Sex Differences in Vascular Reactivity During Acute and Chronic Hypoxia

by

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Abstract

BACKGROUND: Current evidence suggests that males and females regulate resting blood pressure differently. This may be a function of altered sympathetic activity, neurovascular coupling, or vascular smooth muscle receptor sensitivity. In this study, we wished to determine the role of α_1-adrenoreceptors in vascular reactivity in both males and females with acute hypoxia exposure, and with early (day 2-3) and late (day 7-9) acclimatization to high-altitude hypoxia (White Mountain, CA; 3,800m). We hypothesized that 1) α_1-adrenergic sensitivity would be attenuated with exposure to hypoxia, reducing vascular reactivity to sympathetic excitation and 2) females would exhibit lesser reactivity in comparison to males.

METHODS: We studied a convenience sample of 7 males and 6 females at three time points: 1) low altitude (Calgary AB, 1,045m) breathing room air (normoxia; LOW) and acute isocapnic hypoxia ($P_{ET}O_2$ ~48mmHg; $P_{ET}CO_2$ ~37mmHg; ACUTE); 2) during early (day 2-3; EARLY) and 3) late (day 7-9; LATE) acclimatization at high altitude (White Mountain, CA; 3,800m) breathing room air ($P_{ET}O_2$ ~50mmHg) and hyperoxia (100% O_2). Heart rate (HR; bpm), mean arterial pressure (MAP; mmHg), and blood oxygen saturation (S_pO_2; %) were collected beat-by-beat. Participants performed a cold pressor test (CPT) to assess integrated cardiovascular reactivity to a sympathetic stressor. To isolate α_1-adrenergic receptor sensitivity, we delivered a graded series of phenylephrine hydrochloride (PE) injections. Statistical analysis included mixed-model ANOVAs to assess the effect of sex (male, female) and condition (LOW, ACUTE, EARLY, LATE) on vascular responsiveness, with Holm-Sidak post hoc tests when we identified main effects ($\alpha<0.05$).

RESULTS: α_1-Adrenergic specific MAP sensitivity decreased with hypoxia (main effect P=0.0027). Similarly, hypoxic conditions were associated with attenuated maximal responses to CPT (main effect, P=0.0013). Males and females experienced similar blunting of α_1-adrenergic mechanisms with hypoxia (P=0.6675). Though resting MAP and maximal responses to CPT were not statistically different between males and females (both P<0.05), there was an interaction effect between the dynamic response to CPT at LOW (P=0.0275) and ACUTE (P=0.0301), whereby females presented with an attenuated rise in MAP. However, following short-term acclimatization to high altitude, the CPT response patterns converged with those of males (P<0.05 for sex and interaction effects at EARLY and LATE). Hyperoxia did not rescue responses to LOW values for any test (all P>0.05), indicating adrenergic desensitization is the result of adaptive processes and not due to the acute effects of hypoxia.

CONCLUSIONS: Here, we have demonstrated that α_1-adrenergic receptor mediated vascular reactivity is attenuated with high-altitude hypoxia similarly in males and females. This may be due to receptor desensitization caused by increased basal sympathetic nervous activity and resulting plasma neurotransmitter concentrations. However, there is evidence that males and females may adapt to tonically elevated sympathetic activity differently, which warrants further investigation. This study offers insight into resting and reflexive vascular regulation with acclimatization to hypoxia and has implications for males and females ascending to high altitude.

Preface

This thesis is an original work by Emily R. Vanden Berg. No part of this thesis has been previously published. The research conducted for this project received ethics approval from the University of Alberta Human Research Ethics Board, under the project name: "Sex differences in sympathetic activity and vascular reactivity during acute and chronic hypoxia", (Pro00088122), approved August 1st, 2019. This project also received approval for a Health Canada Clinical Trial Application (Protocol # REMO Pro00088122 (Version 4); NOL control number 229503; Appendix III: Health Canada Clinical Trial No Objection Letter (NOL) and is registered on ClinicalTrials.gov (ID: NCT05001048). Expedition ethics were approved by the University of Calgary Conjoint Human Research Ethics Board (Protocol REB18-0374) and the Mount Royal University Research Ethics Board (Protocol 101879).

This is a study from an expedition to the Barcroft Field Research Station, White Mountain, California, USA (3,800m) as part of an international research expedition in August 2019, lead by Dr. Trevor Day of Mount Royal University, Dr. Richard Wilson of the University of Calgary, and Dr. Nicholas Jendzjowsky of the Lundquist Institute for Biomedical Innovation at Harbour-UCLA Medical Center.

Dr. Craig Steinback and I contributed to the study design, data acquisition, analysis, and interpretation. Andrew Steele and Lindsey Berthelsen contributed to data acquisition.

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Thank you to my lab-mates, friends, and family, particularly my parents, for their continued guidance and encouragement. Finally, thank you to Mitch - I am so grateful for our partnership and I could not have done this without your endless reassurance and support (as well as making sure I was always fed and caffeinated).

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List of Abbreviations

AC	Adenylyl cyclase
ACUTE	Low-altitude, acute isocapnic hypoxia condition
AMS	Acute mountain sickness
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
bpm	Beats per minute
BSA	Body surface area
Ca^{2+}	Calcium ion
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic 3',5'-guanosine monophosphate
CI	Cardiac index
CO_2	Carbon dioxide
DBP	Diastolic blood pressure
DEF	Dynamic end-tidal forcing system
E_2	Estradiol
EARLY	High-altitude, room air condition following 2-3 days acclimatization
EARLY-HYP	High-altitude, hyperoxic (100% O_2) condition following 2-3 days acclimatization
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EPI	Epinephrine
ER	Estrogen receptor
HR	Heart rate
HVR	Hypoxic ventilatory response
IP_3	Inositol triphosphate
IV	Intravenous
K_2EDTA	Dipotassium ethylenediaminetetraacetic acid
LATE	High-altitude, room air condition following 7-9 days acclimatization
LATE-HYP	High-altitude, hyperoxic (100% O_2) condition following 7-9 days acclimatization
LOW	Low-altitude, room air condition
MAP	Mean arterial pressure
MSNA	Muscle sympathetic nerve activity
NE	Norepinephrine
NO	Nitric oxide
NOS	Nitric oxide synthase
NPY	Neuropeptide Y
O_2	Oxygen
P2X	Postsynaptic ATP receptor
P2Y	Presynaptic ATP receptor
P_aO_2	Partial pressure of arterial oxygen
$P_{ET}CO_2$	Pressure of end-tidal carbon dioxide
$P_{ET}O_2$	Pressure of end-tidal oxygen
P_iO_2	Partial pressure of inspired oxygen

