxic exposure. However, the increases in sympathetic vasoconstrictor drive serve to restrain local vasodilatory mechanisms during acute hypoxia, as α-adrenergic blockade with phentolamine augments the vasodilator response (Weisbrod et al., 2001). Thus, elevated MSNA during acute hypoxic exposure is important for appropriate BP control. The reduction in systemic vascular resistance, coupled with a large increase in Qc together increase blood flow to maintain convective oxygen delivery, akin to what occurs during exercise. As a result, acute hypoxia of up to one hour is consistently reported to increase MAP (Leuenberger et al., 1991; Davy et al., 1997; Halliwill & Minson, 2002; Halliwill et al., 2003; Steinback et al., 2009; Querido et al., 2011) however, some studies have reported no change (Tamisier et al., 2007) or a decrease in MAP (Saito et al., 1988).

2.6.5 Arterial baroreflex function during acute hypoxia

Arterial baroreflex function during acute hypoxic exposure has been well characterised. Halliwill and Minson (2002) were the first to comprehensively assess the effect of acute hypoxia on both the vascular sympathetic and cardiovagal baroreflex in humans. This

study reported that the operating pressure and operating point (MSNA burst incidence) of the vascular sympathetic baroreflex were higher following 20 minutes of 12% O_2 (~81% SaO_2), with no change in vascular sympathetic baroreflex gain (i.e. responsiveness). Furthermore, the operating pressure (SBP) and operating point (HR) of the cardiovagal limb of the baroreflex were also higher with no change in reflex gain.

Consistent with the findings of Halliwill and Minson (2002), several other studies have also reported resetting of the vascular sympathetic baroreflex to a higher operating pressure and operating point, with no change in reflex gain. These findings have been reported following 5–30 minutes exposure to acute hypoxia that elicited SpO_2 of between 80–85%, and assessed using spontaneous and pharmacologically induced fluctuations in BP (Halliwill and Minson 2002; Halliwill et al., 2003, Steinback et al., 2009; Querido et al 2011). Thus, the vascular sympathetic baroreflex appears to continue to be highly effective in buffering beat-by-beat changes in BP during acute hypoxic exposure (≤ 30 minutes), but is reset to operate around a higher BP and level of MSNA and that is required to maintain convective oxygen delivery.

Consistent with findings from Halliwill and Minson (2002), studies that have assessed cardiovagal baroreflex function, have consistently reported increases in the operating pressure and operating point of the cardiovagal baroreflex. Moreover, some studies have reported no change in cardiovagal baroreflex gain (Halliwill et al., 2003; Hunt et al., 2008) following acute hypoxic exposure of between 20–60 minutes (~85% SpO_2). In contrast, most studies investigating the effects of acute hypoxia on cardiovagal baroreflex function have reported a hypoxia induced reduction in cardiovagal baroreflex gain (Heistad & Wheeler, 1971; Sagawa et al., 1997; Steinback et al., 2009; Niewinski et al., 2014; Mozer et al., 2016).

Such changes in arterial baroreflex operating pressure and operating point during acute hypoxic exposure appear to be primarily mediated by augmented peripheral chemoreflex activation (Halliwill et al., 2003). Furthermore, the reduction in cardiovagal baroreflex gain reported in several studies also appears to be mediated by increased peripheral chemoreflex activation, as significantly reducing peripheral chemoreceptor afferent firing, via low dose dopamine infusion, reverses the hypoxia-induced reduction in cardiovagal baroreflex gain (Niewinski et al., 2014; Mozer et al., 2016)

2.7 High altitude acclimatisation

2.7.1 Integrative physiological responses to sustained high altitude exposure

Sustained exposure to hypoxia of days and weeks is accompanied by further physiological changes that attempt to restore PaO_2 and SaO_2 towards sea level values. Firstly, there is a progressive increase in alveolar ventilation (termed ventilatory acclimatisation), which serves to increase alveolar and arterial PO_2. This is mediated by a progressive carotid body sensitization, increasing afferent discharge under the same hypoxia stimuli (Nielsen et al., 1988; Dempsey et al., 2014;). Whilst the increase in ventilation at high altitude is accompanied by a reduction in $PaCO_2$, which dampens the central and peripheral chemoreceptor drive to breathe, ventilation remains elevated above sea level values during acclimatisation (Powell et al., 1998).

Qc returns towards sea level values during acclimatisation (Grollman, 1930; Stembridge et al., 2018) due to normalisation of CaO_2; however, HR remains elevated and even increases further. High altitude tachycardia is exclusively mediated by parasympathetic withdrawal, as Siebenmann et al., (2017) demonstrated that following two weeks at 3454 m, the rise in HR was unaffected by sympathetic blockade, but abolished by parasympathetic blockade. However, the contribution of the sympathetic and parasympathetic nervous system may change as a function of duration and severity of altitude exposure. Boushel et al., (2001) reported increased vagal cardiac modulation of the heart with chronic high altitude hypoxia, where pharmacological inhibition of muscarinic receptors increased HR more after nine weeks residence at 5260 m than sea level. Nevertheless, altitude acclimatisation is accompanied by marked alterations in autonomic control of the heart. Indeed, the HR response to maximal exercise and β-adrenergic receptor agonist isoproterenol were blunted

during prolonged high altitude exposure, due to a downregulation of β-adrenergic receptors (Richalet et al., 1988; Antezana et al., 1994) and a reduced myocardial β-adrenergic receptor density (Voelkel et al., 1981; León-Velarde et al., 2001).

The increase in HR at high altitude maintains Qc during acclimatisation, as stroke volume is reduced after several days (Vogel & Harris, 1967; Reeves et al., 1987; Stembridge et al., 2018). The decrease in stroke volume during high altitude acclimatisation is primarily attributed to a reduction in circulating total blood volume and elevations in pulmonary arterial pressure, which reduce right ventricular filling and increase right ventricular afterload respectively (Siebenmann et al., 2013; Stembridge et al., 2018). The reduction in blood volume is exclusively mediated by a decrease in plasma volume, which begins to occur within 24 hours of high altitude exposure and is maintained for up to 40 days (Young et al., 2019). This reduction serves to increase haemoglobin concentration early on during high altitude exposure (Ryan et al., 2014). In addition, after seven days at altitude there is a progressive expansion of red blood cell (RBC) volume (Ryan et al., 2014), driven by increases in erythropoietin release from the kidney, secondary to increased hypoxia-inducible factor (HIF) signalling (Wang & Semenza, 1996). This RBC volume expansion further increases haemoglobin concentration and helps to restore arterial oxygen content to levels that often surpass sea level (Hansen & Sander, 2003; Revera et al., 2017). Despite the increase in RBC volume, the decrease in plasma volume is more pronounced; therefore, circulating blood volume is reduced at high altitude, at least during the first four weeks (Siebenmann et al., 2017).

The normalisation of arterial oxygen content with acclimatisation serves to attenuate the magnitude of hypoxic local vasodilation, as skeletal muscle blood flow and vascular

conductance is primarily related to changes in CaO_2, rather than changes in PO_2 (Roach et al., 1999). Furthermore, the vascular responsiveness to vasodilators, specifically ATP and adenosine, are attenuated following 8–12 days at 4559 m (Calbet et al., 2014).

2.7.2 Sympathetic vasomotor activity response to high altitude acclimatisation

Despite an improvement in CaO_2 and SaO_2 during high altitude acclimatisation, compared to acute exposure, high altitude acclimatisation is paradoxically accompanied by elevations in MSNA of greater magnitude than that observed during acute exposure to the same ambient hypoxic stimulus (Duplain et al., 1999; Lundby et al., 2017) (*Figure 7*). Microneurographic studies at high altitude are scarce, due to the technical and logistical issues associated with performing the microneurography technique in the field. At the time of writing, there have been only four publications reporting MSNA during altitude acclimatisation (Duplain et al 1999; Hansen and Sander 2003; Lundby et al 2017; Fisher et al 2018) (*Table 1*).

Duplain and colleagues (1999) were the first to report augmented MSNA during the initial days at high altitude. The authors reported a ~55% increase in MSNA burst frequency in healthy Lowlanders following 20 minutes hypoxic (12% O_2) exposure. However, more prolonged exposure of 24–36 hours to an equivalent altitude (4559 m) was associated with a more exaggerated increase in MSNA burst frequency (~69%), despite similar reductions in SpO_2. Subsequently, Hansen and Sander (2003) demonstrated a tripling in MSNA burst frequency (from 15 to 48 bursts·min^{-1}) following four weeks residence at 5260 m in Lowlanders. In the only study to perform serial measurements of MSNA during high altitude acclimatisation, Lundby et al (2017) reported an increase in MSNA burst frequency from sea level to day ten at 4300 m (15 vs. 42 bursts·min^{-1} respectively) in Lowlanders, with no further

change in MSNA following 50 days at 4300 m (42 vs. 42 bursts·min^{-1}). Taken together, these findings indicate that MSNA increases progressively over the initial days at high altitude, plateaus within ten days of exposure, with no further increases during acclimatisation (*Figure 7*). Elevated MSNA is maintained for the duration of altitude exposure, at least up to 50 days, and also persists for up to three days following descent from high altitude (Hansen and Sander, 2003).

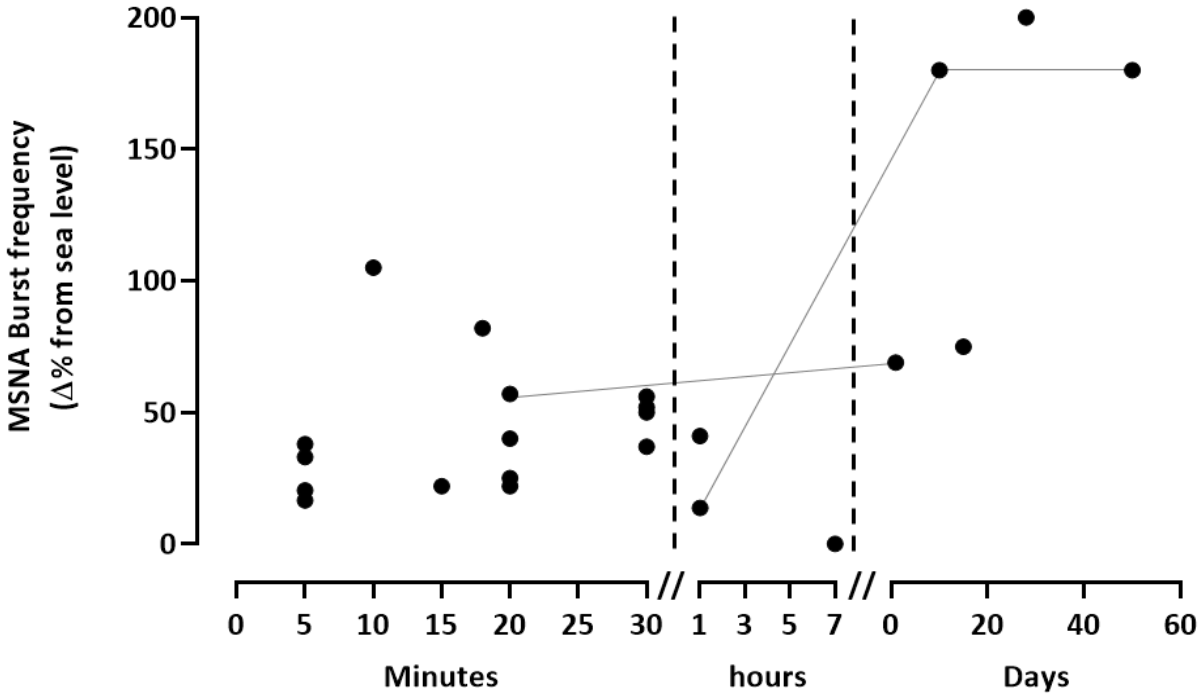

Figure 7. Summary of studies measuring muscle sympathetic nerve activity (MSNA) during hypoxic exposure.

Duration of hypoxic exposure is plotted against the change in MSNA from sea-level values. These studies indicate a trend for the progressive rise in MSNA with longer durations of exposure. The grey lines indicate serial measurements. NB. This is not an exhaustive list of studies conducted under acute hypoxic conditions.

Microneurographic study	Participants studied	Ascent method	Exposure duration	Altitude Studied at	MSNA (Bursts·min^{-1})	Magnitude of change	Mean BP (mmHg)	Magnitude of change
Duplain et al., (1999)	7 Lowlanders (39–41 yrs)	Passive/ Active	24–36 hours	4559 m	**LA:** 22 ± 11 **ALT:** 37 ± 2	69% increase	–	–
	8 HAPE prone Lowlanders (39–41 yrs)	Passive/ Active	24–36 hours	4559 m	**LA:** 16 ± 3 **ALT:** 52 ± 4	225% increase	–	–
Hansen & Sander, (2003)	4 male, 4 female Lowlanders (26 ± 2 yrs)	–	28 days	5260 m	**LA:** 16 ± 2 **ALT:** 48 **± 5**	200% increase	**LA:** 77 ± 2 **ALT:** 87 ± 3	14% increase
Fisher et al., (2018)	9 male, 1 female Lowlanders (26 ± 4 yrs)	Passive	15–17 days	3454 m	**LA:** 16 ± 3 **ALT:** 28 ± 4	75% increase	–	12% increase
Lundby et al., (2018)	6 male, 2 female Lowlanders (22–31 yrs)	Passive	10 days	4100 m	**LA:** 15 ± 2 **ALT:** 42 ± 5	180% increase	**LA:** 72 ± 2 **ALT:** 78 ± 2	8% increase
	As above	As above	50 days	As above	**LA:** 15 ± 2 **ALT:** 42 ± 5	188% increase	**LA:** 72 ± 2 **ALT:** 75 ± 2	14% increase
	7 male Andean Highlanders (26–37 yrs)	N/A	Life-long	4100 m	**LL ALT:** 42 ± 2 HL ALT: 34 ± 4	ns	**LL ALT:** 75 ± 2 **HL ALT:**73 ± 2	3% lower

Table 1. Summary of microneurographic studies at high altitude.

MSNA = muscle sympathetic nerve activity, LA = low altitude, HA = high altitude, LL = Lowlanders, HL = Highlanders., ns = non significant
.

2.7.3 Systemic blood pressure during high altitude acclimatisation

A combination of a reduced vasodilator drive, elevated sympathetic vasoconstrictor drive and, increased blood viscosity during high altitude acclimatisation, mean that calf vascular resistance, forearm vascular resistance and TPR all are elevated compared to both sea level (Hansen & Sander, 2003; Tremblay et al., 2018) and acute hypoxic exposure (Calbet et al., 2014). An elevated TPR is observed alongside a comparable Qc during high altitude acclimatisation, compared to sea level. Therefore, MAP is consistently reported to be elevated during the initial days and weeks at high altitude, when measured both invasively and non-invasively (Hansen & Sander, 2003; Mazzeo et al., 1994; Wolfel et al., 1994). Indeed, a progressive increase in 24-hour ambulatory BP is reported following 19 day residence at 4300 m (SL: 82 mmHg, day 2: 88 mmHg, day 8–10: 91 mmHg, day 17–19: 97mmHg) (Wolfel et al., 1994). Furthermore, a 24 mmHg increase in MAP was reported following 21 days at 4300 m (Mazzeo et al., 1994). This increase in BP was related to urinary NA concentrations (Wolfel et al., 1994) and was attenuated with propranolol administration (Wolfel et al*.,* 1994), suggesting that increased sympathetic activity is a major factor contributing to the elevated BP at high altitude.

All microneurographic studies, which report elevated MSNA at high altitude, also report significantly elevated MAP. However, whilst MSNA is increased by up to 200%, the increase in MAP is relatively modest in comparison (between 3–14%), and BP remains within the normotensive range *(Table 1)*. These findings suggest that elevated MSNA is integral to BP control during altitude acclimatisation and likely restrains the hypoxic local vasodilation described in *section 2.6.3*, providing greater sympathetic support of the vasculature in the face of a reduced total blood volume. However, as high altitude acclimatisation progresses,

the initial rise in MAP is lost. Indeed, Lundby et al., (2017) reported a reduced MAP at day 50 compared to day ten (78 vs 75 mmHg), despite comparable levels of MSNA. Furthermore, in Lowlanders, one and two year residence at 4500 m is associated with a reduction in diastolic and systolic pressure versus sea level (Rotta et al., 1956; Marticorena et al., 1969). A reduction in MAP with no change in MSNA may also suggest a reduction in the effectiveness of a given level of MSNA to elicit vasoconstriction at the level of vasculature (i.e. vascular transduction) during prolonged high altitude exposure, termed hypoxic sympatholysis (Marshall, 2015).

2.7.4 Arterial baroreflex function during high altitude acclimatisation

Whilst arterial baroreflex function during acute hypoxic exposure of up to 60 minutes has been well characterised, the impact of more prolonged hypoxic exposure on arterial baroreflex function is unclear. A single study has reported that seven hours of poikilocapnic hypoxia (~85% SpO_2) is associated with a significant reduction in gain of both the cardiovagal and vascular sympathetic limbs of the baroreflex (Hunt et al., 2008). Furthermore, a study by Yazdani and colleagues (2016) reported a progressive reduction in cardiovagal baroreflex gain, assessed via spontaneous baroreflex analysis, following one and 16 days at 5260 m versus sea level. Thus, cardiovagal baroreflex gain appears to decline during sustained hypoxia of over one hour, which is further exacerbated during the acclimatisation process. Whilst limited studies have characterised cardiovagal baroreflex function during high altitude acclimatisation, vascular sympathetic baroreflex function during high altitude acclimatisation has never been assessed. It is unclear whether the reduction in vascular sympathetic baroreflex gain observed following seven hours of hypoxia is exacerbated during acclimatisation, similar to what is observed in the cardiovagal limb. A reduction in baroreflex gain at high altitude, or incomplete baroreflex resetting may contribute to the increased

incidence of syncope reported following arrival at altitude in otherwise healthy individuals (Nicholas et al., 1992).

2.8 Introduction to highland native populations

In contrast to Lowlanders, populations native to the Tibetan plateau of Asia, Andean Altiplano of South America, and the Ethiopian highlands of East Africa have been exposed to the environmental stress of chronic hypoxia for millennia (Aldenderfer, 2003). Over generations, these populations have undergone natural selection resulting in altered gene expression, which has given rise to distinct cardiorespiratory, haematological, and metabolic adaptations to compensate for the reduction in ambient PO_2. Collectively, such adaptations have facilitated long-term persistence and have allowed these populations to thrive at high altitude. A wealth of evidence demonstrates different patterns of adaptation between these geographically distinct populations (Beall et al., 2001; Beall, 2006, 2007), meaning highland populations have developed different physiological strategies to achieve the same outcome of increased oxygen delivery. However, among high altitude native populations, particularly those of the Andean plateau, there is a proportion of individuals that cannot appropriately adapt to chronic hypoxia and develop maladaptation syndrome chronic mountain sickness (CMS; Monge et al., 1992). The following sections will i) summarise the key physiological adaptations in the two most well studied highland native populations, the Tibetan/Nepalese Sherpa and Andeans, ii) summarise the key features of maladaptation syndrome CMS, before iii) discussing the current understanding of sympathetic vasomotor activity and autonomic regulation of the cardiovascular system in these populations.

2.8.1 Key physiological adaptations in healthy highland natives

Nepalese Sherpa are direct descendants of highland natives from the Tibetan Plateau and have resided at high altitude for approximately 25,000–30,000 years (Aldenderfer, 2011; Zhang et al., 2018). In contrast, natives of the Andean plateau have resided at altitude for a relatively shorter period of time between ~10,000–15,000 years (Aldenderfer, 2003). However, Andean highlanders were the first highland population to be extensively studied and until the late 1960's (Lahiri & Milledge, 1965) the Andean pattern of adaptation was believed to represent human adaptation to high altitude. The Andean pattern of adaptation is characterised by lower resting ventilation, demonstrated by a higher $PaCO_2$, and a blunted hypoxic ventilatory response (an index of peripheral chemoreflex sensitivity) compared to Lowlanders following ascent to high altitude (Severinghaus et al., 1966; Beall et al., 1997). Instead, rather than a high ventilation, Andeans rely on an increased haemoglobin concentration to maintain oxygen delivery to the tissues. Andeans, exhibit an exaggeration of the typical haematological response observed in acclimatising Lowlanders with a greater red cell volume and haemoglobin concentration compared to acclimatising Lowlanders following ten days at high altitude (Stembridge et al., 2019). Because of an increased haemoglobin concentration, Andeans exhibit an arterial oxygen content that is ~16% higher than sea level values (Beall et al., 1997).

In contrast, the Sherpa pattern of adaptation is characterised by high resting ventilation, greater than that observed in Andeans and similar to that observed in acclimatising Lowlanders. A study of 320 Tibetans and 542 Bolivian Aymara residing at 3,800 – 4065 m reported a resting ventilation 1.5 times higher in Sherpa than Andeans, and a hypoxic ventilatory response in Sherpa that was double that of Andeans (Beall et al., 1997). Sherpa also have greater lung volumes and diffusion capacity (Faoro et al., 2014), but

demonstrate a lower haemoglobin concentration compared to both Lowlanders and Andean highlanders at similar altitudes (Beall, 2006; Gassmann et al., 2019). As a result, Sherpa demonstrate a low arterial oxygen content that is ~10% lower than sea level values (Beall et al., 1997). Thus, Sherpa appear to be more hypoxic compared with Lowlanders and Andean highlanders at a similar altitude. The lower haemoglobin concentration in Sherpa, however, is largely a consequence of a greater plasma volume, rather than an absent erythropoietin response (Stembridge et al., 2019). This enables increased oxygen carrying capacity of the blood whilst limiting the increase in blood viscosity that can hinder microcirculatory blood flow (Fan et al., 1980).

Rather than increasing arterial oxygen content, Sherpa appear to rely primarily on an enhanced blood flow, greater diffusion of oxygen from the blood into cells, and downstream metabolic adaptations to increase oxygen utilisation at a cellular level (Beall, 2007; Gilbert-Kawai et al., 2014; Horscroft et al., 2017). Sherpa possess a high peripheral blood flow, evidenced by elevated forearm, femoral and microcirculatory blood flow, a greater capillary density and a decreased pulmonary and systemic vascular resistance versus acclimatising Lowlanders (Groves et al., 1993; Erzurum et al., 2007; Gilbert-Kawai et al., 2017; Tremblay et al., 2018). In fact, Sherpa have more than double the forearm blood flow of Lowlanders, resulting in greater oxygen delivery to tissues (Erzurum et al., 2007). Blood flow is also likely to be greater in Sherpa than Andean highlanders, who exhibit a resting leg blood flow lower than that of acclimatising Lowlanders (Lundby et al., 2006). Furthermore, exhaled NO and circulating NO metabolites, an important regulator of vascular tone and local blood flow, are greater in Sherpa than Andeans and Lowlanders at high altitude (Beall et al., 2001; Erzurum et al., 2007; Horscroft et al., 2017), which likely contributes to the elevated peripheral blood

flow. An overview of the main physiological adaptations observed in Andeans and Sherpa are shown in *Figure 8*.

2.8.2 Chronic mountain sickness

The majority of high altitude natives show excellent adaptation to their environment; however, between 5–10% of highland natives cannot adapt to high altitude and develop CMS (León-Velarde et al., 2005). CMS is characterised by excessive erythrocytosis (haemoglobin concentration [Hb] ≥21g/dL for men, ≥19g/dL for women) (León-Velarde et al., 2005), accentuated arterial hypoxemia for the resident altitude and, in more severe stages of the disease, pulmonary hypertension. Andeans appear to be especially susceptible to CMS, with up to 33% developing CMS (Narvaez-guerra et al., 2018). In contrast, Sherpa are believed to be largely protected, with a lower prevalence of CMS reported in Sherpa (0.9%) at 4,300 m compared with Andean Quechuas (15.6%) at the same altitude (Monge et al., 1992; Wu et al., 1992; León-Velarde et al., 2005).

The pathophysiological mechanisms underlying CMS are incompletely understood; however, a loss of ventilatory acclimatisation and central hypoventilation is proposed as the primary mechanism (León-Velarde et al., 2010). Individuals with CMS exhibit lower levels of resting ventilation and greater $PaCO_2$ compared to their healthy Andean counterparts (Maignan et al., 2009). A depressed ventilation in CMS often results in a lower PaO_2 and accentuated arterial hypoxemia, which leads to an exaggerated erythropoietin response in an attempt to maintain oxygen transport to tissues. Whilst augmented [Hb] has long been considered beneficial at high altitude, excessive erythrocytosis is ineffective in improving tissue oxygenation and is, in fact, detrimental to oxygen delivery (Villafuerte et al., 2004). Excessive erythrocytosis increases packed red blood cell mass, with no change in plasma

volume, versus healthy Andeans, which increases total blood volume and blood viscosity (Claydon et al., 2004). Together, the increase in blood volume and viscosity can increase resistance to blood flow, exacerbate ventilation-perfusion mismatch, and impair pulmonary gas exchange, which further exacerbates arterial hypoxemia. The main physiological differences between CMS and healthy Andeans are summarised in *Figure 8*.

Corante et al*.,* (2018) demonstrated an association between the presence of excessive erythrocytosis and 10-year cardiovascular disease risk in Andean highlanders. This increased risk in CMS is partly mediated by elevated Hct and blood viscosity, which impair endothelial function, microcirculatory blood flow, and increase the likelihood of thrombotic events (Fan et al., 1980; Corante et al., 2018; Tremblay et al., 2019). However, several other clinical conditions characterised by sustained arterial hypoxaemia (e.g. Chronic obstructive pulmonary disease) are often accompanied by arterial baroreflex dysfunction and elevated MSNA (Van Gestel & Steier, 2010; Andreas et al., 2014). Such changes, which can facilitate increased BP variability, elevated BP, increased arterial stiffness and vascular dysfunction (Hijmering et al., 2002; Smit et al., 2002; Świerblewska et al., 2010), can all contribute to the development of cardiovascular disease. However, whether CMS is also accompanied by altered autonomic cardiovascular control is unclear.

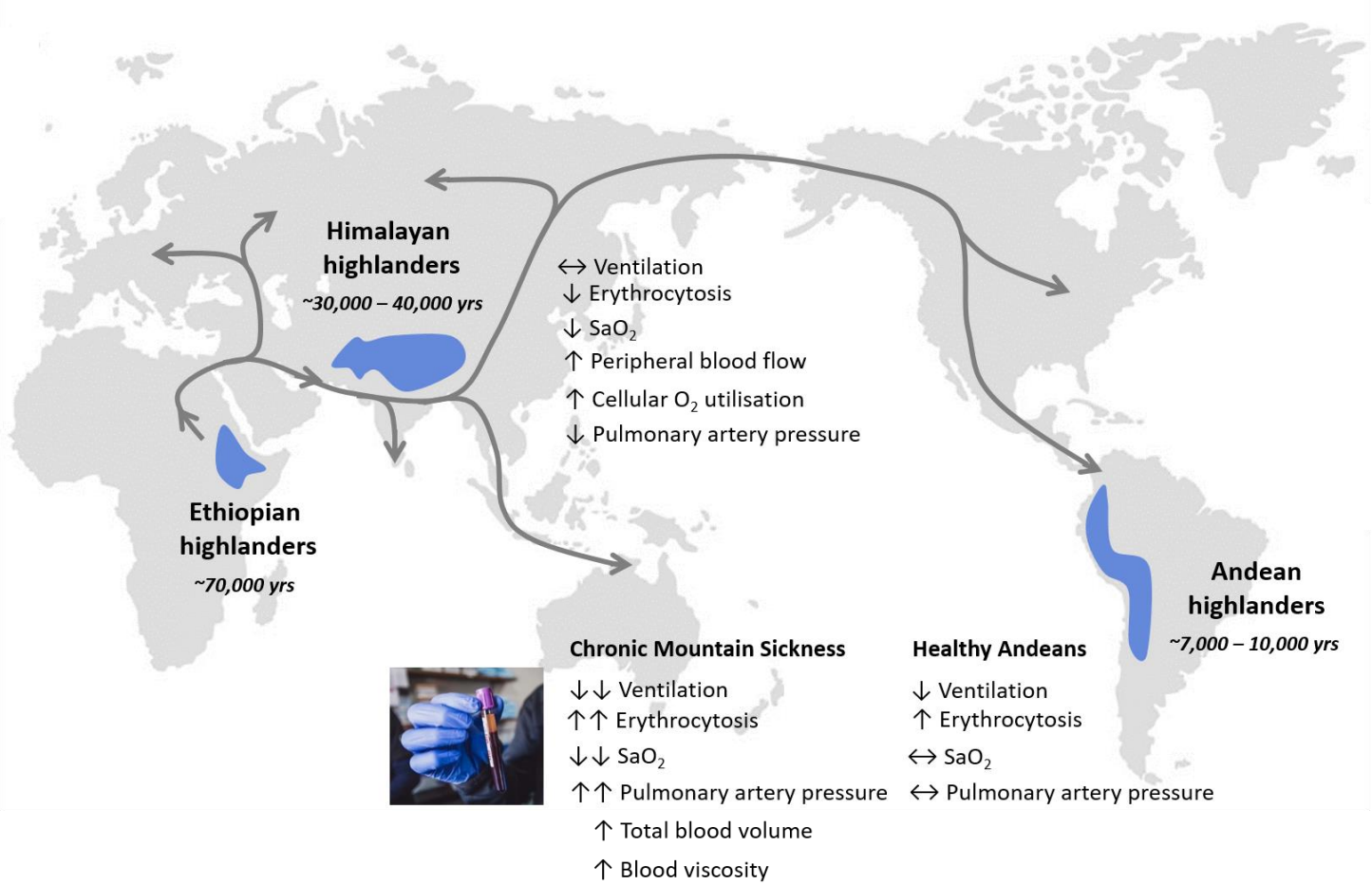

Figure 8. Overview of major physiological adaptations in Himalayan and Andean highland native populations

Geographically distinct highland populations have adapted unique strategies to facilitate successful oxygen delivery, and deal with the stress of ambient hypoxia The major physiological adaptations identified in each population are listed. Sherpa and healthy Andeans are compared to acclimatising Lowlanders, whereas CMS are compared to healthy Andeans (Severinghaus et al., 1966; Beall et al., 1997; Claydon et al., 2004; Beall, 2006; Villafuerte & Corante, 2016).

2.8.3 Sympathetic vasomotor activity in healthy highland natives

Despite extensive research exploring physiological adaptations in highland native populations, very little research exists regarding neural control and autonomic regulation of BP in these populations. The single microneurographic study performed in highland natives reported a comparable MSNA burst frequency in Bolivian (Aymara) Andean highlanders compared to Lowlanders following 10 and 50 days at 4100 m (Lundby et al., 2017). This finding suggests that Andean natives exhibit a higher level of MSNA, compared to values typically reported at sea level (Badrov et al., 2020); even after generational exposure to high altitude. Currently no microneurographic data exists in native Sherpa; nevertheless, indirect measures of sympathetic nervous system activity, would suggest reduced sympathetic nervous system activity in Himalayan natives versus acclimatising Lowlanders (Dhar et al., 2018). Indeed, lower plasma NA concentrations were reported in highland natives of the Indian Himalayan range, compared to acclimatising Indian Lowlanders following both seven days and 15 months at high altitude (Dhar et al., 2018). Furthermore, the values in Indian Himalayan natives were similar to the values observed in Lowlanders at sea level. Whilst Indian Himalayan natives and Sherpa natives likely demonstrate subtle genetic differences, this report suggests that unlike Andean highlanders, natives of the Himalayas may not exhibit increases in MSNA at high altitude. If apparent, the lower sympathetic vasoconstrictor drive may contribute to the reduced vascular resistance and increased blood flow observed in this population compared to both Andeans and acclimatising Lowlanders. However, the increased plasma volume reported in Sherpa (Stembridge et al., 2019) complicates the interpretation of these findings, thus, microneurographic studies are required to confirm this possibility.

2.8.4 Systemic blood pressure in healthy highland natives

Currently, limited research exists regarding systemic BP regulation in highland natives. Nevertheless, it has been reported that healthy Andeans exhibit a lower Qc at altitude (Groepenhoff et al., 2012), compared to acclimatising Lowlanders, as oxygen delivery is primarily supported via increased arterial oxygen content. A lower Qc is associated with a lower MAP compared to acclimatising Lowlanders (Lundby et al., 2017). Whilst vascular resistance in Andeans may be expected to be greater than that reported in acclimatising Lowlanders, due a greater blood viscosity, a concurrent increase in shear stress mediated vasodilation may counteract these effects on vascular resistance. In contrast to Andeans, Stembridge et al, (2014) reported a similar resting Qc in Sherpa compared to acclimatising Lowlanders following 10 days at 5,050 m. A similar Qc, however, is achieved through a lower stroke volume (49 ± 8 vs 63 ± 10 ml) and higher HR (76 ± 14 vs 61 ± 16 bpm) compared to acclimatising Lowlanders (Stembridge et al., 2014). A lower vascular resistance, as previously discussed, and a similar Qc can result in slightly lower MAP in Sherpa, compared to acclimatising Lowlanders (Bernadi et al., 1998; Jansen et al., 2000). However, there are several reports of a similar MAP in Sherpa, compared to acclimatising natives (Faoro et al., 2014; Gilbert-kawai et al., 2014; Stembridge et al., 2014). Overall, despite the limited evidence avaliable, Andeans appear to exhibit lower BP compared to acclimatising Lowlanders. On the other hand, Sherpa appear to exhibit a similar BP compared to acclimatising Lowlanders. Furthermore, these findings imply that the physiological mechanisms regulating BP may be different between populations

2.8.5 Sympathetic vasomotor activity in CMS

No microneurographic study exists examining MSNA in highlanders with CMS. Plasma catecholamine concentrations are reported to be either elevated (Gamboa et al., 2006) or unchanged (Antezana et al., 1995) in CMS, indicating either an increased or comparable global sympathetic activation compared to their healthy Andean counterparts. However, as previously discussed, plasma catecholamine concentrations may not accurately represent sympathetic vasomotor activity (Esler et al., 1984), especially as a greater total blood volume is observed in CMS (Claydon et al., 2004) and a poorer peripheral blood flow.

2.8.6 Systemic blood pressure in CMS

The influence of CMS on systemic BP is inconsistent. Some studies report increased BP, of between 4–13 mmHg, in CMS compared to healthy Andeans and an increased incidence of hypertension is also reported in CMS (Keyl et al., 2003; Richalet et al., 2005; Corante et al., 2018). Indeed, whilst elevated blood viscosity may be expected to decrease venous return and thus Qc (Maigan et al., 2009), it would also be expected to increase TPR (Poiseuille's Law). Despite this, other studies have reported no differences in either Qc, TPR and MAP in CMS compared to healthy Andeans (Penaloza & Sime, 1971; Claydon et al., 2004). The equivocal nature of these findings may be explained by the hetrogeneity of the samples studied. Often, individuals are classified as either CMS positive or CMS negative, which does not account for the non-binary nature of the disease. Whilst excessive erythrocytosis is the defininig feature of CMS, its severity can vary, and excessive erythrocytosis may or may not be accompanied by arterial hypoxemia and clinical symptoms, which also vary in severity if experienced. Potential alterations in physiological mechanisms likely occur as a function of disease severity,

meaning recruitment of Andeans with CMSwith a similar severity is desirable, and study findings should not be over generalised.

2.8.7 Arterial baroreflex function in highland natives

Currently, no comprehensive assessment of arterial baroreflex function exists in highland native populations. Nevertheless, Lundby et al, (2017) did report a comparable MSNA burst incidence, lower HR and lower MAP in healthy Andeans compared to acclimatising Lowlanders. Taken together, these findings indirectly suggest that the arterial baroreflex is reset to operate around a lower operating pressure and HR and similar MSNA operating point in Andeans at high altitude. Despite this, baroreflex responsiveness to BP perturbations was not investigated in this study. One previous study by Gulli et al., (2007) has investigated the cardiovagal baroreflex responsiveness in Andean highlanders and reported a lower spontaneous cardiovagal baroreflex gain in healthy Andean highland natives versus Lowlanders at sea level (9.8 vs 12.3 $m \cdot mmHg^{-1}$); although, no assessment of MSNA and vascular sympathetic baroreflex responsiveness was made. In addition, no data relating to arterial baroreflex function currently exists in Sherpa; therefore, it is unclear if differences in physiological adaptations between highland populations extend to arterial baroreflex control of blood pressure

2.8.8 Arterial baroreflex function in CMS

One previous study, comparing healthy Andeans to CMS, found that the cardiovagal baroreflex operates around a shorter RRI and higher SBP in CMS compared to healthy Andeans (Keyl et al., 2003) with a reduced gain (Keyl et al., 2003). However, a reduction in cardiovagal baroreflex gain is not a consistent finding, as Gulli and colleagues (2007) reported a similar RRI responsiveness in CMS compared to healthy Andeans. Utilising neck collars, no

differences in carotid baroreflex control of vascular resistance were reported in CMS versus healthy Andean controls (Moore et al., 2006), but the reflex operated around a higher arterial pressure and vascular resistance. In contrast, Claydon et al., (2005) reported an attenuated forearm vasoconstrictor response to an orthostatic challenge in CMS versus healthy Andeans at sea level, which may result from a reduced vascular sympathetic baroreflex gain. Nevertheless, like healthy Andeans, no direct measurement of baroreflex control of sympathetic vasomotor activity exists for CMS individuals.

2.9 Mechanisms involved in neural control of the cardiovascular system at high altitude

The mechanisms underlying the sustained elevations in MSNA, in Lowlanders at high altitude, have not been interrogated fully. However, heightened MSNA likely involves the central integration of a variety of inputs, including afferent inputs from several peripheral autonomic reflexes within the cardiovascular system. This section will discuss three candidate mechanisms for sustained sympathoexcitation at high altitude.

2.9.1 Peripheral chemoreceptor activation

In Lowlanders, elevated MSNA during acute hypoxic exposure is primarily mediated by activation of the peripheral chemoreceptors (Marshall, 1994). Thus, the augmented MSNA during high altitude acclimatisation is also widely attributed to the peripheral chemoreceptors (Somers et al., 1989a; Mansukhani et al., 2014; Sander, 2016). Indeed, this possibility appears logical, as the carotid bodies become more responsive (i.e. greater afferent discharge frequency) to the same hypoxic stimulus during longer exposure periods (Nielsen et al., 1988). Peripheral chemoreceptor sensitisation is known to contribute to the progressive increase in ventilation during the initial days at altitude (i.e. ventilatory acclimatisation) (Smith et al., 1986), and thus may also augment MSNA. In support of this,

Lundby et al, (2017) reported no change in MSNA burst frequency during 15 minutes of normobaric hypoxia (FiO_2 0.11, SaO_2 88%); however, demonstrated a 180% increase in MSNA burst frequency after ten days at a comparable altitude 4300 m (SaO_2, 90%). Furthermore, peripheral chemoreceptor sensitisation and elevated minute ventilation persists for several days following return to normoxia (Smith et al., 1986) and similarly MSNA remains elevated three days following descent (Hansen & Sander, 2003; Mitchell et al., 2018). However, despite sharing an afferent pathway, peripheral chemoreceptor activation does not elicit equivalent efferent sympathetic and ventilatory responses (Keir et al., 2019). Indeed, following hypoxic exposures of seven hours, where peripheral chemoreceptor sensitisation is largely complete (Smith et al 1986), minute ventilation is augmented, but MSNA is reduced below normoxia values. This dissociation argues against a role for the peripheral chemoreceptors in sustained hypoxia induced increases in MSNA (Tamisier et al., 2007; Hunt et al., 2008).

The importance of the peripheral chemoreflex to the augmented MSNA at high altitude has been questioned by findings from Hansen and Sander (2003) and Fisher and colleagues (2018) who both acutely attenuated peripheral chemoreceptor drive at high altitude. Hansen and Sander (2003) reported a 22% reduction in MSNA burst frequency in Lowlanders during 25 minutes of hyperoxia following 28 days at 5260 m and Fisher et al., (2018) reported no change in MSNA burst frequency during low-dose intravenous dopamine infusion following 15–17 days at 3454 m. Taken together these studies suggest that whilst peripheral chemoreceptors are important for initiating sympathoexcitation acutely, other mechanisms likely maintain high altitude sympathoexcitation, at least in acclimatizing Lowlanders. Despite this, the peripheral chemoreflex appears to be important in the regulation of arterial baroreflex gain at high altitude. Indeed, Yazdani et al., (2016) reported that hyperoxia reversed the high altitude associated reduction in cardiovagal baroreflex gain

following one day at 5260 m, in Lowlanders. Whether the role of the peripheral chemoreflex differs in highland natives is yet to be determined

2.9.2 Arterial baroreflex unloading

Other autonomic mechanisms have also been investigated as potential mediators of high altitude sympathoexcitation, including the arterial baroreflex. A marked reduction in circulating blood volume is observed during high altitude acclimatisation (Stembridge et al., 2019). Such a reduction decreases stroke volume and pulse pressure, with no significant changes in right atrial pressure and left ventricular filling pressure (pulmonary wedge pressure), at least up to simulated altitudes of 6100 m (Groves et al., 1987; Reeves et al., 1987). These reductions in blood volume may contribute to high altitude sympathoexcitation, via engagement of the arterial baroreflex. Arterial baroreceptors respond to mechanical distension and not arterial pressure *per se* (Angell-James, 1971) and a reduced stroke volume and/or pulse pressure would reduce distension of baroreceptive areas, independent of arterial pressure. Indeed, in healthy normotensive individuals at sea level, an inverse relationship exists between blood volume and MSNA burst frequency (Best et al., 2014) and acute reductions in central blood volume, via LBNP, elicit increases in MSNA (Ryan et al., 2012). Furthermore, the reduction in blood volume at high altitude begins within 24 hours of exposure and progressively decreases during the first one to two weeks at altitude and plateaus thereafter (Siebenmann et al., 2017). A similar temporal relationship is observed in the MSNA response. Despite this, blood volume expansion following four weeks at 5260 m, via infusion of 1000 mL saline, only reduced MSNA by seven bursts·min^{-1}. Accounting for only 25% of the 200% increase in MSNA observed at high altitude (Hansen and Sander, 2003). Although it is possible that saline infusion reduced CaO_2, subsequently increasing peripheral

chemoreflex activation, and thus underestimating the contribution of blood volume changes, combined saline infusion and hyperoxia had no additive effect (a reduction of 10 bursts·min^{-1}) (Hansen and Sander, 2003). Therefore, reductions in blood volume do not appear to play a major role in sympathoexcitation during sustained high altitude exposure, leaving the major underlying mechanism to be elucidated.

2.9.3 Pulmonary arterial baroreceptor activation

Another potential, but unexplored, autonomic mechanism is pulmonary artery baroreceptors. Opposite to the vasodilator response in systemic vessels, hypoxic exposure elicits vasoconstriction in the pulmonary vasculature, referred to as hypoxic pulmonary vasoconstriction (HPV) (Naeije, 1992). HPV results from the inhibition of oxygen sensitive K^+ channels leading to depolarization of the pulmonary artery smooth muscle cells and increased intracellular $Ca^{2+,}$ and thus vasoconstriction (Naeije, 1992; Lumb & Slinger, 2015). HPV is largely an adaptive mechanism that matches regional perfusion and ventilation in the lung to optimise systemic oxygen delivery. However, during exposure to high altitude, hypoxia in the lung is global, causing wide spread HPV, resulting in elevated pulmonary vascular resistance and pulmonary pressure (Groves et al., 1987). HPV occurs within seconds of the onset of hypoxic exposure and plateaus after five minutes. This is followed by a further rise in HPV, and pulmonary artery pressure, that reaches its maximum at around two hours (Dorrington et al., 1997; Talbot et al., 2005) and is maintained thereafter. If the hypoxic stimulus remains for several weeks elevated pulmonary pressure persists despite return to normoxia or inhalation of 100% oxygen (Groves et al., 1987; Talbot et al., 2005; Maufrais et al., 2016), suggesting remodeling of the pulmonary arterioles (Groves et al., 1987).

Duplain et al., (1999) reported a 62% increase in pulmonary artery pressure in healthy Lowlanders following acute (20 minutes) hypoxic (12% O_2) exposure, which was associated with a ~55% increase in MSNA burst frequency. With more prolonged exposure (24–36 hours) to an equivalent altitude (4559 m), pulmonary pressure had increased by 100%, which was associated with a more exaggerated increase in MSNA (~69%), despite similar reductions in SpO_2. Duplain et al., (1999) also demonstrated a positive relationship between pulmonary artery pressure and resting MSNA at high altitude (*Figure 9*). The authors speculated that an exaggerated increase in MSNA contributed to the exaggerated increase in pulmonary pressure and thus the development of high altitude pulmonary edema. However, HPV occurs in excised isolated lungs, thus, elevated pulmonary pressure appears to primarily be a local response to hypoxia and not sympathetically mediated (Lloyd, 1966). Elevated pulmonary artery pressure, therefore, may be the stimulus driving the exaggerated MSNA response. Indeed, as previously described in *section 2.3.2*, isolated elevations in pulmonary arterial pressure, and thus, increased afferent input to pulmonary arterial baroreceptors, results in reflex systemic vasoconstriction and renal sympathoexcitation in anesthetized normoxic animals, (Moore et al., 2004b, 2004a, 2011). However, no studies have investigated the potential role of

pulmonary arterial baroreceptors and elevated pulmonary artery pressure in sympathoexcitation at high altitude in humans.

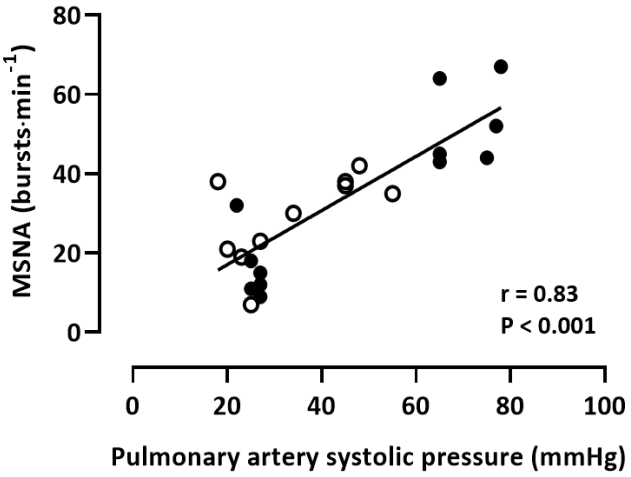

Figure 9. Relationship between pulmonary artery systolic pressure (PASP) and muscle sympathetic nerve activity (MSNA).

Duplain et al (1999) demonstrated a positive correlation between PASP and MSNA burst frequency measured at 580 m and 4559 m in healthy (O) and high altitude pulmonary oedema (HAPE) prone subjects (●). Redrawn from Duplain et al., (1999).

2.10 General summary of literature review

Appropriate regulation of BP is critical to ensure adequate tissue perfusion and oxygen delivery, which is especially important at high altitude, where oxygen availability is reduced. In response to acute hypoxic exposure, the arterial baroreflex is reset to a higher operating pressure and higher HR and level of MSNA, which helps maintain convective oxygen delivery. During high altitude acclimatisation, MSNA increases further and is sustained throughout exposure; however, the effect of high altitude acclimatisation on arterial baroreflex function has not being determined. Furthermore, the mechanism(s) responsible for the sustained elevations in MSNA at high altitude require elucidation. Whilst augmented peripheral chemoreflex activation appears to be the primary mechanism during acute hypoxic exposure, it appears to be less important during sustained exposure, at least for Lowlanders. Elevated pulmonary pressure may play an important role in sympathoexcitation during high altitude acclimatisation; although, this has not been investigated in humans.

In contrast to Lowlanders, highland native populations have adapted to the stress of chronic ambient hypoxia over generations, through natural selection. Consequently, these populations have developed distinct physiological strategies to maintain oxygen delivery. Sherpa appear to rely primarily on an increased blood flow, whereas Andeans rely on an increased haemoglobin concentration, which increases excessively in Andeans with CMS. Such differences likely have important implications for BP control and autonomic control of the cardiovascular system; however, no comprehensive assessment of sympathetic neural activity and arterial baroreflex function exists in highland natives.

2.11 Thesis Aims

The overall aim of this thesis is to comprehensively examine sympathetic neural activity and autonomic control of BP at high altitude in acclimatising Lowlanders and highland natives of the Tibetan and Andean plateau, with life-long exposure to high altitude. Three separate studies were designed to address the following aims. Hypotheses are presented in the introduction to each experimental chapter.

Chapter 4 (Experimental study 1).

Aims: To examine sympathetic neural activity and arterial baroreflex function in healthy Lowlanders during acclimatisation to high altitude and compare to acute exposure to hypoxia. Furthermore, to examine and compare sympathetic neural activity and arterial baroreflex function in healthy Lowlanders during high altitude acclimatisation to Nepalese Sherpa.

Chapter 5 (Experimental study 2)

Aims: To examine sympathetic neural activity and arterial baroreflex function in Andean Quechua with maladaptation syndrome CMS and compare to healthy Andean Quechua. To determine if dysregulation of autonomic cardiovascular control is apparent in individuals who cannot adapt to high altitude. Chapter 4 and 5 also aimed to determine the mechanistic contribution of the peripheral chemoreflex to sympathetic neural activity and arterial baroreflex function in both Lowlanders and highland native populations at high altitude.

Chapter 6 (Experimental study 3)

Aims: To examine the mechanistic role of pressure sensitive receptors in the pulmonary artery to sympathetic neural activity and arterial baroreflex regulation in Lowlanders at high altitude.

CHAPTER 3: GENERAL METHODOLOGY

3.1 Introduction

This chapter describes the general methods of data collection and data analysis employed in all three experimental chapters (Chapter 4, 5 and 6).

3.2 Ethical approval

The described studies in this thesis were performed as part of two high altitude research expeditions, the first to the Ev-K2-CNR Research Facility (5050 m; Khumbu Valley, Nepal) in October 2016, and the second to the Universidad Peruana Cayetano Heredia's Instituto de Investigacions de Altura (4380 m; Cerro de Pasco, Peru) in July 2018 (*Figure 10*). Ethical approval was granted by several separate institutional ethics committees (Appendix I). In Chapter 4, low altitude experiments were completed at the University of British Columbia Okanagan in Kelowna, Canada (344 m); therefore, ethical approval was granted by the Human Ethics Committee of the University of British Columbia (H16-01297/H16-01028). High altitude experiments in Chapter 4 were completed at the Ev-K2-CNR Research Facility in Nepal; with ethical approval granted by the Nepal Health Medical Research Council, Bangor University and the University of Alberta. For Chapter 5 and 6 all experiments were performed at the Universidad Peruana Cayetano Heredia's Instituto de Investigacions de Altura. For Chapter 5, involving Andean highlanders, ethical approval was granted from the Universidad Peruana Cayetano Heredia (101686) and for Chapter 6, involving Lowlanders, ethical approval was granted by the Human Ethics Committee of the University of British Columbia (HS17-02687). All experimental procedures conformed to the latest revision of the *Declaration of Helsinki*, except for registration in a database.

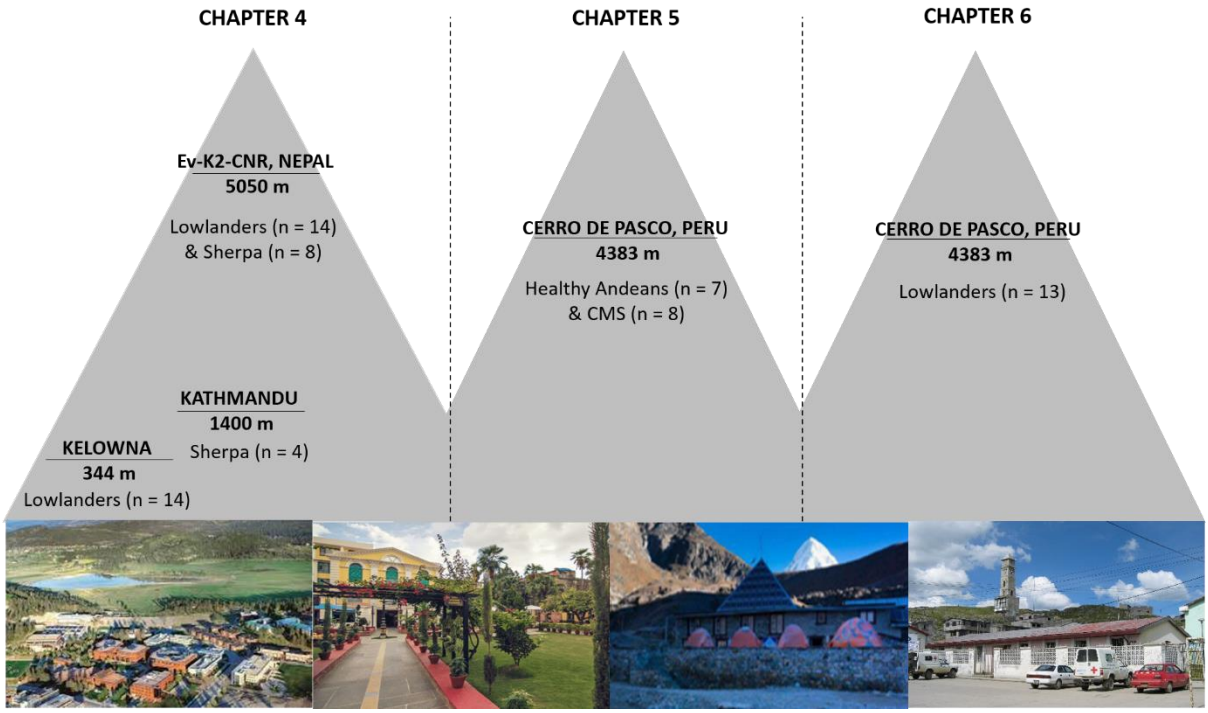

Figure 10. Schematic of thesis experimental design.

3.3 Participants

All Lowlanders were of North American or European ancestry, born at and living below 1000 m, and were members of the expedition team. High altitude natives were of Sherpa and Andean ancestry, who had at least two previous known generations of Sherpa or Andean ancestry, and who were born and living at an altitude above 3250 m. Sherpa were recruited from local villages of the Solokhumbu valley by word of mouth and Andeans were recruited from the Cerro de Pasco region, via a local database set-up by the Universidad Peruana Cayetano Heredia. Prior to participation, all experimental procedures and associated risks were explained to subjects in writing, and verbally, in their native language, and written informed consent was provided (Appendix II). All participants completed a health history questionnaire and had no prior self-reported history of cardiovascular, pulmonary, metabolic, neurological, or renal disease. At the time of participation, none of the participants were taking prescribed or over-the-counter medication. Due to the large scale nature of high

altitude research expeditions, participants took part in several other studies. Considerable care was taken to ensure appropriate recovery between protocols to prevent any potential for confounding results. These mitigations included: 1) a minimum recovery period of five half-lives following pharmacological interventions, to ensure complete drug washout. 2) A minimum recovery period of 24 hours following VO_2 peak assessments, submaximal exercise assessments or any intervention involving end tidal gas manipulation 3) exclusion from further study if participants had taken part in an iron infusion or hemodilution study. Participant demographics and anthropometrics will be presented in the relevant experimental chapters.

3.4 Experimental set-up

All participants were asked to abstain from caffeine, alcohol and vigorous exercise for 12 h before the experimental testing session. Subjects were also asked to arrive at the laboratory a minimum of 2 h after a light meal (Cox et al., 1995) and were asked to void their bladder on arrival (Fagius & Karhuvaara, 1989). Anthropometric measurements were determined (height, weight, Body Mass Index [BMI]) and an antecubital venous cannula was inserted for subsequent drug administration. All participants then rested in the supine position and were instrumented with equipment to measure cardiovascular haemodynamics and MSNA.

3.5 Experimental protocol

The experimental protocol for each experimental study is presented in detail in the relevant experimental chapter.

3.6 Experimental measurements

3.6.1 Cardiovascular haemodynamics

HR was continuously recorded via Lead II electrocardiography. Beat-by-beat peripheral BP was recorded using finger photoplethysmography (Chapter 4, Finometer Pro; Finapres Medical Systems BV, Amsterdam, The Netherlands; Chapter 5 & 6, Finometer MIDI, Finapres Medical Systems BV, Amsterdam, Netherlands). Briefly, arterial diameter of the finger is measured using an infrared photoplethysmograph in an inflatable cuff placed around the second or third finger on the right hand. The finger cuff pressure is automatically adjusted, via an inflatable bladder, to maintain a constant arterial diameter throughout the cardiac cycle. The artery clamped at a diameter where the transmural pressure is zero, and the cuff pressure is equal to the arterial pressure, which is a method known as the volume-clamp method (Penaz 1973). Brachial arterial pressure is then reconstructed from finger arterial pressure. This approach has been shown to be reliable and accurate in assessing beat-by-beat changes in BP during cardiovascular reflex testing, when compared with intra-arterial measurements (Imholz et al., 1998). Systolic (SBP), diastolic (DBP), and mean (MAP) pressures were calculated on a beat-by-beat basis from the finger arterial pressure waveform. Finometer values were calibrated against the average of three brachial artery BP measurements taken during baseline, via manual sphygmomanometry (Chapter 4; Welch Allyn, UK) or automated sphygmomanometry (Chapter 5 and 6; Omron M2, Kyoto, Japan). An example recording of beat-by-beat cardiovascular haemodynamics is shown in *Figure 11*. In Chapter 4 and 5, beat-by-beat stroke volume and Qc were estimated from the arterial pressure waveform using the Model Flow algorithm and subsequently used to estimate total peripheral resistance (TPR = MAP/Qc). In Chapter 6, stroke volume and Qc were determined

via echocardiography, as described below, and subsequently used to estimate TPR. SpO_2 was determined using finger pulse oximetry (Nellcor, Medtronics, USA). All data were sampled at 1000 Hz using commercially available data acquisition software and stored for offline analysis (PowerLab 16SP hardware and LabChart Pro v8.3.1, ADInstruments, Sydney, Australia).

3.6.2 Assessment of muscle sympathetic nerve activity

Microneurography was used to measure multiunit MSNA from the common peroneal nerve. Due to the time constraints associated with high altitude field research, the microneurography technique was performed by an experienced microneurographer (JPM, CDS). First, the leg used for MSNA measurement was elevated and supported in a slightly flexed position (between 30 and 45 degrees), using foam cushions and a mouldable pillow, to facilitate subject comfort and electrode placement. The common peroneal nerve was located through palpation and the path of the nerve was mapped. A high impedance tungsten microelectrode (200 μm diameter, 35 mm long; FHC, Bowdoinham, ME, USA) was then inserted through the skin and into the nerve. A reference microelectrode, which filters out background noise, was then inserted beneath the skin, 1-3 cm from the recording site. Once inserted, the recording microelectrode was manually manipulated until it penetrated a muscle fascicle and a satisfactory MSNA signal was found. Confirmation that the microelectrode was recording from a muscle fascicle, and not a skin fascicle, involved pulse-synchronous bursts, an increase in bursts during end expiratory apnea and a Valsalva manoeuvre with no response to startle stimuli or skin stroking (Delius et al., 1972b, 1972a). Nerve signals were amplified (1000x pre-amplifier and 100x variable gain isolated amplifier), band pass filtered (700-2,000 Hz), rectified, and stored on a computer using commercially available data acquisition and analysis software (PowerLab 16SP hardware and LabChart Pro

v8.3.1, ADInstruments, Sydney, Australia). In Chapter 4, nerve signals were acquired using the model 662C-3; Iowa University Bioengineering; USA; however, in Chapter 5 and 6 nerve signals were acquired using the Neuroamp EX, ADInstruments, Sydney, Australia. The raw MSNA signal was sampled at 10 KHz and integrated in LabChart (ADInstruments, Chart Pro v8.3.1) (*Figure 11*). No adverse events or complications occurred during or following the microneurography procedure in any subject. Baseline data was acquired following a ten minute stabilisation period following acquisition of a satisfactory MSNA signal.

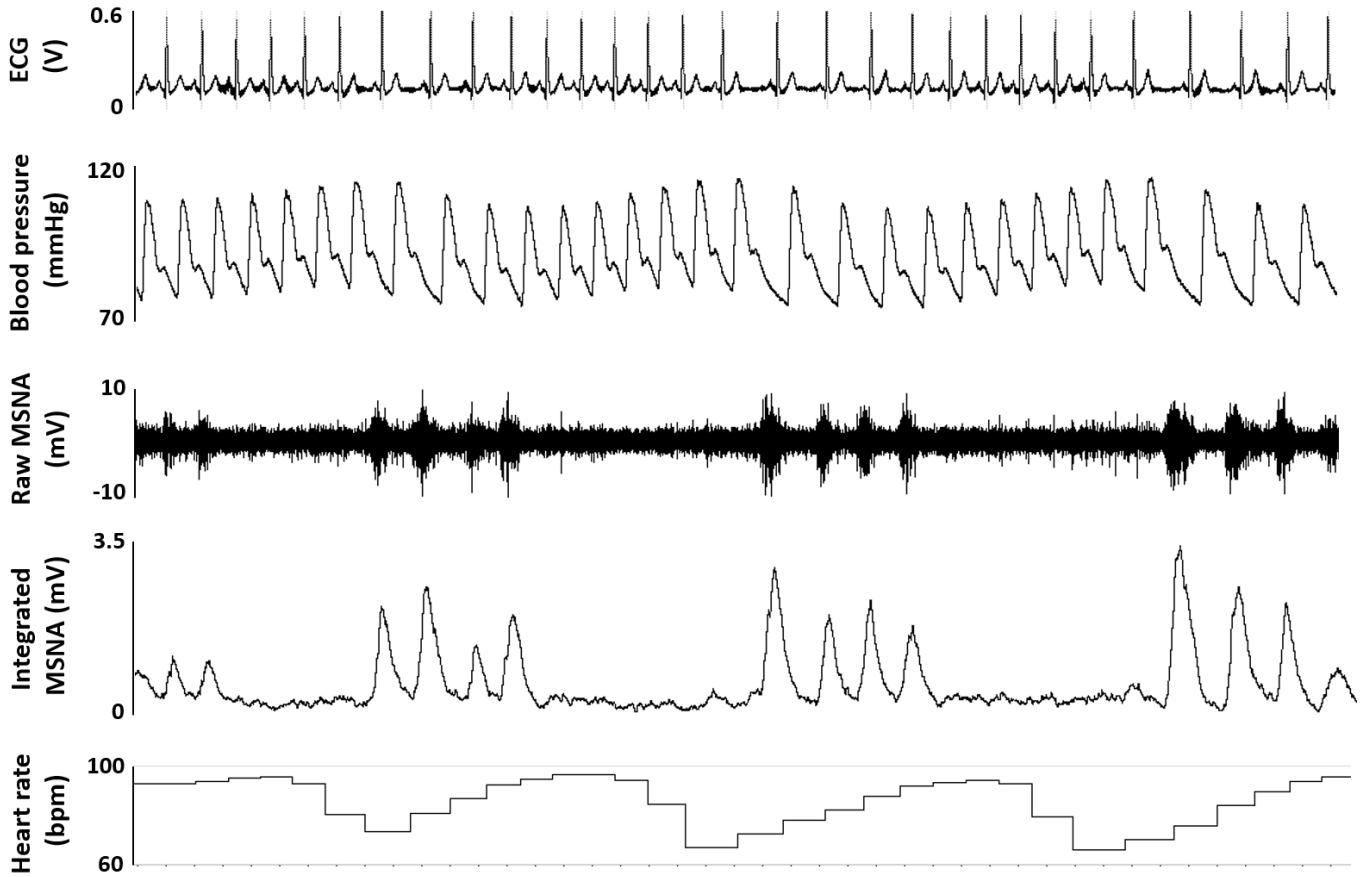

Figure 11. Example recording of beat-by-beat cardiovascular haemodynamic and muscle sympathetic nerve activity (MSNA).

3.6.3 Assessment of arterial baroreflex gain

Arterial baroreflex gain can be determined via multiple methods. The assessment of baroreflex gain using spontaneous fluctuations in BP, typically 5–10 minutes of rest, is widely employed in the literature, due to its non-invasive nature. A criticism of the spontaneous baroreflex approach, however, is that the relationship between MSNA and spontaneous changes in BP may not reflect baroreflex causality (Kamiya et al., 2011). Furthermore, this approach is more likely to be confounded by alterations in respiratory patterns, which are known to be altered with altitude exposure. In addition, the spontaneous baroreflex approach provides an assessment of baroreflex gain over a small BP range, around the operating point, thus limiting the assessment of the full baroreflex curve.

In order to examine the stimulus-response relationship over a wider range of the baroreflex curve, methods involving active perturbation of BP can also be employed. These methods include neck suction and pressure, tilt test, and the modified Oxford method (Elisberg, 1963; Eckberg, 1976; Palmero et al., 1981; Ebert & Cowley, 1992; Fadel et al., 2003). The modified Oxford test is considered the 'gold standard' method to assess arterial baroreflex gain and involves pharmacologically inducing changes in BP through bolus injections of vasoactive drugs (*Figure 12*). This technique was first developed to assess cardiovagal baroreflex gain (Smyth et al., 1969) and involved injections of angiotensin or phenylephrine (PE) to examine the RRI responses to increases in BP. The Oxford method was then modified to include the use of vasodilator sodium nitroprusside (SNP), to enable the baroreflex responses to both increases and decreases in BP to be characterised (Ebert & Cowley, 1992). An advantage of the use of the modified Oxford approach is that it involves large and rapid changes in BP, which allows the larger baroreflex curve to be characterised. Furthermore, pharmacological manipulation of pressure partially opens the closed-loop

system of the baroreflex, whereby RRI and MSNA responses are the direct effect of alterations in BP, which allows baroreflex causality to be assessed (Kamiya et al., 2011). Therefore, in this thesis, the modified Oxford method will be employed to determine vascular sympathetic and cardiovagal baroreflex gain (Rudas et al., 1999).

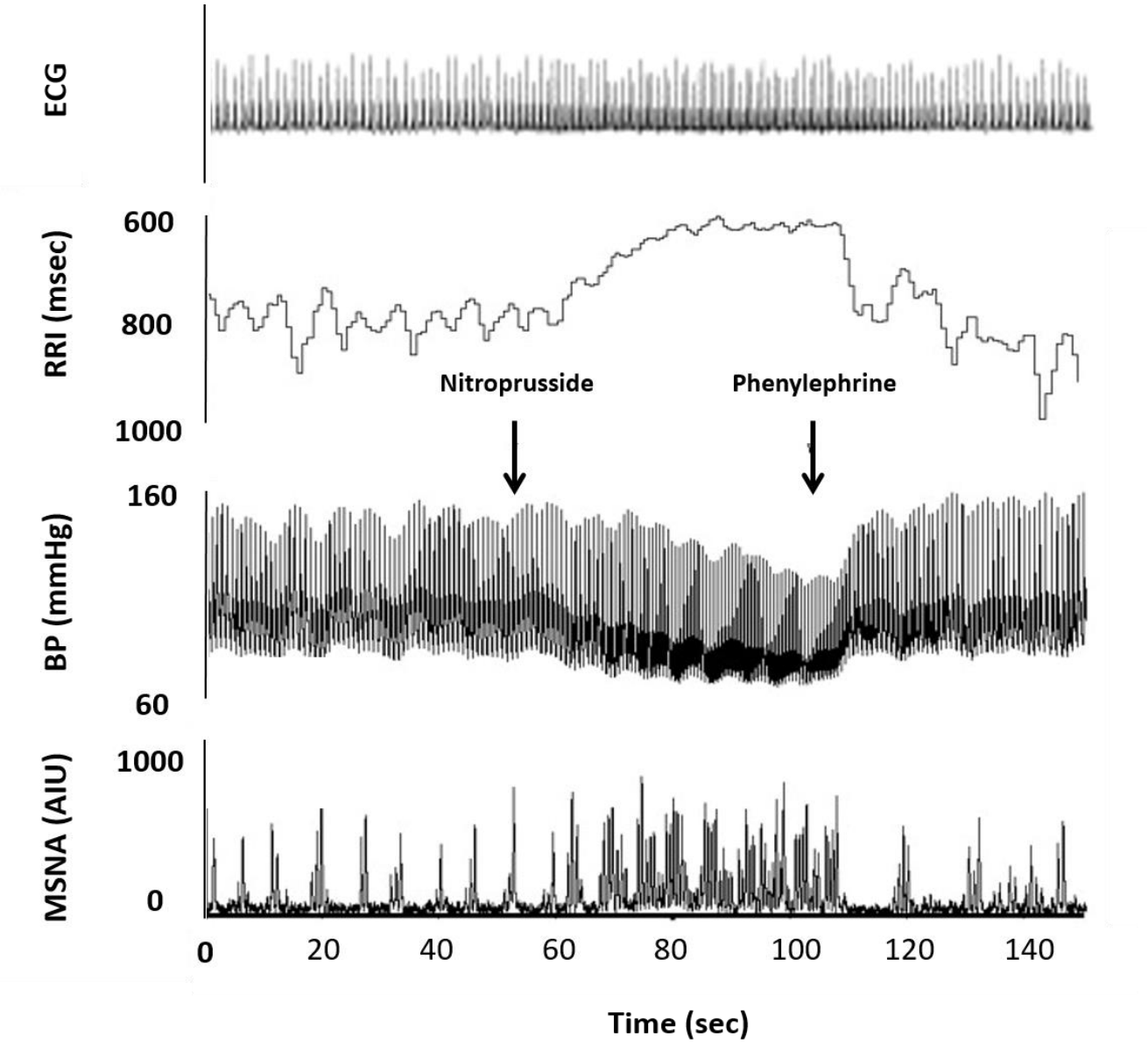

Figure 12. Assessment of arterial baroreflex gain via the modified Oxford method.

Sequential intravenous bolus injections of sodium nitroprusside and phenylephrine evoke ~15 mmHg perturbations above and below resting arterial blood pressure (BP) respectively. BP perturbations elicit baroreflex mediated alterations in muscle sympathetic nerve activity (MSNA) and RR interval (RRI), which attempt to return BP to the homeostatic operating pressure. Modified from Adler et al., 2009.

The modified Oxford test involved bolus injection of nitric oxide donor SNP, followed 90 seconds later by α_1-adrenergic agonist PE. Prior to the start of experimental testing, bolus doses of SNP and PE that evoked ~15 mmHg perturbations above and below resting BP were determined for each individual. Briefly, individualized doses of vasoactive drugs were calculated based on total blood volume, which was estimated using the Nadler equation (shown below) (Nadler et al., 1962), in the ratio of 20 $\mu g \cdot L^{-1}$ SNP; 30 $\mu g \cdot L^{-1}$ PE. These doses were adjusted if insufficient BP perturbations were achieved. Identical drug doses were administered across all trials in the same individual. Doses of vasoactive drugs injected, and the resultant BP perturbations are shown in *Table 2*.

Nadler formula

Males = $(0.3669 * \text{Height}^3) + (0.03219 * \text{weight}) + 0.6041$

Females = $(0.3561 * \text{Height}^3) + (0.03308 * \text{weight}) + 0.1833$

Height = metres, Weight = kilograms

	Lowlanders 344 m	Lowlanders 5050 m	Sherpa 5050 m	Healthy Andeans 4383 m	CMS 4383 m
Sodium nitroprusside dose (ug·kg^{-1})	1.71 ± 0.53	1.72 ± 0.39	1.60 ± 0.40	1.48 ± 0.30	1.66 ± 0.35
Phenylephrine dose (ug·kg^{-1})	2.27 ± 0.21	2.29 ± 0.24	2.10 ± 0.20	2.34 ± 0.42	2.48 ± 0.25
Sodium nitroprusside BP decrease (mmHg)	15 ± 6	15 ± 6	14 ± 4	11 ± 3	11 ± 5
Phenylephrine BP increase (mmHg)	19 ± 9	11 ± 3	15 ± 4	15 ± 3	13 ± 5
Total BP change (mmHg)	35 ± 11	26 ± 8	28 ± 6	25 ± 6	26 ± 5

Table 2 *Doses of vasoactive drugs, administered to Lowlanders, Sherpa, healthy Andeans and CMS during modified Oxford test and the resultant BP perturbations*.

3.6.4 Echocardiography

In Chapter 6, echocardiography was used to determine stroke volume and in Chapter 5 and Chapter 6 echocardiography was used to estimate pulmonary artery systolic pressure (PASP). Briefly, echocardiography involves imaging the heart using ultrasound, which can determine cardiac structure and dimensions, function, and blood flow velocity through the heart (Feigenbaum, 1977). Ultrasound waves, whose frequency exceeds that of human hearing (over 20 KHz), are generated by the vibration of piezoelectric crystals in the ultrasound transducer, when electricity is applied (Armstrong & Ryan, 2012). Ultrasound waves are emitted from the transducer through the bodily tissues, which causes them to oscillate. The transducer receives the reflected ultrasound waves (i.e. the echo), which produces an electrical signal and allows the generation of an ultrasound image (Armstrong & Ryan, 2012). Different echocardiographic imaging modalities are used to assess different aspects of cardiac structure and function. The two modalities employed in the present thesis are two-dimensional (2D) B-mode echocardiography, which is used to assess cardiac dimensions (i.e. diameter of left ventricular outflow tract) and Doppler echocardiography, which is used to determine blood flow velocity across the hearts valves (i.e. the tricuspid valve) and within arteries (i.e. aorta outflow) (Armstrong & Ryan, 2012).

All echocardiography images were obtained by an experienced sonographer (performed by MS or ALD) using a commercially available system (Vivid Q, GE, Fairfield, CT, USA) with a 1.5-4 MHz phased array transducer and stored for subsequent off-line analysis with commercially available software (Echopac, GE Medical, Horten, Norway, version 110.1.3). Echocardiography images were acquired at end-expiration during spontaneous breathing. If participants were required to hold their breath to facilitate image acquisition, a

comment was entered into the data acquisition file and the corresponding haemodynamic and MSNA data were not included in analysis, due to the known sympathoexcitatory effects of breath-holding (Delius et al., 1972b). Three cardiac cycles were measured for each parameter and averaged.

Stroke volume was calculated from the Doppler signal using the velocity-time integral (VTI) and the aortic cross section area (π x aortic radius^2), in line with the American Society of Echocardiography guidelines (Lang et al., 2005, 2015; Rudski et al., 2010). First, the diameter of the left ventricular outflow tract, at the level of the aortic annulus, was determined from the parasternal long axis view. Measurements were taken at the end of systole and the average of three cardiac cycles was taken as the diameter of the aorta. Second, the VTI of the left ventricular outflow tract was obtained from an apical five chamber view by placing a pulsed wave Doppler sample volume within the aortic valve. Pulmonary artery systolic pressure (PASP) was quantified as the peak systolic pressure gradient across the tricuspid valve, added to right atrial pressure estimated from the collapsibility of the inferior vena cava, in line with the American Society of Echocardiography guidelines (Rudski et al., 2010). A regurgitate blood flow jet through the tricuspid value is detectable during systole in 70–75% of individuals. The velocity of this jet is directly related to the systolic pressure difference (ΔP) between the right atrium and right ventricle and can be calculated using the Bernoulli equation (*Figure 13*). The addition of right atrial pressure to this pressure gradient provides an estimation of right ventricular systolic pressure, which equals the systolic pressure in the pulmonary artery. Echocardiography is an accurate and reproducible method for the determination of PASP that correlate closely with invasive measurements obtained via right heart catheterisation at high altitude (Allemann et al., 2000). First, the peak tricuspid regurgitation jet velocity was measured by continuous wave Doppler ultrasound using colour

flow imaging from the apical four-chamber view. Measurements were taken as the average value over three cardiac cycles. Second, right atrial pressure was estimated by the collapsibility of the inferior vena cava in response to a short, sharp sniff, as recommended by the American Society of Echocardiography (Lang et al. 2005). If the inferior vena cava diameter was reduced by ≥ 50%, then a right atrial pressure of 5 mmHg was assumed. This method has been validated against right atrial pressure values obtained directly by right heart catheterization (Yildirimturk et al., 2011). Subjects who did not exhibit a ≥ 50% reduction in inferior vena cava diameter were excluded prior to participation, due to elevated right atrial pressure. Peak systolic pressure difference across the tricuspid valve was then estimated from the modified Bernoulli equation (4 x V^2), where V is the peak systolic velocity of the tricuspid regurgitation jet, and PASP estimated by the addition of right atrial pressure (*Figure 13*).

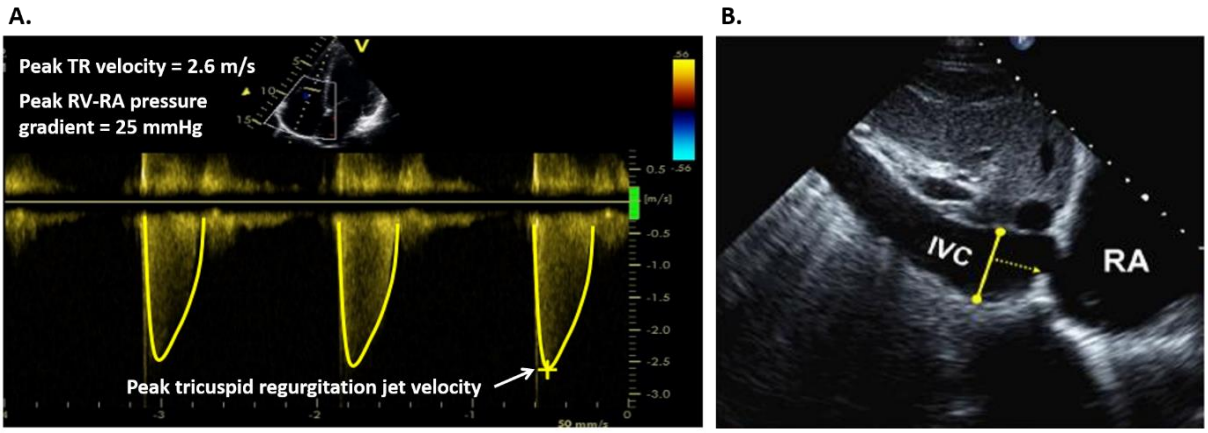

Figure 13. Estimation of pulmonary artery systolic pressure (PASP) via echocardiography.

Panel A. Peak tricuspid regurgitation (TR) jet velocity is measured by continuous wave Doppler ultrasound (2.6 m/s in the example shown). Peak TR velocity is then used to calculate the peak pressure gradient between the right ventricle (RV) and right atrium (RA) using the modified Bernoulli equation (4 x $[2.6]^2$ = 25mmHg). Panel B. RA pressure is estimated by determining the diameter of the inferior vena cava (IVC), in the subcostal view, and determining its percentage collapsibility during a short sharp sniff. If the IVC diameter is reduced by ≥ 50%, then a RA of 5mmHg is assumed. Pulmonary artery systolic pressure is then calculated by adding the peak pressure gradient to the estimated RA pressure.

3.7 Data analysis

3.7.1 Muscle Sympathetic Nerve Activity

Multi-unit bursts of MSNA were identified using a semi-automated detection algorithm (LabChart Pro, version 8.3.1) where a minimum amplitude (2 SD above noise) was set for burst detection, and verified via visual inspection of the integrated neurogram. Inaccurately identified bursts were removed and unidentified bursts were included. MSNA bursts were confirmed using established criteria, including burst morphology and relation to DBP (White et al., 2015; Hart et al., 2017). Bursts were only marked if they exhibited a sharp peak, a 3:1 signal to noise ratio, and if the upstroke of the burst corresponded with the diastolic period of the cardiac cycle. Absolute burst amplitude (mv) is an indication of both the number and size of sympathetic neurons that are firing action potentials (Steinback et al., 2010), but also dependent on the proximity of the microelectrode to the post ganglionic sympathetic neurons it is recording from. Absolute burst amplitude data cannot be used for comparisons between individuals or within an individual when the recording site and microelectrode position has changed (Hart et al., 2017; White et al., 2015). As a result, burst amplitude data was normalized with a value of 100 assigned to the largest MSNA burst observed during the unstimulated baseline period, with all other bursts expressed as a percentage calibrated against the value of the largest burst. The integrated neurogram was then time shifted to account for the baroreflex latency and ensure each MSNA burst was aligned with the diastolic period in which it was initiated. The baroreflex latency represents the time taken for the baroreflex to remove sympathetic inhibition in the brain and the conduction delay of sympathetic outflow to the recording site. Baroreflex latency was calculated as the time interval between the peak of an MSNA burst and the preceding R-wave

of the ECG. In each participant, baroreflex latency was calculated as the average latency of six MSNA bursts (two small, two medium, two large bursts) occurring during the resting period. Average burst latency in Lowlanders at 344 m, -1.3 ± 0.06 s ;Lowlanders at 5050 m, -1.36 ± 0.08 s; Sherpa at 5050 m, -1.28 ± 0.04 s; CMS, -1.23 ± 0.05 s; Healthy Andean highlanders, -1.17 ± 0.05 s.

Quantification of MSNA

Increases in MSNA can be achieved via several mechanisms. First, by increasing MSNA burst frequency (bursts·min^{-1}), which can be achieved through increasing the probability of a burst occurring per cardiac cycle (i.e. burst incidence), or by increasing the opportunities for a burst to occur (i.e. heart rate), and second, by increasing the amplitude of MSNA bursts. Burst frequency, burst incidence and burst amplitude, can all be regulated independently of each other (DiBona & Jones, 1998; Hjemdahl et al., 1989; Malpas et al., 1996); therefore, quantification of all indices of MSNA is necessary to give a comprehensive and accurate quantification of MSNA. In this thesis, resting sympathetic vasomotor outflow was quantified as MSNA burst frequency (bursts·min^{-1}) and total activity (a.u·min^{-1}). These indices are reflective of the neurotransmitter release by sympathetic nerve terminals, and thus the sympathetic vasoconstrictor drive that the vasculature is exposed to in a given time, which subsequently determines vascular tone (Wallin et al., 1992; Notarius et al., 2015). Conversely, baroreflex gating of sympathetic bursts, was quantified as MSNA burst incidence and total MSNA, which represent the probability of a burst occurring per cardiac cycle, independent of time and HR.

3.7.2 Assessment of vascular sympathetic baroreflex gain

Vascular sympathetic baroreflex gain can be estimated by plotting the relationship between DBP and MSNA. DBP is used as it more closely correlates with MSNA than SBP (Sundlof & Wallin, 1978). This is because bursts of MSNA are initiated during diastole, the time when the baroreceptors are unloading. MSNA burst probability or a combination of MSNA burst probability and MSNA burst amplitude (total MSNA) are used in the analysis of vascular sympathetic baroreflex gain. Within an individual, lower DBP are associated with higher MSNA burst probability or total MSNA and vice versa; therefore, an inverse relationship between DBP and MSNA burst probability or total MSNA is observed. The use of MSNA burst amplitude is less successful and reliable (Kienbaum et al., 2001; Keller et al., 2006), as the baroreflex primarily determines the MSNA bursts occurrence with less influence over burst amplitude (Kienbaum et al., 2001).

Vascular sympathetic baroreflex gain

In this thesis, baroreflex control of MSNA was determined from the stimulus response relationship between 1) MSNA burst probability and DBP 2) total MSNA (burst probability x burst amplitude) and DBP during modified Oxford test.

Vascular sympathetic baroreflex gain: Burst probability

Firstly, DBP was related to the probability of a sympathetic burst occurring. DBP values were assigned to a 3 mmHg bin. This binning procedure reduces the statistical impact of the beat-by-beat variability in MSNA from non-baroreflex influences (i.e. respiration) (Eckberg et al., 1985). The percentage (ranging from 0–100%) of cardiac cycles associated with a sympathetic burst was calculated, providing the probability of a burst occurring for each DBP bin.

Vascular sympathetic baroreflex gain: Total MSNA

Secondly, DBP was related to both the probability and amplitude of a sympathetic burst, as changes in both burst occurrence and amplitude are observed with changes in DBP (Sundlof & Wallin, 1978). DBP values were assigned to a 3 mmHg bin. The sum of normalized burst amplitudes for each DBP bin was determined. This value was then divided by the number of bursts within the DBP bin, to calculate mean burst amplitude. Mean burst amplitude was then multiplied by the burst probability to calculate total MSNA.

The slope of the linear portion of the relationship between MSNA burst probability or total MSNA and DBP, was used as an index of vascular sympathetic baroreflex gain (*Figure 14*). As threshold and saturation regions were not observed in all participants, non-linear threshold and saturation regions, if present, were removed through visual inspection of data points. Slope of the linear portion of these relationships were determined by linear regression analysis. Each data point was weighted for the number of cardiac cycles in each diastolic pressure bin, which removes bias that would otherwise occur if bins containing very few cardiac cycles and bins containing a very large number of cardiac cycles have equal contribution to linear regression analysis. Only slopes with $R > 0.5$ were accepted and included in the group mean data (Hart et al., 2011; Taylor et al., 2015). Whilst hysteresis is a recognised characteristic of the vascular sympathetic baroreflex, where the reflex is more responsive to falling BP than rising BP (Sundolf and Wallin 1978, Studinger et al., 2007; Hart et al., 2011), reflex gain to rising and falling pressures were not determined independently. This was due to an insufficient number of data points, in one or both of the split portions, to construct statistically significant baroreflex slopes. An inherent limitation in the assessment of vascular sympathetic baroreflex gain is that it relies upon the occurrence of a burst of MSNA, which does not occur with every cardiac cycle. This is particularly problematic during higher

pressures where there is significant inhibition of sympathetic bursts. This reduces the number of data points in the regression analysis for the estimation of baroreflex gain. Therefore, baroreflex slopes were constructed using both data during decreases in BP following SNP injection and increases in BP following PE injection. The vascular sympathetic baroreflex set-point was taken as the average values for DBP and MSNA burst incidence (bursts·100HB^{-1}) or total MSNA (a.u·100HB^{-1}), respectively, during the resting period before the modified Oxford test.

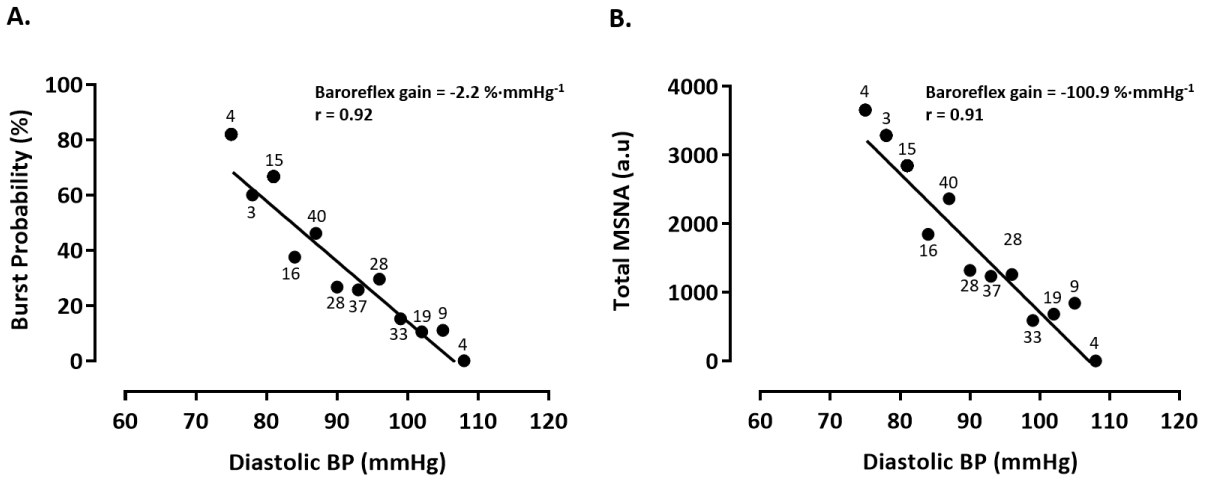

Figure 14. Determination of vascular sympathetic baroreflex gain in a 20-year-old female Lowlander at 5050 m.