PKA	Protein kinase A
PKG	Protein kinase G
PLC	Phospholipase C
Q	Cardiac output
RR	Respiratory rate
SBP	Systolic blood pressure
SD	Standard deviation
sGC	Soluble guanylyl cyclase
SNA	Sympathetic nerve activity
S_pO_2	Peripheral oxygen saturation
TPC	Total peripheral conductance
TPR	Total peripheral resistance
V_E	Minute ventilation
V_T	Tidal volume
Y1	Postsynaptic NPY receptor
Y2	Presynaptic NPY receptor

Chapter 1: Introduction

1.1 Purpose

Approximately 81.6 million people worldwide live at altitudes of 2,500m or higher, and millions travel to high-altitude each year for work or tourism (Tremblay & Ainslie, 2021). The fall in atmospheric pressure results in a reduction in the partial pressure of oxygen and an associated hypoxemia. This hypoxemia causes significant physiological stress resulting in several reflex responses and adaptations across the majority of physiological systems.

Following detection of hypoxia by carotid chemoreceptors, the chemoreflex invokes activation of cardiorespiratory stimulation including an increase in ventilation to counter hypoxia (Somers et al., 1989). Meanwhile, a simultaneous increase in sympathetic nervous system activity occurs (Saito et al., 1988) to translocate blood and oxygen to critical organs and tissues.

There is evidence that sympathetic control of the cardiovascular system is altered during hypoxia. Following exposure, muscle sympathetic nervous activity – which regulates blood vessel diameter – increases substantially (Berthelsen et al., 2020; Busch et al., 2020; Hansen & Sander, 2003; Saito et al., 1988; Simpson et al., 2019). However, with acclimatization to high altitude, this is not accompanied by increases in pressure, which is maintained near sea-level values (Berthelsen et al., 2020; Busch et al., 2020; Simpson et al., 2019). In addition, there is reduced transduction of the neural signal to cardiovascular outcomes in acute (Steele, Skow, et al., 2021) and chronic (Berthelsen et al., 2020) hypoxia. The adaptations underlying this blunted transduction are not clear but may be related to downregulation of α-adrenergic sensitivity. However, few studies have explored the role of adrenoreceptors during acclimatization to high-altitude hypoxia.

Furthermore, the majority of previous research has been conducted with almost exclusively male participants. Nonetheless recent evidence suggests that males and females may respond and adapt differently to hypoxemic stress. For example, females have been shown to have a faster rise in muscle sympathetic nerve activity (Jones et al., 1999), yet an attenuated vascular resistance (Patel et al., 2014) in response to hypoxia compared to males. Differences in adrenergic receptor sensitivity to sympathetic activation may underlie these sex differences in neurovascular control (Hart et al., 2011; Barry J. Kneale et al., 2000; Schmitt et al., 2010). It has been previously documented that α-adrenergic receptors, which couple to the neurotransmitter norepinephrine, are imperative in the communication between the sympathetic nervous system and vascular control (Fairfax et al., 2013). Therefore, we aimed to elucidate the role of these receptors in both females and males in relation to adaptation to high altitude hypoxia.

1.2 Study Aims

The aims of this study were to:

1. Investigate physiological responses to sympathetic stress (cold pressor test) and direct stimulation of α_1-adrenergic receptors using a specific agonist (phenylephrine hydrochloride) during acute hypoxia as well as following more chronic hypoxia exposure during short-term acclimatization to high altitude (2-9 days at 3,800m).
2. Compare vascular reactivity in response to reflex sympathoexcitation and through direct activation of α_1-adrenergic receptors in males and females at low altitude, with acute hypoxia exposure, and during short-term acclimatization to high altitude.

1.3 Hypotheses

We hypothesized that:

1. Males and females would both have attenuated responses to sympathetic stressors and direct α_1-adrenoreceptor stimulation with high-altitude hypoxia and during short-term (<10 days) acclimatization to high altitude.
2. Vascular reactivity would be lower in females in response to the cold pressor test (a sympathetic stressor) and the α_1-adrenergic agonist phenylephrine in comparison to males.

1.4 Significance

Historically, high-altitude physiology research has focused on male participants and has lacked consideration of potential sex differences during acclimatization. The results of this study will further our understanding of differences in vascular responses in males and females, in general and specifically those travelling to high altitude. Additionally, these results may be relevant to female vascular health in clinical conditions that are characterized by hypoxemia (e.g. emphysema, sleep apnea).

Chapter 2: Literature Review

2.*1 Introduction to Systemic Blood Pressure Regulation*

Differences in basal blood pressure between the sexes may be attributable to variations in peripheral sympathetic nerve activity (Hart, Charkoudian, et al., 2009), neurovascular coupling (Hart, Charkoudian, et al., 2009; Keir et al., 2020), or specific receptor sensitivity (Hart et al., 2011; Barry J. Kneale et al., 2000). This may be underpinned by difference in levels of circulating sex hormones observed between males and females (Shearman, 2006). Here, I review the relevant systems that contribute to resting blood pressure regulation, as well as observed differences between males and females and potential mechanistic explanations.

2.1.1 Basal Vascular Regulation

The distribution of cardiac output and systemic blood pressure is regulated through contraction of blood vessels. Though cardiac output can change considerably during stress (e.g. exercise), resistance to flow (represented as total peripheral resistance; TPR) is important for opposing concurrent vasodilatory signals to maintaining adequate blood flow distribution, as well as blood pressure at rest and during stress. There is considerable evidence that vascular regulation differs between males and females. Mean arterial pressure (MAP) is typically lower at rest in females than it is in males (Usselman, Gimon, et al., 2015), and clinically, young females have a lower risk of hypertension (Gudmundsdottir et al., 2012) and a greater risk of developing hypotensive disorders (Robertson, 1999). However, this discrepancy disappears after menopause (Gudmundsdottir et al., 2012) indicating that sex hormones may play a role in these observed differences (Joyner et al., 2016). Peripheral vascular resistance and in turn blood pressure is

controlled via the interactive influences of local, circulating, and neural factors. However, this thesis focuses primarily on vascular regulation via the sympathetic nervous system.

2.1.2 Sympathetic Nervous System Activity

Control of blood pressure and flow is accomplished by changes in blood vessel diameter, particularly resistance vessels. These changes in diameter are controlled in part by the sympathetic nervous system, which directly innervates vascular smooth muscles cells. Direct measurement of 'bursts' of muscle sympathetic nerve activity (MSNA) can be measured through microneurography (Macefield, 2021). Sympathetic nerve activity directly raises resting blood pressures through tonic vasoconstrictive effects on blood vessels (Seals, 1989). This is also true for transient increases in sympathetic activation, which occurs in response to stressors. For example, the cold pressor test (CPT) elicits increases in sympathetic nerve activity, increased vascular resistance and ultimately elevated MAP (Hartwich et al., 2010; Victor et al., 1987). Combined, evaluation of basal and reflex activation of the sympathetic nervous system provides a comprehensive understanding of how MSNA may differentially contribute to vascular function between groups or conditions.

There are several differences between males and females in terms of the neurovascular control of blood pressure. At rest, mean MSNA burst frequency is lower in females than in males (Hart, Charkoudian, et al., 2009). Though there does not appear to be a relationship between resting MSNA levels and basal MAP, young males demonstrate a significant positive relationship between resting levels of MSNA and TPR, and a negative relationship with cardiac output (Hart, Joyner, et al., 2009). Conversely, this relationship between basal MSNA and the determinants of MAP is absent in young females; resting MSNA does not appear to be related to

TPR nor cardiac output (Hart et al., 2012; Hart, Charkoudian, et al., 2009). This suggests that young males and females rely on different mechanisms for blood pressure control at rest, and specifically that MSNA provides a lesser contribution in females.

It has also been demonstrated that resting MSNA increases steeply with aging in both males and females (Keir et al., 2020). Notably, this increase is more pronounced in females, who exhibit similar MSNA levels to males past the age 50 (Keir et al., 2020). In contrast to the relationship between MSNA, TPR, and cardiac output described above in young individuals, older females develop a positive relationship between MSNA and TPR (Hart et al., 2011, 2012). Since the females in this study were postmenopausal, when sex hormone levels drop off steeply, it suggests that estrogen plays a potent role in sympathetic vascular regulation in young (but not older) females (Joyner et al., 2015, 2016).

Sympathetic transduction describes the coupling of individual (or groups of) bursts of sympathetic activity and the resulting change in blood pressure (Wallin & Nerhed, 1982). This analytical approach models the relationship between systemic reactivity and prevailing sympathetic signalling. Like resting blood pressure, there is evidence suggesting differences in neurovascular transduction in males and females. At rest, females have been found to display attenuated (Briant et al., 2016) or similar (Coovadia et al., 2022; Robinson et al., 2019) neurovascular transduction as males in response to bursts of sympathetic activity. Yet, they have smaller reductions in metrics of vascular resistance (e.g. drops in blood pressure) in the absence of sympathetic bursts (Coovadia et al., 2022; Robinson et al., 2019). This suggest that females may be better able to maintain pressure with lower tonic levels of MSNA (Hissen & Taylor, 2020).

It has been established that the magnitude of spontaneous fluctuations in beat-by-beat vascular resistance is inversely related to resting levels of MSNA (Wallin & Nerhed, 1982). However, these relationships were recognised without considering possible sex differences in neurovascular balance, which is worth attention given the decreased reliance on sympathetic mechanisms in the maintenance of blood pressure in females. But sympathetic neural activity is only the first step in neurovascular communication, and there may be other downstream adaptations influencing neurovascular transduction. The frequency of MSNA bursts has been found to be inversely related to vascular reactivity to vasoconstrictive agonists, indicating adaptation across the neurovascular junction to maintain systemic blood pressure (Charkoudian et al., 2006; Hart, Charkoudian, et al., 2009). Thus, consideration must also be given to post-junctional neuroeffector mechanisms, including the specific receptors that the sympathetic nervous system utilizes in coupling to vascular control.

2.1.3 Neuroeffector and Receptor Influences

When activated, postganglionic sympathetic neurons innervating the vascular smooth muscle release various neurotransmitters which interact with specific receptors. The activation of these receptors regulates intercellular calcium, smooth muscle contractility, vascular cross-sectional area, and ultimately vascular resistance and blood pressure (**Figure 1**).