Panel A. illustrates the burst probability method and Panel B. illustrates the total MSNA method. The linear regression lines between diastolic BP and MSNA are weighted to the number of cardiac cycles in each 3 mmHg bin, which is indicated next to data each point

Whilst the vascular sympathetic and cardiovagal limbs of the baroreflex share the same afferent pathway, the effectiveness of their efferent limbs may differ. It should not be assumed that an individual with low cardiovagal baroreflex gain also possesses poor vascular sympathetic baroreflex gain (Dutoit et al., 2010; Rudas et al., 1999; Taylor et al., 2015). Furthermore, the cardiovagal and vascular sympathetic limb of the baroreflex can be

differentially affected under several conditions (Grassi et al., 1998; Narkiewicz et al., 1998; Monahan et al., 2001; Usselman et al., 2015); therefore, it is important that both limbs of the arterial baroreflex are investigated. Moreover, both the vascular sympathetic and cardiovagal limbs contribute to the correction of blood pressure by the baroreflex.

3.7.3 Assessment of cardiovagal baroreflex gain

Cardiovagal baroreflex gain can be estimated by plotting the relationship between SBP and RRI. Within an individual, lower systolic blood pressures are associated with shorter RRI and vice versa; therefore, a positive relationship between SBP and RRI is observed. Cardiovagal baroreflex gain can also be estimated by plotting SBP against HR. Assessment of RRI and HR responses during rapid BP perturbations induced by the modified Oxford test will examine parasympathetic modulation of the heart, rather than sympathetic modulation. Indeed, reflex RRI/HR responses to pharmacologically induced increases and decreases in BP are abolished with parasympathetic blockade (atropine) and unaffected by sympathetic blockade (propranolol) (Pickering et al., 1972). This is because parasympathetic control exhibits almost no time lag in its response to changes in arterial pressure, whereas sympathetic control exhibits a long time lag and rapid changes in blood pressure do not allow sufficient time to observe the slower sympathetic component (Pickering et al., 1971; Scher et al., 1972; Sagawa., 1978).

Cardiovagal Baroreflex gain

In this thesis, baroreflex control of the heart was determined from the relationship between SBP and RRI during modified Oxford trial (*Figure 15*). RRI values were averaged over 3 mmHg bins to reduce non-baroreflex influences. Cardiovagal baroreflex delays were accounted for by associating SBP values with concurrent heartbeat (resting RRI >800 ms, HR

<75 bpm) or subsequent heartbeat (resting RRI <800 ms, HR >75bpm; Eckberg & Eckberg, 1982). If the resting RRI is less than 800 ms a given SBP will not have time to influence the concurrent RRI therefore that SBP will influence the subsequent RRI. If the resting RRI is greater than 800 ms, there is sufficient time to influence the concurrent RRI. The slope of the linear portion of the relationship between RRI and SBP was used as an index of cardiovagal baroreflex gain. Non-linear saturation and threshold regions were excluded through visual inspection of data points and linear regression analysis was used to determine slope of linear portion of relationship. Only slopes with $R > 0.8$ were accepted and included in the group mean data and each data point was weighted for the number of cardiac cycles in each diastolic pressure bin. Linear regression between HR and SBP was also determined. SBP was used as HR correlates closely with SBP but not DBP. As every cardiac cycle has an RRI and instantaneous HR, assessment of the cardiovagal baroreflex does not suffer from the same lack of data points as the vascular sympathetic baroreflex; therefore, it is possible to limit the effects of hysteresis. To minimize the potential effects of hysteresis, data analysis was restricted to the rising arm of SBP and used values from the nadir to the peak SBP response during the Modified Oxford test (Hunt & Farquhar, 2005). Thus, only the cardiovagal baroreflex gain to increasing pressure was determined. Cardiovagal baroreflex set-point was taken as the average SBP and RRI or HR, respectively, during the resting period immediately prior to the modified Oxford test.

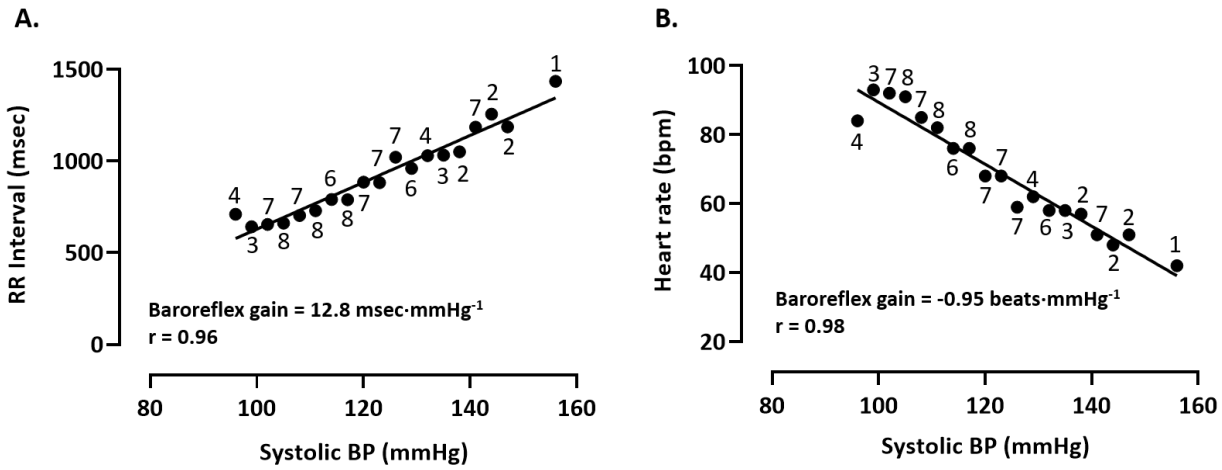

Figure 15. Determination of cardiovagal baroreflex gain in a 20-year-old female Lowlander at 5050m.

Panel A. illustrates cardiovagal baroreflex gain calculated from the stimulus-response relationship between systolic BP and RRI and Panel B. illustrates cardiovagal baroreflex gain calculated from the relationship between systolic BP and HR. Linear regression lines are weighted to the number of cardiac cycles in each 3 mmHg bin, which is indicated next to each data point. As every cardiac cycle has an RRI and instantaneous HR, the linear regression lines are calculated from a large number of data points, compared to the vascular sympathetic baroreflex

3.8 Statistical analysis

Differences between groups and conditions were assessed using pre-planned contrasts (dependent and independent T-tests). Statistical analyses were performed using Graphpad (GraphPad Prism, Version 8.3.0, San Diego, California, USA) with *a priori* alpha (α). set at 0.05. In Chapters 4 and 5, calculated *P* value was adjusted (α') to account for the number of multiple comparisons (*c*), using the experiment-wise error rate (α_e) (Hinkle et al., 2003).

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

$$\text{Adjusted } a \text{ priori alpha} = c/\alpha_e$$

$$\alpha' = \alpha(P \text{ value} / \text{adjusted } a \text{ priori alpha})$$

Detailed description of the precise statistical tests employed for each experimental study will be described in the methodology section of each experimental chapter.

CHAPTER 4: EXPERIMENTAL STUDY 1

Baroreflex Control of Sympathetic Vasomotor Activity and Resting Arterial Pressure at High Altitude: Insight from Lowlanders and Sherpa

A version of this chapter has been published in the Journal of Physiology

Simpson LL, Busch SA., Oliver SJ., Ainslie PN., Stembridge M., Steinback CD & Moore JP (2019). Baroreflex control of sympathetic vasomotor activity and resting arterial pressure at high altitude: insight from Lowlanders and Sherpa. **597(9),** 2379–2390 doi: 10.1113/JP277663

4.1 Abstract

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

4.2 Introduction

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

Several studies have characterised MSNA and BP responses to sustained high altitude exposure in healthy Lowlanders (Hansen & Sander, 2003; Lundby et al., 2017; Fisher et al., 2018). Despite a greater probability of a burst of MSNA at rest (i.e. MSNA burst incidence; 64% as opposed to 26%) (Hansen and Sander, 2003), any accompanying change in BP is relatively modest, at least for exposures lasting 10–50 days. These observations imply chronic resetting of the vascular sympathetic baroreflex, which attempt to maintain BP, presumably to balance local vasodilator mechanisms and maintain haemodynamic stability. However, arterial baroreflex control of MSNA has never been investigated at high altitude.

Relatively little is known regarding the consequences of lifelong high altitude hypoxia on sympathetic vasomotor activity and BP regulation. A single microneurographic study found similar basal MSNA (i.e. MSNA burst frequency), but lower MAP, for Bolivian highlanders

compared to well-acclimatised Lowlanders (Lundby et al., 2017); arterial baroreflex function was not tested. This suggests sustained sympathetic activation may be an evolutionary adaptation for those living permanently under high altitude hypoxia. However, distinct differences in physiological adaptation are known to exist between natives of the South American Andes, Himalaya plateau and Ethiopian highlands (Beall, 2006, 2007; Erzurum et al., 2007), with the suggestion that the Sherpa (Himalayan) adaptation represents the most effective phenotype for chronic hypoxia (Gilbert-kawai et al., 2014; Horscroft et al., 2017). However, due to a lack of microneurographic data for highlanders, other than Ayamara of the Bolivian Andes, it is unclear whether differences in the patterns of adaptation extends to sympathetic neural activity and autonomic control of the cardiovascular system and arterial pressure homeostasis.

Therefore, this study aims to i) to examine sympathetic vasomotor activity and arterial baroreflex regulation of BP (resetting and gain) in healthy Lowlanders at 5050 m and, ii) compare to highland native Sherpa. Based upon previous reports for acute hypoxia (Halliwill & Minson, 2002; Halliwill et al., 2003; Steinback et al., 2009; Querido et al., 2011), it is hypothesised that the 'operating pressure' (i.e. DBP) and 'operating point' (i.e. MSNA burst incidence) of the vascular sympathetic baroreflex will shift to higher values, during high altitude acclimatisation, with no change in reflex 'gain' (i.e. slope). It is further hypothesised that Sherpa, who cope extremely well with chronic hypoxia, will have a similar operating pressure but a lower MSNA operating point, compared with Lowlanders at 5050 m. A secondary aim is to determine the contribution of the peripheral chemoreflex to basal MSNA and arterial baroreflex function in acclimatising Lowlanders and Sherpa.

4.3 Methodology

4.3.1 Participants

Fourteen Lowlanders (12 male; mean ± SD: age, 27 ± 6 yrs; height, 1.77 ± 0.8 m; weight, 72.2 ± 10.1 kg) and nine male Sherpa (age, 33 ± 12 yrs; height, 1.68 ± 0.07 m; weight, 65.3 ± 10.3 kg) participated. Five Sherpa were self-reported smokers (1–5 cigarettes per day). None of the Lowlanders experienced clinical acute mountain sickness (AMS) at the time of testing, as assessed by the Lake Louise questionnaire (LLQ score ≤3); however, one Lowlander was tested 48 hours following an intramuscular injection of dexamethasone (half-life, 3 hours) (Ritschel et al., 1998).

4.3.2 Experimental design

This experiment was carried out within the framework of the 2016 UBC Nepal Expedition to the Ev-K2-CNR Research Facility (Willie et al., 2018). All participants underwent two testing sessions (*Figure 16*). Pre-expedition, low altitude (LA), testing of Lowlanders was conducted at 344 m (Kelowna, Canada, barometric pressure, 758 ± 8 mmHg) four weeks prior to departure. Pre-expedition testing of Sherpa was conducted at 1400 m (Kathmandu [KT], Nepal; barometric pressure, 652 ± 3 mmHg) that was performed a minimum of 4 days following descent from their resident altitude. All high altitude (HA) testing was performed at 5050 m (barometric pressure, 431 ± 44 mmHg). All participants flew from Kathmandu (1400 m) to Lukla (2860 m) and then trekked to the Ev-K2-CNR Research Facility (5050 m) over a 9–10 day period. This conservative ascent profile was chosen to aid acclimatisation and minimize the risk of altitude related illness, which included rest days at both 3400m and 4371 m (*Figure*

16). Sherpa were studied on days 1–4 at 5050 m (i.e. 10–14 days above 2860 m), and Lowlanders were studied on days 1–10 (i.e. 10–20 days above 2860 m).

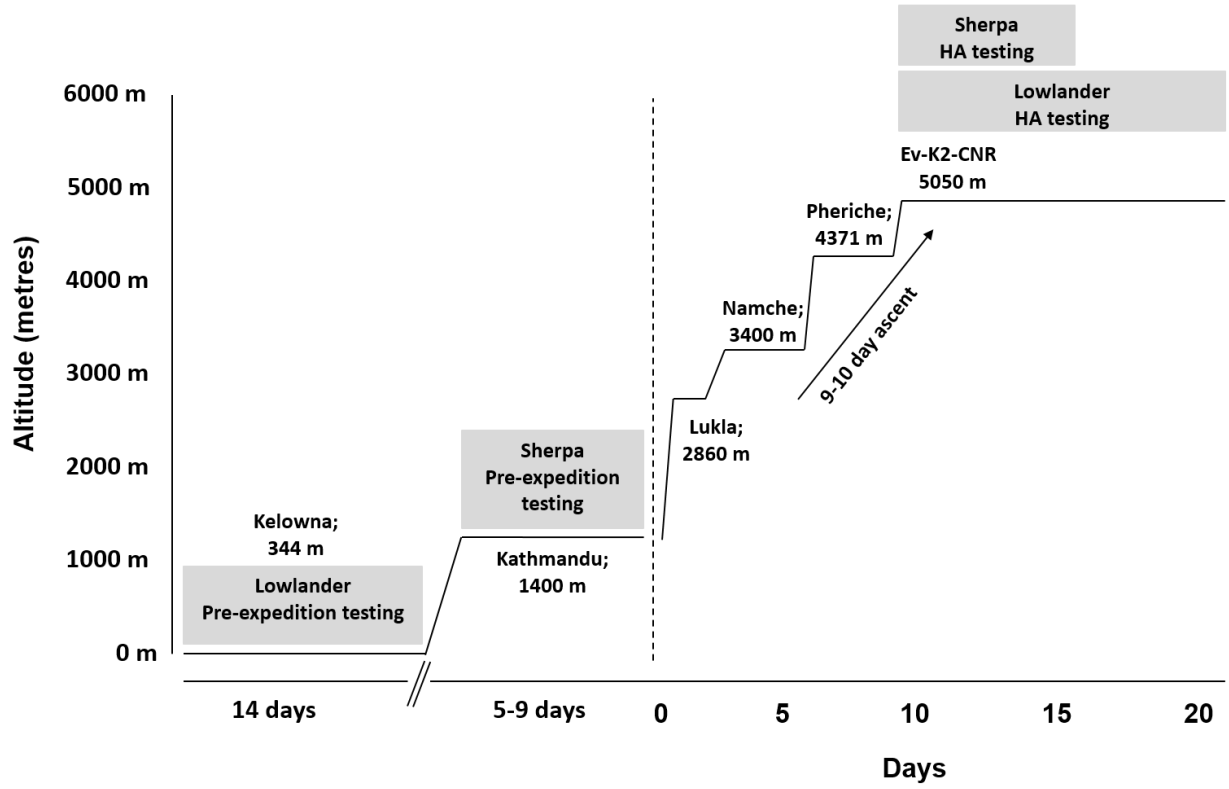

Figure 16. Schematic of experimental design and ascent profile for Experimental study 1.

4.3.3 Experimental measurements

Beat-by-beat cardiovascular haemodynamics (RRI, HR, SBP, DBP, MAP, stroke volume, Qc, TPR), SpO_2 and MSNA were continuously recorded, as described in Chapter 3.

4.3.4 Experimental protocol

Schematic showing experimental protocol is shown in *Figure. 17.* Following instrumentation, acquisition of an acceptable MSNA signal and a period of stabilisation, ten minutes of baseline data were recorded to determine resting cardiovascular haemodynamics and MSNA during ambient air breathing. A modified Oxford test was then performed to assess arterial baroreflex function during ambient air breathing.

Arterial baroreflex–peripheral chemoreflex interaction

At LA, a modified Oxford test was also performed whilst breathing a gas mixture containing 11% oxygen (FiO_2 0.11, equivalent to 5050 m), to increase peripheral chemoreceptor drive (Acute hypoxia; AH). At both LA and HA, a single modified Oxford test was also performed whilst participants breathed 100% oxygen (Hyperoxia; LA + 100% O_2, HA + 100% O_2), to acutely eliminate peripheral chemoreceptor drive. Participants breathed each of the gas mixtures for a period of 5 minutes and then a modified Oxford test was performed. No attempt was made to control ventilation or end tidal CO_2 during manipulation of peripheral chemoreceptor drive. At least 20 minutes separated the modified Oxford tests, but the order of trials at LA were not randomized as persistent alterations in MSNA and vascular sympathetic baroreflex function have been shown following acute hypoxia stimulus (Querido et al., 2011).

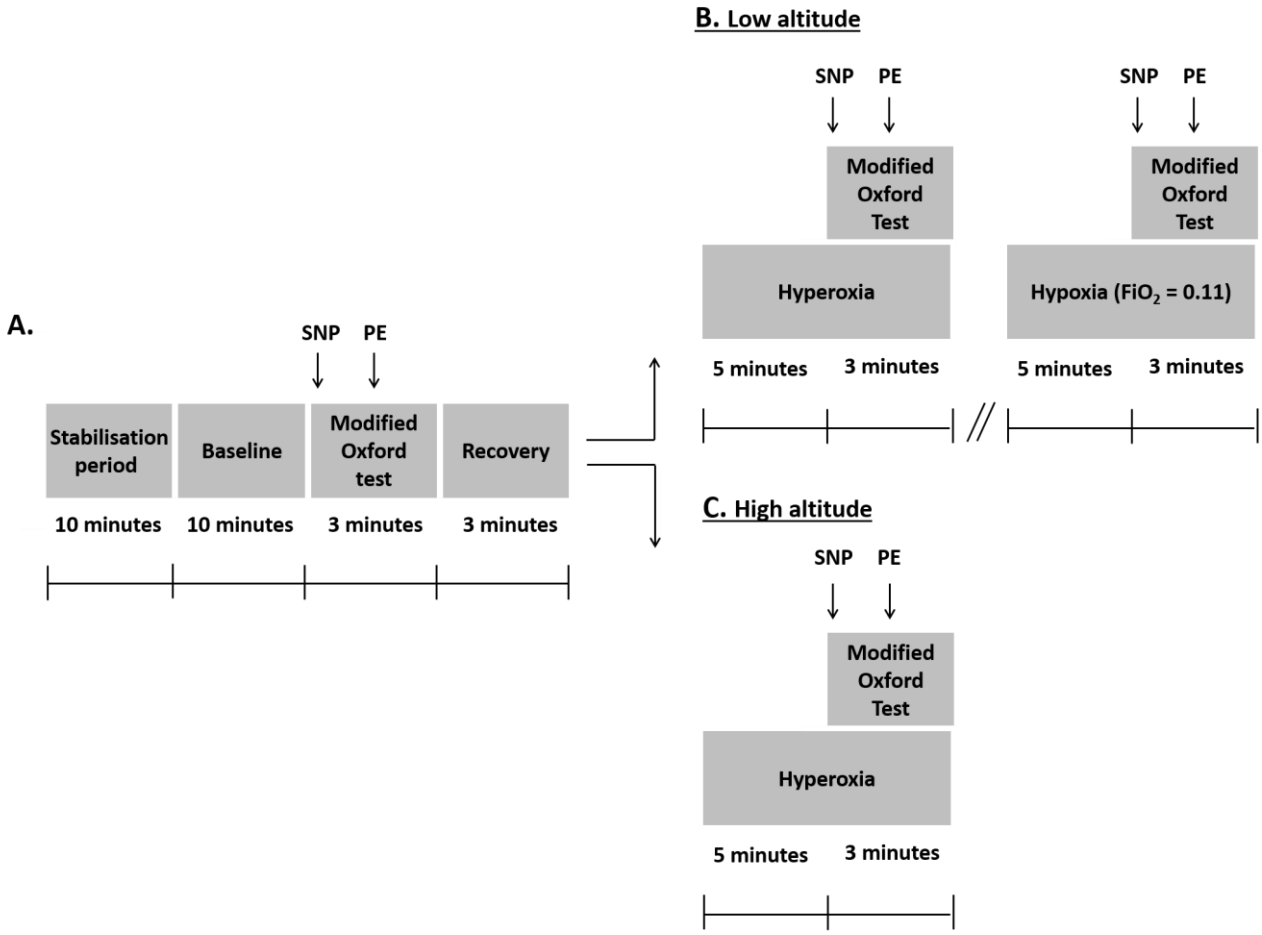

Figure 17. Schematic of experimental protocol for Experimental study 1.

A. Baseline cardiovascular haemodynamics, MSNA and arterial baroreflex gain were assessed, during ambient air breathing, at both low altitude and high altitude. B. Cardiovascular haemodynamics, MSNA and arterial baroreflex gain were also assessed during acute hyperoxia and acute hypoxia at low altitude and C. during acute hyperoxia at high altitude.

4.3.5 Data analysis

Beat-by-beat data for RRI, HR, SBP, DBP, MAP, stroke volume, CO, TPR and MSNA were extracted from Labchart (ADInstruments, Chart Pro v8.3.1). Resting values for cardiovascular haemodynamics and sympathetic vasomotor outflow were calculated by averaging values over the ten-minute baseline period. Cardiovascular haemodynamics and sympathetic vasomotor outflow during acute manipulation of peripheral chemoreceptor drive were calculated by averaging values over the last five minutes of hypoxia and hyperoxia at LA, respectively, and hyperoxia at HA. Arterial baroreflex gain during ambient air breathing and during acute manipulation of peripheral chemoreceptor drive was assessed from the slope of linear regression analyses relating MSNA burst probability and total MSNA to corresponding DBP (vascular sympathetic baroreflex) and RRI or HR to corresponding SBP (cardiovagal baroreflex) during the modified Oxford test performed under each condition. The vascular sympathetic baroreflex set-point was taken as the average value for DBP and MSNA burst incidence or Total MSNA during the ten minutes baseline period, and during the last five minutes of hypoxia and hyperoxia respectively. Cardiovagal baroreflex set point was taken as the average value for SBP and RRI or HR over the same period.

4.3.6 Statistical analyses

Differences between conditions (LA vs. AH, LA vs. HA, LA vs LA + 100% O_2, HA vs HA + 100% O_2) and between groups (Lowlanders vs. Sherpa) were assessed using pre-planned contrasts. The effects of HA acclimatisation in Lowlanders were assessed using dependent t-tests, whereas differences between Lowlanders and Sherpa at HA and Sherpa at HA and Lowlanders at LA were assessed using independent t-tests. To address the secondary aim of the study and examine the effects of manipulating peripheral chemoreceptor drive on arterial

baroreflex function, sympathetic vasomotor activity and arterial pressure at LA (AH, LA + 100% O_2) and HA (HA + 100% O_2) dependent t-tests were used. Due to technically challenging conditions during pre-expedition testing at KT, resting sympathetic vasomotor activity could only be obtained in four out of eight Sherpa, and one of these four Sherpa was not re-tested at HA. Therefore, no statistical comparisons were performed between Sherpa KT and Sherpa HA; however, these data are included for completeness. Significant vascular sympathetic baroreflex slopes ($R \leq 0.5$) and cardiovagal baroreflex slopes ($R \leq 0.8$) were not obtained in four Lowlanders at HA; therefore, repeated measures comparisons of sympathetic and cardiovagal baroreflex function were performed in ten Lowlanders (nine males, one female). Modified Oxford tests were performed successfully in seven out of eight Sherpa investigated at HA; no baroreceptor tests were performed in KT. Due to the loss of MSNA signals in three participants, data analyses for arterial baroreflex–peripheral chemoreflex interactions at LA are for eleven Lowlanders. Multiple t-tests were chosen to maximize the number of subjects included in statistical analyses. To correct for multiple comparisons A *priori* alpha was adjusted, using the experiment-wise error rate (Hinkle et al., 2003), as previously used (Busch et al., 2017). Statistical significance was set at $P \leq 0.05$. Normality was assessed using Shapiro-Wilk test, and data that was not normally distributed underwent $\log_{10}$ transformation prior to analysis. Values presented are means ± SD.

4.4 Results

4.4.1 Resting cardiovascular haemodynamic and basal sympathetic vasomotor activity in Lowlanders and Sherpa

Examples of MSNA and haemodynamic data recorded in one Lowlander and one Sherpa, under each experimental condition, are presented in *Figure 18*. In Lowlanders (n = 14), SpO_2 is decreased and HR is increased with HA acclimatisation, whereas Qc, TPR, and MAP are similar compared to LA. All indices of MSNA are greater in Lowlanders at HA compared to LA (*Table 3*).

At HA, Sherpa (n = 8) and Lowlanders have a similar SpO_2 and resting cardiovascular haemodynamics; however, Sherpa exhibited significantly lower MSNA total activity than Lowlanders (*Table 3*). The reduction in total activity is mediated by a reduction in MSNA burst frequency with no difference in mean burst amplitude. Compared to Lowlanders at LA, Sherpa have lower SpO_2 and higher HR, but similar Qc, TPR and MAP. Sherpa exhibit significantly greater MSNA total activity, MSNA burst frequency, and mean burst amplitude, versus Lowlanders at LA.

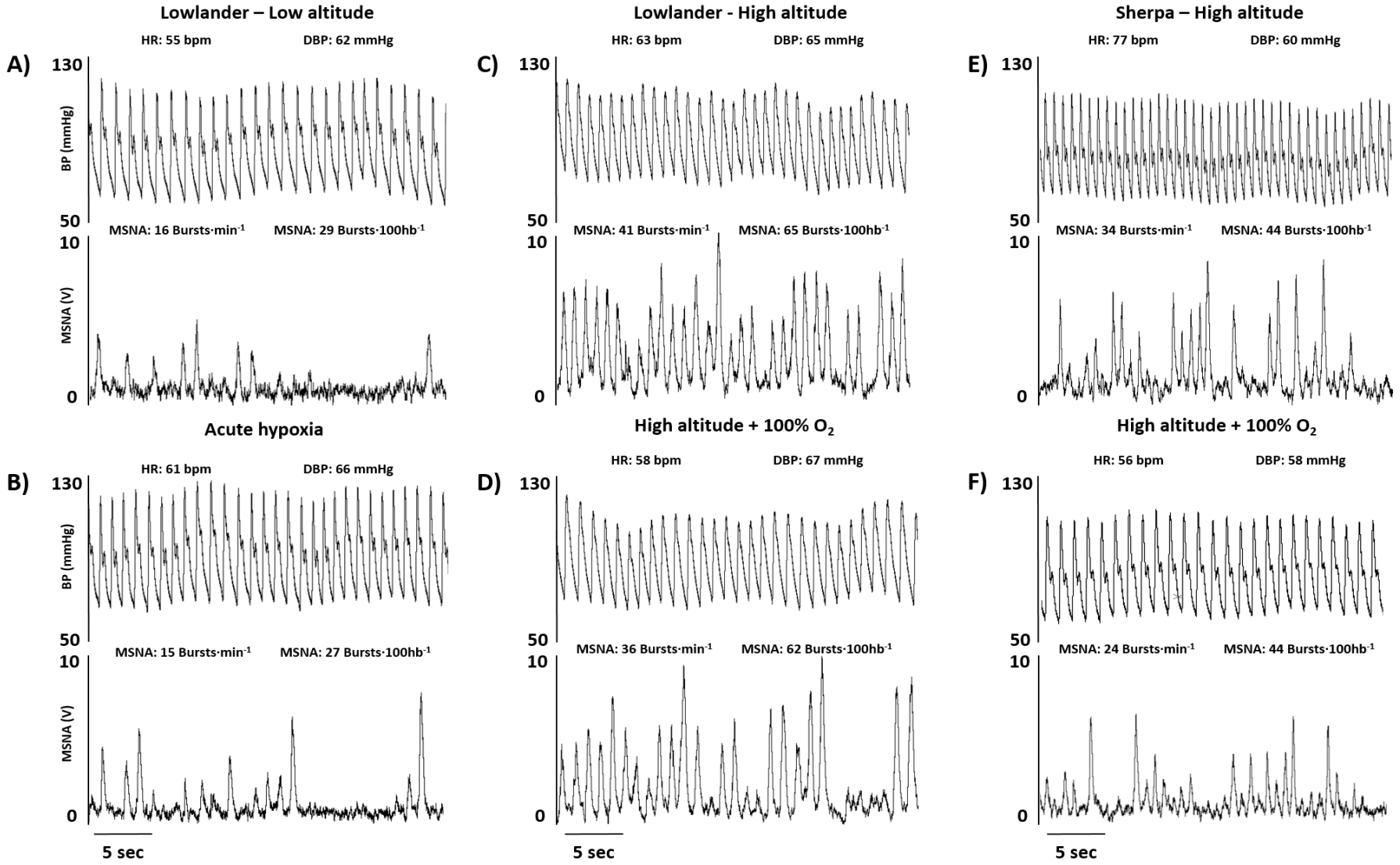

Figure 18. Example recordings of beat-by-beat muscle sympathetic nerve activity (MSNA) and blood pressure (BP) from one representative Lowlander and one representative Sherpa.

A 29 year old male Lowlander at A. low altitude, B. during acute hypoxia, C. following 8 days at high altitude, D. during 100% oxygen breathing at high altitude and a 26 year old Sherpa E. following 3 days at high altitude and F. during 100% oxygen breathing at high altitude

	Lowlanders			Sherpa			
						HA *vs* Lowlanders HA	HA *vs* Lowlanders LA
	LA (n=14)	**HA (n=14)**	***P* Value**	**KT (n=4)**	**HA (n=8)**	***P* Value**	
Haemodynamic variables							
SpO_2 (%)	98 ± 1	82 ± 3	**0.001**	96 ± 1	81 ± 4	0.6	**0.001**
Heart rate (bpm)	54 ± 10	64 ± 13	**0.006**	63 ± 7	73 ± 7	0.16	**0.002**
Qc ($L \cdot min^{-1}$)	5.2 ± 1.0♦	5.2 ± 1.2♦	0.46	4.9 ± 1.2	5.8 ± 1.7	0.42	0.41
TPR ($mmHg \cdot L \cdot min^{-1}$)	16.7 ± 3.3♦	17.5 ± 3.9♦	0.94	19.8 ± 4.9	16.3 ± 6.8	0.73	0.9
MAP (mmHg)	84 ± 8	85 ± 10	0.84	92 ± 3	84 ± 9	0.84	0.9
Systolic BP (mmHg)	117 ± 11	111 ± 12	0.19	117 ± 3	110 ± 9	0.97	0.15
Diastolic BP (mmHg)	67 ± 7	70 ± 9	0.46	77 ± 5	66 ± 8	0.47	0.9
Muscle Sympathetic Nerve Activity							
Burst frequency ($bursts \cdot min^{-1}$)	11 ± 5	30 ± 9	**0.001**	11 ± 2	22 ± 11	**0.05**	**0.003**
Burst incidence ($bursts \cdot 100HB^{-1}$)	22 ± 12	48 ± 16	**0.001**	18 ± 6	30 ± 13	**0.02**	0.16
Mean burst amplitude (a.u)	43 ± 8	50 ± 5	**0.02**	49 ± 7	53 ± 4	0.2	**0.003**
Total activity ($a.u \cdot min^{-1}$)	461 ± 194	1508 ± 548	**0.001**	521 ± 140	1168 ± 540	0.2	**0.002**
Total MSNA ($a.u \cdot 100HB^{-1}$)	898 ± 462	2440 ± 908	**0.001**	860 ± 325	1579 ± 663	**0.03**	**0.01**
Arterial Baroreflex Function							
Vascular sympathetic baroreflex gain ($\% \cdot mmHg^{-1}$)	-2.3 ± 0.7	-2.6 ± 1.2	0.33	-	-2.6 ± 0.9	0.98	0.94
Vascular sympathetic baroreflex gain ($a.u \cdot mmHg^{-1}$)	-126 ± 50	-143 ± 72	0.37	-	-151 ± 65	0.95	0.45
Cardiovagal baroreflex gain ($ms \cdot mmHg^{-1}$)	20.6 ± 5.0	16.2 ± 8.2	**0.007**	-	12.9 ± 5.4	0.60	**0.01**
Cardiovagal baroreflex gain ($beats \cdot mmHg^{-1}$)	-1.3 ± 0.4	-1.2 ± 0.5	0.68	-	-1.3 ± 0.5	0.72	0.86

Table 3. Cardiovascular haemodynamics, muscle sympathetic nerve activity (MSNA) and arterial baroreflex function in Lowlanders and Sherpa at 344m (LA), 1400m (KT) and 5050m (HA). Data are presented as mean (± SD). ♦ Qc and Total Peripheral Resistance for Lowlanders, n = 10. Statistical comparisons performed using dependent and independent t-test. Note: No intragroup comparison for Sherpa at HA versus KT, as only three Sherpa were tested at both altitudes.

4.4.2 Arterial baroreflex function in Lowlanders and Sherpa

HA acclimatisation has no effect on the vascular sympathetic baroreflex diastolic operating pressure for Lowlanders, but the MSNA operating point (i.e. MSNA burst incidence) is increased compared to LA. Vascular sympathetic baroreflex gain (i.e. slope) is not different at LA and HA (*Figure 19*). HA acclimatisation results in a downward shift of the cardiovagal baroreflex, reflected by a shortening of RRI (increased HR), with no change in prevailing SBP. Cardiovagal baroreflex gain was reduced in Lowlanders at HA versus LA when assessed from the relationship between RRI and SBP; however, cardiovagal baroreflex gain is unchanged when assessed using HR (*Figure 20*).

At HA, the diastolic operating pressure for Sherpa is similar to that of Lowlanders, but the MSNA operating point was lower for Sherpa. There was no difference in vascular sympathetic baroreflex gain (*Figure 19*). Furthermore, when compared to Lowlanders at LA, operating pressure, operating point and vascular sympathetic baroreflex gain for Sherpa is similar. The cardiovagal baroreflex gain in Sherpa is similar to that for Lowlanders at HA, but less than that of Lowlanders at LA. The cardiovagal baroreflex operating SBP was similar for Sherpa, compared to Lowlanders at both HA and LA, whereas RRI is similar to Lowlanders at HA, but lower than that at LA (*Figure 20*). In Sherpa, cardiovagal baroreflex gain is similar to that of Lowlanders at HA, but less than that of Lowlanders at LA.

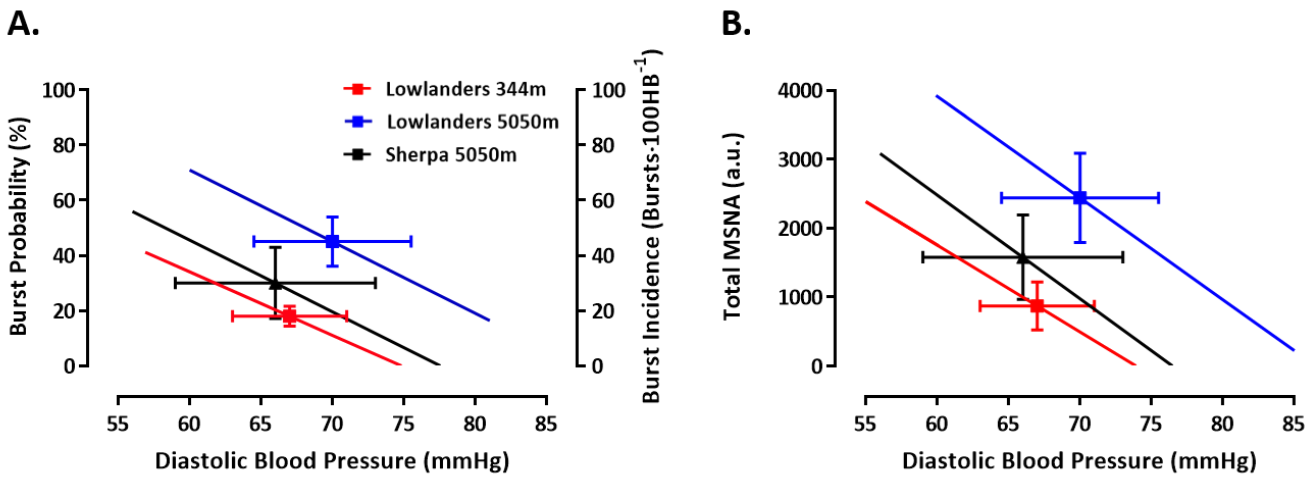

Figure 19. Vascular sympathetic baroreflex function in Lowlanders and Sherpa.

Group average regressions between A. MSNA burst probability and DBP and B. total MSNA and DBP in Lowlanders (n = 10) at 344 m (LA) and 5050 m (HA) and in Sherpa at HA (n = 7). The operating points are indicated by symbols and error bars (mean ± SD). Operating diastolic blood pressure is similar in Lowlanders at LA and HA and in Sherpa at HA. MSNA operating point is significantly elevated in Lowlanders at HA, relative to Lowlanders at LA. MSNA operating point is lower in Sherpa relative to Lowlanders at HA, but greater than Lowlanders at LA. This indicates an upward resetting of the vascular sympathetic baroreflex following ascent to HA in Lowlanders. The slope of the relationships are similar in Lowlanders at LA and HA and similar in Sherpa at HA compared to Lowlanders at both HA and LA, regardless of whether DBP is plotted against burst probability or total MSNA. This indicates no differences in vascular sympathetic baroreflex gain. Statistical comparisons performed using dependent and independent t-tests.

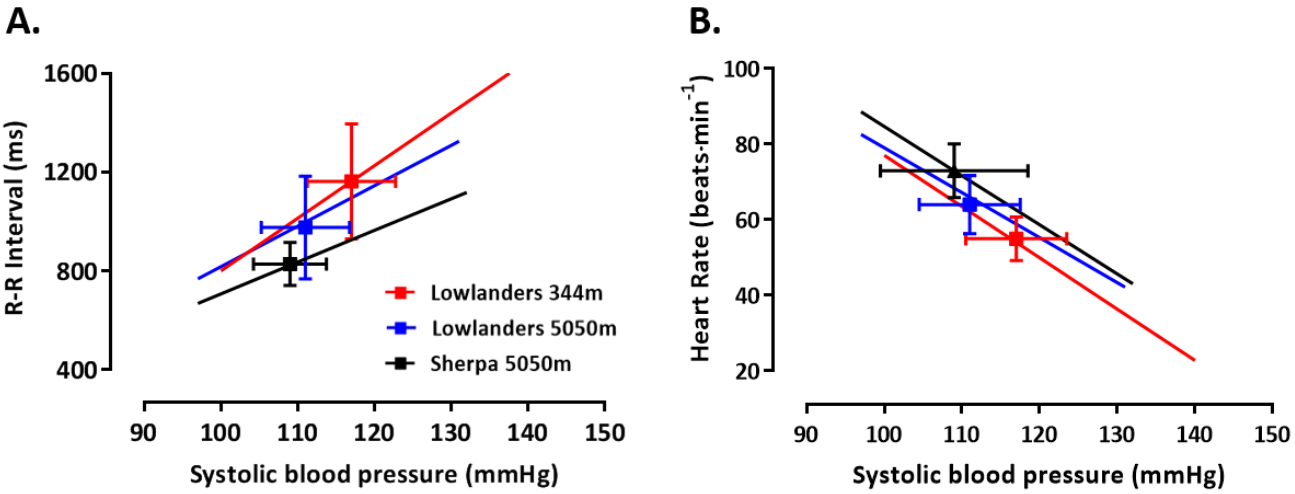

Figure 20. Cardiovagal baroreflex function in Lowlanders and Sherpa.

Group average regressions between A. RRI and SBP and B. HR and SBP in Lowlanders (n = 10) at 344 m (LA) and 5050 m (HA) and in native Sherpa at HA (n = 7). The operating points are indicated by symbols and error bars (mean ± SD). RRI is significantly decreased in Lowlanders at HA, relative to Lowlanders at LA, but is similar in Sherpa relative to Lowlanders at HA. Operating systolic blood pressure is similar. This indicates a downward resetting of the cardiovagal baroreflex in Lowlanders following ascent to HA. The slope of the relationship between SBP and RRI is less steep in Lowlanders at HA versus LA, and Sherpa at HA versus Lowlanders at LA, indicating a reduction in cardiovagal baroreflex gain at HA. The slope of the relationship between systolic blood pressure and RRI is similar in Sherpa at HA relative to Lowlanders at HA. However, there are no differences in cardiovagal baroreflex gain between LA and HA and between Lowlanders and Sherpa when assessed using HR. Statistical comparisons compared using dependent and independent t-tests.

4.4.3 Arterial baroreflex–peripheral chemoreflex interaction at low altitude

AH reduces SpO_2, increases HR, MAP and Qc, and decreases TPR. MSNA burst frequency is unchanged, but total MSNA is increased, due to an augmented burst amplitude (*Table 4*). Vascular sympathetic baroreflex gain is reduced during AH, with no change in baroreflex diastolic operating pressure, or the MSNA operating point. Administration of 100% oxygen at LA has no effect on baseline haemodynamics, MSNA burst frequency, burst amplitude, or indices of vascular sympathetic and cardiovagal baroreflex function.

	Lowlanders				
	LA (n=11)	AH (n=11)	*P* Value	LA + 100% O_2 (n=9)	# *P* Value
Haemodynamic Variables					
SpO_2 (%)	98 ± 1	84 ± 4	**0.001**	-	
Heart Rate (bpm)	53 ± 10	68 ± 15	**0.001**	56 ± 11	0.55
R-R Interval (ms)	1164 ± 226	933 ±248	**0.001**	1177 ±245	0.78
Qc ($L \cdot min^{-1}$)	5.1 ± 1.0♦	6.7 ± 1.8♦	**0.002**	5.7 ± 0.9	0.27
Total Peripheral Resistance ($mmHg \cdot L \cdot min^{-1}$)	16.8 ± 3.5♦	14.2 ± 3.7♦	**0.01**	15.9 ± 2.7	0.67
Mean Arterial Pressure (mmHg)	84 ± 7	89 ± 5	**0.05**	88 ± 5	0.09
Systolic blood pressure (mmHg)	117 ± 10	123 ± 7	0.12	121 ± 10	0.32
Diastolic blood pressure (mmHg)	67 ± 7	70 ± 4	0.09	70 ± 9	0.14
Muscle Sympathetic Nerve Activity					
Burst frequency ($bursts \cdot min^{-1}$)	11 ± 4	13 ± 6	0.43	11 ± 6	0.90
Mean burst amplitude (a.u)	40 ± 7	52 ± 13	**0.01**	46 ± 14	0.06
Total activity ($a.u \cdot min^{-1}$)	502 ± 183	789 ± 489	**0.06**	549 ± 305	0.49
Burst incidence ($bursts \cdot 100HB^{-1}$)	20 ± 6	19 ± 8	0.68	20 ± 9	0.88
Total MSNA ($a.u \cdot 100HB^{-1}$)	819 ± 347	1093 ± 534	**0.05**	789 ± 384	0.10
Arterial Baroreflex Function					
Vascular sympathetic baroreflex gain ($\% \cdot mm^{Hg^{-1}}$)	-2.7 ± 1.0	-1.9 ± 0.6	**0.02**	-2.3 ± 0.9	0.35
Vascular sympathetic baroreflex gain ($a.u \cdot mm^{Hg^{-1}}$)	-162 ± 59	-120 ± 40	**0.05**	142 ± 55	0.69
Cardiovagal baroreflex gain ($ms \cdot mmHg^{-1}$)	21.2 ± 8.4	23.5 ± 16.2	0.51	27.2 ± 17.4	0.24
Cardiovagal baroreflex gain ($beats \cdot mmHg^{-1}$)	-1.2 ± 0.34	-1.6 ± 0.65	0.11	-1.2 ± 0.3	0.59

Table 4. *Effects of manipulating peripheral chemoreceptor drive on cardiovascular haemodynamics, MSNA and arterial baroreflex function in Lowlanders at 344 m (LA).* Data are presented as mean (± SD). ♦Qc and Total Peripheral Resistance, n = 10. #Intragroup comparison for nine participants, LA versus LA + 100 % O_2. Statistical comparisons performed using dependent t-tests. AH = acute hypoxia

4.4.4 Arterial baroreflex–peripheral chemoreflex interaction at high altitude

As a result of MSNA signal losses, these comparisons are performed for nine Lowlanders and four Sherpa (*Table 5*). In Lowlanders exposed to 100% O_2, SpO_2 and MAP increases, and HR decreases, with no effect on any other cardiovascular haemodynamic or MSNA. Vascular sympathetic baroreflex function gain is also unchanged. There is no change in cardiovagal baroreflex gain. For Sherpa breathing 100% oxygen, SpO_2 increases and HR decreases with no effect on other cardiovascular haemodynamics. MSNA burst frequency, burst incidence, total activity and total MSNA are unchanged; however, mean burst amplitude decreases. Vascular sympathetic baroreflex gain is unchanged. Breathing 100% oxygen reduces RRI, with no change in cardiovagal baroreflex gain.