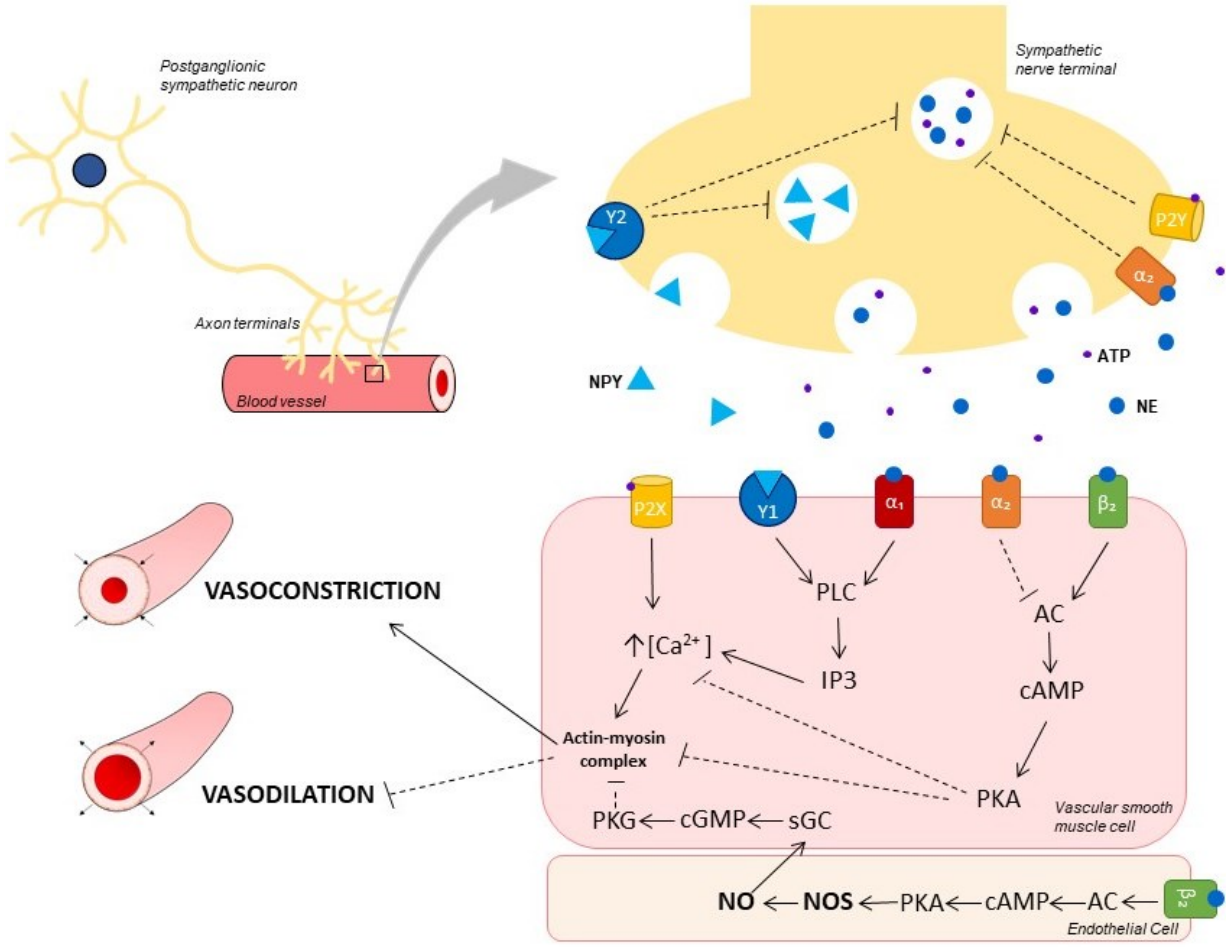

Figure 1. Sympathetic neurotransmitter signalling cascades. Sympathetic activity induces release of adenosine triphosphate (ATP), norepinephrine (NE) and neuropeptide Y (NPY) from the synaptic cleft. Subsequent downstream vasoconstriction or vasodilation of vascular smooth muscle by enacting signalling cascades through specific receptor subtypes. ATP couples with post-synaptic P2X receptors which are associated with ligand gated ion channels allowing influx of calcium (Ca^{2+}). Calcium interacts with actin, allowing for binding of myosin light chains and inducing vasoconstriction. Through activation of Y1 and α_1-adrenergic receptors by NPY and NE respectively, phospholipase C (PLC) is activated, generating inositol triphosphate (IP_3), and inducing increases in intracellular Ca^{2+} concentrations and vasoconstriction. NE may also activate β_2-adrenoreceptors increases cyclic adenosine monophosphate (cAMP) through adenylyl cyclase (AC) activation, thereby activating protein kinase A (PKA) which both inhibits myosin de-phosphorylation and decreases intracellular calcium, inhibiting counteracting constrictive effects. Conversely, α_2 receptors inhibit this process, favouring vasoconstriction. Additionally, β_2 adrenoreceptors can be found on vascular endothelium. Stimulation promotes vasodilation through activation of nitric oxide synthase (NOS), generating nitric oxide (NO). NO promotes production of cyclic 3',5'-guanosine monophosphate (cGMP) through soluble guanylyl cyclase (sGC), inactivating myosin through protein kinase G (PKG). Pre-synaptic receptors for NE, NPY, and ATP (α_2, Y2, and P2Y, respectively) are located on the sympathetic nerve terminal, where stimulation inhibits further release of neurotransmitters.

Activity of efferent sympathetic neurons causes the release of neurotransmitters, including adenosine triphosphate (ATP), neuropeptide Y (NPY), and norepinephrine (NE) (Bradley et al., 2003; Lundberg et al., 1982; Pernow et al., 1987). NPY acts as a neuromodulator and is preferentially released during high frequency nervous stimulation, usually during stressors (Kennedy et al., 1997). It enacts its post-junctional vasoconstrictive effects through Y1 receptors, which activate phospholipase C (PLC), causing increases in inositol triphosphate (IP_3), thereby increasing cellular calcium concentrations (Pons et al., 2008). It also interacts with Y2 receptors on the pre-synaptic cleft, inhibiting further release of NPY and other sympathetic neurotransmitters (Smith-White et al., 2001). ATP is a fast-acting molecule that binds to P2X ligand gated calcium channels on the vascular smooth muscle cells causing immediate influx of calcium and promoting muscle contraction (McLaren et al., 1998). On the SNS side of the synaptic cleft, G-protein coupled P2Y receptors cause inhibition of further neurotransmitter release (Gonçalves & Queiroz, 1996).