	Lowlanders			Sherpa		
	HA (n=9)	HA + 100% O_2 (n=9)	*P* Value	HA (n=4)	HA + 100% O_2 (n=4)	*P* Value
Haemodynamic variables						
SpO_2 (%)	82 ± 4	97 ± 2	**0.001**	82 ± 5	99 ± 1	**0.008**
Heart rate (bpm)	70 ± 12	61 ± 8	**0.01**	74 ± 6	62 ± 5	**0.02**
R-R Interval (ms)	882 129	994 139	**0.006**	790 64	960 80	**0.04**
Qc ($L \cdot min^{-1}$)	5.4 ± 1.0	5.2 ± 1.0	0.60	6.2 ± 1.7	5.7 ± 1.8	0.34
Total Peripheral Resistance ($mmHg \cdot L \cdot min^{-1}$)	17.1 ± 3.7	18.3 ± 4.1	0.26	14.4 ± 7.4	16.5 ± 10.7	0.26
Mean arterial pressure (mmHg)	88 ± 9	93 ± 9	**0.008**	78 ± 8	79 ± 11	0.79
Systolic blood pressure (mmHg)	113 ± 11	119 ± 9	**0.01**	103 ± 7	111 ± 12	0.10
Diastolic pressure (mmHg)	72 ± 9	74 ± 10	0.06	63 ± 8	65 ± 12	0.34
Muscle Sympathetic Nerve Activity						
Burst frequency ($bursts \cdot min^{-1}$)	30 ± 10	27 ± 11	0.35	22 ± 8	17 ± 6	0.14
Mean burst amplitude (a.u)	50 ± 5	46 ± 13	0.36	53 ± 5	46 ± 6	**0.01**
Total activity ($a.u \cdot min^{-1}$)	1495 ± 614	1289 ± 729	0.18	1158 ± 330	786 ± 250	0.08
Burst incidence ($bursts \cdot 100HB^{-1}$)	44 ± 16	45 ± 16	0.62	29 ± 10	29 ± 12	0.93
Total MSNA ($a.u \cdot 100HB^{-1}$)	2189 ± 887	2080 ± 933	0.77	1520 ± 396	1314 ± 446	0.42
Arterial Baroreflex Function						
Vascular sympathetic baroreflex gain ($\% \cdot mmHg^{-1}$)	-2.6 ± 1.2	-2.5 ± 1.0	0.16	-2.8 ± 1.2	-3.0 ± 1.3	0.69
Vascular sympathetic baroreflex gain ($a.u \cdot mmHg^{-1}$)	-159 ± 68	-156 ± 100	0.95	-156 ± 73	-146 ± 70	0.79
Cardiovagal baroreflex gain ($ms \cdot mmHg^{-1}$)	21.5 ± 5.5	21.2 ± 11.4	0.92	13.2 ± 3.5	18.4 ± 9.1	0.20
Cardiovagal baroreflex gain ($beats \cdot mmHg^{-1}$)	-1.1 ± 0.5	-1.3 ± 0.5	0.36	-1.3 ± 0.3	-1.0 ± 0.5	0.72

Table 5. Effect of manipulating peripheral chemoreceptor drive on cardiovascular haemodynamics, MSNA and arterial baroreflex function in Lowlanders and Sherpa at 5050 m (HA). Data are presented as mean (± SD). Statistical comparisons performed using dependent and independent t-tests.

4.5 Discussion

Principal novel findings of Experimental study 1 are as follows: i) baroreflex control of MSNA is preserved in Lowlanders following 10–20 days at high altitude; ii) the MSNA operating point of the vascular sympathetic baroreflex is upwardly reset with no change in operating pressure for Lowlanders at high altitude; iii) Sherpa have lower basal MSNA burst frequency compared to Lowlanders at high altitude, but similar resting BP; iv) Sherpa have similar vascular sympathetic baroreflex gain, but a lower MSNA operating point when compared with Lowlanders at high altitude. Finally, v) eliminating peripheral chemoreceptor drive at high altitude does not influence the vascular sympathetic baroreflex set point or gain for both Lowlanders and Sherpa. Taken together, these findings provide important new insight into reflex control of the vasoconstrictor drive and BP at high altitude, and highlight a novel adaptation in Sherpa.

4.5.1 Sympathoexcitation at high altitude

Following 10–20 days of high altitude exposure, an almost three-fold increase in MSNA burst frequency is observed in Lowlanders at 5050 m; this is consistent with previous microneurographic studies at high altitude (Hansen & Sander, 2003; Lundby et al., 2017). Furthermore, for the first time, this study demonstrates that basal MSNA burst frequency in Sherpa is lower than that of Lowlanders at the same altitude, despite similar peripheral oxygen saturation in both groups. The observation for Sherpa contrasts with that of the only previous study of highlanders (Lundby et al., 2017), which found that Bolivian Aymara have basal MSNA that is comparable with Lowlanders after 10 and 50 days of high altitude exposure. Although many factors may influence basal sympathetic outflow, the present study raises the importance of ethnicity. The divergent pathways of physiological adaptation

observed in geographically distinct high altitude populations might extend to sympathetic nervous system activation, whereby adaptation in Sherpa appears to favour lower basal sympathetic activity. However, basal MSNA in Sherpa at high altitude is higher than that for Lowlanders at low altitude. Furthermore, for three Sherpa studied four days following descent to 1440 m, basal MSNA burst frequencies are approximately 30% lower than those observed when they were re-tested at 5050 m. Taken together these findings suggest that hypoxia remains a significant physiological stressor for Sherpa despite generations of adaptation and lifelong exposure.

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

4.5.2 Arterial baroreflex function at high altitude

These data indicate an upward resetting of the vascular sympathetic baroreflex in Lowlanders at 5050m. This occurs without a change in the ability of the reflex to increase or

decrease MSNA in response to a baroreceptor challenge i.e. the gain is unchanged. Furthermore, the ability of the baroreflex to regulate MSNA in Sherpa and Lowlanders is similar, but the likelihood of a burst of MSNA at a given diastolic pressure is lower for Sherpa. Vascular sympathetic baroreflex function at high altitude had not been assessed prior to this study. Previous reports of heightened MSNA burst incidence (Hansen & Sander, 2003; Lundby et al., 2017; Fisher et al., 2018) indirectly support an upward resetting of the vascular sympathetic baroreflex operating point for Lowlanders exposed to chronic high altitude hypoxia. However, in contrast to this study, higher MSNA burst incidence was accompanied by an increase in resting MAP (Hansen & Sander, 2003; Lundby et al., 2017; Fisher et al., 2018). This may be due to methodological differences across studies in relation to the ascent profile, physical activity levels whilst at altitude, and the final elevation achieved. In the present study, the ascent to 5050 m was conservative (i.e. 9–10 days), whereas rate of ascent in previous studies was more rapid (one to three days), which has been shown to influence cardiovascular responses to high altitude (Vogel et al., 1967). In addition, a temporal relationship may exist between elevated sympathetic vasomotor activity and MAP in Lowlanders. Arterial baroreflex resetting and heightened sympathetic outflow initially may be homeostatic during early acclimatisation; however, over time other cardiovascular changes and alterations in constricting and dilating factors acting on the vasculature (Calbet et al., 2014; Bruno et al., 2016) could contribute to elevated MAP at high altitude. Future studies at high altitude should incorporate serial measurements of arterial baroreflex control of MSNA and other factors that modulate arterial pressure.

The secondary effects of increased ventilation at high altitude may complicate the effects of hypoxia on baroreflex control of the heart (Angell James & De Burgh Daly, 1969; Eckberg et al., 1980). Nevertheless, this study determined how the cardiovagal component of

the arterial baroreflex is affected. At high altitude, cardiovagal baroreflex gain is similar for Lowlanders and Sherpa, but gain is lower than Lowlanders at low altitude. Interestingly, acute hyperoxia at 5050 m does not reverse the reduction in cardiovagal baroreflex gain for Lowlanders. Taken together, these data suggest that altitude acclimatisation has differential effects on the responsiveness of the vascular sympathetic and cardiovagal limbs of the arterial baroreflex. When cardiovagal baroreflex gain was assessed from the relationship between SBP and HR, rather than RRI, cardiovagal baroreflex gain was comparable to low altitude.

4.5.3 Vascular sympathetic baroreflex–peripheral chemoreflex interactions

In the present study, for Lowlanders exposed to acute hypoxia, there is no change in basal MSNA burst frequency, although there is a modest increase in mean burst amplitude and thus a modest increase in MSNA total activity. These findings imply that MSNA burst frequency and amplitude can be regulated independently of each other under hypoxic conditions, as previously demonstrated under non-hypoxic conditions (Kienbaum et al., 2001; Salmanpour et al., 2011; Steinback & Shoemaker, 2012). Furthermore, the operating point of the vascular sympathetic baroreflex (i.e. MSNA burst incidence) is not significantly different during acute hypoxia, while vascular sympathetic baroreflex gain is reduced. These findings for acute hypoxia contrast with those for high altitude, and suggest different mechanisms contribute to activation of sympathetic outflow during acute and chronic hypoxic exposure. Vascular sympathetic baroreflex resetting in Lowlanders at 5050 m is not reversed during acute administration of 100% oxygen. Furthermore, MSNA burst frequency is not reduced, a finding that is consistent with previous studies that attempted to reduce peripheral chemoreflex drive at high altitude (Hansen & Sander, 2003; Fisher et al., 2018). Therefore, mechanisms other than the peripheral chemoreflex likely play a role in vascular sympathetic

baroreflex resetting at high altitude. Interestingly, the peripheral chemoreflex may be more important in mediating high altitude sympathoexcitation in Sherpa. Although the vascular sympathetic baroreflex operating point is not changed during 100% oxygen administration, a reduction in mean burst amplitude and total activity was observed. This possibility, however, requires further investigation.

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

4.6 Experimental limitations

This is the first study to record sympathetic neural discharges from Sherpa at high altitude. However, technically challenging conditions in Kathmandu limited the study to only four participants at a lower elevation. Furthermore, around half of Sherpa were light to moderate smokers and it is reported that tobacco smoking leads to increased basal MSNA and attenuates vascular sympathetic baroreflex gain (Middlekauff et al., 2013, 2014). However, smoking status was not a significant covariate for any indices in this study. It was not possible for groups of participants to arrive on separate days to minimize any confounding effects of the varying time course of acclimatisation once at 5050m. While it is acknowledged that a difference between day 10 and day 20 may have influenced these results, analysis

indicates that test day was not a significant covariate. Furthermore, whilst the MSNA response over the initial days of high altitude exposure remains unclear, Lundby et al, (2017) reported similar basal MSNA values on day 10 and day 50 at 4300 m. This indicates a plateauing of the MSNA response after 10 days, with no apparent change thereafter.

Arterial baroreflex gain was assessed over a smaller pressure range at high altitude (26 ± 8 mmHg) versus low altitude (35 ±11 mmHg; *P* = 0.02), as an identical dose of phenylephrine elicited a smaller pressor response. Vascular sympathetic and cardiovagal baroreflex gain is at its greatest around the operating range of the reflex, therefore assessing the baroreflex over a smaller pressure range, closer to the operating pressure, at high altitude may have influenced the results. However, spontaneous vascular sympathetic and cardiovagal baroreflex gain were also assessed during five minutes of rest. This enabled baroreflex gain to be assessed over a similar BP range at both low altitude and high altitude (24 ± 4 vs. 21 ± 6 mmHg; *P* = 0.29). Spontaneous vascular sympathetic baroreflex gain was comparable in Lowlanders at low altitude and high altitude (-2.8 ± 2.0 vs. -2.7 ± 1.1 %·mmHg^{-1}; *P* = 0.88), and was comparable to Sherpa at HA (-3.1 ± 1.8%·mmHg^{-1}; *P* = 0.5). Furthermore, spontaneous cardiovagal baroreflex gain was significantly lower in Lowlanders at high altitude versus low altitude (18.0 ± 11.2 vs. 27.4 ± 11.4 ms·mmHg^{-1}) suggesting that the differences in pressure range over which the arterial baroreflex was assessed did not influence the results observed.

4.7 Conclusions

This study demonstrates highly effective arterial baroreflex control of sympathetic vasomotor activity in healthy humans during sustained hypoxia. Chronic resetting of the vascular sympathetic baroreflex supports elevated sympathetic vasoconstrictor drive in

Lowlanders during early acclimatisation to high altitude, but without an increase in resting arterial pressure. Sherpa, by comparison, have a lower vascular sympathetic baroreflex operating point and lower vasoconstrictor drive, but similar vascular resistance and arterial pressure. For Lowlanders, vascular sympathetic baroreflex resetting and heightened sympathetic vasomotor activity may protect against orthostatic hypotension at high altitude. In contrast, Sherpa may have adapted to high altitude to require lower sympathetic vasomotor outflow for homeostatic control of blood pressure. Such a difference may represent another example of a beneficial hypoxic adaptation in this highland population.

4.8 Contribution to this chapter

For this chapter, I contributed to the data collection, data analysis, data interpretation, preparation of figures, chapter drafting and chapter revisions.

Author Name	Conception and Design	Data Collection	Data Analysis	Data Interpretation	Preparation of Figures	Manuscript Drafting	Manuscript Revisions
Lydia L Simpson		X	X	X	X	X	X
Stephen A Busch		X	X				X
Samuel J Oliver							X
Philip N Ainslie	X						X
Mike Stembridge	X	X				X	X
Craig D Steinback	X	X					X
Jonathan P Moore	X	X	X	X		X	X

Table 6. – Authorship summary for Chapter 4

CHAPTER 5: EXPERIMENTAL STUDY 2

Andean Highlanders, Chronic Mountain Sickness and the Integrative Regulation of Resting Blood Pressure.

A version of this chapter has been published online in the Journal of Experimental Physiology

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5.1 Abstract

High altitude maladaptation syndrome CMS is characterised by excessive erythrocytosis and is frequently accompanied by accentuated arterial hypoxaemia. Whether altered autonomic cardiovascular regulation is also apparent in CMS is unclear. Therefore, integrative control of BP was assessed, by determination of basal sympathetic vasomotor outflow and arterial baroreflex function in eight Andean natives with CMS ([Hb] 22.6 ± 0.9g/dL) and seven healthy Andeans ([Hb] 19.3 ± 0.8g/dL) at their resident altitude (Cerro de Pasco, Peru; 4383 m). RRI (electrocardiogram), beat-by-beat BP (photoplethysmography) and MSNA (microneurography) were recorded at rest and during pharmacologically induced changes in BP (modified Oxford test). Although [Hb] and blood viscosity (7.8 ± 0.7 vs 6.6 ± 0.7cP; d = 1.7, P = 0.01) are elevated in CMS compared to healthy Andeans, Qc, TPR and MAP are similar between groups. The vascular sympathetic baroreflex MSNA operating point (i.e. MSNA burst incidence) and reflex gain (i.e. responsiveness) are also similar between groups (MSNA operating point; d = 0.75, P= 0.16, gain; d = 0.2, P = 0.69). In contrast, in CMS the cardiovagal baroreflex operates around a longer RRI (960 ± 159 vs 817 ± 50 ms; d = 1.4, P = 0.04) with a greater reflex gain (17.2 ± 6.8 vs 8.8 ± 2.6 ms·mmHg^{-1}; d = 1.8, P = 0.01) versus healthy Andeans. There is also a trend for basal sympathetic vasomotor activity to be lower in CMS, compared to healthy Andeans (33 ± 11 vs 45 ± 13 bursts·min^{-1}; d = 1.0, P = 0.08). In conclusion, these findings indicate that adaptive differences in basal sympathetic vasomotor activity and HR compensate for the haemodynamic consequences of excessive erythrocyte volume and contribute to integrative BP regulation in mild CMS.

5.2 Introduction

Globally, between 5–10% of the ~140 million people living at high altitude (>3000 m) lack the ability to adapt to chronic hypoxia and develop a progressively incapacitating maladaptation syndrome CMS (León-Velarde et al., 2005). CMS, which is most prevalent in natives of the Andean plateau, is characterised by excessive erythrocytosis (haemoglobin concentration [Hb] ≥21 g/dL for men, ≥19 g/dL for women), is frequently accompanied by accentuated arterial hypoxaemia for the resident altitude; and, in more severe stages of the disease, pulmonary hypertension occurs (León-Velarde et al., 2005). In addition, highlanders with CMS may present with a number of clinical symptoms including headache, breathlessness, sleep disturbances, and cognitive impairment (León-Velarde et al., 2005; Villafuerte & Corante, 2016). Importantly, CMS is also associated with an increased cardiovascular disease risk, which increases with disease severity (Corante et al., 2018). Specifically, an increased prevalence of thrombotic events, stroke, coronary heart disease and systemic and pulmonary hypertension, which can give rise to cardiac hypertrophy and congestive heart failure, have all been reported in CMS (Monge, 1942; Peñaloza & Sime*.,* 1971; Leon-Velarde and Arregui, 1994; Leon-Velarde et al., 2014). Excessive erythrocyte volume and the resulting elevations in hematocrit and blood viscosity are known to contribute to this increased risk (Corante et al., 2018; Tremblay et al*.,* 2019). However, several other clinical conditions characterised by sustained hypoxaemia (i.e. Chronic Obstructive Pulmonary Disease) are often accompanied by arterial baroreflex dysfunction and elevated MSNA (Van Gestel & Steier, 2010; Andreas et al., 2014). Such changes, which can facilitate increased BP variability, elevated BP, increased arterial stiffness and vascular dysfunction (Hijmering et al., 2002; Smit et al., 2002; Świerblewska et al., 2010), contribute to the

development of cardiovascular disease. However, whether arterial baroreflex dysfunction and elevated MSNA are also apparent in CMS is unclear.

The arterial baroreflex plays a fundamental role in the control of BP through its regulation of RRI and sympathetic vasomotor outflow. Previous research has found impaired baroreflex control of RRI in Andean highlanders with CMS compared to Healthy Andeans (Keyl et al., 2003); however, this is not a consistent finding (Gulli et al., 2007). Baroreflex control of arterial pressure also occurs via alterations in sympathetic vasomotor outflow. Previously, no difference in maximum gain (i.e. responsiveness) of carotid baroreflex control of forearm vascular resistance (index of sympathetic vasomotor activity) was reported for CMS compared to healthy Andean highlanders (Moore et al., 2006). Nevertheless, no direct measurement of sympathetic vasomotor outflow exists for Andean highlanders with CMS. Whilst plasma catecholamine concentrations may not accurately represent sympathetic nervous system activity (Esler et al., 1988), they are reported to be either elevated (Gamboa et al., 2006) or unchanged (Antezana et al., 1995) in CMS, indicating either an increased or comparable global sympathetic activation compared to their healthy Andean counterparts. On one hand, elevated sympathetic activity might be predicted in CMS if exaggerated arterial hypoxaemia is present; thus augmenting tonic peripheral chemoreflex activation. On the other hand, a larger blood volume in CMS (Claydon et al., 2004) might have a sympathoinhibitory effect on basal MSNA, as shown in healthy individuals at sea level (Charkoudian et al., 2004; Best et al., 2014).

In light of the equivocal findings, and absence of microneurographic data for CMS, it is unclear what effect CMS has on sympathetic neural control and arterial baroreflex regulation of BP in Andean highlanders. The present study, therefore, aims to

comprehensively assess integrative regulation of resting BP in Andean highlanders with CMS, and compare them with Healthy Andean highlanders. To achieve this, blood volume, basal MSNA, and arterial baroreflex control of RRI and MSNA were assessed. Based upon limited previous reports, it is hypothesised that i) the vascular sympathetic baroreflex operates around a higher set point (i.e. MSNA operating point and DBP) for CMS compared to Healthy Andeans, with no difference in reflex gain (i.e. responsiveness) ii) the cardiovagal baroreflex operates around a shorter RRI and higher SBP in CMS, with a concurrent reduction in reflex gain, therefore, iii) basal sympathetic vasomotor outflow and arterial pressure will be elevated in CMS. A secondary aim is to determine the contribution of the peripheral chemoreflex to basal MSNA and arterial baroreflex function in CMS.

5.3 Methodology

5.3.1 Participants

Twenty Andean men born at an altitude above 3250 m, permanently residing in the Cerro de Pasco area and who had at least two previous known generations of high altitude Andean ancestry were recruited for the study. None of the subjects had travelled to an altitude lower than 3000 m in the previous six months and did not have a history of working in the mining industry.

5.3.2 Experimental design

This study was part of the Global REACH high altitude research expedition to the Universidad Peruana Cayetano Heredia's Instituto de Investigacions de Altura (4380 m; Cerro de Pasco, Peru) in July 2018. Participants attended the laboratory on two occasions, with a minimum of 24 hours between visits 1) preliminary screening visit, and 2) experimental visit. During the preliminary visit, participants provided a detailed clinical history and history of high altitude residence and ancestral background. A venous blood sample was drawn from the antecubital vein to measure [Hb], hematocrit and blood viscosity. An arterial blood sample was also drawn, from the radial artery (performed by CG), following local anaesthesia (2% lidocaine), to determine arterial blood gases (PaO_2 and $PaCO_2$) and arterial oxygen saturation (SaO_2). In addition, resting PASP was determined using Echocardiography, as described in Chapter 3. Total blood volume (packed cell volume and plasma volume) was determined via the modified carbon monoxide (CO) rebreathing method as previously described in detail (Schmidt & Prommer, 2005) and used previously in Lowlanders and highland natives at high altitude (Stembridge et al., 2018, 2019). Participants also performed an incremental exercise test (20 Watts/min) to exhaustion, in the semi recumbent position,

on an electronically braked cycle ergometer (Lode Angio; Lode). Breath-by-breath respiratory data were collected throughout (Oxycon Mobile; Carefusion) to determine peak oxygen consumption (VO_{2peak}).

CMS scores were calculated using the Qinghai CMS questionnaire based on the presence and severity of eight signs and symptoms of CMS, as agreed by international consensus (León-Velarde et al., 2005); excessive erythrocytosis, heart palpitations, difficulty sleeping, cyanosis, parathesia, headache, tinnitus and dilated veins. A value of zero was assigned to negative answers. Positive answers were categorised as light, moderate, or severe and assigned values of one, two and three respectively. The sum of assigned values constituted the CMS score. Subjects were diagnosed with CMS by a score ≥5 in the presence of excessive erythrocytosis ([Hb] ≥21g/dL) and individuals not meeting these criteria were categorised as Healthy Andeans. The sum of the score defines CMS severity as absent (0–5), mild (6–10), moderate (11–14) or severe (≥ 15). Two highlanders (both CMS) were current smokers, but refrained from smoking on the day of testing.

5.3.3 Experimental measurements

Beat-to-beat cardiovascular haemodynamics (RRI, HR, SBP, DBP, MAP, stroke volume, Qc, TPR) and MSNA were continuously recorded as described in Chapter 3.

5.3.4 Experimental protocol

Schematic of experimental protocol is shown in *Figure 21*. Following instrumentation, acquisition of an acceptable MSNA signal and a period of stabilisation, ten minutes of baseline data were recorded to determine resting cardiovascular haemodynamics and MSNA. A modified Oxford test was then performed to assess vascular sympathetic and cardiovagal baroreflex function during ambient air breathing. Following baseline measurements,

participants were transferred to breathing 100% O_2, via a mouthpiece with noseclip, in an attempt to eliminate peripheral chemoreceptor drive. Participants breathed hyperoxia for a period of five minutes before a second modified Oxford test was performed, to investigate arterial baroreflex–peripheral chemoreflex interactions. No attempt was made to control ventilation or end tidal CO_2 during manipulation of peripheral chemoreceptor drive. Due to the unknown time course of recovery from hyperoxia, the order of conditions was not randomised. A minimum of 20 minutes separated each modified Oxford test.

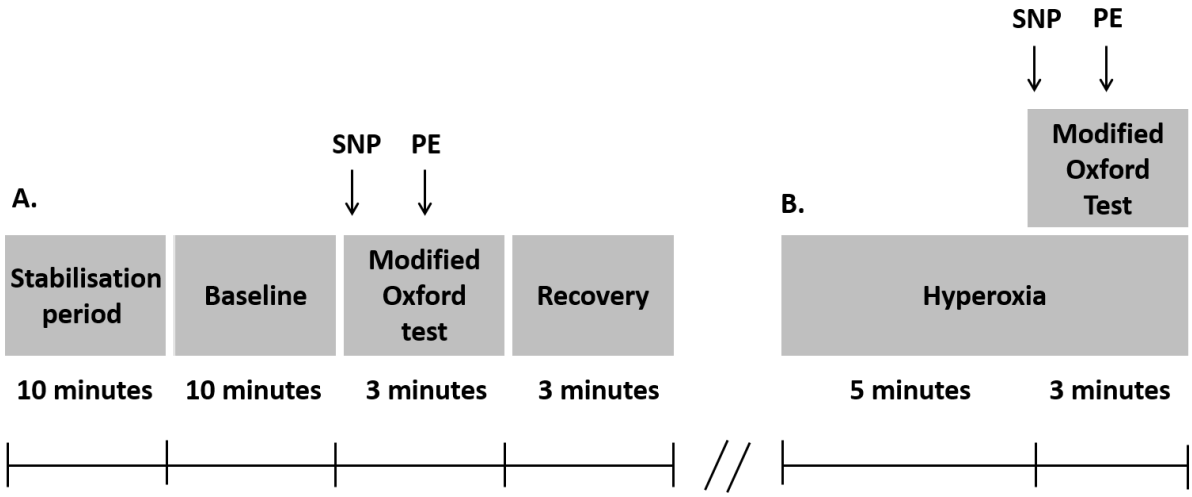

Figure 21. Schematic of experimental protocol for Experimental study two.

A. Assessment of baseline cardiovascular haemodynamics, MSNA and arterial baroreflex function during ambient air breathing. B. Assessment of cardiovascular haemodynamics, MSNA and arterial baroreflex function arterial baroreflex during acute hyperoxia. SNP = Sodium nitroprusside, PE = Phenylephrine.

5.3.5 Data analysis

Beat-by-beat data for RRI, HR, SBP, DBP, MAP, stroke volume, Qc, TPR and MSNA were extracted from Labchart (ADInstruments, Chart Pro v8.3.1). Resting values for cardiovascular haemodynamics and sympathetic vasomotor outflow for CMS and healthy Andeans were calculated by averaging values over the 10 minute baseline period. Cardiovascular haemodynamics and sympathetic vasomotor outflow during acute reductions in peripheral chemoreceptor hyperoxia were calculated by averaging values over the last minute of hyperoxia. These values were compared to the one minute of rest immediately preceding the start of hyperoxia, when subjects were breathing through a mouthpiece. Sympathetic vasomotor outflow was quantified as MSNA burst frequency and MSNA total activity. Arterial baroreflex gain during ambient air breathing and during hyperoxia was assessed from the slope of linear regression analyses relating MSNA burst probability and total MSNA to corresponding DBP (vascular sympathetic baroreflex) and RRI or HR to corresponding SBP (cardiovagal baroreflex) during the modified Oxford test performed under each condition. The vascular sympathetic baroreflex set-point was taken as the average value for DBP and MSNA burst incidence or Total MSNA during the ten minutes baseline period, during the one minute immediately preceding the start of hyperoxia and the last minute of hyperoxia respectively. Cardiovagal baroreflex set-point was taken as the average value for SBP and RRI or HR over the same period.

5.3.6 Statistical analyses

Differences between groups (CMS vs. Healthy Andeans) and between conditions (baseline vs. hyperoxia) were assessed using pre-planned contrasts. To address hypotheses 1, 2 and 3, differences in arterial baroreflex function, basal sympathetic vasomotor outflow and

arterial pressure, between CMS and Healthy Andeans, were assessed using independent t-tests. To address the secondary aim and examine the contribution of the peripheral chemoreflex mechanism, differences in arterial baroreflex function, sympathetic vasomotor outflow and arterial pressure in CMS and Healthy Andeans between baseline and hyperoxia were assessed using dependent t-tests. Significant cardiovagal baroreflex slopes ($R \leq 0.8$) were not obtained in one CMS participant and one healthy highlander; therefore cardiovagal baroreflex gain analyses at baseline were based on seven CMS participants and six Healthy Andeans. As a result of MSNA signal losses, repeated measures comparisons for cardiovascular haemodynamics and sympathetic neural outflow during hyperoxia are performed on six CMS participants and six Healthy Andeans. Furthermore, during hyperoxia, cardiovagal baroreflex slopes did not meet the inclusion criteria ($R \leq 0.8$) in one out of six Healthy Andeans; therefore, repeated measures comparisons for cardiovagal baroreflex gain are limited to five Healthy Andeans and six Andeans with CMS. Multiple t-tests were chosen to maximize the number of subjects included in statistical analyses. To correct for multiple comparisons, *a priori* alpha was adjusted, using the experiment-wise error rate (Hinkle et al., 2003) as described in Chapter 3. Statistical significance was set at $P \leq 0.05$. Furthermore, due to a small sample size, Cohen's *d* effect sizes are also reported with $d \geq 0.8$ indicative of large effects (Cohen, 1988). Cohen's $d = (Mean_{healthy\ Andeans} - Mean_{CMS}) / SD_{pooled}$, where $SD_{pooled} = \sqrt{((SD_{CMS}^2 + SD_{healthy\ Andeans}^2)/2)}$. Normality was assessed using Shapiro-Wilk test, and data that was not normally distributed underwent $\log_{10}$ transformation prior to analysis. All statistical analyses were performed using Prism 7.03 (GraphPad software, USA). Data are presented as means ± SD. Differences between groups and conditions are also reported as mean difference and (95% confidence interval).

5.4 Results

5.4.1 Participant characteristics

Although twenty participants were recruited for the study; an MSNA signal could not be obtained in five of them; therefore data are presented for fifteen participants. Eight Andeans with CMS were tested with a mean ± SD CMS score of 8 ± 2 (range 5–11) and seven Healthy Andeans with a CMS score of 1 ± 1 (range 0–3). Seven CMS participants were classified as having mild CMS and one was classified as having moderate CMS. CMS participants were similar in age, height, weight, and BMI to Healthy Andeans (*Table 7*). VO_{2peak} values were also similar in CMS and Healthy Andeans (*Table 7*).

5.4.2 Resting cardiovascular haemodynamic and basal sympathetic vasomotor outflow in CMS and healthy Andeans

Example recordings of beat-by-beat muscle sympathetic nerve activity (MSNA) and BP from one representative CMS and Healthy Andean is shown in *Figure 22*. SaO_2 and PaO_2 are lower and $PaCO_2$ is higher in CMS compared to Healthy Andeans (*Table 7*). As expected, haemoglobin concentration, haematocrit and blood viscosity (7.8 ± 0.7 vs 6.6 ± 0.7 cP; d = 1.7, P = 0.01) are all higher in CMS (*Table 7*). Although not statistically significant, total blood volume tends to be greater in CMS compared to Healthy Andeans (101 ± 25 vs 85 ± 16 $mL \cdot kg^{-1}$; d = 0.8, P = 0.2), which is due to a larger total red blood cell volume, with a similar plasma volume between groups (*Table 7* and *Figure 23*). CMS also tend to exhibit a greater stroke volume (76 ± 13 vs 64 ± 19 mL; d = 0.8, P = 0.32,) and have a lower HR (64 ± 10 vs 74 ± 4 bpm; d = 1.4, P = 0.03) compared to Healthy Andeans, with a similar Qc in both groups (4.8 ± 0.7 vs 4.7 ± 1.3 $L \cdot min^{-1}$; d = 0.1, P = 0.83). TPR is also similar between CMS and Healthy Andeans (19.2 ± 6.2 vs 19.9 ± 5.5 $mmHg \cdot L^{-1} \cdot min^{-1}$); however CMS exhibit a trend for lower MSNA burst

frequency (33 ± 11 vs 45 ± 13 burst·min^{-1}; d = 1.0, P = 0.08) compared to Healthy Andeans (*Figure 23*). Because Qc and TPR are comparable, MAP is also similar in CMS compared to Healthy Andeans (*Figure 23*). Unfortunately, due to the difficulty in identifying the tricuspid value regurgitant jet in four participants, PASP measurements could only be obtained in seven CMS and four Healthy Andeans (*Table 7*).

	CMS	Healthy Andeans	*P* value	Cohen's *d*	Mean difference (95% CI)
Participant characteristics					
Age (yrs)	40 ± 12	45 ± 12	0.39	0.4	-5 (-19 to 7)
Height (m)	1.61 ± 0.06	1.61 ± 0.03	0.97	0	0 (-0.05 to 0.06)
Weight (kg)	69 ± 12 kg	71 ± 11	0.79	0.2	-2 (-15 to 11)
BMI ($kg \cdot m^{-2}$)	26.4 ± 4.9	26.5 ± 3.8	0.74	0.2	-0.9 (-5.8 to 4.0)
$VO_{2\ peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)*	32.9 ± 10.5	28.7 ± 8.8	0.49	0.4	4.1 (-7.8 to 16.2)
CMS score	8 ± 2	1 ± 1	**<0.01**	4.7	-6 (-9 to - 4)
Haematological variables					
Haemoglobin (g/dL)	22.6 ± 0.9	19.3 ± 0.8	**<0.01**	3.9	3.3 (2.2 to 4.2)
Haematocrit (%)	65 ± 5	57 ± 3	**<0.01**	2.0	8 (3 to 13)
SaO_2 (%)†	82 ± 2	87 ± 3	**0.01**	2.0	-5 (-9 to -2)
PaO_2 (mmHg)†	47 ± 2	51 ± 4	**0.05**	1.4	-4 (-8 to 0)
$PaCO_2$ (mmHg)†	34 ± 1	29 ± 4	**0.02**	1.9	5 (1 to 9)
RBC volume ($mL \cdot kg^{-1}$)	57 ± 14	48 ± 8	0.19	0.8	9 (-5 to 21)
Plasma volume ($mL \cdot kg^{-1}$)	41 ± 9	42 ± 9	0.87	0.1	-1 (-11 to 9)
Pulmonary haemodynamics					
PASP (mmHg) ♦	29 ± 7	33 ± 7	0.4	0.6	-4 (-14 to 6)

Table 7. Haematological variables and resting pulmonary haemodynamics in CMS and healthy Andeans.

Data presented as mean ± SD for CMS (n = 8) and Healthy Andeans (n = 7). * Values based on seven CMS and six healthy Andeans, † Values based on six CMS and five Healthy Andeans ♦ Values based on seven CMS and four Healthy Andeans. BMI = body mass index, CMS = chronic mountain sickness, RBC = red blood cell, PASP = pulmonary artery systolic pressure. Statistical comparisons performed using independent t-tests.

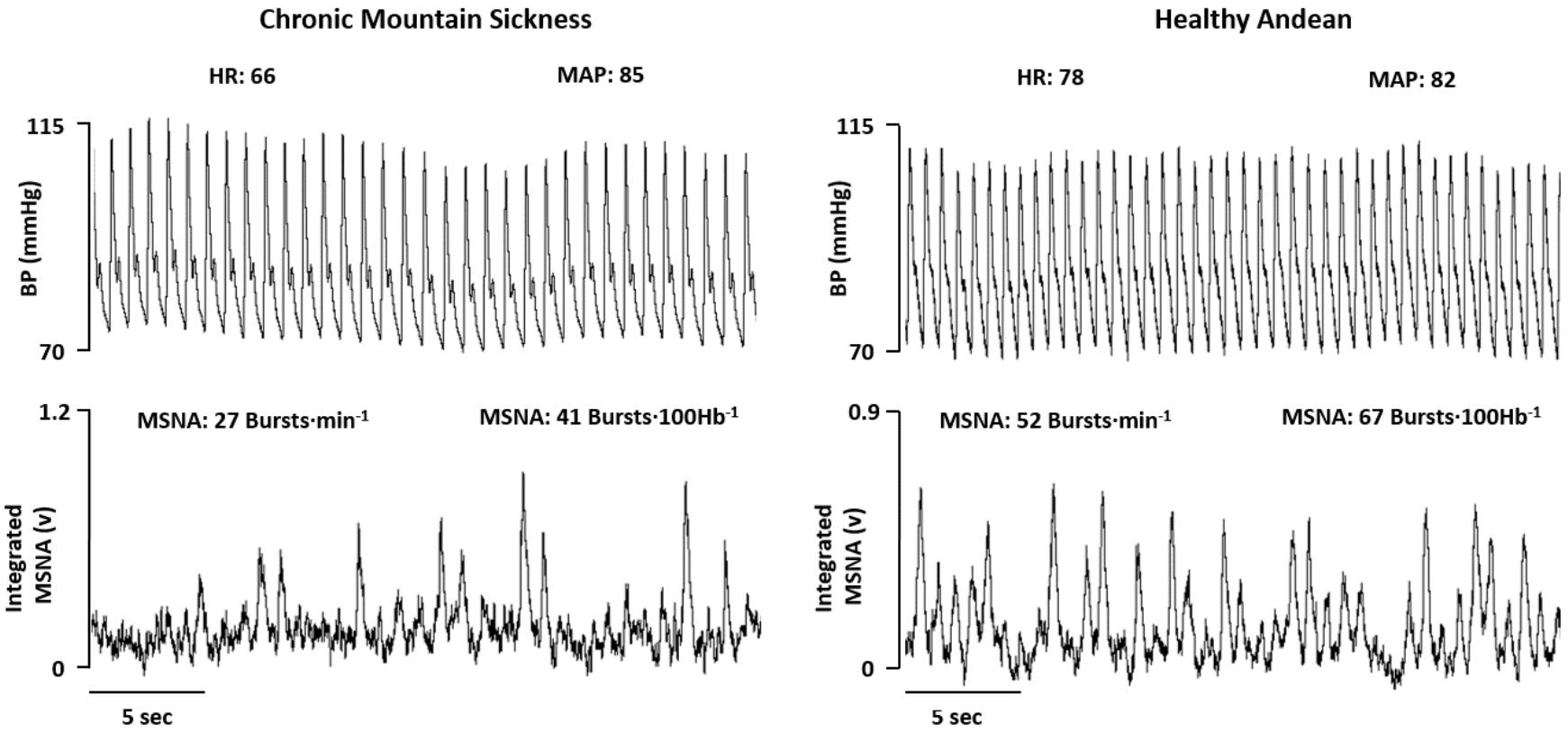

Figure 22. Example recordings of beat-by-beat muscle sympathetic nerve activity (MSNA) and BP from one representative CMS (aged 53 yrs) and Healthy Andean (aged 48 yrs) at 4383 m

HR, heart rate, MAP , mean arterial pressure.

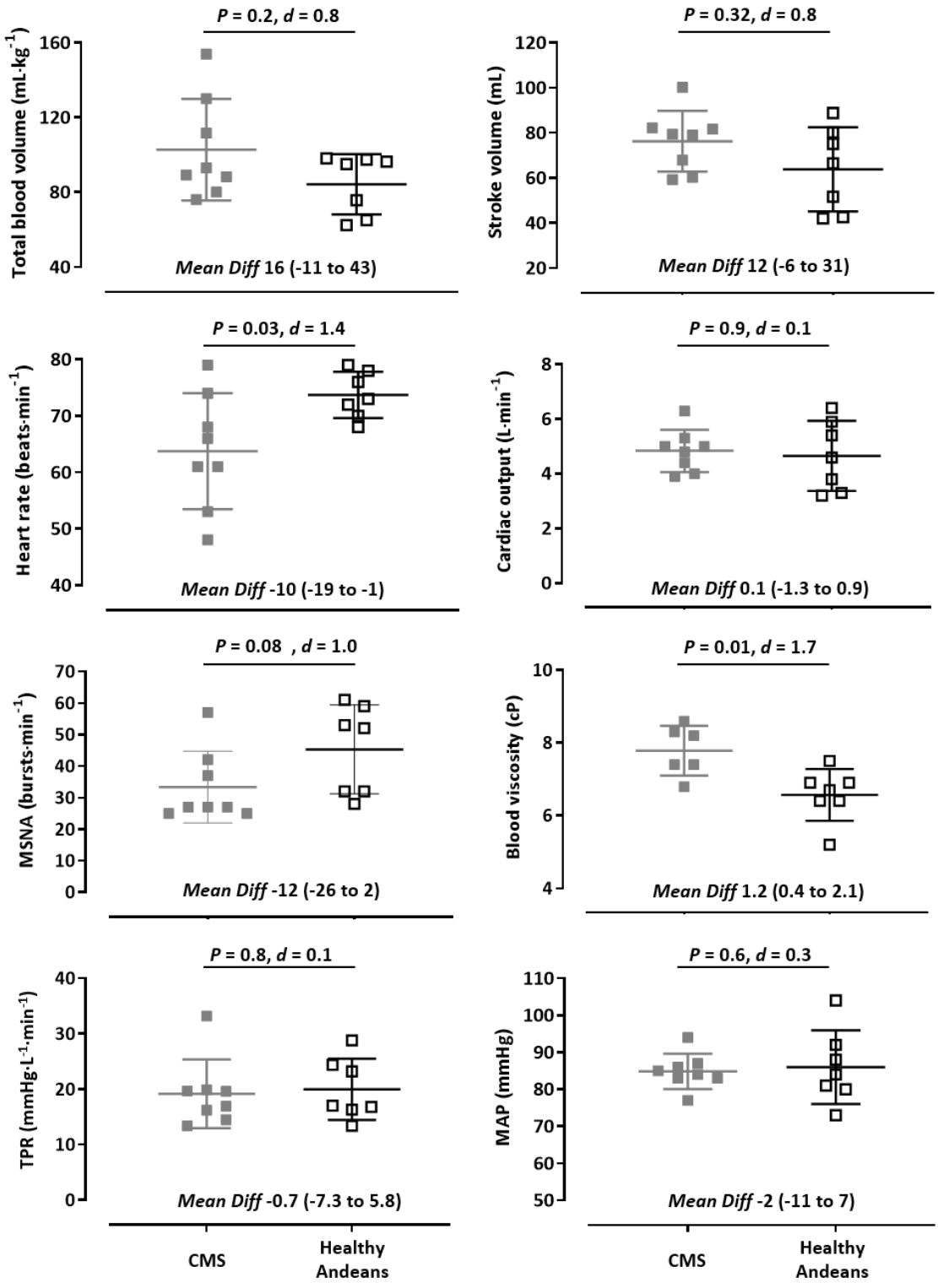

Figure 23. Haematological, cardiovascular haemodynamics and basal MSNA in CMS (n = 8) and healthy Andeans (n = 7).

Group mean (± SD) and individual data presented. Blood viscosity values based on six CMS and five Healthy Andeans. Statistical comparisons performed using independent t-tests. P values are reported with Cohen's *d* effect sizes (*d*) and mean differences (95% confidence intervals).

5.4.3 Arterial baroreflex function in CMS and healthy Andeans

Vascular sympathetic baroreflex gain (i.e. slope of the DBP-MSNA relationship) is comparable in CMS and Healthy Andeans (*Figure 24*). The operating DBP is also similar in both groups (71 ± 4 vs 74 ± 9; d = 0.5, P = 0.41, mean diff -3 [-11 to 4]).The MSNA operating point is lower in CMS compared to Healthy Andeans (51 ± 12 vs 62 ± 17 bursts·100HB^{-1}; d = 0.75, P = 0.16, mean diff -11 [-28 to 8]), although this is not statistically significant (*Figure 24*).

Cardiovagal baroreflex gain (i.e. slope of the relationship between RRI and SBP) is greater in CMS compared to Healthy Andeans (17.2 ± 6.8 vs 8.8 ± 2.6 ms·mmHg^{-1}; d = 1.8, P < 0.01, mean diff 8.4 [2.7 to 15.0]; *Figure 25*). These findings are similar regardless of whether RRI or HR was used. Operating SBP is similar in both groups (CMS, 109 ± 8 vs Healthy Andeans, 113 ± 15 mmHg; d = 0.4, P = 0.5, mean diff – 4 [-17 to 8]); however CMS participants operate around a longer RRI (960 ± 159 vs 817 ± 50 ms; d = 1.4, P = 0.04, mean diff -143 [-7 to -279]; *Figure 25*).

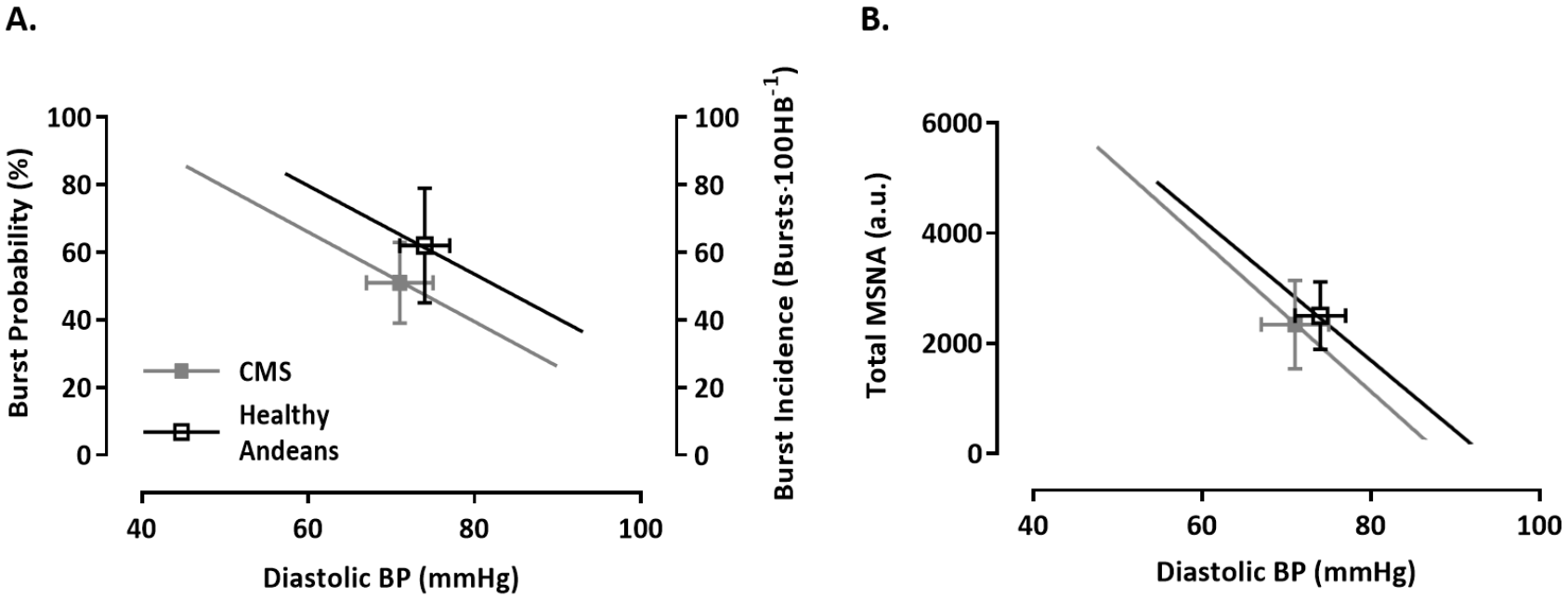

Figure 24. Vascular sympathetic baroreflex function in CMS and healthy Andeans.

Panel A shows the group average regressions between diastolic BP and MSNA burst probability and Panel B shows the group average regressions between diastolic BP and total MSNA in CMS (n = 8) and Healthy Andeans (n = 7). The set-point of the vascular sympathetic baroreflex are indicated by the symbols and error bars (mean ± SD). The vascular sympathetic baroreflex set-point is similar between CMS and Healthy Andeans. The slope of the relationship between diastolic BP and MSNA is also similar between groups regardless of whether MSNA was quantified as burst probability (-2.5 ± 0.9 vs -2.7 ± 1.1 %·mmHg^{-1}; *d* = 0.2, *P* = 0.69, mean diff 0.2 [-0.9 to 1.3]) or total MSNA, indicating no differences in reflex gain. Statistical comparisons performed using independent t-tests.

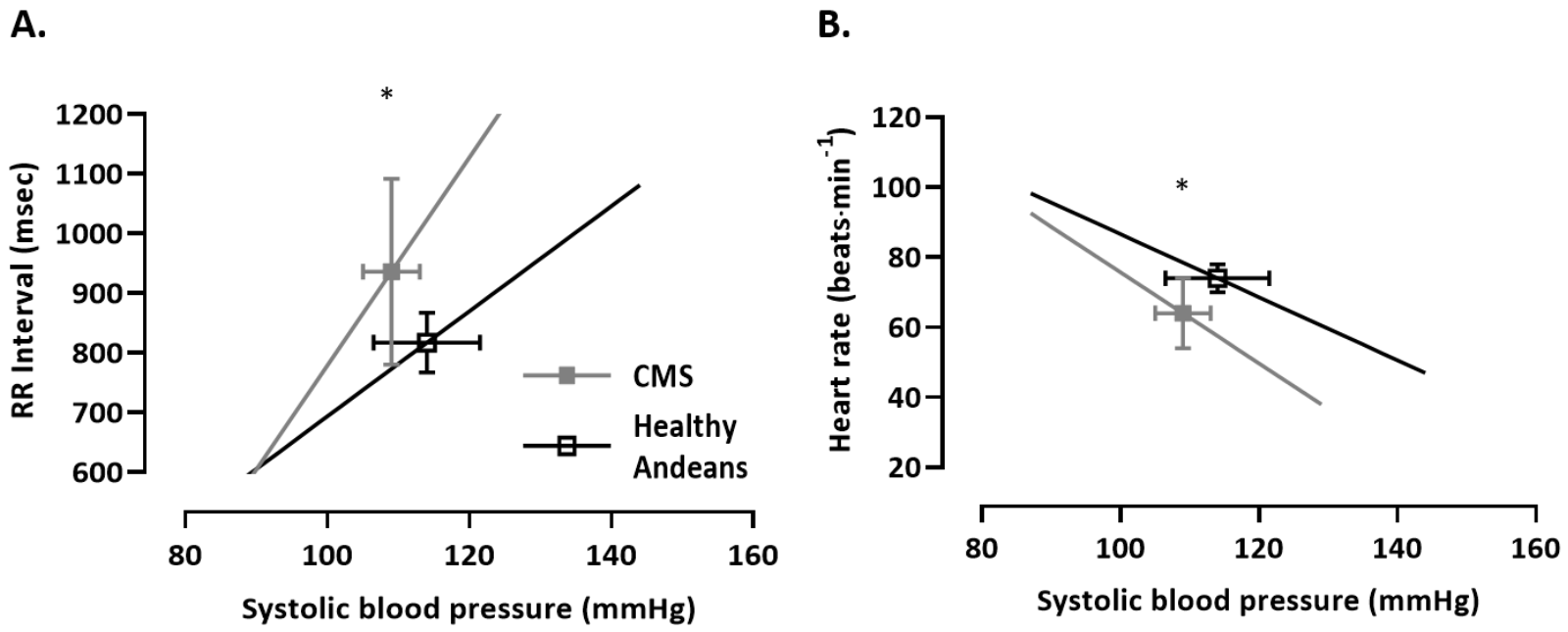

Figure 25. Cardiovagal baroreflex function in CMS and healthy Andeans.

Panel A shows the group average regressions between systolic BP and RRI and Panel B shows the group average regressions between systolic BP and HR in CMS (n = 7) and Healthy Andeans (n = 6). The set-point of the cardiovagal baroreflex are indicated by the symbols and error bars (mean ± SD).The cardiovagal baroreflex operates around a similar systolic BP, but a greater RRI/lower HR set-point in CMS compared to Healthy Andeans. The slope of the relationship between systolic BP and RRI/HR is also greater in CMS compared to healthy Andeans regardless of whether systolic BP was regressed against RRI (17.2 ± 6.8 vs. 8.8 ± 2.6 ms·mmHg^{-1}; *d* = 1.8, P < 0.01, mean diff 8.4 [2.7 to 15.0]) or HR (-1.3 ± 0.3 vs. -0.9 ± 0.3 beats·mmHg^{-1}; *d* = 1.33, P = 0.04, mean diff 0.4 [-0.8 to -0.02]), indicating a greater reflex gain. * P <0.05 versus Healthy Andeans. Statistical comparisons performed using independent t-tests.

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5.4.4 Arterial baroreflex–peripheral chemoreflex interactions

In CMS participants exposed to 100% O_2, HR is significantly reduced and Qc also decreases, although this did not achieve significance (*d* = 0.6, *P* = 0.07). Oxygen administration has no effect on any other cardiovascular haemodynamic variable in CMS. HR and Qc both significantly decrease in Healthy Andeans exposed to 100% O_2. This reduction in Qc, is accompanied by an increase in TPR, with no significant effect on BP. The reduction in HR in both groups are accompanied by a lowering of MSNA burst frequency, with no effect on burst amplitude (*Table 8*).

Administration of oxygen has no significant effect on vascular sympathetic baroreflex gain, operating DBP or MSNA set-point in either CMS or Healthy Andeans. Administration of oxygen has no effect on cardiovagal baroreflex gain (18.8 ± 9.7 to 20.3 ± 7.4 ms·mmHg^{-1}; *d* = 0.2, *P* = 0.7; *Figure 26*) or operating SBP in CMS, but RRI is lengthened. In Healthy Andeans administration of oxygen also lengthens RRI; cardiovagal baroreflex gain is greater (8.0 ± 2.6 to 14.1 ± 4.9 ms·mmHg^{-1}; *d* = 1.6, *P* = 0.01), with no change in operating SBP (*Figure 26*)

	CMS					Healthy Andeans				
	Baseline	**Hyperoxia**	***P* value**	**Cohen's *d***	**Mean difference (95% CI)**	**Baseline**	**Hyperoxia**	***P* value**	**Cohen' s*d***	**Mean difference (95% CI)**
Haemodynamic variables										
Heart rate (bpm)	60 ± 10	54 ± 14	**<0.01**	0.5	-6 (-10 to -3)	69 ± 7	61 ± 6	**0.03**	1.2	-8 (-14 to -1)
R-R interval (ms)	1019 164	1175 263	**0.01**	0.7	156 (52 to 262)	880 ± 89	905 ± 100	**0.03**	0.3	25 (17 to 207)
Stroke volume (mL)	92 ± 6	93 ± 12	0.95	0.1	0.6 (-16 to 18)	80 ± 21	82 ± 26	0.72	0.1	1.8 (-8 to 12)
Qc ($L \cdot min^{-1}$)	5.6 ± 1.1	4.9 ± 1.2	0.07	0.6	-0.7 (-1.4 to 0.1)	5.4 ± 1.1	4.9 ± 1.4	**0.05**	0.4	-0.5 (-0.9 to -0.2)
TPR (mmHg·L·min)	16.0 ± 3.2	17.9 ± 4.9	0.18	0.5	1.9 (-1.3 to 5.1)	17.6 ± 5.7	20.5 ± 7.9	**0.01**	0.4	2.9 (0.3 to)
MAP (mmHg)	89 ± 9	89 ± 5	0.15	0.2	-2 (-4 to 1)	87 ± 11	92 ± 7	0.11	0.5	3 (-1 to 8)
Diastolic BP (mmHg)	73 ± 12	71 ± 12	0.20	0.2	-2 (-4 to 1)	74 ± 5	77 ± 6	0.15	0.5	3 (-1 to 6)
Systolic BP (mmHg)	118 ± 8	115 ±10	0.13	0.3	-3 (-6 to 1)	115 ± 7	120 ± 12	0.13	0.5	5 (-2 to 13)
Muscle Sympathetic Nerve Activity										
Burst frequency ($burst \cdot min^{-1}$)	32 ± 13	29 ± 13	**0.02**	0.7	-3 (-6 to -1)	41 ± 16	33 ± 13	**0.04**	0.7	-8 (-16 to -0.7)
Burst incidence ($bursts \cdot 100HB^{-1}$)	51 ± 14	53 ± 13	0.37	0.2	2 (-2 to 5)	60 ± 20	54 ± 21	0.15	0.2	-6 (-13 to 3)
Normalized burst amplitude	56 ± 8	55 ± 13	0.99	0.2	2 (-10 to 9)	57 ± 5	54 ± 13	0.78	0.2	2 (-10 to 13)
Total activity ($a.u \cdot min^{-1}$)	1780 ± 801	1618 ± 922	0.19	0.4	-162 (-473 to 149)	2335 ± 900	1992 ± 1026	0.08	0.4	-343 (-728 to 42)
Total MSNA ($a.u \cdot 100HB^{-1}$)	2876 ± 1030	2989 ±1359	0.66	0.1	113 (-515 to 740)	3356 ± 1127	2984 ± 1745	0.33	0.3	-372 (-1260 to 515)

Table 8. Cardiovascular haemodynamics, MSNA and arterial baroreflex function assessed at baseline and during acute hyperoxia in CMS and Healthy Andeans.

Data presented as mean ± SD in CMS (n = 6) and Healthy Andeans (n = 6). Statistical comparisons performed using dependent t-tests.

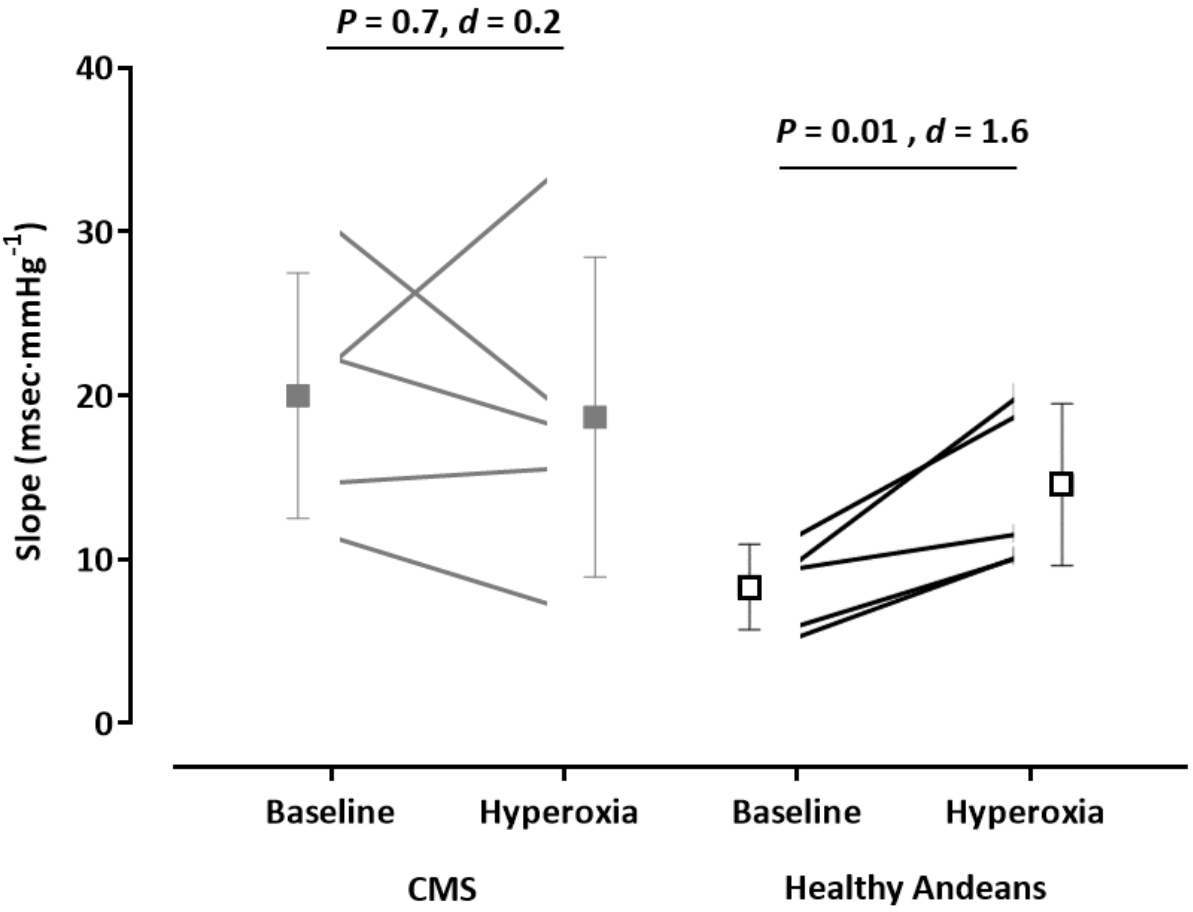

Figure 26. Effect of manipulating peripheral chemoreceptor drive on cardiovagal baroreflex gain in CMS and healthy Andeans.

Individual and group average slopes for the relationship between RRI and systolic BP at baseline and during hyperoxia in CMS (n=5) and Healthy Andeans (n=6). Administration of oxygen has no effect on cardiovagal baroreflex gain in CMS but, increases cardiovagal baroreflex gain (8.0 ± 2.6 to 14.1 ± 4.9 ms·mmHg^{-1}; *d* = 1.6, *P* = 0.01) in Healthy Andeans. Statistical comparisons performed using dependent t-tests.

5.5 Discussion

The major findings of the present study are threefold: i) Andeans with CMS and Healthy Andeans exhibit similar vascular sympathetic baroreflex gain (i.e. responsiveness), operating diastolic pressure, and MSNA operating point (i.e. MSNA burst incidence); ii) however, in mild CMS, the cardiovagal baroreflex operates around a longer RRI (lower HR) with a greater reflex gain; iii) Andeans with CMS have comparable Qc, total peripheral resistance, and thus, BP compared to Healthy Andeans. However, Andeans with CMS exhibit a greater haemoglobin concentration, total blood volume and blood viscosity, and lower basal vasomotor sympathetic activity (i.e. MSNA burst frequency) compared to Healthy Andeans. Taken together, these findings indicate adaptive changes in autonomic regulation of BP homeostasis in Andean highlanders with mild CMS.

5.5.1 Basal sympathetic vasomotor outflow in Andeans

The one previous study that has assessed resting sympathetic vasomotor outflow in Andean high altitude natives found comparable basal MSNA in healthy Bolivian Andeans (Aymara) and acclimatising Lowlanders (Lundby et al., 2017). However, this study is the first to assess basal sympathetic vasomotor outflow in Peruvian (Quechua) Andeans, including individuals with CMS. A 25% *lower* basal MSNA was observed in CMS compared to Healthy Andeans, as indicated by a lower MSNA burst frequency (*Figure 23*). This finding is in contrast to the hypothesis that basal sympathetic vasomotor outflow would be greater in CMS, which was based upon previous studies reporting either comparable (Antezana et al., 1995) or elevated (Gamboa et al., 2006) plasma noradrenaline levels in individuals with CMS. A reduced glomerular filtration rate (Lozano & Monge, 1965) and thus noradrenaline clearance, in CMS could overestimate sympathetic activation using this method, and potentially explain

these contradictory findings. Despite this, it might be anticipated that sympathetic vasomotor outflow would be elevated in Andeans with CMS due to several factors. These factors include: exaggerated arterial hypoxaemia (lower PaO_2), reports of increased inflammation and oxidative stress (Bailey et al., 2013, 2019); and, a reduced NO bioavailability, all of which exert known sympathoexcitatory effects (Patel et al., 2001). Sympathetic vasomotor outflow, however, is the net effect of the integration of both excitatory and inhibitory inputs to the cardiovascular control centres in the brainstem. For example, elevations in blood volume exert a sympathoinhibitory influence on basal MSNA, likely through increased distension of arterial baroreceptors (Charkoudian et al., 2004, Best et al., 2014). Notably, Andeans with CMS in the present study exhibited a 20% greater blood volume compared to Healthy Andeans (*Figure 23*). Whilst not statistically significantly different, the effect was large (Cohen's d = 0.8) and the differences were comparable to those previously reported in this population (Claydon et al., 2004). Recently, evidence suggests that the CO rebreath technique may underestimate blood volume in CMS (Wachsmuth et al., 2019). Thus, lower basal sympathetic vasomotor outflow in CMS could be mediated by an increase in circulating blood volume. Indeed, high altitude native Sherpa demonstrate a lower basal sympathetic outflow compared to acclimatising Lowlanders (Chapter 4), with Sherpa also shown to exhibit a greater total blood volume (Stembridge et al., 2019). Despite this, there is no significant correlation between these factors in the present study. It is also important to note, however, that individuals with CMS were on average ~5 years younger than Healthy Andeans. Although, the reported ~3 bursts·min^{-1} increase in basal MSNA per decade of life (Narkiewicz et al., 2005) would not exclusively explain the observed 12 burst·min^{-1} difference in basal MSNA.

5.5.2 Arterial baroreflex function in Andeans

This is the first study to assess baroreflex control of MSNA in Andean high altitude natives. In addition, it is the first to simultaneously assess the vascular sympathetic and cardiovagal limbs of the arterial baroreflex in the same group. Both CMS and Healthy Andeans exhibited a similar ability to increase and decrease MSNA in response to transient, pharmacologically induced changes in BP (i.e. the vascular sympathetic baroreflex gain was unchanged). This is consistent with one previous report of a similar reflex gain for carotid baroreflex control of forearm vascular resistance (Moore et al., 2006) in both CMS and Healthy Andeans. Contrary to what was hypothesised, resting heart rate was lower, and the ability to alter RRI during the modified Oxford test was greater in CMS compared to Healthy Andeans. The operating diastolic pressure for the vascular sympathetic baroreflex and the operating systolic pressure for the cardiovagal baroreflex were similar in CMS and Healthy Andeans. Furthermore, MSNA burst incidence (i.e. vascular sympathetic baroreflex set-point) was also not significantly different between groups, meaning the probability of a burst occurring per cardiac cycle was similar between CMS and Healthy Andeans. Therefore, these data indicate that CMS does not influence the arterial baroreflex control and gating of sympathetic bursts. Importantly, however, a lower resting HR in CMS reduces the opportunities (i.e. cardiac cycles) for a burst to occur. Thus, the interaction of a similar MSNA burst incidence (i.e. vascular sympathetic baroreflex operating point) and lower resting heart in CMS reduces MSNA burst frequency (i.e. basal sympathetic vasomotor activity). It should be noted that, in contrast to the findings presented here, several studies report higher, rather than lower, resting HR and/or BP for CMS (Keyl et al., 2003; Claydon et al., 2004; Richalet et al., 2005, Corante et al., 2018). The reasons for these differences are unclear, although differences in posture, measurement technique, time of day for measurement, and the duration of the measurement period, all

might contribute. In addition, CMS severity may also be important; indeed, studies that report higher resting heart rates and blood pressures for CMS also report greater average Hct and CMS scores than those observed in the present study. A positive correlation has also been observed between [Hb] and BP in the Cerro de Pasco population (Gonzales & Tapia, 2013). Importantly in the present study, Qc, total peripheral resistance, and thus, arterial pressure for CMS are comparable to Healthy Andeans; this is despite greater blood viscosity and total blood volume, secondary to an increase in red blood cell volume, in CMS. Thus, the lower sympathetic vasomotor outflow and heart rate in CMS appear to balance the haemodynamic effects of EE, which maintains BP homeostasis, at least in mild CMS studied here.

There are several possible explanations for the observed differences in cardiovagal baroreflex set-points. One possibility is a larger stroke volume in CMS (Charkoudian et al., 2004), secondary to an increase in blood volume. Arterial baroreceptors respond to mechanical distension and not arterial pressure *per se* (Angell-James, 1971). Therefore, a greater stroke volume would increase distension of baroreceptive areas at a given BP, eliciting a reduction in sympathetic and/or increase in vagal outflow to the heart on a beat-by-beat basis. Chronically, this may lead to resetting of the RRI set-point in CMS. Furthermore, a greater blood viscosity in CMS and a subsequent increase in shear stress at the level of the carotid baroreceptors may also increase baroreceptor afferent firing for a given BP (Hajduczok et al., 1988). Altered afferent input at the level of the baroreceptors, however, may also be expected to reset the vascular-sympathetic operating point, which was not observed (although MSNA burst incidence was lower, this was not statistically significant). Thus, these changes may be independent of autonomic outflow. Whilst reductions in HR and basal MSNA may be expected in severe CMS, for the same reasons as above, increasing oxidative-inflammatory stress, metabolic dysregulation, (Corante et al., 2018) and endothelial

dysfunction (Tremblay et al., 2019) with greater disease severity, may tip the balance to a state of sympathoexcitation and hypertension.

5.5.3 Influence of peripheral chemoreflex on arterial baroreflex function in Andeans

Lower PaO_2 in Andeans with CMS would be expected to increase peripheral chemoreflex activation and potentially reset the arterial baroreflex to operate at higher heart rates, arterial pressures and level of MSNA (Halliwill and Minson et al., 2002, Steinback et al., 2009). Importantly, however, peripheral chemoreceptor ventilatory responsiveness to hypoxia is reported to be blunted in Andeans with CMS (Severinghaus et al., 1966; León-Velarde & Richalet, 2006), contributing to alveolar hypoventilation (higher $PaCO_2$) reported in this population (León-Velarde & Richalet, 2006). Despite a blunted ventilatory responsiveness reported in CMS the peripheral chemoreflex mechanism does not appear to contribute to the lower HR in CMS, as acutely eliminating peripheral chemoreceptor drive, via 100% oxygen administration, had comparable effects on HR in both groups. Interestingly, MSNA burst incidence remains unchanged for CMS during acute hyperoxia, whilst it was reduced (~ 6 bursts·100HB^{-1}) for Healthy Andeans. However, this reduction in MSNA burst incidence occurs alongside a small increase in both arterial pressure (~3 mmHg) and stroke volume; therefore, such reductions are likely arterial baroreflex-mediated. In addition, whilst other haemodynamic responses to hyperoxia were comparable between groups, there was a significant increase in TPR in Healthy Andeans; not observed in CMS. This may indicate different local control and regulation of vascular tone; that is Healthy Andeans possess a greater vascular responsiveness to hypoxia (i.e. greater vasodilator response) compared with CMS. This could contribute, in part, to the observed difference in basal MSNA under ambient hypoxic conditions, where healthy Andeans require a greater basal MSNA to restrain local

vasodilation and thus maintain BP. Any potential difference in local control, however, cannot be determined from the data presented here.

An inhibitory relationship exists between the peripheral chemoreflex and baroreflex mechanisms (Somers et al., 1991), whereby an acute increase in peripheral chemoreflex activation is consistently shown to inhibit baroreflex control of the heart (Heistad & Wheeler, 1971; Sagawa et al., 1997; Steinback et al., 2009; Niewinski et al., 2014; Mozer et al., 2016) with inconsistent effects on baroreflex control of MSNA (Halliwill & Minson, 2002; Simpson et al., 2019). In the present study, during ambient air breathing, a greater cardiovagal baroreflex responsiveness is observed for CMS compared to Healthy Andeans. Furthermore, no change in cardiovagal baroreflex responsiveness is observed for CMS during acute hyperoxia, but Healthy Andeans demonstrated a 75% increase in reflex gain. These findings indicate a peripheral chemoreflex-mediated inhibition of cardiovagal baroreflex responsiveness in healthy Andeans at high altitude, which does not appear to be present in CMS. This raises an interesting possibility that whilst a blunted peripheral chemoreflex responsiveness may contribute to the exaggerated arterial hypoxaemia in CMS, it may, paradoxically, prevent the reduced cardiovagal baroreflex gain normally observed during sustained high altitude exposure, observed in Chapter 4 and by others (Yazdani et al., 2016; Bourdillon et al., 2018).

5.6 Experimental limitations

There are several limitations in the present study that should be acknowledged. First, due to time constraints, only small opportunistic samples could be studied. Therefore, meaningful differences between groups may not have been detected due to low statistical power. Indeed, insufficient statistical power likely prevented a meaningful 20% difference in

total blood volume between groups from being detected, despite a similar magnitude of difference to previous studies (Claydon et al., 2004). Second, two Andeans with CMS were light to moderate smokers. It is reported that tobacco smoking leads to increased basal MSNA and attenuates vascular sympathetic baroreflex gain (Middlekauff et al., 2013, 2014), which may have influenced these results. However, this would have potentially overestimated resting MSNA in CMS, which would not have altered the interpretation of these results (i.e. lower sympathetic vasomotor outflow in CMS). Last, due to a lack of CMS positive female volunteers, and since women are protected from CMS until menopause (León-Velarde et al., 1997) only males were studied. Thus, these findings cannot be generalised to females, who may exhibit differences in BP control mechanisms.

5.7 Implications and significance

Our findings imply that elevated sympathetic vasomotor outflow and arterial baroreflex dysfunction do not contribute to the elevated cardiovascular disease risk reported in mild CMS, since autonomic control of BP is well maintained in the group studied here. Therefore, other factors may predispose individuals with CMS to cardiovascular disease. However, the possibility that elevated sympathetic vasomotor outflow and/or arterial baroreflex dysfunction may develop in more severe CMS, cannot be excluded. If autonomic dysfunction were to occur in more severe cases, this may contribute to the greater cardiovascular disease risk reported in moderate and severe CMS (Corante et al., 2018).

5.8 Conclusion

Contrary to the hypotheses, elevated sympathetic vasomotor outflow and arterial baroreflex dysfunction are not apparent in mild CMS. In fact, basal sympathetic vasoconstrictor drive and heart rate are lower in CMS, with enhanced cardiovagal baroreflex

gain, compared to Healthy Andeans. Such changes appear to be adaptive physiological responses to the elevations in red blood cell volume, which allow BP homeostasis to be maintained. Furthermore, whilst a blunted peripheral chemoreflex is reported to be a possible mechanism responsible for accentuated arterial hypoxaemia in CMS, it may, paradoxically, augment cardiovagal baroreflex responsiveness compared to Healthy Andeans.

5.9 Contribution to this chapter

For this chapter, I contributed to the conception and design of the study, data collection, data analysis, data interpretation, preparation of figures, chapter drafting and chapter revisions

Author Name	Conception and Design	Data Collection	Data Analysis	Data Interpretation	Preparation of Figures	Manuscript Drafting	Manuscript Revisions
Lydia L Simpson	X	X	X	X	X	X	X
Victoria L Meah		X					X
Andrew R Steele		X					X
Christopher Gasho		X					X
Connor A Howe		X	X				X
Tony G Dawkins		X	X				X
Stephen A Busch			X				X
Samuel J Oliver				X			X
Gilberto Moralez		X					X
Justin S Lawley		X					X
Michael M Tymko	X						X
Gustavo A Vizcardo-Galindo		X					X
Romulo J Figueroa-Mujica		X					X
Francisco C Villafuerte							X
Philip N Ainslie	X						X
Mike Stembridge	X	X		X		X	X
Craig D Steinback	X	X					X
Jonathan P Moore	X	X		X		X	X

Table 9. – Authorship summary for Chapter 5

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CHAPTER 6: EXPERIMENTAL STUDY 3

Evidence for a physiological role of pulmonary arterial baroreceptors in sympathetic neural activation in healthy humans at high altitude

This chapter has been published in the Journal of Physiology

Simpson LL., Meah VL., Steele AR., Thapamagar S., Gasho C., Anholm JD., Drane AL., Dawkins TG., Oliver SJ., Lawley JS., Tymko MM., Ainslie PN., Steinback CD., Stembridge M & Moore JP (2020). New physiological evidence for a role of pulmonary arterial baroreceptors in sympathetic activation. **598(5)**, 955-965 doi: 10.1113/JP278731

6.1 Abstract

In animal models, distension of baroreceptors located in the pulmonary artery induces a reflex increase in sympathetic outflow; however, this has not been examined in humans. Therefore, this study investigates whether reductions in pulmonary arterial pressure influence sympathetic outflow and baroreflex control of MSNA. Healthy lowlanders (n=13; 5 females) were studied 4–8 days following arrival at high altitude (4383 m; Cerro de Pasco, Peru), a setting that increases both pulmonary arterial pressure and sympathetic outflow. MSNA (microneurography) and BP (photoplethysmography) were measured continuously during 1) ambient air breathing (Amb) and 2) a six minute inhalation of the vasodilator nitric oxide (iNO; 40 ppm in 21% O_2), to selectively lower pulmonary arterial pressure. A modified Oxford test was performed under both conditions. Pulmonary artery systolic pressure (PASP) was determined using Doppler echocardiography. iNO reduces PASP (24 ± 3 vs 32 ± 5 mmHg; *P* <0.001) compared to Amb, with a similar reduction in MSNA total activity (1369±576 to 994±474 a.u·min^{-1}; *P* = 0.01). iNO also reduces the MSNA operating point (burst incidence; 39 ± 16 to 33 ± 17 bursts·100 Hb^{-1}; *P* = 0.01) and diastolic operating pressure (82 ± 8 to 80 ± 8 mmHg; *P* < 0.001) compared to Amb, without changing HR (*P* = 0.6) or vascular-sympathetic baroreflex gain (*P* = 0.85). In conclusion, unloading of pulmonary arterial baroreceptors reduces basal sympathetic outflow to the skeletal muscle vasculature and reset vascular-sympathetic baroreflex control of MSNA downward and leftward in healthy humans at high altitude. These data suggest the existence of a lesser-known reflex input involved in sympathetic activation in humans.

6.2 Introduction

In animal models, distension of pulmonary arterial baroreceptors, whilst carefully controlling the distending pressure to other major reflexogenic areas, elicits reflex sympathoexcitation and systemic vasoconstriction with no effect on HR (Ledsome and Kan, 1977; Mcmahon et al., 2000; Moore et al., 2011). Strikingly, these baroreceptors, located at the pulmonary artery bifurcation and in the extrapulmonary artery branches, elicit sympathetic responses that contrast with reflex sympathoinhibition and the resulting systemic vasodilation elicited by isolated distension of carotid sinus baroreceptors in the same experimental preparation (Moore et al., 2011). Moreover, an interaction exists between pulmonary arterial and carotid sinus baroreceptors, whereby altering pressure within the pulmonary arteries acutely resets the vascular limb of the carotid sinus baroreflex with unaltered reflex gain (Moore et al., 2011). In contrast to these studies in experimental animals, a role for pulmonary arterial baroreceptors in sympathetic activation in humans has been largely unexplored, likely due to the difficulty in isolating a physiological stimulus to the pulmonary circulation.

Some indirect support for a sympathoexcitatory reflex originating from baroreceptors in the pulmonary circulation of humans is evident in the literature. First, although a causal relationship has not been established, pulmonary arterial pressure is positively related to basal MSNA under conditions of elevated pulmonary arterial pressure, including exposure to high altitude hypoxia (Duplain et al., 1999), in heart failure (Ferguson et al., 1990) and in pulmonary arterial hypertension (Velez-Roa et al., 2004). Second, approximately a quarter of single-unit muscle sympathetic efferent fibres display an increase in neural activity during non-hypertensive lower body positive pressure (LBPP), a manoeuvre that increases central venous pressure and right-heart filling, and a decrease in sympathetic neural activity during

non-hypotensive lower body negative pressure (LBNP), which reduces right-sided filling pressure (Millar et al., 2013; Millar et al 2015; Incognito et al., 2018). Together, given the strong evidence from animal studies and supporting evidence in humans, the potential role for pulmonary arterial baroreceptors in sympathetic neural activation in humans requires investigation.

Therefore, the aim of the present study is to investigate the mechanistic role of pressure sensitive receptors in the pulmonary circulation in control and regulation of sympathetic outflow in humans. To investigate this, a novel experimental paradigm was employed to isolate the pulmonary baroreceptors. Healthy Lowlanders were studied at high altitude, a setting known to increase pulmonary arterial pressure (Naeije, 1992). Basal MSNA and baroreflex control of MSNA were assessed whilst breathing ambient air and during inhalation of the selective pulmonary vasodilator NO (iNO) (Frostell et al., 1991; Pepke-Zaba et al., 1991), to reduce the pressure stimulus to baroreceptors in the pulmonary circulation, without altering the stimulus to systemic arterial baroreceptors. Based on available evidence in animals, it is hypothesised that reducing pulmonary artery pressure will reduce basal MSNA in healthy humans at high altitude and reset baroreflex control of MSNA.

6.3 Methodology

6.3.1 Participants

Thirteen Lowlanders (five females) mean age (28 ± 7 yrs), height (1.7 ± 0.1 m) and weight (71 ± 7 kg) free from cardiovascular, respiratory, metabolic and neurological disease were recruited. Participants rapidly ascended from sea level (Lima, Peru) to 4383 m over the course of 9–10 h by motor vehicle and were studied 4-8 days (5 ± 2 days) following arrival at 4383 m (Cerro de Pasco, Peru; barometric pressure, 455 ± 0.7 mmHg). On the day of testing,

participants completed the Lake Louise Acute Mountain Sickness (AMS) questionnaire (Roach et al., 2018) to evaluate symptoms of AMS. Twelve participants reported a Lake Louise Score (LLS) ≤3 and, therefore, did not have clinically defined AMS. One subject was administered medication for treatment of AMS four days following arrival at high altitude (two doses of 125 mg acetazolamide over 24 hours]), but was tested following a 72 hour washout period. The washout allowed sufficient clearance time (i.e. >48 hours), since the reported half-life for acetazolamide is ∼10 hours (Ritschel et al., 1998) and this low-dose quantity is reported to be 90–100% excreted within 24 hours of administration (Richalet et al., 2005).This subject reported a LLS score of 5 on the day of testing and was, therefore, experiencing mild AMS at the time of participation. Based upon self-reporting, three females were tested in the early-follicular phase, one in the late-follicular phase of their menstrual cycle and one in the low-hormone phase of oral contraceptive use.

6.3.2 Experimental protocol

Schematic of experimental protocol is shown in *Figure 27*. Following instrumentation, acquisition of an acceptable MSNA signal, and a period of stabilisation, a single modified Oxford test was performed to determine arterial baroreflex function during ambient air breathing (Amb). Participants were then transferred to breathing room air via a mouthpiece and noseclip. Following a period of stabilisation, subjects rested for five minutes to determine baseline cardiovascular and pulmonary haemodynamics and sympathetic vasomotor activity. Subjects were then switched to breathing a gas mixture containing 21% oxygen and 40 ppm nitric oxide from a Douglas bag. Following six minutes of iNO, a second modified Oxford test was performed. During the modified Oxford test, subjects continued to breathe iNO. No

attempt was made to control ventilation or end tidal CO_2 during iNO. Modified Oxford tests were separated by a minimum of 20 minutes.

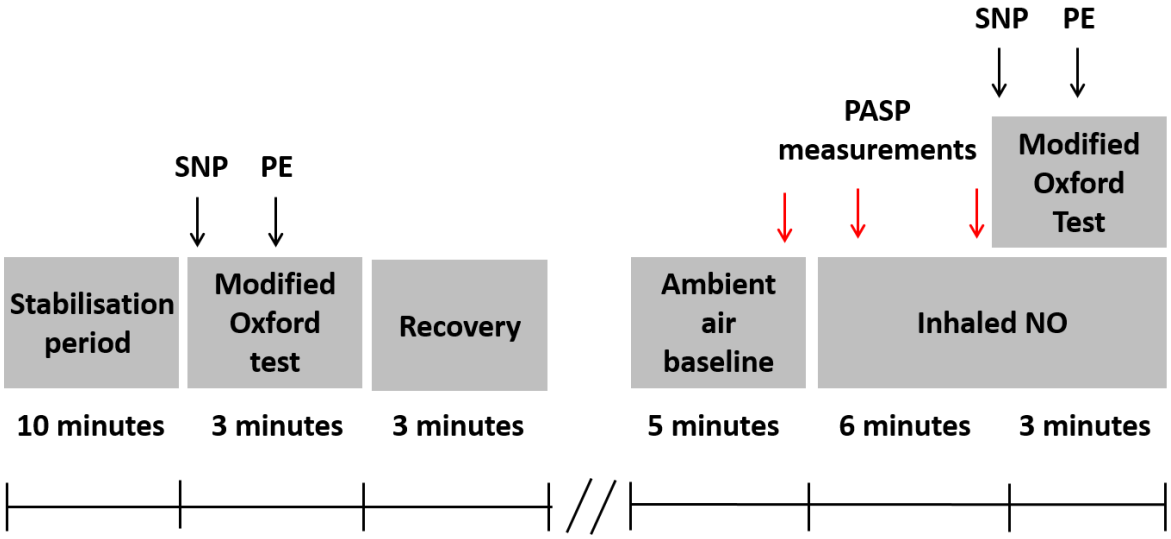

Figure 27. Schematic of experimental protocol for Experimental study 3.

SNP = sodium nitroprusside, PE = phenylephrine, PASP = pulmonary artery systolic pressure, NO = nitric oxide.

Inhaled Nitric Oxide

Inhaled NO rapidly diffuses across the alveolar epithelial membrane into the pulmonary vascular smooth muscle where it induces vascular smooth muscle relaxation (Steudel et al*.,* 1999) (*Figure 28*). Any NO that diffuses into the intravascular space rapidly binds to haemoglobin, which serves to inactivate NO (Rimar & Gillis, 1993) and prevent systemic vasodilation (Frostell et al., 1991; Pepke-Zaba et al*.*, 1991). Therefore, inhaled NO acts as a selective pulmonary vasodilator. NO was diluted in 100% nitrogen (N_2) in a Douglas bag, to prevent the production of nitrogen dioxide (NO_2). Immediately prior to inhalation, the NO/N_2 gas mixture was titrated with 100% Oxygen (O_2) to obtain a gas mixture containing 21% O_2, 79% N_2 and 40 ppm NO (ML206; ADInstruments, Colorado Springs, CO, USA). O_2 and NO gas concentrations were verified and NO_2 concentration was measured to ensure levels remained below 5 ppm..