Increases in sympathetic nervous system activity are correlated with increases in plasma catecholamines (Victor et al., 1987) which includes NE, the predominant SNS neurotransmitter eliciting vasoconstriction (Johnson et al., 2001). NE is co-released with ATP and interacts with various subtypes of G-protein coupled adrenergic receptors. Adrenergic receptors cause vasoconstriction or vasodilation of the smooth muscle of blood vessels depending on the receptor subtype: α- and β-adrenergic receptors cause contraction and relaxation of the vascular smooth muscle, respectively. A summary of the actions of these receptor subtypes and their mechanisms of action is depicted in **Figure 1**.

α_1-Adrenergic Receptors. Vasoconstriction is mediated by sympathetic stimulation of α-adrenergic receptors located on vascular smooth muscle cells (Fairfax et al., 2013). These

receptors bind to catecholamines NE and epinephrine (EPI), but NE is the main effector of sympathetic nerve terminals (Taylor & Cassiagnol, 2021). α_1-Adrenoreceptors are located on the vascular smooth muscle cells, and interaction with NE leads to activation of PLC and generation of IP_3, resulting in an increase in cellular calcium and therefore in smooth muscle contraction (Wier et al., 2009). This results in the reduction of the diameter of blood vessels, increasing vascular resistance and influencing whole-body blood pressure.

α_2-Adrenergic Receptors. Like α_1-adrenergic receptors, α_2's located on vascular smooth muscle cells also contribute to vasoconstriction. A study by Dinenno and colleagues (2002) found that they have a greater contribution to resting vascular resistance than α_1-adrenoreceptors. Following activation, α_2-adrenergic receptors work to inhibit formation of cyclic adenosine monophosphate (cAMP) through adenylyl cyclase (AC), which normally prevents the association of myosin and actin for smooth muscle contraction through protein kinase A (PKA) activity, and decreases intracellular calcium (Durkee et al., 2019). α_2-adrenoreceptors are also located pre-junctional varicosities of the post-ganglionic sympathetic neurons and bind NE to inhibit further release of the neurotransmitter (Shepherd & Vanhoutte, 1985).

β_2-Adrenergic Receptors. In contrast to α-adrenergic receptors, β_2 receptors cause a cellular cascade that restricts vasoconstriction, subsequently inducing dilation of resistance vessels (Queen & Ferro, 2006). These receptors preferentially bind circulating EPI, but will also bind NE, inducing vasodilatory influences that are in competition with α-adrenergic constrictive mechanisms (Westfall & Westfall, 2015). When activated, these receptors elicit relaxation of the vascular smooth muscle though AC activation and increasing cAMP within the cell, thereby activating PKA (Somlyo & Somlyo, 1994). This lowers the affinity of myosin for calmodulin by phosphorylation, inducing relaxation (Murray, 1990). There is also evidence to suggest that β_2-

adrenergic receptors enact a considerable portion of their vasodilatory activity through increases in nitric oxide (NO) (Ferro et al., 1999). β_2-adrenoreceptors are also found on vascular endothelial cells and induce a cAMP mediated cascade similar to that observed in vascular smooth muscle cells, activating nitric oxide synthase (NOS) (Ferro et al., 1999). Once generated, NO acts on the vascular smooth muscle through soluble guanylyl cyclase (sGC) (Farrell & Blake, 1996), which converts guanosine triphosphate (GTP) to cyclic 3',5'-guanosine monophosphate (cGMP), ultimately causing relaxation of resistance vasculature through inactivation of myosin by protein kinase G (PKG) (Furchgott & Zawadzki, 1980; Palmer et al., 1987; Queen & Ferro, 2006).

Sex differences. It is important to recognize the role of each type of receptor when considering blood pressure control between sexes. It has been suggested that the sensitivity and/or density of α- and β-adrenergic receptors is altered in females in comparison to males, contributing to differences in vascular control between the sexes. A study by Schmitt et al. (2010) demonstrated that females have a smaller decrease in MAP in response to an α-adrenergic receptor blockade than males, suggesting these receptors play a lesser role in basal blood pressure regulation in females. It has also been demonstrated that the sympathetic nervous system mediates basal blood pressure through α_1-adrenoreceptor stimulation in males (Fairfax et al., 2013). However, sex differences in α_1-adrenergic reactivity to sympathetic stressors is less understood.

Notably, there is evidence that β-adrenergic receptor stimulation plays a greater role in vascular regulation in young females when compared to males. For example, females have an augmented increase blood flow in response to local infusions of the β-adrenergic agonist

salbutamol (Barry J. Kneale et al., 2000). Females also display attenuated increases in vascular resistance with NE infusion, which activates both α- and β-adrenergic receptors (Hart et al., 2011; Barry J. Kneale et al., 2000). However, when co-infused with the β-antagonist propranolol, females subsequently displayed augmented vasoconstriction to norepinephrine in comparison to the non-antagonist control condition (Hart et al., 2011; Barry J. Kneale et al., 2000). Taken together, these studies suggest that the greater degree of vasodilation induced by the β_2-adrenergic receptors in young females is likely offsetting α_1-adrenoreceptor mediated vasoconstriction (Hart et al., 2011; Joyner et al., 2016).

2.1.4 Insights into Effects of Sex Hormones

There is considerable evidence to suggest that estrogen influences vascular resistance, and could exert cardioprotective effects regardless of sex (Shearman, 2006). Importantly, estradiol receptors exist both on the endothelium and smooth muscle cells of blood vessels, pointing to their possible role in vascular regulation (Mendelsohn & Karas, 1999). Indeed, estrogen exerts vasodilatory effects and appears to play a crucial role in endothelium-dependant vasodilation through upregulation of NOS (V. M. Miller & Mulvagh, 2007; Rubanyi et al., 1997). Additionally, estrogen receptors are found on vascular smooth muscle cells, where activation leads to upregulation of β_2-adrenoreceptors, (Machuki et al., 2018), and downregulation of α_1 receptors (González-Arenas et al., 2006). **Figure 2** illustrates the effects of estradiol on the vasculature.