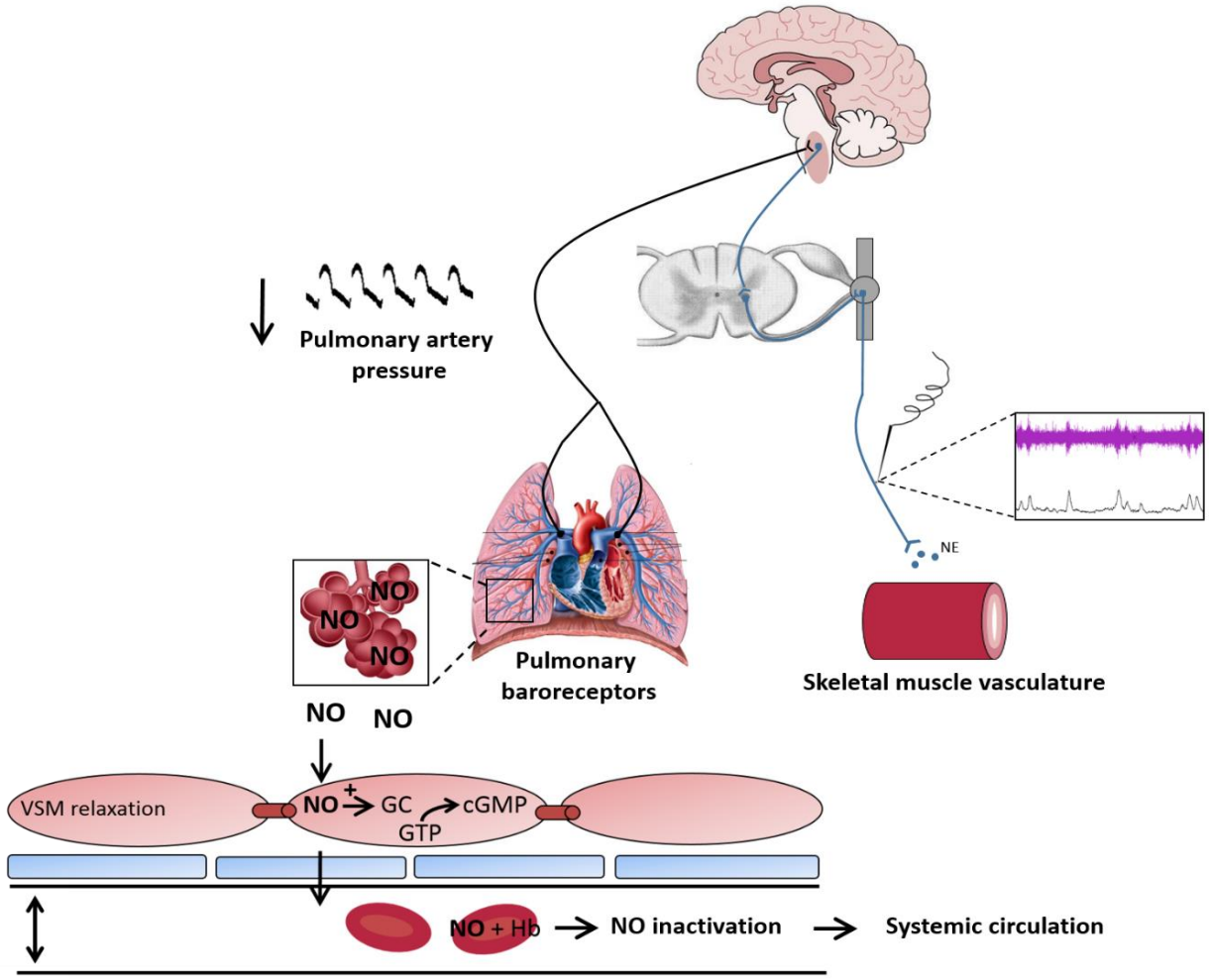

Figure 28. Action of inhaled nitric oxide (NO).

During inhalation, NO diffuses from the alveolar, through the epithelial cells, to the vascular smooth muscle (VSM). Within the VSM, NO binds to and activates soluble guanylate cyclase (GC), which in turn catalyses the dephosphorylation of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). cGMP induces relaxation of the VSM (Steudel et al., 1999). Any NO that diffuses into the blood binds to haemoglobin, becomes inactivated and is subsequently broken down. As a result, iNO elicits VSM relaxation in the pulmonary circulation, lowering pulmonary arterial pressure, without directly influencing the systemic circulation. Therefore, iNO serves to reduce afferent input to pressure sensitive receptors in the pulmonary artery, which allows investigation of the pulmonary arterial baroreceptors on reflex control of MSNA

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6.3.3 Experimental measurements

Beat-by-beat cardiovascular haemodynamic (RRI, heart rate, SBP, DBP, MAP and SpO_2) and MSNA were continuously recorded as described in Chapter 3. Left ventricular stroke volume and PASP were assessed via echocardiography, as described in Chapter 3, and subsequently used to calculate Qc (stroke volume X·HR) and TPR (MAP/Qc).

6.3.4 Data analyses

Beat-by-beat data for RRI, HR, SBP, DBP, MAP, SpO_2 and MSNA were extracted from Labchart (ADInstruments, Chart Pro v8.3.1). Values for cardiovascular haemodynamic and sympathetic vasomotor activity during Amb were calculated by averaging values over the five minutes ambient air breathing immediately preceding the start of NO inhalation. These values were compared to the average values over the last five minutes of NO. Sympathetic vasomotor activity was quantified as MSNA burst frequency and MSNA total activity. Cardiovascular haemodynamics (i.e. stroke volume, Qc, TPR) and pulmonary haemodynamics (i.e. PASP) were assessed between four and five minutes of Amb and compared to the average values across iNO. Cardiovascular haemodynamics and pulmonary haemodynamics during iNO were determined between one and two minutes, and between five and six minutes of inhalation. The duration of inhalation had no effect on cardiovascular and pulmonary haemodynamics (i.e. values determined after one minute were not different from those measured after five minutes). Therefore, values for PASP are the average values for all measurements taken across time-points. Arterial baroreflex gain during Amb and iNO was assessed from the slope of linear regression analyses relating MSNA burst probability and total MSNA to corresponding DBP (vascular sympathetic baroreflex) and RRI or HR to corresponding SBP (cardiovagal baroreflex) during the modified Oxford test performed under

each condition. Furthermore, arterial baroreflex gain was also assessed from the slope of linear regression analyses relating MSNA burst probability and total MSNA to corresponding spontaneous fluctuations in DBP during each of these five minute periods. Only values that met the previously described criteria were included in subsequent statistical analyses. The vascular sympathetic baroreflex set-point was taken as the average value for DBP and MSNA burst incidence or Total MSNA during the five minutes of the Amb immediately prior to the start of iNO, and during the last five minutes of iNO. Cardiovagal baroreflex set-point was taken as the average value for SBP and RRI or HR over the same period. Participants breathed with a mouthpiece during both periods.

6.3.5 Statistical analyses

Significant vascular sympathetic and cardiovagal baroreflex slopes were not obtained in 2 out of 13 participants ($R \geq 0.5$) and a modified Oxford test was not performed in one participant during iNO; therefore, repeated measure comparisons for vascular sympathetic and cardiovagal baroreflex gain are limited to 10 participants. Spontaneous baroreflex slopes were not obtained in 5 out of 13 participants ($R \geq 0.5$); therefore, repeated measure comparisons for spontaneous vascular sympathetic baroreflex gain are limited to eight participants. The effects of iNO on cardiovascular and pulmonary haemodynamics, MSNA, and vascular sympathetic baroreflex gain were assessed using paired t-tests. Normality was assessed using Shapiro-Wilk test, and data that was not normally distributed underwent $\log_{10}$ transformation prior to analysis. All statistical analyses were performed using Prism 7.03 (GraphPad software, USA) and statistical significance was set at $P \leq 0.05$ *a priori*. Group data are reported as means (± SD).

6.4 Results

6.4.1 Effect of iNO on pulmonary, cardiovascular haemodynamics and basal sympathetic vasomotor activity

By design, inhalation of NO reduces PASP compared to Amb (*Table 10*). MSNA total activity is also significantly reduced during iNO. An example of MSNA and haemodynamic data recorded in one subject during Amb and iNO is presented in *Figure 29*. The relationship between MSNA total activity and PASP during Amb and iNO is shown in *Figure 30*. There is, no significant relationship between the ΔPASP and ΔMSNA total activity (r = 0.32, *P* = 0.29), or ΔPASP and ΔMSNA burst frequency (r = 0.27, *P* = 0.38). The reduction in total activity is mediated by a reduction in MSNA burst frequency with no change in mean burst amplitude compared to Amb (*Table 10*). There is a small, but significant, reduction in SBP, DBP and MAP during iNO compared to Amb, with no change in HR, stroke volume, Qc or SpO_2 (*Table 10*).

	Amb (n=13)	iNO (n=13)	*P* Value
Pulmonary haemodynamics			
Pulmonary systolic artery pressure (mmHg)	32 ± 5	26 ± 4	**<0.001**
Pulmonary vascular resistance(Woods units)	6.3 ± 1.4	4.8 ± 1.5	**0.001**
Cardiovascular haemodynamics			
SpO_2 (%) ♦	82 ± 3	85 ± 5	0.07
RRI (ms)	830 ± 173	863 ± 184	0.5
Heart rate (bpm)	75 ± 18	73 ± 17	0.6
Systolic BP (mmHg)	130 ± 17	128 ± 17	**0.002**
Diastolic BP (mmHg)	82 ± 8	80 ± 8	**<0.001**
MAP (mmHg)	101 ± 11	100 ± 11	**0.0034**
Stroke volume (mL)	71.4 ± 16.3	73.9 ± 18.3	0.13
Qc ($L \cdot min^{-1}$)	5.3 ± 1.3	5.3 ± 1.2	0.81
TPR ($mmHg \cdot L \cdot min^{-1}$)	20.9 ± 5.6	19.9 ± 4.8	0.76
Muscle sympathetic nerve activity			
Burst frequency ($bursts \cdot min^{-1}$)	29 ± 13	23 ± 13	**0.008**
Burst incidence ($bursts \cdot 100HB^{-1}$)	39 ± 15	33 ± 17	**0.01**
Mean burst amplitude (au)	48 ± 11	46 ± 16	0.17
Total activity ($au \cdot min^{-1}$)	1369 ± 576	994 ± 474	**0.01**
Total MSNA ($au \cdot 100HB^{-1}$)	1882 ± 862	1479 ± 891	**0.02**

Table 10. Haematological, cardiovascular and pulmonary haemodynamics and muscle sympathetic nerve activity (MSNA) during ambient air breathing (Amb) and during inhalation of nitric oxide (iNO).

Group data are presented as mean (± SD) ♦ SpO_2 for n = 11. Statistical comparisons performed using paired t-tests.

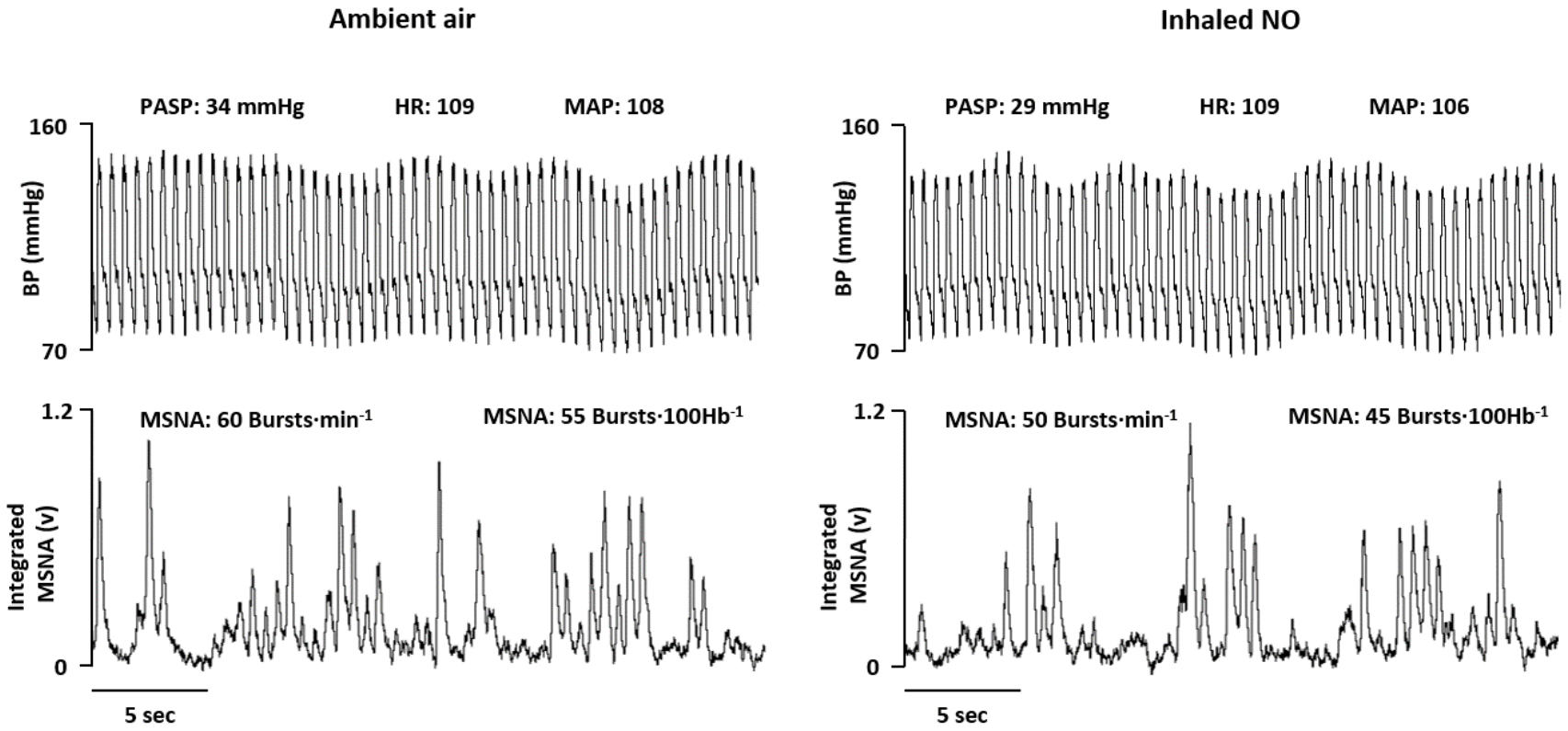

Figure 29. Example recording of beat-by-beat muscle sympathetic nerve activity (MSNA) and blood pressure (BP) in one representative subject during ambient air breathing and inhalation of nitric oxide (NO).

PASP = pulmonary artery systolic pressure, HR = heart rate, MAP = mean arterial pressure.

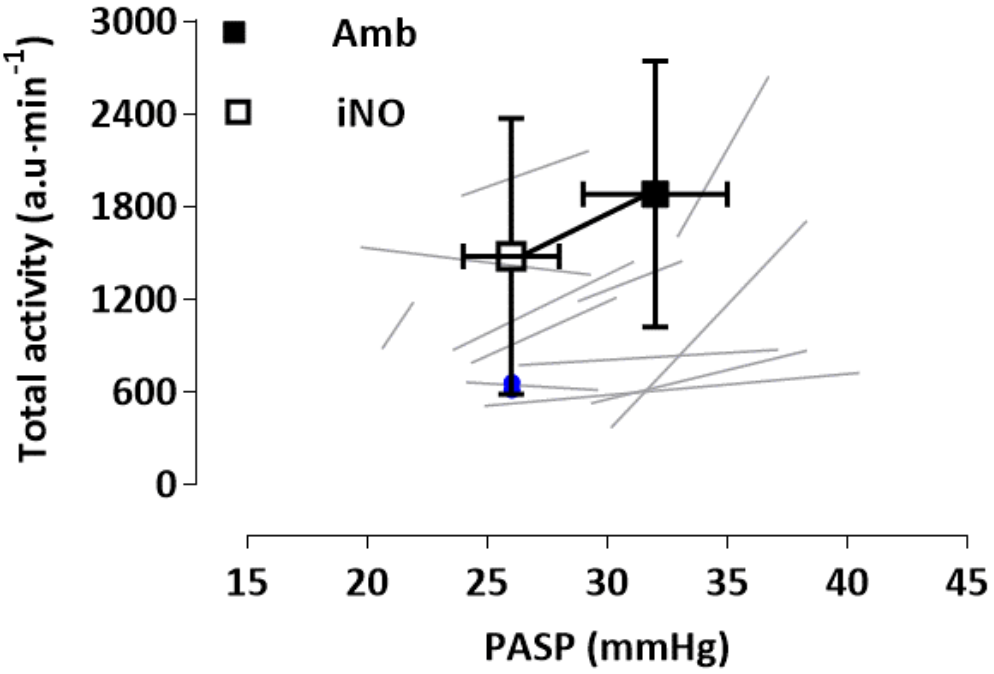

Figure 30. Group average pulmonary arterial systolic pressure (PASP) and corresponding muscle sympathetic nerve activity (MSNA) total activity during ambient air breathing (Amb) and inhalation of nitric oxide (iNO).

Grey lines represent each individual's MSNA response to changes in PASP and the black line represents average group MSNA response to changes in PASP. Data from one participant who did not display a change in PASP during iNO is highlighted in blue. Statistical comparisons performed using paired t-tests

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6.4.2 Effect of iNO on vascular sympathetic baroreflex function

Inhalation of NO significantly reduces diastolic operating pressure (*Table 10*) and MSNA operating point, indicating a leftward and downward resetting of the vascular sympathetic baroreflex during iNO (*Figure 31*). The mean slope of the linear portion of the baroreflex curve is similar during iNO and Amb, regardless of whether MSNA is quantified as burst probability (-3.4 ± 1.7 to -3.5 ± 1.8 %·mmHg^{-1}; *P* = 0.85) or total MSNA (-204 ± 108 to -216 ± 83 au·mmHg^{-1}; *P* = 0.7), indicating no differences in vascular sympathetic baroreflex gain (*Figure 31*). Similarly, the mean slope of the linear portion of the relationship between MSNA and spontaneous changes in DBP, is comparable during the iNO and Amb conditions, regardless of whether MSNA is quantified as burst probability (-3.0 ± 1.5 %·mmHg^{-1} to -3.2 ± 0.9 %·mmHg^{-1}; P = 0.84) or total MSNA (-186 ± 135 to -197 ± 144 au·mmHg^{-1}; *P* = 0.88) (*Figure. 31*). In addition, inhalation of NO significantly reduces systolic operating pressure (*Table 10*), with no significant change in RRI/HR operating point, or cardiovagal baroreflex gain (17.3 ± 10.4 to 16.4 ± 8.8 ms·mmHg^{-1}; *P* = 0.49; *Table 10*).

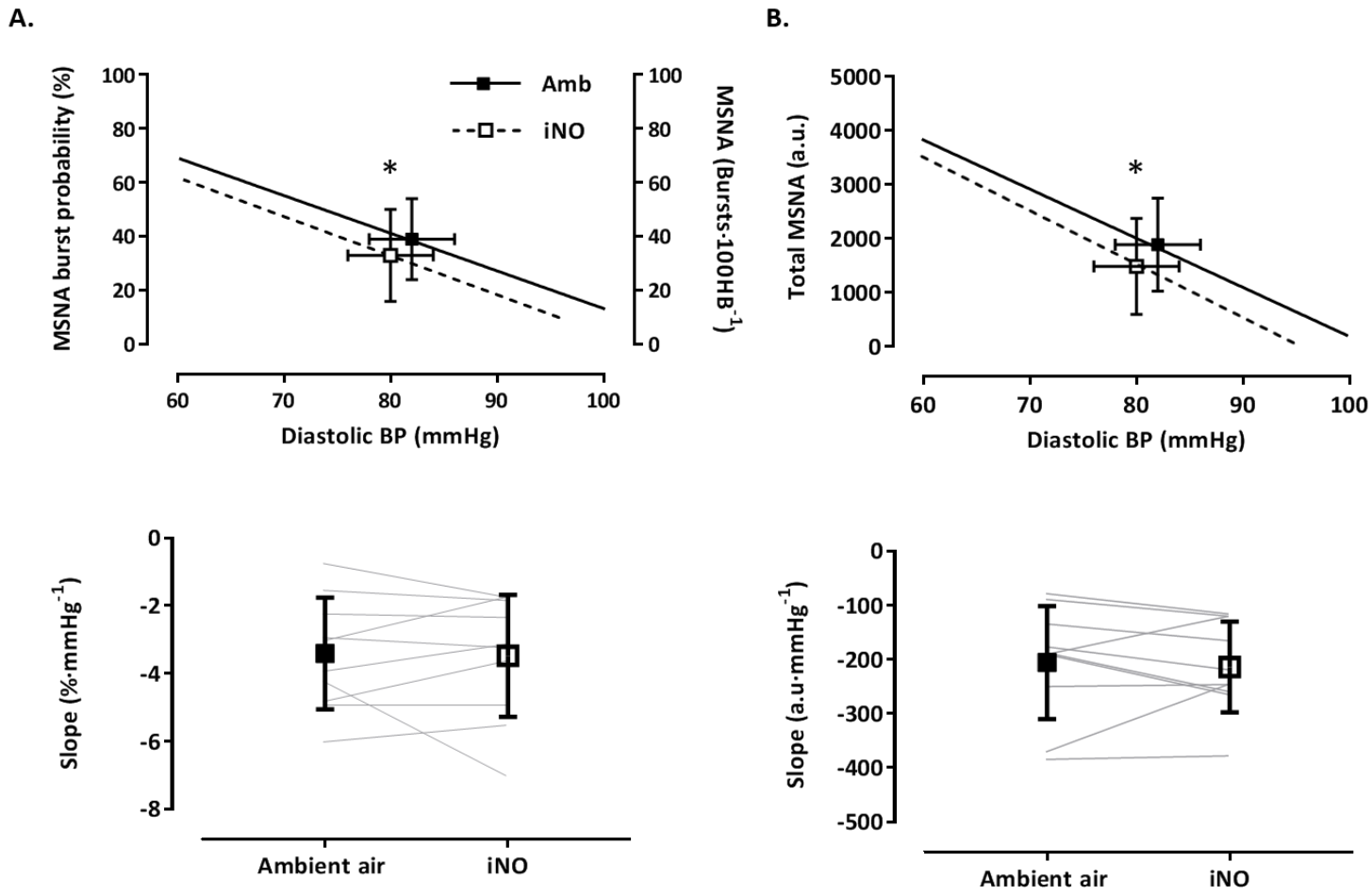

Figure 31. Vascular sympathetic baroreflex function during ambient air (Amb) and following reductions in pulmonary artery systolic pressure during inhaled nitric oxide (iNO).

Average regression line for relationships (n = 10) between DBP and MSNA burst probability (Panel A) and DBP and total MSNA (Panel B). The operating points are indicated by symbols and error bars (mean ± SD) * Vascular sympathetic baroreflex set-point P ≤ 0.05 versus Amb. The vascular sympathetic baroreflex is reset downward during iNO. The slope of the relationship between DBP and MSNA is similar during Amb and iNO, indicating no difference in vascular sympathetic baroreflex gain. B) Individual and mean (n = 10) slopes for the relationship between DBP and MSNA burst probability and DBP and Total MSNA. Statistical comparisons performed using independent t-test.

6.5 Discussion

The key novel findings from this study are twofold: i) lowering arterial pressure in the pulmonary circulation alters the prevailing level of MSNA, providing first-in-human evidence of afferent input from pulmonary arterial baroreceptors regulating sympathetic neural outflow; and ii) pulmonary arterial pressure influences the set-point of the vascular sympathetic baroreflex, providing a mechanism contributing to vascular sympathetic baroreflex resetting during high altitude exposure.

6.5.1 Sympathetic neural activation by pulmonary arterial baroreceptors

The existence of vagal afferent fibres whose firing is related to pulmonary arterial pressure changes, has been known for a long time (Bianconi & Green, 1959; Coleridge et al., 1961). In anesthetised non-hypoxic animals, isolated distension of these baroreceptors, located at the pulmonary artery bifurcation and in the extrapulmonary artery branches, elicit increases in renal sympathetic neural activity with no effect on heart rate (Ledsome and Kan, 1977; Moore et al., 2011). Furthermore, pulmonary arterial baroreceptor distension resets carotid baroreflex control of the peripheral vasculature (Moore et al., 2011) to operate at higher levels of sympathetic nerve activity and systemic pressure. Until now, however, the physiological role of these receptors in humans has not been investigated, presumably due to the technical difficulty in isolating a physiological stimulus to the pulmonary vasculature in a closed-loop system. In an attempt to overcome this challenge, healthy Lowlanders were studied at high altitude, before and during inhalation of nitric oxide (iNO), an intervention shown to reduce pulmonary arterial pressure without altering systemic arterial pressure (Frostell et al., 1991; Pepke-Zaba et al., 1991). With this approach, pulmonary arterial systolic pressure was lowered by 20%, which was accompanied by a 25% reduction in MSNA total

activity. However, the magnitude of the response (i.e. ΔMSNA) was not related to the magnitude of stimulus (i.e. ΔPASP) between individuals, which is likely due to individual differences in both the location of the operating point on the stimulus-response curve and the responsiveness of the reflex (i.e. gain). Whilst the reduction in MSNA during iNO also occurred in parallel to a small reduction in systemic arterial pressure, this would have been expected to evoke an increase in MSNA via arterial baroreflex buffering. Strikingly, MSNA total activity was remarkably stable in one subject who did not display a reduction in PASP during iNO. Therefore, the reduction in MSNA observed during iNO was likely mediated by unloading of the pulmonary arterial baroreceptors. Notably, this response is directionally opposite to the sympathetic response elicited during unloading of the systemic arterial baroreceptors.

As the human circulation is an integrated closed-loop system, changes to reflexogenic areas other than the pulmonary artery, may have influenced the MSNA response. Thus, several alternative interpretations of these data require discussion. First, an increase in atrial pressure is proposed to activate single-unit MSNA in healthy humans (Millar et al., 2013; Incognito et al., 2018). However, in contrast, when a stimulus is localized precisely to the left atria-pulmonary vein junction of anaesthetized dogs, atrial receptor activation has no effect on sympathetic efferent activity in lumbar nerves, which is an analogue of human MSNA (Karim et al., 1972). Notably, neither atrial receptor stimulation (Carswell et al., 1970) nor changes in ventricular filling (Drinkhill et al., 2001) affect systemic vascular resistance in anaesthetized dogs. Thus, whilst differences may exist between anaesthetized dogs and conscious humans, the intrathoracic receptors most likely to elicit the observed MSNA response are those located in the pulmonary artery and its bifurcation (Moore et al., 2004b, 2004a). Furthermore, studies that have employed invasive haemodynamic measurements report no change in right atrial pressure and pulmonary capillary wedge pressure from sea

level to high altitude (6100m), despite an increase in pulmonary artery mean pressure from 15 to 24 mmHg (Reeves et al., 1987; Groves et al., 1987). Therefore, right and left atrial pressure would not necessarily be expected to change during the reduction in PASP observed during iNO in the present study. Second, some contribution by the arterial baroreflex cannot be excluded. Notably, arterial baroreceptors respond to deformation of the arterial wall and not arterial pressure *per se* (Angell-James, 1971). Therefore, a small change in stroke volume, independent of arterial pressure, could alter baroreceptor afferent activity and influence the observed MSNA response (Fu et al., 2008; Lacolley et al., 1992; Taylor et al., 1995). Closer inspection of the data, however, reveals a variable change in stroke volume during iNO; that is, stroke volume increased in eight participants, and decreased in five. Despite this, MSNA was reduced in 11 out of 13 participants. Along with the previously discussed small decrease in arterial blood pressure, this variability in the stroke volume response suggests that the arterial baroreflex did not mediate the observed decrease in MSNA.

Inhalation of NO resulted in a small, non-significant increase in SpO_2; therefore, a reduced peripheral chemoreceptor drive may have mediated the reduction in MSNA. Whilst this possibility must not be ignored, previous studies and data from Chapter 4 demonstrated no change in MSNA with administration of 100% oxygen at high altitude (Hansen & Sander, 2003). Furthermore, a significant relationship between the change in SpO_2 and change in MSNA ($r = -0.16$, $P = 0.61$) was not observed in the present study. Finally, whilst most of the NO that enters the circulation is rapidly inactivated (Rimar & Gillis, 1993), an increase in NO metabolites may increase central NO bioavailability, via the nitrate-nitrite-NO pathway, which may have influenced the MSNA response (Owlya et al*.,* 1997; Young et al*.*, 2009; Notay et al*.*, 2017). However, no change in plasma nitrite levels were previously reported in healthy subjects during a 60 minute inhalation of 25 ppm iNO (Westfelt et al., 1995).

6.5.2 Interaction between pulmonary arterial baroreceptors and arterial baroreflex

Interestingly, the reduction in MSNA total activity was mediated by a reduction in burst occurrence, with no change in burst amplitude. Kienbaum et al, (2001) proposed two central sites for modulation of sympathetic outflow, where the arterial baroreflex determines the occurrence of sympathetic bursts, however, other peripheral inputs largely determine the size of those bursts. As such, it appears that afferent input from pulmonary arterial baroreceptors alter sympathetic outflow by modulating baroreflex control of MSNA. Indeed, the present study demonstrated an interaction between the two groups of baroreceptors, whereby unloading of the pulmonary arterial baroreceptors (i.e. lowering pulmonary arterial systolic pressure) did not change the MSNA responsiveness to acute increases and decreases in arterial pressure (i.e. gain), but reset sympathetic neural activity and diastolic pressure to lower levels. Thus, there was a downward and leftward resetting of the arterial baroreflex control of MSNA. This is consistent with work in experimental animals that report an upward and rightward resetting of the carotid baroreflex control of vascular resistance, with no change in gain, during increases in pulmonary arterial pressure (Moore et al., 2011).

In the present study, a small, but significant, reduction in diastolic BP (~2 mmHg) was observed during reductions in pulmonary arterial systolic pressure. Using individual stimulus-response relationships, it was estimated that such a reduction in diastolic pressure should increase the probability of a burst by around 5 to 6% (i.e. 5 – 6 bursts·100HB^{-1}), via engagement of the arterial baroreflex. In fact, the opposite was observed, a reduction of 6 bursts·100HB^{-1}. A lack of reflex sympathoexcitation is interpreted as further confirmation that the set-point of the vascular sympathetic baroreflex is reset when the prevailing arterial pressure in the pulmonary circulation changes. In Chapter 4, there was an upward resetting of the vascular-sympathetic limb of the arterial baroreflex during chronic exposure (10–21

days) of healthy Lowlanders to high altitude. Furthermore, reducing peripheral chemoreceptor drive, via administration of 100% oxygen, does not reverse this resetting, leaving the potential mechanism(s) unclear. The mechanism presented and discussed here, therefore, likely plays a role; that is, afferent feedback from pulmonary arterial baroreceptors contributes to resetting of arterial baroreflex regulation of MSNA and BP control at high altitude.

6.6 Experimental limitations

First, the present study characterises the neural response to a reduction in pulmonary arterial pressure only. Primarily, this was due to the difficultly in independently elevating pulmonary arterial pressure in conscious humans. All the same, a study performed under non-hypoxic conditions observed marked suppression of MSNA during rapid volume infusion, which acutely raised pulmonary arterial pressure (Pawelczyk et al., 2001); however, sympathoinhibition in that setting is the net effect of the integration of multiple reflex inputs, most notably the arterial baroreflex. In contrast, experiments in non-hypoxic animals demonstrated sympathoexcitation in response to incremental increases in pulmonary arterial pressure over a physiological range. Notably, careful control of pressure to the aortic and carotid baroreceptors eliminated baroreflex buffering of measured response (Moore et al., 2011), something which very difficult to accomplish in humans. Second, only sympathetic vasomotor outflow to the skeletal muscle vasculature was investigated in the present study, and outflow to other vascular beds may exhibit differential reflex responses (Morrison, 2001). Indeed, lowering afferent input to pulmonary arterial baroreceptors did not influence heart rate, suggesting no influence on autonomic outflow to the heart, despite a reduction in MSNA. Third, under ambient conditions, a modified Oxford test was performed prior to a participant

being placed on the mouthpiece, whereas during the iNO intervention the test was performed whilst the subject breathed with a mouthpiece. Therefore, breathing through a mouthpiece, alone, may have influenced the vascular sympathetic baroreflex. However, MSNA operating point was determined from a period when the participants were spontaneously breathing through a mouthpiece for both conditions; furthermore, an index of spontaneous vascular sympathetic baroreflex gain was determined during these same periods. Notably, inhalation of NO did not alter vascular sympathetic baroreflex gain, regardless of the method of determination. Therefore, it is unlikely that breathing via a mouthpiece confounds the interpretation. Fourth, ventilation was not measured, which has known influences on MSNA (Hagbarth & Vallbo, 1968; Somers et al., 1989) and arterial baroreflex gain (Van De Borne et al., 2000). However, Scherrer and colleagues (1996) demonstrated that a 12 minute inhalation of NO (40 ppm), in healthy Lowlanders at a similar altitude (4559 m), had no effect on ventilation or end tidal CO_2. Fifth, due to the unknown time course of recovery iNO and potential long lasting, central effects of NO (Notay et al., 2017), the order of conditions was not counterbalanced in the present study; therefore, a potential order effect on these results cannot be excluded.

6.7 Implications and significance

The present study represents an important first step to bridge a gap in evidence between human and animal studies. Consistent with data in non-hypoxic anaesthetized dogs, MSNA was greater when pulmonary arterial pressure was higher in conscious humans, albeit under high altitude hypoxia. From the findings of this study, it is proposed that a sustained increase in pulmonary arterial pressure evokes a reflex input to CNS areas controlling sympathetic vasoconstrictor outflow. This input, therefore, acts as signal for sympathetic

restraint of hypoxic vasodilatation, thus preserving arterial pressure homeostasis at high altitude. The same pathway could link increased right ventricular outflow and elevated pulmonary arterial pressure during exercise to sympathetic restraint of muscle blood flow, so that BP is maintained. Still, this speculation requires investigation. Furthermore, it is uncertain how this input pathway contributes to beat-by-beat control of the vasoconstrictor outflow when pulmonary arterial pressure is normal. More study, therefore, is required to distinguish low-pressure pulmonary baroreceptor control of sympathetic outflow from classical negative feedback reflex control originating from arterial baroreceptors located in the systemic circulation.

6.8 Conclusion

This study provides evidence that supports a physiological role for pulmonary arterial baroreceptors in sympathetic activation in humans, at least when there is a sustained elevation in arterial pressure in the pulmonary circulation. Taking advantage of a novel experimental approach, a reduction in basal MSNA was observed during acute lowering of pulmonary arterial pressure; this is opposite to the sympathoexcitatory effect when there is a reduction of systemic arterial pressure. Furthermore, lowering arterial pulmonary pressure influences the set-point of the vascular sympathetic baroreflex. Finally, this study illustrates some of the technical challenges encountered when studying sympathetic neural responses to stimulation of different reflex inputs in conscious humans, and highlights the importance of developing experimental approaches to overcome such challenges.

6.9 Contribution to this chapter

For this chapter, I contributed to the conception and design of the study, data collection, data analysis, data interpretation, preparation of figures, chapter drafting and chapter revisions.

Author Name	Conception and Design	Data Collection	Data Analysis	Data Interpretation	Preparation of Figures	Manuscript Drafting	Manuscript Revisions
Lydia L Simpson	X	X	X	X	X	X	X
Victoria L Meah		X					X
Andrew R Steele		X					X
Suman Thapamagar		X					X
Christopher Gasho	X	X					X
James D Anholm	X						X
Tony G Dawkins		X	X				X
Stephen A Busch			X				X
Samuel J Oliver	X						X
Justin S Lawley		X					X
Michael M Tymko	X						X
Philip N Ainslie							X
Mike Stembridge	X	X	X	X		X	X
Craig D Steinback	X	X					X
Jonathan P Moore	X	X		X		X	X

Table 11. – Authorship summary for Chapter 6

CHAPTER 7: GENERAL DISCUSSION

7.1 Introduction

The main aim of this thesis was to examine sympathetic neural activity and autonomic control of resting BP at high altitude, in Lowlanders and highland natives of the Tibetan and Andean plateau. Chapter 4 examined basal MSNA and arterial baroreflex function in Lowlanders following 10–20 days of acclimatisation to high altitude and compared these to Nepalese Sherpa. Chapter 5 examined basal MSNA and arterial baroreflex function in a group of Andean Quechua with maladaptation syndrome CMS and compared these to healthy Andean Quechua. Chapter 4 and 5 also examined the mechanistic contribution of the peripheral chemoreflex to MSNA and arterial baroreflex function in both Lowlanders and highland native populations. Chapter 6 aimed to extend these findings by investigating the mechanistic role of pressure sensitive receptors in the pulmonary circulation to MSNA and arterial baroreflex regulation in Lowlanders at high altitude. This chapter summarises the main findings of the thesis (*Figure. 32*) and discusses sympathetic neural activity and autonomic control of resting BP during acclimatisation to high altitude and following life-long high altitude exposure, and compares these distinct populations. Lastly, the broad significance and implications of the findings are highlighted before recommendations for future research directions are made, and limitations acknowledged.

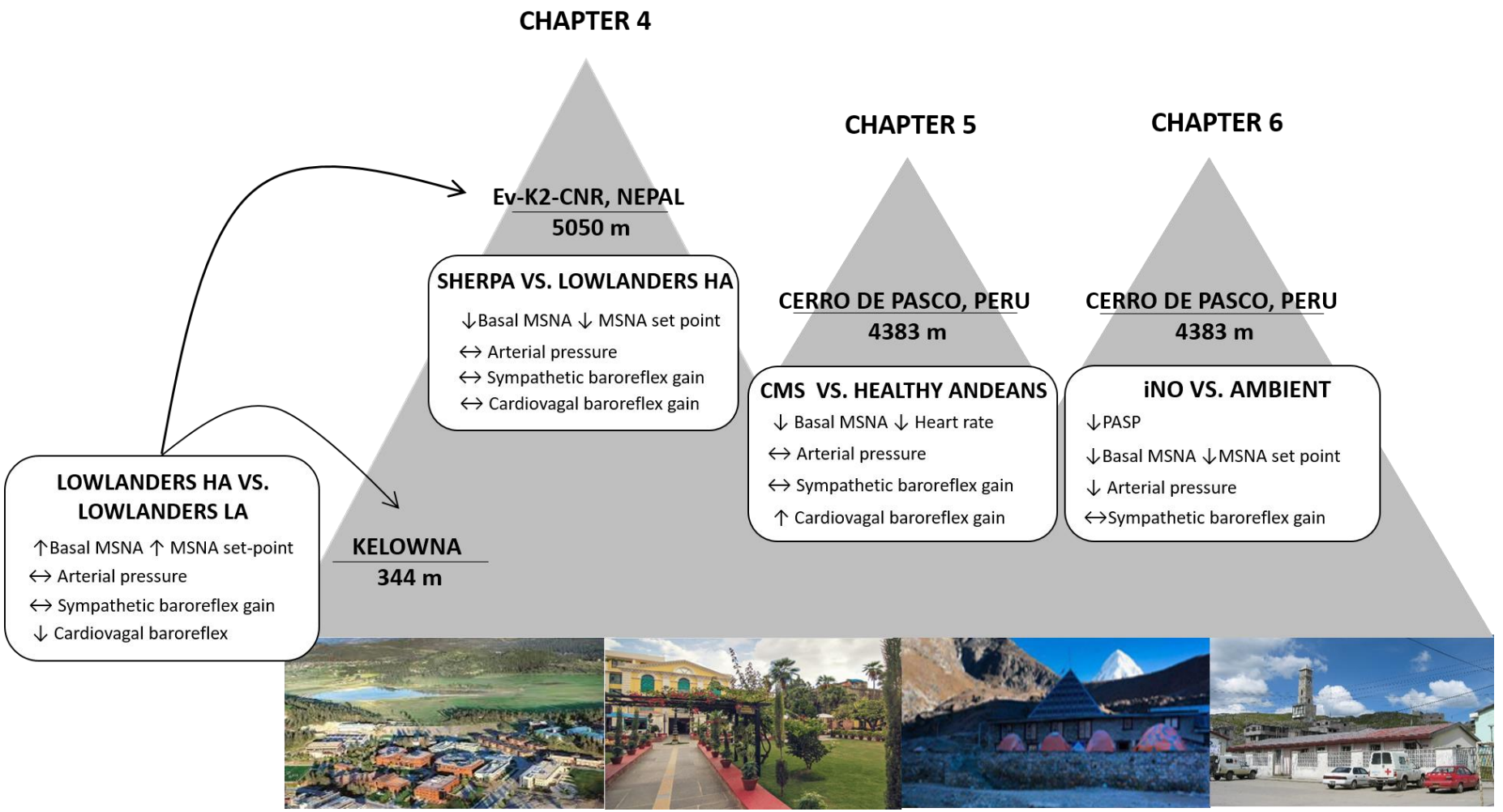

Figure 32. Schematic summarising main findings of this thesis.

LA = low altitude, HA = high altitude, iNO = inhaled NO, PASP = Pulmonary artery systolic pressure.

7.2 Summary of main findings

In agreement with previous literature (Hansen and Sander 2003), this thesis reported an increase in basal MSNA (i.e. MSNA burst frequency/total activity) during acclimatisation to high altitude in Lowlanders. This thesis extends these findings and demonstrates, for the first time, that basal MSNA is lower in highland native Nepalese Sherpa compared to Lowlanders and Andeans at high altitude. Notably, basal MSNA in Nepalese Sherpa at high altitude is still greater than that observed in both Lowlanders and Sherpa at low altitude. Furthermore, this thesis demonstrates, for the first time, that Andean highlanders with excessive erythrocytosis and mild CMS do not exhibit elevated basal MSNA. In fact, CMS demonstrate a lower basal MSNA, versus healthy Andeans, despite greater arterial hypoxemia. Importantly, despite variability in basal MSNA across populations, resting arterial pressure is similar, suggesting population differences in integrative regulation of resting BP.

This thesis also comprehensively examined arterial baroreflex control of resting BP at high altitude, by simultaneously assessing baroreflex control of MSNA (vascular sympathetic baroreflex) and RRI (cardiovagal baroreflex), in Lowlanders and highland natives. In this thesis, acute exposure to hypoxia (FiO_2 0.11) reduces vascular sympathetic baroreflex gain (i.e. slope of the linear relationship between DBP and MSNA) in Lowlanders; however, vascular sympathetic baroreflex gain is restored to low altitude values during acclimatisation. Furthermore, vascular sympathetic baroreflex gain was well preserved in Nepalese Sherpa and Andean Quechua, with and without CMS, as values observed are similar to those observed in Lowlanders at low altitude. Despite preserved vascular sympathetic baroreflex gain, cardiovagal baroreflex gain (i.e. slope of the linear relationship between SBP and RRI) is blunted at high altitude. A smaller RRI response is observed for a given change in arterial

pressure in Lowlanders during acclimatisation, with a similar reflex gain observed in Nepalese Sherpa. Interestingly, Andeans with CMS exhibit a greater RRI responsiveness, and thus cardiovagal baroreflex gain, versus healthy Andeans at high altitude, which is comparable to that observed in Lowlanders at low altitude.

This thesis also examined the mechanistic role of the peripheral chemoreflex to the regulation of MSNA and arterial baroreflex function at high altitude, in Lowlanders and highland natives. An attempt to eliminate peripheral chemoreceptor drive, via administration of 100% oxygen for five minutes, does not affect any index of vascular sympathetic baroreflex function (i.e. operating pressure, MSNA operating point or gain) or basal MSNA in Lowlanders. Furthermore, administration of oxygen does not affect any index of vascular sympathetic baroreflex function, and has minimal effects on basal MSNA, in both Nepalese Sherpa or Andeans. Thus, for the first time, this thesis provides evidence that the peripheral chemoreflex does not play a major role in sympathoexcitation in either Lowlanders or highlanders at altitude. Reducing peripheral chemoreceptor drive, however, did reduce HR in both Lowlanders and Highlanders at altitude. Thus, the peripheral chemoreflex does appear to be important in hypoxia-induced tachycardia. Lastly, this thesis provides the first evidence of a mechanistic role for elevated pulmonary arterial pressure in vascular sympathetic baroreflex resetting, and sympathoexcitation, in Lowlanders at high altitude.