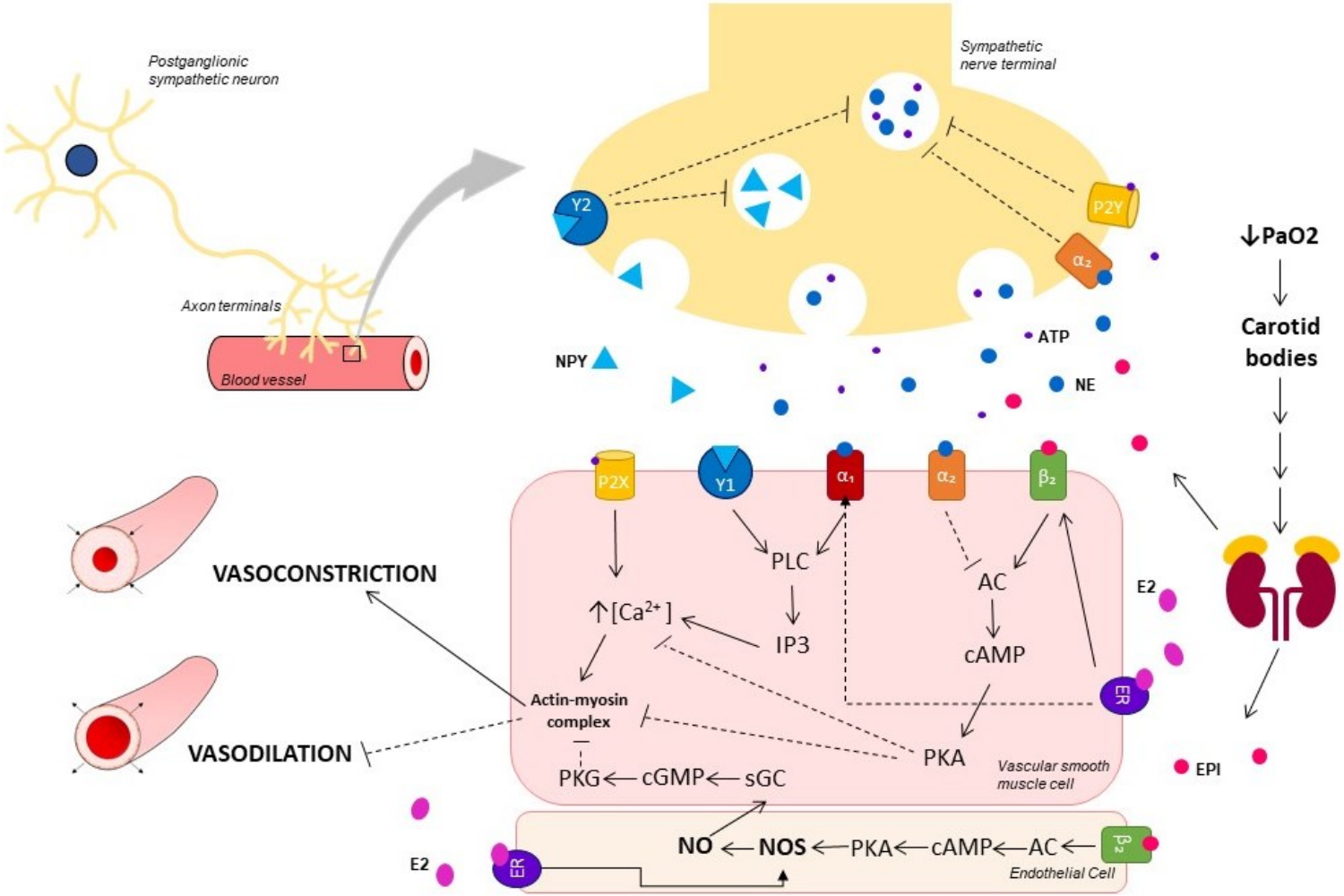

Figure 2. Effects of systemic hypoxia (P_aO_2) and estradiol (E2) on sympathetic neurotransmitter signalling cascades. Through binding to estrogen receptors (ERs) on the vascular endothelium, E2 promotes production of NOS, thereby increasing NO which leads directly to vasodilation. There are also ERs present on vascular smooth muscle cells, where stimulation causes up-regulation of β_2-adrenergic receptors, and downregulation of α_1-adrenoreceptors. Hypoxia is sensed by the carotid bodies, which activate the chemoreflex and through a series of steps stimulates the sympathoadrenal system to produce EPI. This binds to β_2-adrenergic receptors on the endothelium and vascular smooth muscle cells to promote vasodilatory mechanisms.

It has also been shown that menstrual cycle phase, which involves the natural fluctuation of sex hormones including estrogen and progesterone, has an impact on resting sympathetic activity. Though resting MAP remains similar, basal MSNA burst frequency and incidence as well as total MSNA are higher during the midluteal (high hormone) phase of the menstrual cycle than during the early follicular (low hormone) phase (Minson et al., 2000; Usselman, Gimon, et al., 2015). Though burst incidence was always higher in men, burst frequency was only higher in men compared to women during the early follicular phase, not midluteal (Usselman, Gimon, et al., 2015). Additionally, plasma concentrations of NE are significantly higher during the midluteal phase (Minson et al., 2000), indicating heightened sympathetic activity. This is also reflected in females taking cyclic oral contraceptives, with those in the high hormone phase having higher MSNA burst frequency and incidence than the low hormone phase (Usselman et al., 2013). These parameters throughout the menstrual cycle are summarized in **Figure 3**. The dissociation between fluctuation in MSNA and MAP suggests another adaptive mechanism that dampens the influence of MSNA on the vasculature.

In post-menopausal females (who have less circulating estrogen), ganglionic block (attenuating sympathetic outflow) induced a larger decrease in blood pressure compared to young women, and this was also proportional to resting MSNA levels (Jones et al., 1999). Post-menopause, the vaso-protective effects of estrogen appear to be diminished, as MSNA and vascular resistance become closely related, which is similar to the relationship observed in young females, but not males (Joyner et al., 2016). Together, these studies support the vasodilatory influence of estrogen. Therefore, sex hormone concentrations may be an important contributor to the variations observed in resting sympathetic control of blood pressure in females.

Sympathetic responsiveness to external stressors also appears to be dependant on circulating hormone levels. In response to chemoreflex stress, females undergoing the low-hormone phase of oral contraceptive use experienced augmented MSNA reactivity than during their high-hormone phase (Usselman et al., 2013). This is in contrast to the changes in baseline MSNA through the menstrual cycle, but may instead indicate a ceiling effect in sympathetic recruitment during stress (Usselman et al., 2013). Though differences were observed in sympathetic reactivity, MAP responsiveness was unaltered by oral contraceptive phase, indicating transduction of sympathetic signals may be attenuated with high hormone concentrations (Usselman et al., 2013).

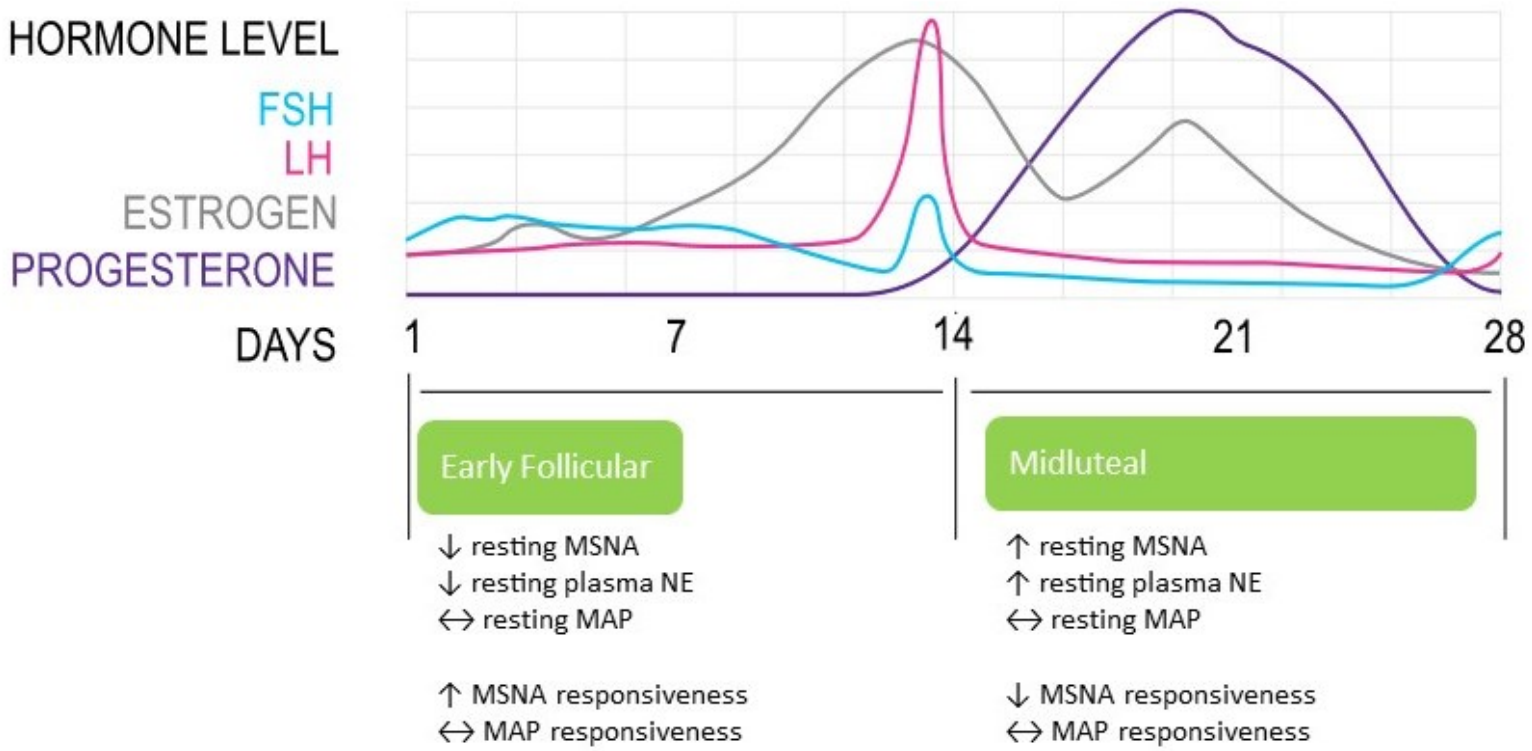

Figure 3. Changes in neurovascular parameters throughout the menstrual cycle. During the early follicular phase (low hormone), resting muscle sympathetic nervous activity (MSNA), as well as plasma concentrations of norepinephrine (NE), are attenuated in comparison to midluteal (high hormone), whereas resting mean arterial pressure (MAP) remains relatively unchanged. However, MSNA responsiveness is augmented during early follicular, though MAP reactivity is also consistent throughout the cycle. (From MenstrualCycle2 en, by Isometrik, 2010, Wikimedia Commons (https://commons.wikimedia.org/wiki/File:MenstrualCycle2_en.svg#file). CC BY-SA 3.0).