7.3 Sympathetic neural activity at high altitude

During high altitude acclimatisation, there is an almost three-fold increase in MSNA burst frequency for Lowlanders at 5050 m (*Figure 33*); consistent with previous microneurographic studies at high altitude (Hansen & Sander, 2003; Lundby et al., 2017). MSNA burst frequency in Nepalese Sherpa is, however, less than that of Lowlanders at 5050 m, despite similar peripheral oxygen saturation in both groups. Thus, it appears that Sherpa have adapted to favour a lower sympathetic vasomotor activity compared to acclimatising Lowlanders. The consequences of lower MSNA in Sherpa are unclear. Nevertheless, heightened MSNA in Lowlanders at altitude is associated with deleterious alterations in vascular structure and function, including increased arterial stiffness and reduced endothelial function (Świerblewska et al., 2010; Bruno et al., 2012; Lewis et al., 2014; Revera et al., 2017; Tremblay et al., 2018; Tymko et al., 2020). Lower basal MSNA in Sherpa may, therefore, represent a beneficial adaptation to minimize the cardiovascular consequences of chronically elevated sympathetic vasomotor activity. Indeed, Sherpa do not exhibit the progressive reduction in endothelial function, measured via brachial artery flow mediated dilation (FMD), observed in Lowlanders during ascent to 5050 m (Tremblay et al., 2018). However, this is not a consistent finding, as others (Bruno et al., 2014; Lewis et al., 2014) have reported comparable FMD in Sherpa and Lowlanders at 5050 m, which are both lower than the values observed for Lowlanders at low altitude.

Interestingly, basal MSNA for Sherpa at high altitude was greater than that observed for Lowlanders at low altitude (*Figure 33*). Moreover, for three Sherpa studied four days following descent to 1440 m, basal MSNA burst frequencies were approximately 30% lower than those observed when they were re-tested at 5050 m. Taken together these findings

suggest that hypoxia remains a significant sympathetic stressor for all populations, regardless of ethnicity.

The observation of a lower basal MSNA for Sherpa contrasts with that of healthy Andean highlanders, who exhibited a MSNA burst frequency that was 104% greater than that of Sherpa, and was also 70% greater than that of Lowlanders at high altitude (*Figure 33*). This was despite Andeans residing at a lower altitude (4383 m vs. 5050 m) and exhibiting a greater arterial oxygen saturation (Lowlanders at high altitude, 82 ± 3%; Nepalese Sherpa, 81 ± 4%; Healthy Andeans, 87 ± 3%). Thus, healthy Andean Quechua appear to have maintained a high level of sympathetic vasomotor activity, despite generational exposure to high altitude. This is in agreement with one previous study of healthy Andean highlanders (Lundby et al., 2017), which found that basal MSNA in Bolivian Aymara was comparable to Lowlanders following 10–50 days of high altitude exposure. Taken together, these findings provide the first evidence that the sympathetic neural response to high altitude differs between Nepalese Sherpa and Andean highlanders, providing further evidence of divergent pathways of physiological high altitude adaptation.

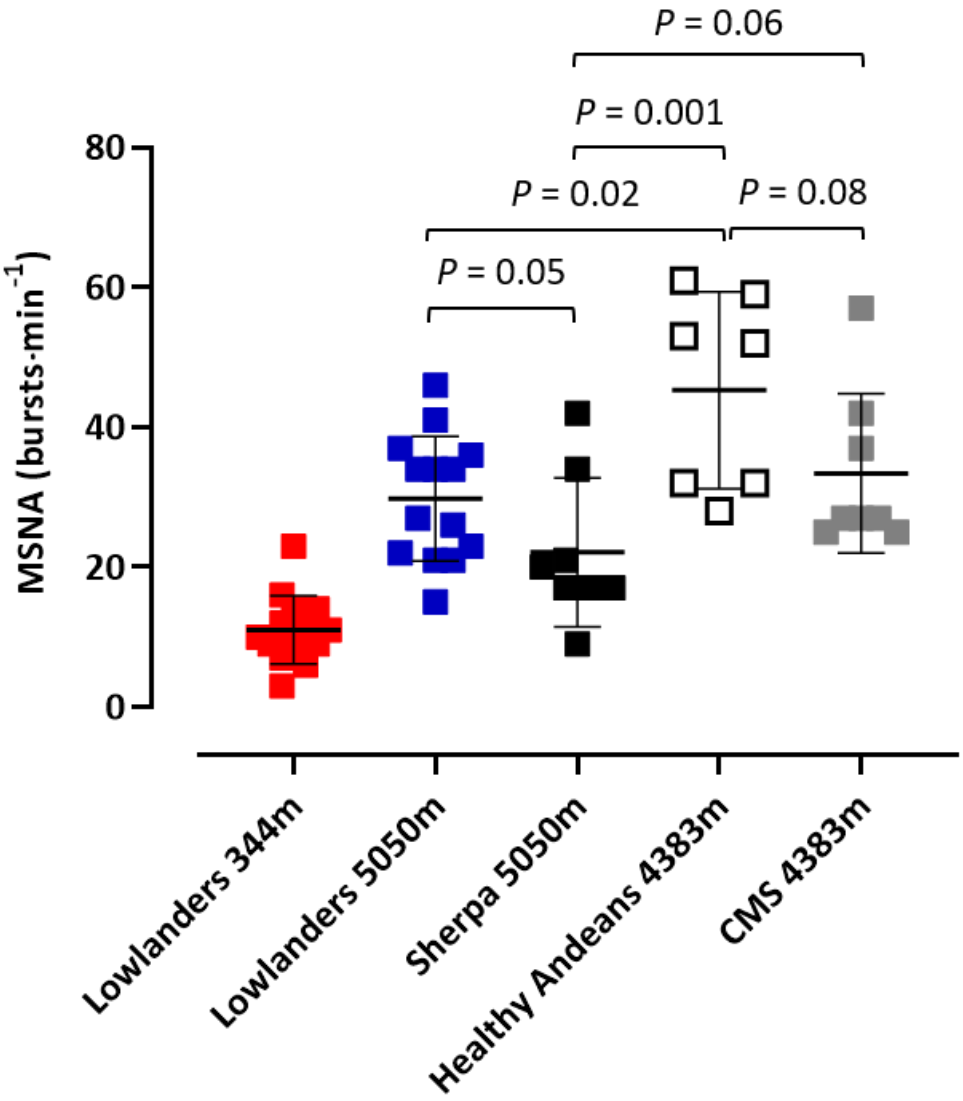

Figure 33. Basal MSNA in Lowlanders, Nepalese Sherpa, healthy Andeans and CMS across experimental studies.

Basal MSNA in all groups at high altitude is significantly greater than that for Lowlanders at 344 m ($P \leq 0.05$, P values not shown on figure). Basal MSNA for Lowlanders at high altitude is greater than that for Sherpa at high altitude, similar to CMS and, lower than that for healthy Andeans. Basal MSNA for Sherpa at high altitude is lower than that for both Lowlanders at and healthy Andeans at high altitude, with a trend for lower basal MSNA compared to CMS. Statistical comparisons were performed using a one way ANOVA, with Tukey post hoc comparisons, and dependent t-tests.

The variability in basal MSNA between Lowlanders, Sherpa, and Andeans could be influenced by genotypic differences between populations (Wallin et al., 1993); nevertheless, many other factors can affect basal MSNA, including age, obesity, and physical activity (Ng et al., 1993; Jones et al., 1997;Matsukawa et al., 1998b; Narkiewicz et al., 2005; Lambert et al., 2010). Notably, Andeans studied here are, on average, older than both Lowlanders and Sherpa, and exhibit a greater BMI. Basal MSNA is positively correlated with both age (Ng et al., 1993; Matsukawa et al., 1998a; Narkiewicz et al., 2005) and BMI (Jones et al., 1997; Lambert et al., 2010), at least in lowland populations at sea level. Whilst it is unclear if these relationships are similarly observed in highland populations, the greater age and BMI in Andeans may contribute to the greater basal MSNA compared to Lowlanders and Sherpa at high altitude. Indeed, there was a trend for a significant correlation between basal MSNA and BMI ($P = 0.06$) across all groups at high altitude; although, there was no significant relationship between basal MSNA and age ($P = 0.15$).

7.4 Regulation of resting blood pressure at high altitude

Despite marked differences in basal MSNA (i.e. sympathetic vasoconstrictor drive), TPR and MAP were remarkably similar for all groups (*Figure 34*). Thus, greater basal MSNA did not coincide with a greater TPR, in contrast to what is observed in healthy males at sea level (Charkoudian et al., 2005; Charkoudian et al., 2006). The lack of relationship between basal MSNA and TPR at high altitude may reflect alterations in vascular control (i.e. release of vasoactive substances and/or vascular sensitivity to local and neural factors at high altitude), which moderate the vasoconstrictor effects of MSNA.

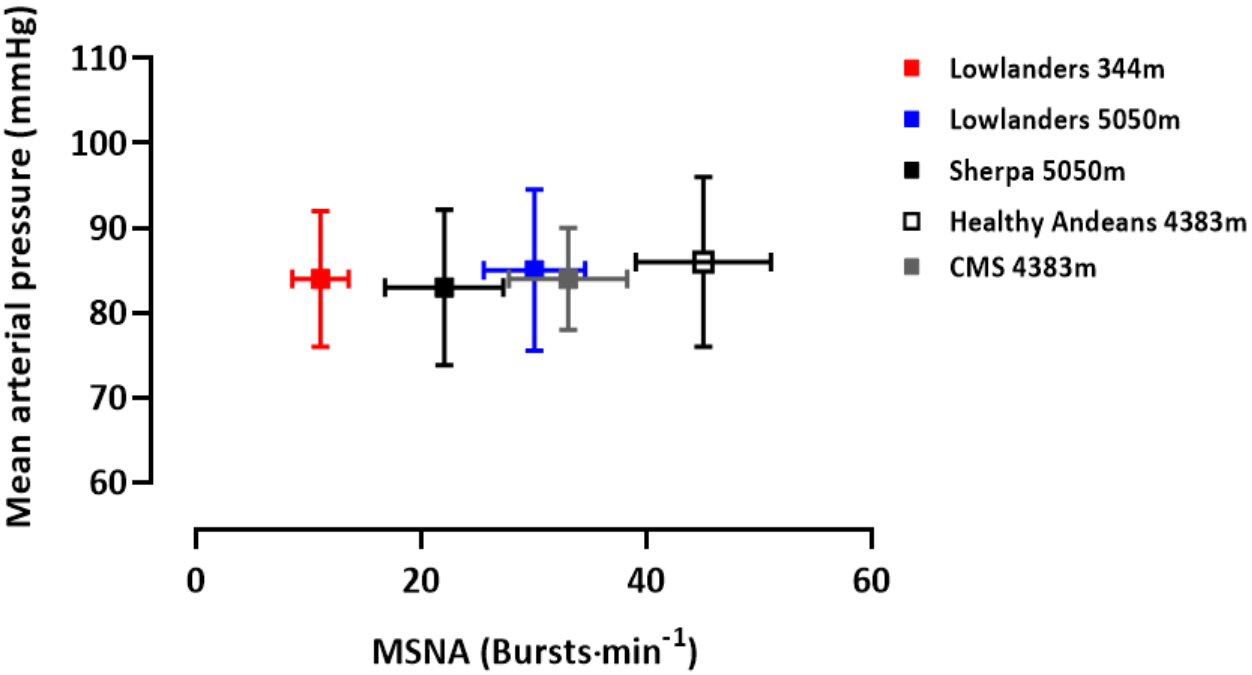

Figure 34. Group average basal MSNA and corresponding mean arterial pressure in Lowlanders, Nepalese Sherpa, healthy Andeans and CMS across experimental studies.

Despite large variability in basal MSNA between groups, no significant differences are observed between groups. Data are presented as mean (± SD). Statistical comparisons performed using a one-way ANOVA.

7.4.1 Influence of vasodilatory signalling

Exposure to systemic hypoxia increases the local release of potent vasodilatory factors, (Heistad & Wheeler, 1970; Rowell & Blackmon, 1986; González-Alonso et al., 2002), which counteract the vasoconstrictor effects of heightened MSNA. Thus, at high altitude, a greater basal MSNA is required to restrain local vasodilation, and maintain vascular tone (Weisbrod et al., 2001). Indeed, studies at sea level have shown that plasma nitrate levels (indicator of NO release) are positively correlated with basal MSNA (Skarphedinsson et al., 1997). Furthermore, systemic infusion of NO synthase inhibitor leads to greater increases in BP in individuals with higher basal MSNA (Charkoudian et al., 2006). Thus, individuals with greater NO release, require higher basal MSNA to maintain resting BP. Contradictory to this, greater

exhaled NO and circulating NO metabolites are reported in Sherpa compared to both Andean highlanders (Beall et al*.,* 2001) and Lowlanders (Erzurum et al., 2007). These findings suggest that, Sherpa exhibit greater NO signalling, despite exhibiting the lowest basal MSNA. Thus, differences in NO release, independent of alterations in vascular sensitivity, do not necessary explain population differences in MSNA.

Other vasodilatory signalling pathways, in addition to NO, are also involved in hypoxia-induced vasodilation, including ATP, adenosine, prostaglandins, and endothelium-derived hyperpolarizing factor (Weisbrod et al., 2001; Marshall, 2015; Dinenno, 2016). Acute administration of hyperoxia attenuates the release of local vasodilators during ambient hypoxia (Casey & Joyner, 2011), independent of mechanism. In this work, acute hyperoxia induces the largest increase in TPR in Andean highlanders (16%), with Sherpa exhibiting the smallest increase in TPR (4%), despite similar relative changes in sympathetic vasoconstrictor drive (i.e. MSNA burst frequency) in both groups. This may indicate greater hypoxia-induced vasodilator drive at rest in healthy Andeans. Thus, comparable TPR across groups at rest, despite marked differences in basal MSNA, may be related to differences in the local vasodilator milieu. Non-metabolic pathways could also contribute, where higher haematocrit in Andean highlanders may elicit greater shear stress-mediated vasodilation compared to Sherpa, who have adapted with lower haematocrit; however, further study is required to determine these possibilities.

7.4.2 Altered neurovascular transduction

Another possible explanation for a similar TPR across groups, despite large variability in basal MSNA, are differences in neurovascular transduction (i.e. the ability of the vasculature to respond to bursts of MSNA). The vasoconstrictor effect of a greater basal MSNA may be

offset by a reduced neurovascular transduction. Indeed, there is evidence in animals that sympathetically mediated vasoconstriction in the skeletal muscle vasculature is blunted during systemic hypoxia, a phenomenon referred to as hypoxic sympatholysis (Coney & Marshall, 2007; Marshall, 2015). Data collected during the same testing session as the data presented in this thesis (Busch et al., 2020; Berthelsen et al., 2020, *under review*), does indeed provide evidence of differences in neurovascular transduction between populations. Analysis of spontaneous fluctuations in MSNA and the related changes in RRI and MAP (Berthelsen et al., 2020, *under review*), as described by (Steinback et al., 2019), demonstrates three principal findings. First, Lowlanders exhibited a blunted increase in MAP following a burst of MSNA at high altitude compared to low altitude. Second, Sherpa exhibited a greater increase in MAP following a burst of MSNA at high altitude, and third, Andeans exhibited a reduced MAP response compared to both Lowlanders and Sherpa at high altitude. Moreover, the overall magnitude of the neuro-cardiovascular transduction was inversely related to the basal level of MSNA, independent of population. In addition, Busch et al., (2020) reported elevated neurovascular transduction (ΔMSNA/ΔMAP) in Sherpa, compared to Lowlanders at high altitude, during maximal apnea. Whilst these measures only provide indirect measures of neurovascular transduction, as they do not directly quantify the vascular response to MSNA via vascular ultrasound, they do suggest differences in vascular responsiveness to vasoconstrictor signals.

It is unclear what level of the transduction pathway may be altered at high altitude; nevertheless, a blunted vascular α-adrenergic receptor responsiveness has been reported during acute systemic hypoxia (Heistad and Wheeler 1970; Heistad & Wheeler 1971; Coney and Marshall., 2007), although, this is equivocal (Dinenno et al., 2003; Tan et al., 2013; Wilkins et al., 2006). Characterisation of the vascular resistance responses to infusion of different

doses of either α-adrenergic agonist PE or exogenous/endogenous noradrenaline is required to examine α-adrenergic receptor responsiveness (Charkoudian et al., 2005). However, in this thesis, the dose of PE, a α_1-adrenergic agonist, required to elicit a sufficient pressor response varied between groups (*shown in Table. 2, Chapter 3*), implying differences in α_1-adrenergic receptor responsiveness. Subsequently, an indirect index of α_1-adrenergic receptor responsiveness was determined from these data (*Figure 35*) and calculated as the increase in BP per unit dose [$ug \cdot kg^{-1}$] of PE. These data suggest a blunted α_1-adrenergic receptor responsiveness in Andeans, with and without CMS, at high altitude versus Lowlanders and Sherpa. Thus, a greater basal MSNA in Andeans appears to be required to overcome a blunting of the responsiveness to vasoconstrictor signals, and maintain TPR and arterial pressure.

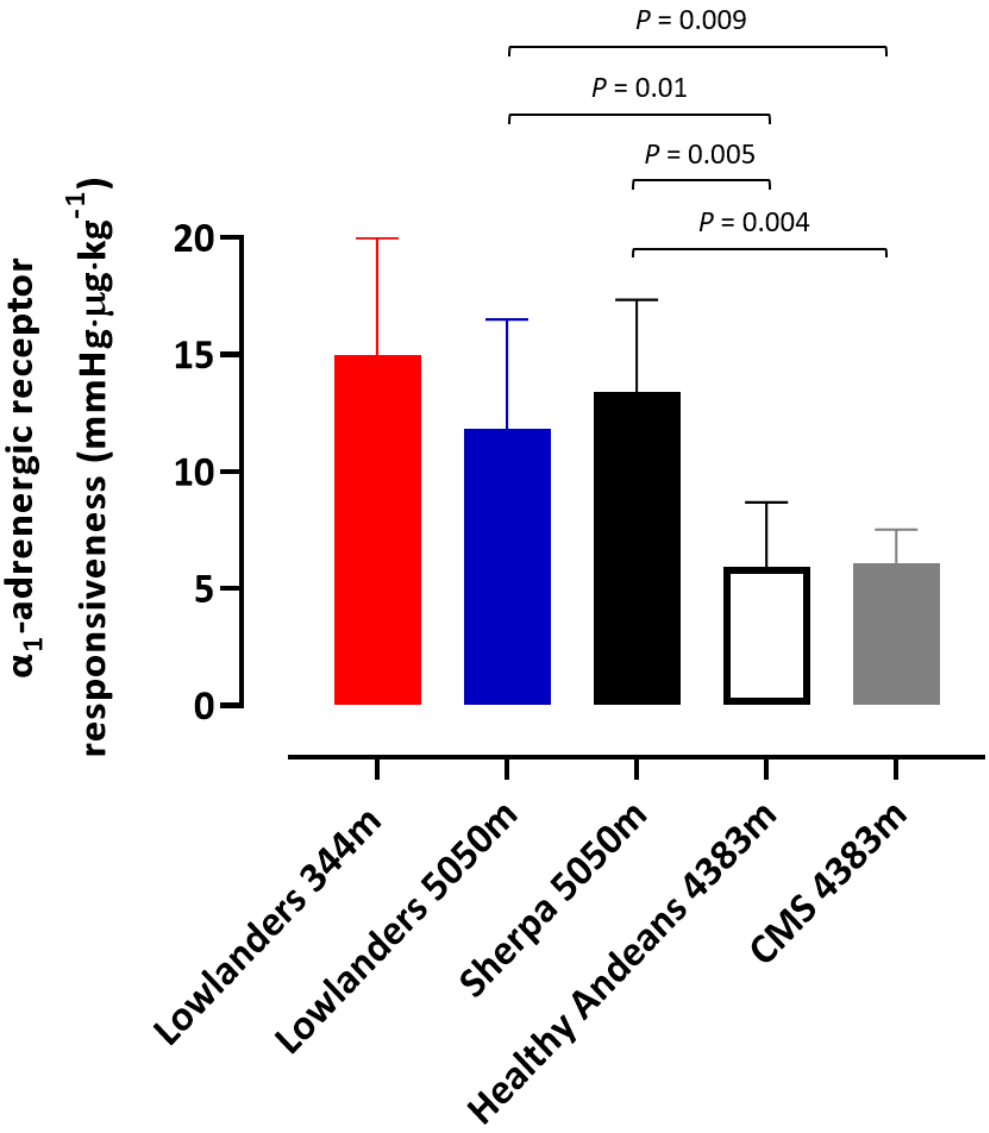

Figure 35. Indirect index of α_1-adrenergic receptor responsiveness in Lowlanders, Nepalese Sherpa, healthy Andeans and CMS across experimental studies.

α_1-adrenergic receptor responsiveness is calculated as the increase in BP, per unit dose [ug·kg^{-1}] of phenylephrine. α_1-adrenergic receptor responsiveness is not significantly different between Lowlanders at low altitude and Lowlanders at high altitude. α_1-adrenergic receptor responsiveness is not significantly different in Sherpa, compared to Lowlanders at high altitude. However, α_1-adrenergic receptor responsiveness is significantly lower in healthy Andeans and CMS compared to both Lowlanders and Sherpa at high altitude. Statistical comparisons performed using a one-way ANOVA, with Tukey post-hoc comparisons, and independent t-tests.

7.5 Arterial baroreflex function at high altitude

The studies in this thesis are the first to comprehensively assess arterial baroreflex control of BP at high altitude, by simultaneously assessing the MSNA and RRI responses to pharmacologically induced changes in BP in Lowlanders and highlanders.

7.5.1 Vascular sympathetic baroreflex function

Firstly, this thesis demonstrates that acclimatising Lowlanders, Sherpa and Andeans, with and without CMS, exhibit a similar ability to increase and decrease MSNA in response to perturbations in BP (i.e. the vascular sympathetic baroreflex gain) (*Figure 36*). Moreover, vascular sympathetic baroreflex gain, in all groups at high altitude, is comparable to Lowlanders at low altitude. Thus, vascular sympathetic baroreflex responsiveness is maintained under the physiological stress of ambient hypoxia and heightened basal MSNA does not alter the ability of the SNS to appropriately respond to moderate BP challenges. Neurovascular transduction is important when considering the baroreflex ability to counteract beat-by-beat fluctuations in BP, and an interaction between vascular sympathetic baroreflex gain and neurovascular transduction exists in healthy men at sea level (Hissen et al., 2019). This thesis only examined the 'neural arc' of the vascular sympathetic baroreflex i.e. the efferent MSNA response to a pressure input at the baroreceptor. As discussed in *section 7.4.2*, neurovascular transduction may be blunted in Andean highlanders. Thus, a similar change in MSNA for a given change in BP in all groups may result in a reduced vasoconstriction response in Andeans at high altitude. Consequently, there may be a reduced ability to counteract fluctuations in pressure, and maintain BP at its homeostatic set point.

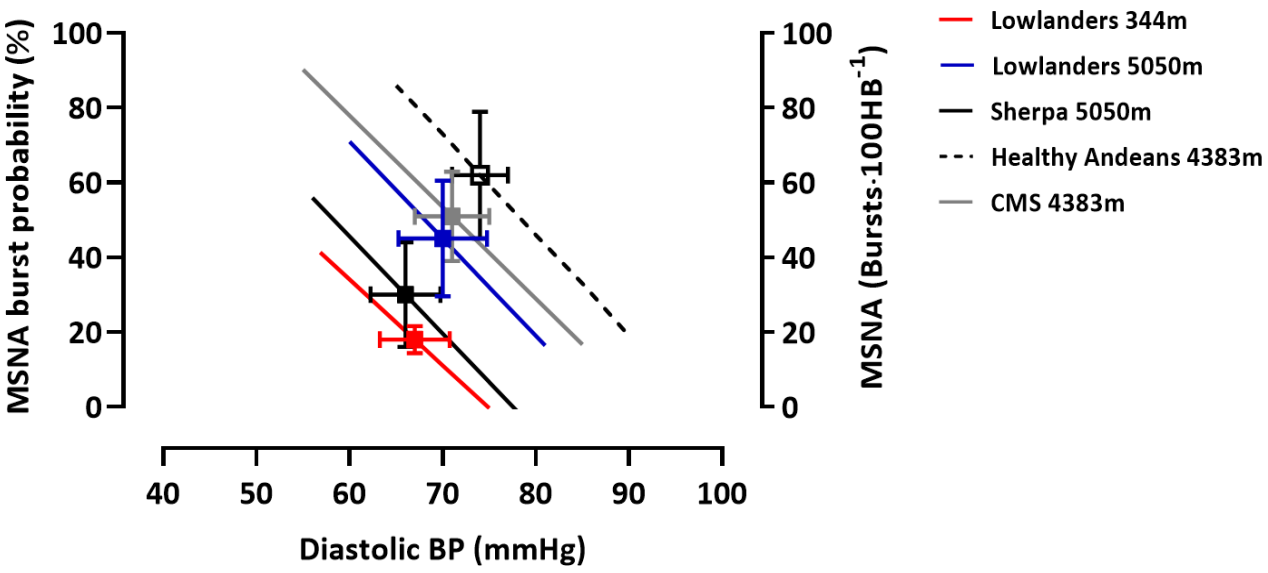

Figure 36. Vascular sympathetic baroreflex function in Lowlanders, Nepalese Sherpa, healthy Andeans and CMS across experimental studies.

Group average regression lines between MSNA burst probability and diastolic BP. The operating points are indicated by symbols and error bars (mean ± SD).The slope of the relationship between diastolic BP and MSNA is similar between all groups at high altitude (ANOVA, $P = 0.98$), and similar to Lowlanders at low altitude (ANOVA, $P = 0.85$; t-test, $P = 0.33$), indicating a similar vascular sympathetic baroreflex gain. Statistical comparisons performed using a one-way ANOVA, with Tukey post-hoc comparisons, and independent t-tests.

7.5.2 Cardiovagal baroreflex function

The arterial baroreflex not only influences MSNA, but also influences RRI. Investigating one limb of the arterial baroreflex in isolation, therefore, limits the understanding of the arterial baroreflex ability to buffer beat-by-beat changes in BP as a whole (Dutoit et al., 2010; Taylor et al., 2015). Indeed, despite a preserved vascular sympathetic baroreflex gain at high altitude, acclimatising Lowlanders exhibit a reduced cardiovagal baroreflex gain. Moreover, cardiovagal baroreflex gain was lower in Nepalese Sherpa and healthy Andeans compared to Lowlanders at low altitude (*Figure 37*). Taken together, these findings indicate that high altitude exposure reduces RRI responsiveness to changes in BP. Interestingly, however,

Andeans with CMS exhibited a cardiovagal baroreflex gain at high altitude, which was comparable to that observed in Lowlanders at low altitude (*Figure 37*).

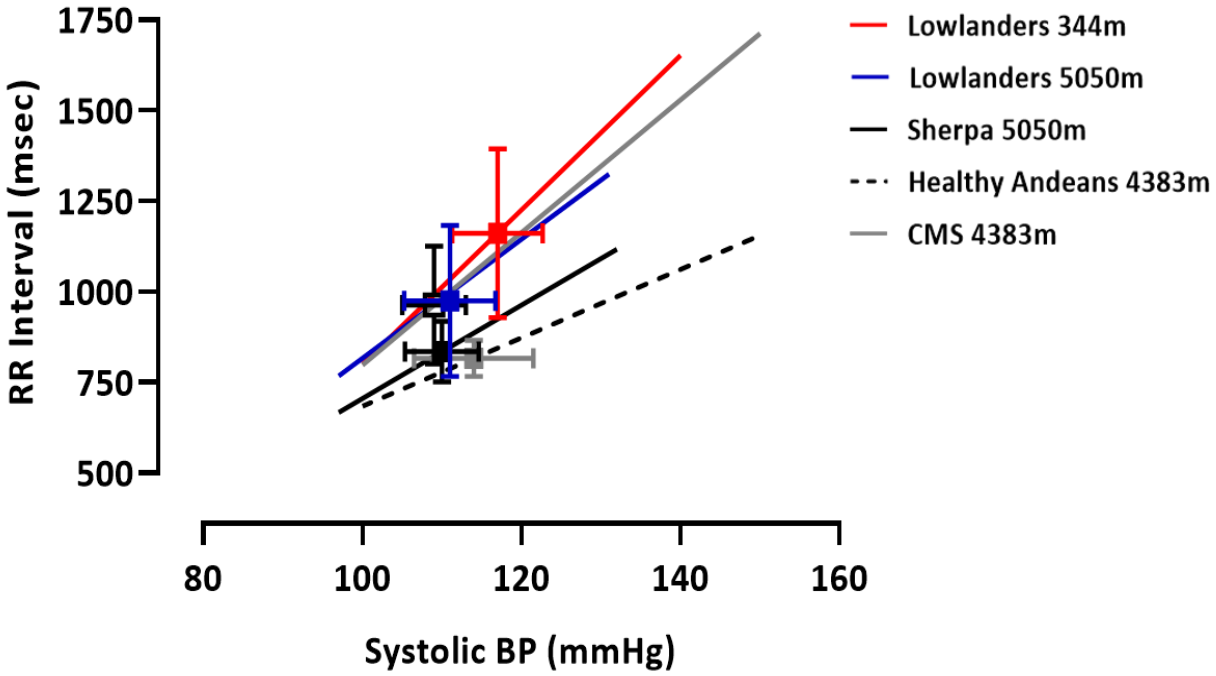

Figure 37. Cardiovagal baroreflex function in Lowlanders, Nepalese Sherpa, healthy Andeans and CMS across experimental studies.

Group average regressions between RRI and SBP. The operating points are indicated by symbols and error bars (mean ± SD). The slope of the relationship between systolic BP and RRI is lower in Lowlanders (P = 0.007), Sherpa (P = 0.01) and healthy Andeans (P <0.001) at high altitude, compared to Lowlanders at low altitude, indicating a reduced cardiovagal baroreflex gain. However, cardiovagal baroreflex gain in CMS is comparable to Lowlanders at low altitude (P = 0.68). Statistical comparisons performed using a one-way ANOVA, with Tukey post-hoc comparisons, and independent t-tests.

Due to the rapid and transient nature of the changes in BP during the modified Oxford test, the RRI responses are predominantly mediated by alterations in parasympathetic outflow to the heart, rather than sympathetic outflow (Pickering et al., 1972; Scher et al., 1972; Sagawa., 1978). Thus, specifically, the responsiveness of the efferent parasympathetic limb of the baroreflex appears to be reduced at high altitude, which may explain the differential

effects of high altitude on the vascular sympathetic and cardiovagal limb of the baroreflex. In contrast to the assessment of vascular sympathetic baroreflex gain, the assessment of cardiovagal baroreflex gain encompasses both the neural arc of the reflex (i.e. efferent vagal response to a pressure input at the baroreceptor), and the transduction of alterations in parasympathetic activity into an end-organ response (i.e. RRI). Previous research has reported a reduced RRI response to alterations in sympathetic nerve activity, during maximal exercise and isoproterenol infusion, following prolonged high altitude hypoxia (~21 days), due to a reduced sensitivity (Richalet et al., 1988; Antezana et al., 1994) and density (Voelkel et al., 1981; León-Velarde et al., 2001) of cardiac β-adrenergic receptors. If similar changes are observed in cardiac muscarinic receptors, a reduced RRI response to parasympathetic nerve activity may also occur. However, Kacimi et al., (1993) reported a 49% *increase* in muscarinic receptor density, in rats, following 30 days at a simulated altitude of 5500 m. Furthermore, animal studies have demonstrated no interference with the effectiveness of the transmission of parasympathetic activity at the level of the heart, suggesting that a reduced RRI response is instead mediated by a reduced efferent parasympathetic response to BP changes (Pisarri & Kendrick, 1984).

A further possibility for the reduced cardiovagal baroreflex gain at high altitude is an increased arterial stiffness and reduced arterial distensibility (Monahan et al., 2001; Steinback et al., 2005; Lewis et al., 2014; Klassen et al., 2016; Revera et al., 2017). Such changes within the barosensory vessels (i.e. carotid artery and aorta) would reduce baroreceptor distension for a given change in BP, and thus reduce the capacity for baroreceptors to encode changes in pressure (i.e mechanotransduction). Whilst altered mechanotransduction may also be expected to reduce vascular sympathetic baroreflex gain (Okada et al., 2013), a compensatory increase in the MSNA response, for a given afferent input, may maintain vascular sympathetic

baroreflex gain, as previously reported in healthy aging (Studinger et al., 2009). Altered vascular mechanics, however, would not explain the greater cardiovagal baroreflex gain in CMS, compared to healthy Andeans, as arterial stiffness is reported to be greater in CMS (Rimoldi et al., 2012).

Elevated peripheral chemoreceptor drive at high altitude may also contribute to the depressed cardiovagal baroreflex gain in Lowlanders, Nepalese Sherpa, and healthy Andeans at high altitude. Indeed, an inhibitory relationship exists between the peripheral chemoreflex and baroreflex mechanisms (Somers et al., 1991). An acute increase in peripheral chemoreflex activation, via acute hypoxic exposure, is consistently shown to inhibit baroreflex control of the heart (Heistad & Wheeler, 1971; Sagawa et al., 1997; Steinback et al., 2009; Niewinski et al., 2014; Mozer et al., 2016), with no effect on baroreflex control of MSNA (Halliwill & Minson, 2002; Halliwill et al., 2003). In agreement, Andeans with CMS, who are reported to have a blunted peripheral chemoreceptor sensitivity (Severinghaus et al., 1966), have the greatest cardiovagal baroreflex gain at high altitude, which is similar to Lowlanders at low altitude. Furthermore, hyperoxic breathing did not affect cardiovagal baroreflex gain in CMS, but increased reflex gain by ~75% in healthy Andeans, and by ~ 39% in Sherpa (although this was not significant). Five minutes of hyperoxia did not increase cardiovagal baroreflex gain in Lowlanders, although, six minutes of hyperoxia has previously been shown to restore reflex gain to low altitude values (Yazdani et al., 2016). Whilst the peripheral chemoreflex may be important, the hypoxia-induced reduction in cardiovagal baroreflex responsiveness may also be partly mediated by a central mechanism. Indeed, isolated perfusion of the cerebral circulation with hypoxic blood, in dogs, reduced cardiovagal baroreflex responsiveness (Pisarri & Kendrick 1984) and demonstrated a direct action of hypoxia on the CNS.

Interestingly, cardiovagal baroreflex gain is inversely related to resting HR across groups at high altitude in this thesis (*Figure 38*). Thus, individuals with higher resting HRs exhibited a smaller RRI responsiveness to changes in blood pressure. This inverse relationship may be a function of the non-linear relationship between RRI and HR. At a higher HR, a smaller reduction in RRI will produce the same change in HR versus someone with a lower HR, who will require a greater change in RRI to produce the same change in HR (O'Leary, 1996).

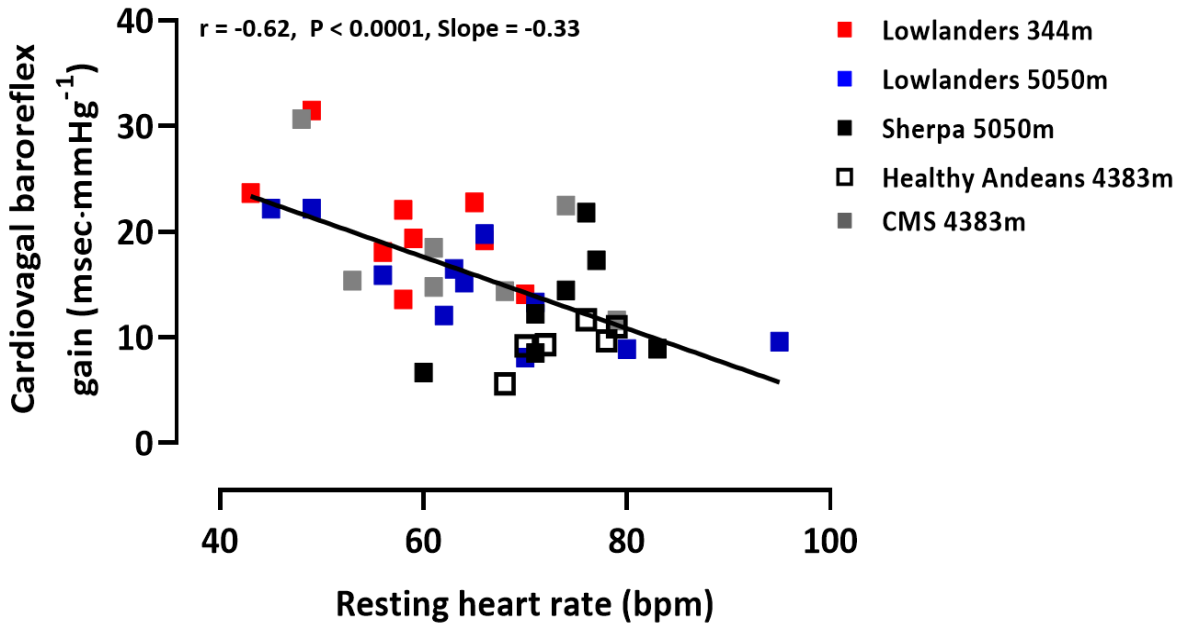

Figure 38. Linear regression analysis of the relationship between cardiovagal baroreflex gain and resting heart rate across all experimental groups.

Despite a reduced RRI responsiveness to pressure changes in Lowlanders at high altitude, compared to low altitude, HR responsiveness to pressure changes in Lowlanders at high altitude was in fact similar to that at low altitude. Whilst the baroreflex does not regulate HR *per se*, it is the change in HR that is linearly related to Qc, which subsequently relates to the correction of pressure by the baroreflex. Therefore, whilst the baroreflex mediated

efferent vagal response appears to be reduced in hypoxia (Pisarri & Kendrick, 1984), this does not appear to translate to a reduced ability to correct changes in BP.

7.6 Mechanisms involved in neural control of the cardiovascular system at high altitude

Despite no differences in vascular sympathetic baroreflex gain and operating pressure (i.e. DBP) between populations, the MSNA operating point (i.e. MSNA burst incidence) of the vascular sympathetic baroreflex is markedly different between groups (*Figure 39*). MSNA operating point is greater at high altitude in all groups, compared to Lowlanders at low altitude, meaning the likelihood of a sympathetic burst occurring at a given DBP was increased. Therefore, the MSNA operating point is upwardly reset at high altitude. Similarly, the cardiovagal baroreflex operated around a greater resting HR at high altitude for a given SBP.

Together, both a greater probability of a burst (i.e. MSNA burst incidence) and greater number of opportunities for a burst (i.e. greater resting HR) explain why basal sympathetic vasomotor activity (i.e. burst frequency) is markedly elevated at high altitude. Resetting of both the vascular sympathetic baroreflex, and to a lesser extent the cardiovagal baroreflex, appears to be required to maintain normal resting arterial pressure at high altitude, likely compensating for hypoxia-induced local vasodilation, changes in blood volume and potential alterations in neurovascular transduction.

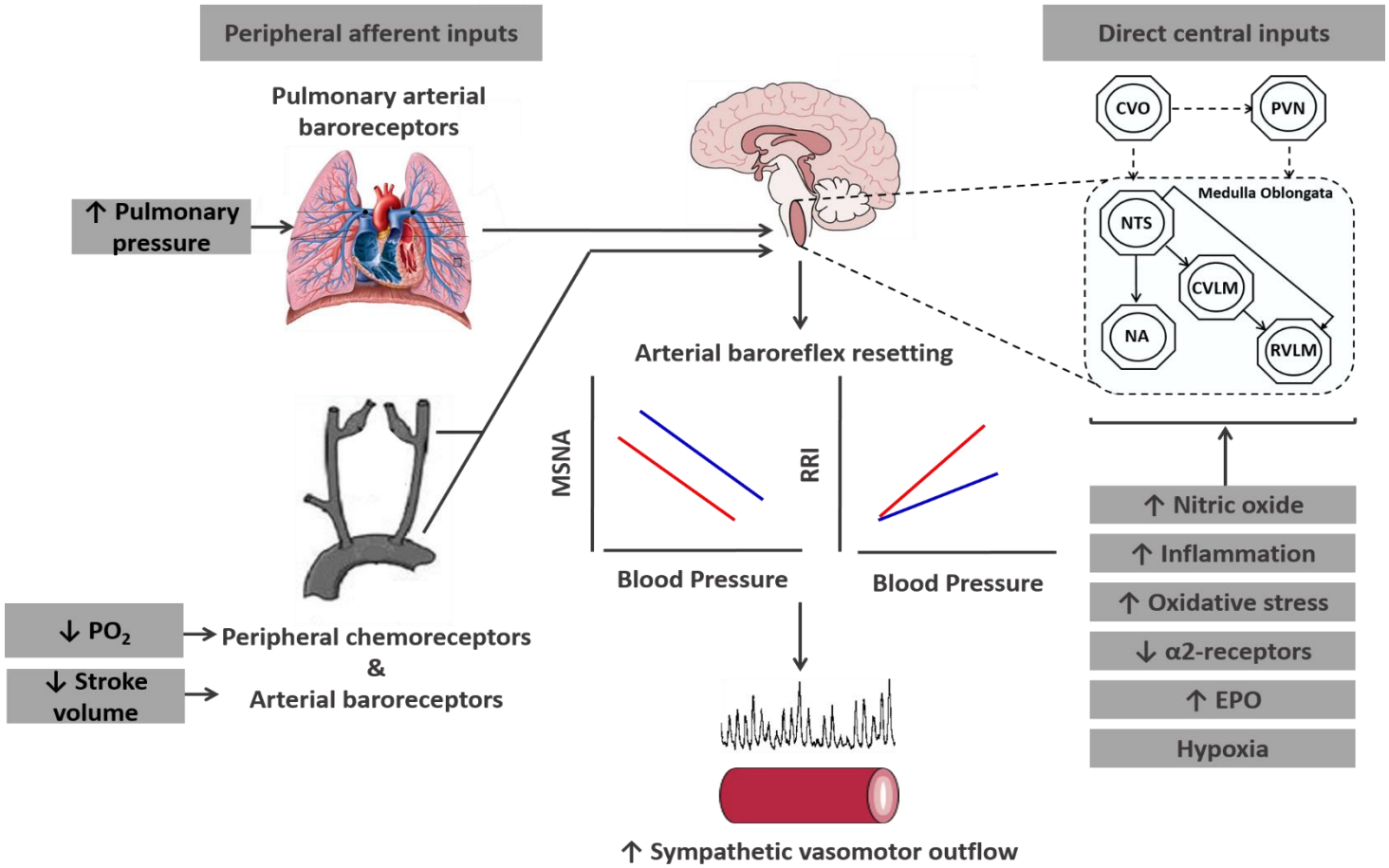

Figure 39. Mechanisms contributing to neural control and arterial baroreflex function at high altitude.

Arterial baroreceptor unloading, via a reduction in stroke volume, accounts for ~25% of the increase in MSNA at high altitude (Hansen & Sander, 2003). Increased peripheral chemoreceptor drive accounts for between ~ 0–20% of the increase in MSNA (Hansen & Sander, 2003; Fisher et al 2018; Chapter 4), and elevated pulmonary pressure may accounts for ~25% (Chapter 6). The influence of direct central inputs are yet to be investigated in humans at high altitude.

7.6.1 Peripheral chemoreflex mechanism

This thesis investigated the mechanistic role of the peripheral chemoreflex to sympathetic neural activity and arterial baroreflex function at high altitude. At high altitude, administration of 100% oxygen for five minutes, in an attempt to eliminate peripheral chemoreceptor drive, did not significantly alter the vascular sympathetic baroreflex MSNA set-point or gain in either Lowlanders, Nepalese Sherpa or Andean highlanders, with or without CMS. These findings imply that the peripheral chemoreflex mechanism does not play a major role in the resetting of the vascular sympathetic limb of the baroreflex at high altitude in either acclimatising Lowlanders or highlanders. However, the peripheral chemoreflex mechanism does appears to play a role in the regulation of resting HR and the resetting of the cardiovagal limb of the baroreflex at high altitude, as hyperoxia significantly reduced HR in all groups. Therefore, whilst reducing peripheral chemoreceptor drive does not appear to alter the probability of a sympathetic burst occurring per cardiac cycle, via the vascular sympathetic baroreflex, hyperoxia reduces the number of opportunities for a burst of sympathetic activity to occur, via the cardiovagal baroreflex. Consequently, MSNA burst frequency (i.e. sympathetic vasomotor activity) tended to be reduced, although only in the order of 9–22%. Whilst the peripheral chemoreflex may be involved in elevated sympathetic vasomotor activity at high altitude, through its effect on HR, its contribution appears to be minor. This is consistent with two previous studies reporting minimal changes in MSNA burst frequency during peripheral chemoreflex deactivation, via 25 minutes of hyperoxia or low-dose dopamine infusion, in Lowlanders following 18–30 days at high altitude (Hansen and Sander 2003; Fisher et al., 2018). Taken together, the findings in this thesis provide further evidence challenging the widely held view that augmented peripheral chemoreceptor activation is the major contributing mechanism for elevated basal MSNA observed during sustained high altitude

exposure. It should be acknowledged that silencing peripheral chemoreceptor activity via hyperoxia may have underestimated the peripheral chemoreflex contribution. Concurrent increases in $PaCO_2$ and central chemoreceptor activation, secondary to reductions in ventilation, may have increased sympathetic vasomotor outflow and masked a greater reduction in MSNA. Nevertheless, Muza and colleagues (2004) reported no change in ventilation during 10 minutes of hyperoxic breathing in Lowlanders following 12 days at 4300 m; although, future studies should maintain end tidal CO_2 via dynamic end-tidal forcing.

7.6.2 Pulmonary arterial baroreceptors

This thesis also investigated the, previously unexplored, contribution of pulmonary arterial baroreceptors to sympathetic neural activity and arterial baroreflex function in Lowlanders at high altitude. In response to the reduced PO_2 at high altitude, the pulmonary vascular smooth muscle contracts, elevating pulmonary arterial vascular resistance and pressure (Groves et al., 1987), with a correlative relationship between pulmonary artery pressure and basal MSNA previously observed (Duplain et al., 1999). This thesis demonstrates that lowering pulmonary arterial pressure, in Lowlanders following 4–9 days at high altitude, reduces basal MSNA. This reduction in basal MSNA (i.e. MSNA burst frequency/total activity) was mediated by a resetting of the vascular sympathetic baroreflex. Indeed, lowering pulmonary arterial pressure elicits a downward and leftward resetting of the vascular sympathetic baroreflex, to a lower operating pressure and MSNA operating point with no change in cardiovagal baroreflex regulation. Therefore, for the first time in humans, this thesis demonstrates that the increased afferent input from pulmonary arterial baroreceptors is a potential mechanism contributing to sympathoexcitation and resetting of vascular sympathetic baroreflex at high altitude, at least in Lowlanders. It is possible that incomplete

reversal of elevations in pulmonary arterial pressure following return to low altitude (Groves et al., 1987; Talbot et al., 2005; Maufrais et al., 2016), may also explain the maintained elevations in basal MSNA up to three days following descent (Hansen and Sander 2003; Mitchell et al., 2018).

The potential role of pulmonary arterial baroreceptors to sympathetic regulation in highlanders remains unclear. Life long hypoxic exposure in Andean highlanders is associated with pulmonary vascular remodelling, including muscularisation of the distal pulmonary arteries (Arias-Stella & Saldana, 1963) adventitial thickening and lumen narrowing, which serve to increase pulmonary vascular resistance and elevate pulmonary arterial pressure. However, in contrast to Andeans, Sherpa appear to be largely protected against pulmonary hypertension, displaying minimal hypoxic pulmonary vasoconstriction and normal or only minimally elevated mean pulmonary arterial pressures, compared to low altitude values (Groves et al., 1993; Faoro et al., 2014). Moreover, Tibetans exhibited mean pulmonary arterial pressures that were on average 28% lower than those in Andean highlanders at their resident altitudes (Antezana et al., 1998; Hoit et al., 2005). Therefore, it is possible that differences in afferent input to the pulmonary arterial baroreceptors contribute to the observed differences in basal MSNA between populations.

Importantly, whilst MSNA was lowered during reductions in pulmonary arterial pressure, the magnitude of this reduction was ~25%, which when compared to the magnitude of the increase in MSNA previously observed in Lowlanders from low altitude (173%), is relatively small. Whilst it is possible that a greater reduction in pulmonary arterial pressure (i.e. to sea level values), would have elicited a greater reduction in MSNA; elevated pulmonary

arterial pressure is likely one of several mechanisms mediating high altitude sympathoexcitation.

7.6.3 Additional mechanisms involved in neural control of the cardiovascular system at high altitude

In this thesis, the role of afferent input from the peripheral chemoreceptors and pulmonary arterial baroreceptors was investigated. Reductions in blood volume, and thus stroke volume, also contribute to elevated basal MSNA in Lowlanders at high altitude. Whether differences in stroke volume contribute to the population differences in basal MSNA is unclear. Whilst the smallest stroke volume (64 ml) and greatest basal MSNA was observed in healthy Andean highlanders, and the largest stroke volume (82 ml) and lowest basal MSNA was observed in Sherpa, there was no correlation between these factors across our groups at high altitude (p = 0.14). Furthermore, Lowlanders exhibited a similar stroke volume to Sherpa (81 ml), despite a greater basal MSNA. Taken together, these imply that differences in stroke volume do not account for differences in basal MSNA; however, these results should be interpreted with caution, as stroke volume measurements were estimated using the ModelFlow.

In addition to the contribution from peripheral autonomic reflexes, elevated basal MSNA at high altitude may also be mediated by factors acting centrally, including direct excitatory influences acting on the 'sympathetic vasomotor centre' the RVLM. RVLM neurons act as central oxygen sensors, and are progressively activated by a reduction in PO_2 below 50 mmHg, which elicits increases in sympathetic nerve activity (Sun & Reis, 1994). Additionally, RVLM neurons are directly activated by centrally produced erythropoietin (EPO), which is elevated at high altitude (Oshima et al., 2018). Further support for a role of EPO in the regulation of MSNA at high altitude is highlighted by Sherpa exhibiting lower serum EPO

concentrations compared to both Lowlanders and Andeans studied at the same altitude (Samaja et al., 1993; Winslow et al., 1989). Other excitatory signals could also be involved, including increased pro- inflammatory cytokines and oxidative stress, and reduced central NO bioavaliability (Patel et al., 2001; Hirooka et al., 2010: Hirooka et al., 2011), which may all elicit increases in sympathetic nerve activity, via their actions on RVLM and PVN (Patel et al 2001).

A reduction in α2-adrenergic receptor density may also occur within the CNS, specifically the NTS and RVLM, where α2-adrenergic receptors are heavily expressed (Punnen et al., 1987; Glass et al., 2001). Stimulation of α2-adrenergic receptors within the RVLM increases parasympathetic outflow and reduces sympathetic outflow; thus a reduction in receptor density may facilitate parasympathetic withdrawal and sympathoexcitation. Indeed, Fischetti et al., (2000) and Zaccaria et al., (1997) reported a 21–40% reduction in platelet α2-adrenergic receptor density in healthy Lowlanders following 15–30 days at high altitude.

Overall, the major mechanisms responsible for arterial baroreflex resetting, and thus elevated basal sympathetic outflow, at high altitude are incompletely understood. However, such resetting likely involves a complex integration of several peripheral and central mechanisms, whose contributions may vary with duration of exposure; however, further investigation is warranted.

7.7 Experimental limitations

In addition to the study specific limitations presented within each experimental chapter, there are several general limitations to the work presented in this thesis that should be acknowledged. Due to the logistical difficulty in performing chronic hypoxic exposures in an altitude or hypobaric chamber, these studies were performed on two high altitude field research expeditions. Field research has high ecological validity, allowing an altitude exposure

profile reflective of what is commonly experienced by Lowlander visitors, and also, allows the study of highland populations. However, field studies are inherently less well controlled compared to laboratory based studies. In addition to inspired oxygen, other factors simultaneously change, including barometric pressure, temperature, diet, exercise / physical inactivity and solar radiation exposure; therefore, the effects of hypoxia per se cannot be isolated. Furthermore, due to the time constraints associated with expedition research, the time of day participants were tested was not controlled. Whilst vascular sympathetic baroreflex gain appears to be unaffected by time of day (Hissen et al., 2015), there is significant diurnal variation in cardiovagal baroreflex (Taylor et al., 2011) that may have influenced these results. Nevertheless, our analysis indicates that time of day was not a significant covariate for the main outcome variables in this thesis. Second, compared with Lowlanders, who were members of the expedition team, and thus familiar with physiological testing, Sherpa and Andean subjects were naive to the microneurographic technique. Highlanders may have, therefore, experienced some anxiety during testing, leading to an overestimation of basal MSNA (Wallin et al., 1992). This possibility would not have altered the major findings of this thesis, which is that i) Sherpa exhibit lower basal MSNA at high altitude versus Lowlanders and ii) Andeans exhibited greater basal MSNA than Sherpa. Nevertheless, heightened anxiety in highlanders may have influenced indices of arterial baroreflex function in this work, as mental stress has been shown to alter baroreflex gain (Al-Kubati et al., 1997; Kanbar et al., 2007). Third, vascular sympathetic and cardiovagal baroreflex gain were estimated from a single Oxford test rather than the average of multiple tests. The arterial baroreflex is a dynamic mechanism, which is constantly adapting and reacting to small changes in the internal environment. Indeed, Studinger et al., (2007) reported a large within subject variability in cardiovagal baroreflex gain between consecutive Oxford trials, where the

baroreflex does not appear to generate the same pattern of response each time it is engaged. Fourth, we did not assess vascular sympathetic baroreflex gain to rising and falling pressure independently and we acknowledge that this fails to take baroreflex hysteresis into account (Rudas et al., 1999). A change in baseline MSNA may have influenced responsiveness of the vascular sympathetic baroreflex to both rising and falling pressures independently (Hart et al., 2011). Specifically, there is a possibility that MSNA responsiveness may be reduced during severe baroreceptor unloading at high altitude, due to a reduced sympathetic reserve and potential ceiling effect (Busch et al., 2020); although, this possibility requires investigation. Lastly, the investigations in this thesis predominantly studied male Lowlander subjects, and exclusively studied male highlanders; thus, these findings cannot be generalised to females. Known sex differences exist in autonomic cardiovascular regulation, at least in Lowlanders at sea level (Hart & Charkoudian, 2014). Indeed, compared to males, females exhibit reduced basal MSNA, BP and neurovascular transduction (Hart & Charkoudian, 2014), and exhibit a greater vasodilatory response to acute hypoxia (Casey et al., 2014), which likely impact the autonomic regulation of BP at high altitude.

7.8 Significance and implications of thesis findings

The findings from this thesis further our understanding of sympathetic neural activity and autonomic control of BP during exposure to high altitude hypoxia. This research is relevant to the 100 million individuals globally who travel to high altitude for leisure each year, and the many military personnel who are deployed to mountainous regions. In addition, understanding the normal physiology of highland populations, whose physiology has been altered over generations, is of direct importance to local healthcare. Moreover, this work provides insight into the fundamental physiological mechanisms facilitating adaptation, or

maladaptation, to the high altitude environment, which may be relevant to the understanding and treatment of diseases (Martin & Windsor, 2008; Martin et al., 2013).

Chronically elevated sympathetic neural activity is a feature of many cardiovascular and respiratory disease states (e.g. pulmonary hypertension, COPD, sleep apnoea, chronic heart failure; Ferguson et al., 1999; Narkiewicz et al,. 2003; Velez-Roa et al., 2004; Andreas et al., 2014), and is an independent predictor of clinical deterioration (Ciarka et al., 2010), adverse outcome (Andreas et al., 2014) and mortality (Barretto et al., 2009). The mechanisms responsible for elevated sympathetic neural activity at high altitude likely overlap with those in disease. This thesis highlights increased afferent input to pulmonary arterial baroreceptors as a mechanism for sympathoexcitation at high altitude, which may also be relevant in the initiation or perpetuation of basal sympathetic overactivity in disease states where sustained elevations in pulmonary pressure exist.

The findings of this thesis also have wider significance to the field of human autonomic cardiovascular control. This thesis demonstrates that pulmonary arterial baroreceptors exert positive feedback control over sympathetic neural activity in humans, which had previously only been demonstrated in experimental animals (Moore et al., 2004, 2011). This positive feedback control by pulmonary baroreceptors is opposite to the negative feedback control exerted by arterial baroreceptors. Positive feedback control is also opposite to the negative feedback control that is commonly attributed to all baroreceptors in the cardiopulmonary circulation. Thus, pulmonary arterial baroreceptors may play an important, but previously underappreciated, role in human cardiovascular control. Moreover, these findings challenge the notion that all baroreceptors in the cardiopulmonary circulation constitute a single cardiopulmonary baroreflex, which elicit a sympathoinhibitory response. Indeed, studies in

experimental animals, which allow isolated stimulation of discrete groups of receptors in the thoracic region, indicate distinct reflex responses. Similar heterogeneity likely in humans, therefore, it is important to develop experimental approaches to allow sympathetic neural responses to stimulation of different reflex inputs in conscious humans.

7.9 Future research directions

The obvious missing piece of the jigsaw from the present thesis is how the ANS has adapted in Ethiopian Amhara highlanders native to the Simien Mountains. Significantly less is known about the Ethiopian Amhara pattern of adaptation, but, the limited study in this population suggest they exhibit [Hb] concentrations, resting ventilation and oxygen saturations similar to those observed in Lowlanders at sea level (Beall et al., 2002; Beall, 2006). Thus, Ethiopians appear to offset ambient hypoxia and, consequently, Ethiopians may be expected to exhibit similar basal MSNA and arterial baroreflex function to Lowlanders at sea level, due to a reduced hypoxic stimulus. On the other hand, Ethiopians exhibit elevated pulmonary artery pressure compared to Lowlanders at sea level (Hoit et al., 2011), which, based off the findings in this thesis, may be expected elicit vascular sympathetic baroreflex resetting and elevated sympathetic neural activity.

This thesis represents an important first step towards understanding the physiological role of pulmonary arterial baroreceptors in humans. Pulmonary arterial baroreceptors may also be important during other physiological and pathophysiological situations, including exercise and disease. Indeed, increases in pulmonary arterial pressure, secondary to elevations in venous return and right ventricular volume loading, occur during exercise (Wright et al., 2016). Thus, stimulation of pulmonary arterial baroreceptor afferents could be linked to sympathetic activation, and contribute to central resetting of arterial control of

MSNA during exercise (Raven et al., 2009). In addition, an exaggerated increase in both pulmonary arterial pressure and MSNA are observed during exercise in individuals with heart failure (Lewis et al., 2011; Notarius et al., 2015). It is possible that the exaggerated MSNA response to exercise, which contributes to the exercise limitation in this population (Notarius et al., 2015), is mediated by the exaggerated input to pulmonary arterial baroreceptors. Indeed, inhalation of NO during exercise increases exercise tolerance in individuals with HF (Koelling et al., 1998), however, there was no simultaneous assessment of MSNA.

7.8 Conclusions

The work presented in this thesis demonstrates that resting arterial blood pressure is remarkably similar across healthy Lowlanders at 344 m and 5050m, healthy Sherpa at 5050m, and Andeans – with and with CMS - at 4383m. Furthermore, this thesis highlights some important changes to neural control and autonomic regulation of BP in Lowlanders and highlanders in response to the physiological challenge of ambient hypoxia. This work confirms that sustained sympathoexcitation in Lowlanders is a feature of high altitude acclimatisation. Notably, a key novel finding is that sympathoexcitation is accompanied by upward resetting of the vascular sympathetic baroreflex; which occurs without any change in the ability to respond to increases or decreases in BP. Another key finding is that Nepalese Sherpa display a lower level of sympathetic outflow than Lowlanders, when studied at the same elevation. Despite this, MSNA for Sherpa at high altitude is higher than for Lowlanders at low altitude, suggesting that sustained sympathoexcitation is a feature of evolutionary adaptation to high altitude hypoxia. Notably, basal MSNA for Andean Quechua is greater than Lowlanders at high altitude, despite studying Andeans at a lower elevation; although, Andeans studied here were older and had higher a BMI – factors known to influence basal MSNA. Nevertheless,

irrespective of basal MSNA, vascular sympathetic baroreflex responsiveness is remarkably similar across all groups studied. Something else remarkable is that CMS does not appear to interfere with the integrated regulation of BP at high altitude, although they may be more reliant on baroreflex control of the heart. Together these findings demonstrate integrated adaptations of BP regulation in Lowlanders recently exposed to altitude, and two geographically and genetically distinct highland populations. In addition, the role of the peripheral chemoreflex is delineated, and evidence of an important contribution from pulmonary arterial baroreceptors, at least in Lowlanders at high altitude, is presented.

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APPENDICES

Appendix I - Ethical Approval Documents

I.a. Ethical approval for Chapter 4 (Experimental study 1).

The University of British Columbia
Office of Research Ethics
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: RENEWAL

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC CREB NUMBER:
Philip Ainslie	UBC/UBCO Health & Social Development/UBCO Health and Exercise Sciences	H16-01028

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
UBC	Okanagan

Other locations where the research will be conducted:
Ev-K2-CNR Research Pyramid, Khumbu Valley, Nepal.

CO-INVESTIGATOR(S):
Luca Ruggiero
Geoffrey Hartley
Christopher Willie
Nia Lewis
Lindsey M. Boulet
Mypinder Singh Sekhon
Ali McManus
Ryan Leo Hoiland
Michael Tymko
Anthony Bain
Daniela Flueck
Joshua Tremblay
Brad Monteleone
Chris McNeil
Mathew Rieger
Alexander Hansen

SPONSORING AGENCIES:
- Canada Research Chairs - "Canada Research Chair in Cerebrovascular Physiology for Dr. Philip Ainslie"
- Natural Sciences and Engineering Research Council of Canada (NSERC) - "Development of new algorithms in the Hexoskin smart shirt for precise measurement of respiration "
- Natural Sciences and Engineering Research Council of Canada (NSERC) - "Exercise Oxidative Metabolism in Children "

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

EXPIRY DATE OF THIS APPROVAL: July 7, 2018

APPROVAL DATE: July 7, 2017
(H16-01028-A002) Nepal 2016 - lowlander and Sherpa, 2017

CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

Approval of the Clinical Research Ethics Board by:

Dr. Stephen Hoption Cann,
Chair

I.b. Ethical approval for Chapter 5 (Experimental study 2)

UNIVERSIDAD PERUANA
CAYETANO HEREDIA
Vicerrectorado de Investigación
Dirección Universitaria de Investigación, Ciencia y Tecnología (DUICT)

CONSTANCIA 101 - 04-18

El Presidente del Comité Institucional de Ética en Investigación (CIEI) de la Universidad Peruana Cayetano Heredia hace constar que el proyecto de investigación señalado a continuación fue **APROBADO** por el Comité de Ética.

Título del Proyecto	: **"Expedición global de investigación sobre la salud crónica relacionada con la altura (Global Reach): Una expedición científica a las montañas de los andes".**
Código de inscripción	: **101686**
Investigador principal	: **Villafuerte Castrillón, Francisco Carlos; Macarlupu Bernuy, José Luis**

La aprobación incluyó los documentos finales descritos a continuación:

1. **Protocolo de investigación,** versión recibida en fecha 12 de febrero del 2018.
2. **Consentimiento informado,** versión recibida en fecha 12 de febrero del 2018.

La **APROBACIÓN** considera el cumplimiento de los estándares de la Universidad, los lineamientos Científicos y éticos, el balance riesgo/beneficio, la calificación del equipo investigador y la Confidencialidad de los datos, entre otros.

Cualquier enmienda, desviaciones, eventualidad deberá ser reportada de acuerdo a los plazos y normas establecidas. El investigador reportará cada seis meses el progreso del estudio y alcanzará un informe al término de éste. La aprobación tiene vigencia desde la emisión del presente documento hasta el **19 de febrero del 2019**.

Si aplica, los trámites para su renovación deberán iniciarse por lo menos 30 días previos a su vencimiento.

Lima, 20 de febrero del 2018.

COMITÉ INSTITUCIONAL DE ÉTICA - CIE
UNIVERSIDAD PERUANA CAYETANO HEREDIA DUICT

Dra. Frine Samalvides Cuba
Presidenta
Comité Institucional de Ética en Investigación

/ silm

Peruvian University

Cayetano Heredia

Constancy 101-04.18

The President of the Institute Ethics Committee for Research at the Peruvian University, Cayetano Heredia has approved the research project.

Project Title: Expedition for research on the Chronic Health in Altitude (GLOBAL REACH).

A scientific expedition in the Andes mountains.

Inscription Code: 101686

Principal Investigator: Villafuerte Castrillon, Francisco Carlos,

Mascarlupu Bernuy, Jose Luis

The authorisation includes the final following documents:

1- Investigation protocol: Version received on the 12/02/2018

2- Consent information: version received on the 12/02/2018

The authorisation follows standard University requirements, the scientific ethics guidelines, the risks/benefits, the investigation team's qualifications and the data confidentiality among others.

Any amendments made will need to be reported and accepted in accordance with the agreement made.

The Investigator will report every 6 months the study progress and will write a report at the end of the study.

This authorisation is valid from the issue date, to February 19th, 2019.

If applicable, the procedure for renewal should start at least 30 days before expiration.

Lima, February 20th, 2018

Dra Frine Salmavides Cuba

President,

Of the institute of Etic Comity for Research.

I.c. Ethical approval for Chapter 6 (Experimental study 3)

The University of British Columbia
Office of Research Ethics
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF FULL BOARD APPROVAL: AMENDMENT

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC CREB NUMBER:
Philip Ainslie	UBC/UBCO Health & Social Development/UBCO Health and Exercise Sciences	H17-02687

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
UBC	Okanagan

Other locations where the research will be conducted:
High Altitude Research Laboratory affiliated with the Universidad Peruana Cayetano Heredia, Lima, Peru. This research lab is located in Cerro de Pasco, Peru, at 4300m.

CO-INVESTIGATOR(S):
Glen E. Foster
Alexander M D Patrician
Geoff Coombs
Lindsey M. Boulet
Tyler D. Vermeulen
Mypinder Singh Sekhon
Ali McManus
Ryan Leo Hoiland
Michael Tymko
Hannah Caldwell
Daniela Flueck
Connor Howe
Alexander Hansen

SPONSORING AGENCIES:
- Canada Research Chairs - "Canada Research Chair in Cerebrovascular Physiology for Dr. Philip Ainslie"

PROJECT TITLE:
Global Research Expedition on Altitude related Chronic Health (Global REACH): a scientific expedition to the Andean mountains

REMINDER: The current UBC CREB approval for this study expires: November 14, 2018

AMENDMENTS BELOW REVIEWED AT REB FULL BOARD MEETING DATE:
January 23, 2018

AMENDMENT(S):	AMENDMENT APPROVAL DATE:
(H17-02687-A001) Peru Lowlanders 2018 - Amendment	January 29, 2018

Document Name	Version	Date

Protocol:			
Protocol	6	January 23, 2018	
Consent Forms:			
Consent Form	6	January 23, 2018	
Investigator Brochures:			
Dopamine	1	April 24, 2013	

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the University of British Columbia Clinical Research Ethics Board, as presented in the documentation, and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects

Approval of the Clinical Research Ethics Board by:

Dr. Stephen Hoption Cann,
Chair

Appendix II – Participant information sheet and informed consent documents

II.a. – Participant information sheet and informed consent Chapter 4 (Experimental study 1)

THE UNIVERSITY OF BRITISH COLUMBIA

OKANAGAN

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
[5] Cardiff Metropolitan University, Cardiff, UK
[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.

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. 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

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.

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.

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.

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.

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).

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

II.b. – Participant information sheet and informed consent Chapter 6 (Experimental study 3)

PARTICIPANT CONSENT FORM

Mechanisms for sympathoexcitation at high altitude: Role of elevations in pulmonary pressure.

Aims: The purpose of the present study is to determine the mechanisms responsible for the progressive increase in activity of the sympathetic 'fight or flight' branch of the nervous system at high altitude.

Synopsis: In lowlanders, ascent to high altitude progressively increases activity of the sympathetic nervous system and serves to reset the vascular sympathetic baroreflex upward; however the mechanisms responsible are unclear. This study aims to determine the contribution of increased pressure in the blood vessels in the lungs to this increased sympathetic nervous system activation and subsequent vascular sympathetic baroreflex resetting.

You will visit the laboratory on one occasion following 7 days acclimitisation at 4380m in Cerro de Pasco. The approximate time commitment will be 3 hours.

General protocol

a. *You will be asked to lay supine on a hospital bed and an intravenous catheter will be inserted.*
b. *An experienced microneurographer will then search for a nerve signal in your leg and you will rest for a period of 15 minutes.*
c. *A bolus dose of vasoactive drugs sodium nitroprusside and phenylephrine will then be sequentially administered to decrease and increase your BP respectively*
d. *You will then breathe room air mixed with Nitric oxide through a breathing mask and nose clip, for 6 minutes.*
e. *A bolus dose of vasoactive drugs sodium nitroprusside and phenylephrine will then be administered whilst you continue to breathe nitric oxide from a mouthpiece.*

Specific methods:

Placement of catheters: *A sterile catheter will be inserted into a vein in your arm to allow infusion of sodium nitroprusside and phenylephrine during the experiment. A highly experienced physicians (Drs David MacLeod, Myp Sekhon or Chris Gasho) will complete this procedure and strict adherence to full sterile procedures will be followed at all times to minimise risk of infection.*

Microneurography: *A thin tungsten needle, similar to an acupuncture needle, will be inserted into a nerve in your leg, just below your knee. This allows for the measurement of sympathetic nervous activity that is travelling to your blood vessels. Insertion of the needle may cause slight*

pain or discomfort. Application of local anaesthetic (similar to what a dentist would use), prior to the insertion of the needle, will minimise this. Manipulation of the needle when trying to find a nerve signal may cause tingling, heaviness, pins and needles and cramp sensations in your lower leg or foot. These sensations should subside within 1-2 seconds. The needle will remain in place for the duration of the experiment and be removed after the experiment is complete or at any time you wish. Microneurography will be performed by two co-investigators with extensive experience (Dr. Craig Steinback and Dr. Jonathan Moore). Dr. Steinback and Moore performed all microneurography procedures during our Nepal expedition in 2016. They have both been trained and have published peer reviewed manuscripts as microneurographer.

Cardiovascular Measurements: *Heart rate and BP will be recorded continuously throughout the experimental protocol. To do this you will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.*

Baroreflex function*: Baroreflex function will be assessed by characterizing the relationship between sympathetic nerve activity and BP during a modified Oxford test. Modified Oxford test involves the sequential, bolus, administration of sodium nitroprusside and phenylephrine which will cause a small decrease and increase resting BP respectively.*

Doppler Ultrasound: *B Pin the vessels of your lungs will be assessed non-invasively using Doppler Ultrasound images of your heart. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. Images of your heart, to determine the BP in the vessels of your lungs will be taken at regular intervals throughout the experimental protocol.*

Risks

Microneurography: Insertion of the needle into a peripheral nerve may cause temporary "pins and needles" sensation or increased sensitivity to touch in the leg following the test. However, this feeling will likely go away within a day or two, and virtually always with 1 to 2 weeks. Subjects will be given a questionnaire to fill out and mail back one week after the study detailing any symptoms they may feel. Subjects will be advised not to perform any vigorous exercise in the 24 hours following the procedure to minimize symptoms.

Infusion of vasoactive drugs: There is a minimal risk of evoking a vasovagal reaction (a faint) during these changes in blood pressure, however your heart rate and BP will be continuously monitored, and the test will be terminated if you feel light-headed, nauseated, or experience any other adverse sign or symptoms. There is also a minimal risk of a local or systemic allergic reaction to the drugs: however, a physician will be present during testing and determine the appropriate course of action. All laboratory personnel are also certified emergency first aid CPR/AED, if required. The modified Oxford test is a commonly used technique is commonly employed and considered the 'gold standard' technique to assess baroreflex function.

Nitric Oxide: Nitric oxide is a naturally occurring gas produced by our bodies. When additional nitric oxide is given, it combines with hemoglobin to form methemoglobin, which does not transport oxygen. Long term usage can lead to methemoglobinemia, but this usually takes several hours and occurs with much higher doses of nitric oxide than will be used in this study.

Inhaled nitric oxide lasts only a very short time (a few seconds) in the body, but people have occasionally reported dizziness, chest discomfort, dry throat, or headache after inhaling nitric oxide. In healthy people, nitric oxide at the dose and duration used in the study, is not known to have harmful side effects.

Please tick boxes

1 I confirm that I have read and understand the information for the above study and what will be required of me. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. ☐

3 I agree to take part in the above study. ☐

Name of Participant …….

Signature ……. Date ……..

Name of Person taking consent…….

Signature ……. Date ……..

Appendix III - Conference abstracts specifically relating to this thesis

Extreme Environmental Physiology – Life at the limits, UK, Sept 2019

NB. Winner of the Michael J Rennie Early Career Researcher Prize for best oral presentation

Baroreflex Function in Andean High Altitude Natives With and Without Chronic Mountain Sickness

[1]Lydia L Simpson, [2]Victoria L Meah, [2]Andrew Steele, [2]Stephen A Busch [1]Samuel J Oliver, [4]Justin S Lawley [5]Michael M Tymko, [6]Gustavo A. Vizcardo-Galindo, [6]Rómulo J. Figueroa-Mujíca, [6]Francisco C. Villafuerte, [5] Phillip N Ainslie, [2]Craig D Steinback, [3]Mike Stembridge, [1]Jonathan P Moore.

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High altitude native populations have adapted to the environmental stress of chronic hypoxia over generations, often demonstrating superior hypoxia tolerance. Up to a third of Andean high altitude natives, however, lose their ability to cope with chronic hypoxia and develop maladaptation syndrome chronic mountain sickness (CMS), which is associated with an increased risk of cardiovascular disease. Autonomic dysfunction has been implicated in the development and progression of many cardiovascular diseases; therefore we investigated whether autonomic function is impaired in CMS sufferers. We assessed baroreflex function in 7 Andean natives with CMS (CMS+; Hb 19.3g/dL) and 7 Andean natives without CMS (CMS-; 22.6g/dL) at their resident altitude (Cerro de Pasco, Peru; 4383m). R-R interval (RRI; Electrocardiogram), beat-by-beat arterial BP(BP; photoplethysmography) and muscle sympathetic nerve activity (MSNA; microneurography) were recorded at rest and during pharmacologically induced changes in arterial BP(modified Oxford method). The responsiveness (i.e gain) of the vascular-vascular sympathetic baroreflex was determined from the slope of the linear relationship between diastolic BPand MSNA burst incidence, and the responsiveness of the cardiovagal baroreflex was determined from the slope of the linear relationship between RRI and systolic blood pressure. Values are means (±SD) and were compared using unpaired T-tests. Resting mean arterial pressure was similar in CMS+ (83 ±

7mmHg) and CMS- (86 ± 10mmHg; *P* 0.58) and resting RRI was higher in CMS+ (936 ± 156msec) compared with CMS – subjects (817 ± 50; *P*=0.07). Vascular-vascular sympathetic baroreflex gain was similar in both CMS + (-2.7 ± 1.1 %·mmHg^{-1}) and CMS – subjects (-2.5 ± 1.0%·mmHg^{-1}; *P* = 0.72). Cardiovagal baroreflex gain, however, was greater in CMS+ subjects (17.2 ± 6.8msec·mmHg^{-1}) versus their CMS- counterparts (8.8 ± 2.6msec·mmHg^{-1}). Our data show that responsiveness of the vascular-vascular sympathetic baroreflex is preserved in CMS sufferers and the responsiveness of the cardiovagal baroreflex is in fact enhanced, compared to CMS- subjects. In conclusion, maladaptation to chronic hypoxia in CMS does not impair baroreflex control of blood pressure.

Experimental Biology, Florida, USA – April 2019

NB. Finalist in trainee awards session run by the Neural Control and Autonomic Regulation (NCAR) Section of the American Physiological Society.

Selective Reductions in Pulmonary Artery Pressure Lowers Sympathetic Neural Activity in Healthy Humans at High Altitude.

Lydia L Simpson[1], Andrew Steele[3], Victoria L Meah[3], Suman Thapamagar, Christopher Gasho, Aimee L Drane[2], Samuel J Oliver[1], Phillip N Ainslie[4], Craig D Steinback[3], Mike Stembridge[2] Jonathan P Moore[1]

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Chronically elevated sympathetic neural activity is a characteristic of many disease states. Tonic chemoreflex activation has been implicated in the development of sympathetic overactivity; however, other reflexes may also contribute. For example, in an animal model, stimulation of pulmonary baroreceptors induces a reflex increase in sympathetic neural activity. In humans, pulmonary pressure has been shown to be related to basal sympathetic activity; however a critical step in determining a causal link is to establish whether changing pulmonary pressure alters sympathetic outflow. In this pilot study, we exposed thirteen healthy humans (5 females, 28 [±7] years) to high altitude hypoxia (HA; 4383m), a setting known to increase both pulmonary pressure and sympathetic activation, before selectively lowering pulmonary pressure via inhalation of the pulmonary vasodilator nitric oxide. Muscle sympathetic nerve activity (MSNA; Microneurography), BP(BP; Photoplethysmography), heart rate (HR; Electrocardiogram), and peripheral oxygen saturation (SPO_2; Pulse oximetry) were continually measured during ambient air breathing (Amb) and during a 6-minute inhalation of nitric oxide (iNO; 40ppm in 21% O_2). Pulmonary artery systolic pressure (PASP), pulmonary vascular resistance (PVR) and stroke volume were determined via cardiac ultrasound. iNO reduced PASP (-23 ± 12%) and PVR (-21 ± 16%) compared to Amb (Table 1). iNO also reduced MSNA burst frequency (-18 ± 18%), burst incidence (-16 ± 17%) and total activity (-21 ± 20%) compared to Amb. MSNA changes during iNO were not related to changes in SPO_2 (r=-0.16, *P*=0.61) or MAP (r=-0.28, P=0.35). Our data demonstrate that selectively reducing pulmonary pressure reduces sympathetic nerve activity in healthy humans; therefore, it provides evidence for a causal link in the relationship between pulmonary pressure and basal sympathetic activity Further investigation is warranted to establish

whether elevated pulmonary pressure is a mechanism contributing to chronically elevated sympathetic outflow in disease.

	Amb (n=13)	**iNO (n=13)**	***P* Value**
Pulmonary haemodynamic s			
PASP (mmHg)	32 ± 6	24 ± 3	**<0.001**
PVR (Woods units)	6.3 ± 1.8	4.8 ± 1.5	**0.005**
Muscle sympathetic Nerve Activity			
Burst frequency (bursts·min^{-1})	28 ± 12	23 ± 13	**0.004**
Burst incidence (bursts·100HB^{-1})	38 ± 16	33 ± 17	**0.006**
Total activity (au·min^{-1})	1553 ± 519	1220 ± 514	**0.002**
Cardiovascular haemodynamic			
SpO_2 (%)	82 ± 3	85 ± 5	0.06
Heart Rate (bpm)	76 ± 18	73 ± 17	**0.02**
Qc (L·min^{-1})	5.2 ± 1.4	5.1 ± 1.3	0.71
Total Peripheral Resistance (mmHg·L·min^{-1})	21 ± 6	21 ± 5	0.76
Mean Arterial Pressure (mmHg)	101 ± 11	100 ± 11	**<0.001**

Table 1. Values are presented as mean ± SD

The influence of high altitude exposure on vascular sympathetic baroreflex function in humans: a comparison of lowlanders and Nepalese Sherpa

Lydia L Simpson[1], Stephen A Busch[3], Mike Stembridge[2], Samuel J Oliver[1], Phillip N Ainslie[4], Craig D Steinback[3], Jonathan P Moore[1]

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Muscle sympathetic neural activity (MSNA) contributes to BPregulation. Microneurographic studies have shown sustained high altitude hypoxia is accompanied by heightened MSNA; however, the influence on baroreflex control of MSNA and BPremains unknown. We investigated vascular sympathetic baroreflex function in 10 lowlanders and 7 Nepalese Sherpa. Lowlanders were studied at low altitude (LA; 344m) and high altitude (HA; 5050m), with Sherpa only studied at HA. Indices of vascular sympathetic baroreflex function (i.e. 'operating pressure', 'set-point' and 'gain') were determined from beat-by-beat changes in diastolic BP(DBP, photoplethysmography) and corresponding MSNA (microneurography) during supine rest, and during boluses of vasodilator sodium nitroprusside and vasoconstrictor phenylephrine (modified Oxford test). Values are means and 95% confidence intervals, compared using paired and unpaired T-tests. In lowlanders, the vascular sympathetic baroreflex 'operating' DBP was unchanged at HA compared to LA (71 [64-78] vs 68 [64-73] mmHg, *P*=0.56); however, the corresponding MSNA burst frequency (29 [22-36] vs 10 [7-12] bursts/min, *P*=0.0004) and MSNA burst incidence (i.e. 'set-point,' 45 [34-56] vs 18 [13-24] bursts/100HB, *P*=0.0007) were increased. Vascular sympathetic baroreflex gain was unchanged (-2.6 [-1.8- -3.5] vs -2.3 [-1.8- -2.8] %/mmHg, *P*=0.30). In lowlanders, breathing 100% oxygen for 5 minutes at HA (n=9), to silence peripheral chemoreceptor drive, did not influence DBP (74 [66-82] vs 72 [65-79] mmHg, *P*=0.22), MSNA burst frequency (26 [18-35] vs 30 [22-38] bursts/min, *P*=0.33), MSNA burst incidence (45 [33-57] vs 44 [31-56] bursts/100HB, *P*=0.78), or vascular sympathetic baroreflex gain (-2.5 [-1.7- -3.2] vs -2.8 [-2.0- -3.6] %/mmHg, *P*=0.16). Compared to lowlanders at HA, Sherpa had a similar baroreflex operating DBP (66 [59-73] mmHg, *P*=0.28); however MSNA burst frequency (22 [12-33] bursts/min, *P*=0.05) and MSNA burst incidence (30 [17-43] bursts/100HB, *P*=0.02) were lower. Vascular sympathetic baroreflex gain was similar (-2.6 [-1.8- -3.4] %/mmHg, *P*=0.94) to lowlanders at HA. Our data show that sustained HA exposure in lowlanders is accompanied by an upward resetting of the vascular sympathetic baroreflex, with no difference in the operating pressure, or the responsiveness of MSNA to changes in blood pressure. In Sherpa, the vascular sympathetic baroreflex set-point is lower, with a similar operating pressure and reflex responsiveness,

compared to lowlanders at HA. Furthermore, the inability of acute hyperoxia to influence vascular sympathetic baroreflex set-point, suggests that mechanisms other than the peripheral chemoreflex could play a role in baroreflex resetting in lowlanders at HA. In conclusion, sustained HA exposure modifies vascular sympathetic baroreflex function in lowlanders; however, the underlying mechanism(s) remain to be delineated.

Okanagan Cardiovascular and Respiratory Symposium, Canada – February 2018

Vascular sympathetic baroreflex Resetting with Unchanged Gain in Acclimatizing Lowlanders at High altitude

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The mechanisms contributing to sympathoexcitation during high altitude (HA) acclimatisation remain unclear. We investigated the potential role of alterations in baroreflex control of sympathetic outflow. In 14 healthy lowlanders, we measured beat-by-beat BP(BP; photoplethysmography) and muscle sympathetic nerve activity (MSNA; microneurography) during supine rest and during sequential infusion of nitroprusside and phenylephrine (modified Oxford method) at sea level (SL; 344m), under acute normobaric hypoxia (AH; SaO_2 84±4%) and after 10-20 days at high altitude (HA; SaO_2 82±3%). Vascular sympathetic baroreflex gain (sBRG) was determined as the slope of the linear relationship between MSNA and diastolic BP. Compared to SL, although AH had no effect on resting MSNA (19±8 vs. 20±6 bursts·100heartbeats^{-1}; *P*=0.64), mean arterial pressure (MAP) was increased (89±8 vs. 83±7mmHg; *P*=0.05) and sBRG was reduced (-1.9±0.6 vs. -2.3±0.7%·mmHg; *P*=0.016). In contrast, when compared to SL, HA increased MSNA (48±16 bursts·100heartbeats^{-1}; *P*=0.0001), with no change in MAP (86±8mmHg; *P*=0.80) or sBRG (-2.6±1.3%·mmHg; *P*=0.33). To further explore the effects of prolonged HA exposure, we investigated sympathetic regulation in 8 HA native Sherpa. Sherpa had similar MAP (84±10mmHg) and sBRG (-2.6±0.9 %·mmHg) compared to lowlanders at HA (*P*>0.05); however, Sherpa had lower MSNA (30±13 bursts·100heartbeats^{-1}; *P*=0.02 vs lowlanders). In summary, the reduction in sBRG observed under acute hypoxia is not evident with HA acclimatisation . Upward resetting of the vascular sympathetic baroreflex during HA acclimatisation facilitates sustained sympathoexcitation, with no change in blood pressure. In contrast to lowlanders, the vascular sympathetic baroreflex operated at lower levels of MSNA, with a similar gain, in Sherpa, indicating a unique adaptation to their environment.

Appendix IV - Co-author publications during PhD.

Peer-reviewed publications

1. Tymko MM., Hoiland RL., Tremblay JC., Stembridge M., Dawkins TG., Coombs GB., Patrician A., Howe CA., Gibbons TD., Moore JP., **Simpson LL**., Steinback CD., Meah VL., Stacey BS., Bailey DM., Gasho C., Anholm J., Bain AR., Lawley JS., Villafuerte FC., Vizcardo-Galindo & Ainslie PN. The 2018 Global Research expedition on altitude related chronic health (REACH) to Cerro de Pasco, Peru: An experimental overview. *Experimental Physiology*

2. Busch S., **Simpson LL**., Sobierajski F., Riske L., Willie CK., Ainslie PN., Stembridge M., Moore JP., & Steinback CD (2019) Muscle sympathetic reactivity to metabolic and apneic stress in high altitude Sherpa. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. In Press.*

3. Busch SA., Van Diepen S., Steele AR., Meah VL., **Simpson LL.**, Figueroa-Mujíca RJ., Vizcardo-Galindo G., Villafuerte FC., Tymko MM., Ainslie PN., Moore JP., Stembridge M & Steinback CD (2020) Global REACH: Assessment of brady-arrhythmias in Andeans and lowlanders during apnea at 4330m. *Frontiers of Physiology*. 10:1603. doi: 10.3389/fphys.2019.01603

4. Wakeham DJ., Lord RN., Talbot JS., Lodge FM., Curry BA., Dawkins TG., **Simpson LL**., Shave R., Pugh CJA., & Moore JP (2019). Upward resetting of the vascular sympathetic baroreflex in middle-aged runners. *American Journal of Physiology – Heart & Circulatory Physiology* **317***,* H181-H189.

5. Busch SA., Davies HE., Van Diepen S., **Simpson LL.**, Sobierajski F., Riske L, Stembridge M., Ainslie PN., Willie CK., Hoiland RL., Moore JP & Steinback CD (2017). Chemoreflex mediated arrhythmia during apnea at 5050m in low but not high altitude natives. *Journal of Applied Physiology* **124,** 930-937.