As described previously, though resting MSNA fluctuates throughout the menstrual cycle, resting MAP does not. This indicates a possible compensatory mechanism for heightened sympathetic activity resulting in lessened neurovascular transduction. There is evidence that sex hormones have direct effects on adrenergic receptor activity. In addition to promoting basal release of the nitric oxide (V. M. Miller & Duckles, 2008; Sudhir et al., 1996), there is an indication that estrogen augments the vasodilatory influences of β-adrenergic receptors (Joyner et al., 2016). β-adrenoreceptor mediated vasodilation appears to be blunted in females post-menopause in comparison to young females, when levels of estrogens are diminished (Hart et al., 2011), suggesting that estrogen may up-regulate β-sensitivity. Further, the differences in vasoreactivity observed between males and females disappear post-menopause (Joyner et al., 2015; Patel et al., 2014). More specifically, the vasodilatory influences of estrogen are also evidenced by the fact that estrogen supplementation is associated with decreased sensitivity to NE (Sudhir et al., 1997). Further, acute infusion of estrogens in post-menopausal females up-regulates vasodilatory reactivity (Gilligan et al., 1994). Taken together, these studies demonstrate the importance of estrogen in the regulation of blood pressure in females, and a possible mechanism contributing to observed differences.

2.2 Reflex Control of Blood Pressure

Apart from tonic SNA regulating basal blood pressure, the sympathetic nervous system becomes more active in response to stress. This includes changes in blood gasses (i.e. chemoreflex) or pressure (i.e. baroreflex), and in response to metabolites (i.e. metaboreflex) or pain to maintain homeostasis. Following detection of an imbalance or stimulus, efferent nerve signals are integrated in the brainstem where sympathetic activity is heightened or supressed. This allows for fine-tuned alterations in vascular resistance and cardiac output to modify blood

flow in relation to the stimulus. Specific to this thesis, the following considers the chemoreflex and the nociceptive (generalized pain) reflexes.

2.2.1 The Peripheral Chemoreflex

The peripheral chemoreceptors, or the carotid bodies, are located bilaterally at the bifurcation of the internal and external carotid arteries. These organs contain glomus cells that are extremely sensitive to changes in blood oxygen and pH levels (Nurse, 2005). Central chemoreceptors also exist in the ventral medulla oblongata and are sensitive to carbon dioxide. Fluctuations in blood gases, such as hypoxia or hypercapnia, will invoke the chemoreflex in order to ensure proper oxygen delivery to organs and tissues. Though the chemoreflex is also sensitive to hypercapnia (high P_aCO_2), this review will focus on the effects of hypoxemia (low P_aO_2) as experienced acutely and at high altitude.

Following stimulation of the chemoreceptors in the carotid bodies, afferent signals are translated to the brainstem for integration through the carotid sinus nerve. These efferent signals travel to the nucleus of the solitary tract, and then to the respiratory pattern generator and rostral ventrolateral medulla (Guyenet, 2014). Pre-ganglionic efferent sympathetic nerve signals receive input from these centres and relay sympathetic impulses to post-ganglionic nerves, which exert excitatory effects on their target organs. In the case of low P_aO_2 tension, ventilation will immediately increase in an attempt to correct blood gasses to baseline tensions (Loeppky et al., 2001; Somers et al., 1989). Though hypocapnia as a result of this hyperventilation has a small effect during chemoreflex stimulation, reductions in oxygen appear to be the main driver of changes in vascular reactivity (Heistad & Wheeler, 1970). Most relevant to this study, the

increase in sympathetic outflow will induce vasoconstriction and a redistribution of blood flow to critical organs and tissues (Marshall, 2015).

Though changes in respiration can modulate sympathetic outflow (Guyenet, 2014), and ventilation is generally higher in females, there does not appear to be a difference in terms of the ventilatory response to the peripheral chemoreflex in males and females (Loeppky et al., 2001; Sayegh et al., 2022). It has been demonstrated that chemoreflex activation is associated with less vasoconstriction in females compared to males (Patel et al., 2014). However, changes in MSNA appear to be similar between males and females in response to peripheral chemoreceptor activation (Sayegh et al., 2022). This indicates that there are possible downstream sympathetic-vascular communication differences. Therefore, it is important to also consider other methods whereby sympathoexcitation can be elicited.

2.2.2 Sympathoexcitatory Tests

Sympathetic stress tests, such as the cold pressor test (generalized pain) can be utilized to examine the mechanisms surrounding the cardiovascular response to sympathetic stress (Fagius et al., 1989; Fonkoue & Carter, 2015; Victor et al., 1987). This sympathoexcitation causes release of NE and produces a pressor response, and causes increases in MAP (Hartwich et al., 2010; Victor et al., 1987). These pressure increases are accomplished by vasoconstriction of peripheral vasculature, as evidenced by decreased forearm blood flow and increases in forearm vascular resistance (Hartwich et al., 2010).

However, there appear to be sex differences in the pressor response to sympathetic stimulation, despite a similar rise in MSNA. In response to CPT, females have been shown to display cutaneous vasodilation, whereas males vasoconstrict (Patel et al., 2014). Females also

present with opposing lower limb vasodilation, offsetting vasoconstrictive responses to sympathetic activation (A. J. Miller et al., 2019). Additionally, females exhibit attenuated increases in MAP and common carotid artery diameter compared to males (Stone et al., 2019). Together, this evidence indicates differential regulation of blood pressure and vessel resistance in response to acute sympathetic activation in females.

2.3 Introduction to High-Altitude Hypoxia

As of 2021, 81.6 million people live at or above 2,500m of elevation (Tremblay & Ainslie, 2021), and millions more travel to high-altitude locations every year for employment and tourism. Exposure to low oxygen, or hypoxia, causes profound physiological adaptations in order to maintain adequate oxygen delivery to tissues. Some of these adaptations occur within minutes of low-oxygen exposure, and further adaptations occur over days, weeks, years, and even generations of high-altitude hypoxia exposure.

2.3.1 Oxygen Delivery

As individuals ascend to high altitude, they will experience decreases in barometric pressure. Though the percentage of atmospheric oxygen available remains consistent, the partial pressure of inspired oxygen (P_iO_2; mmHg) decreases along with increases in elevation. The relationship between barometric pressure and inspired oxygen tension can be represented by *Equation 1*.

Equation 1. Relationship between barometric pressure (mmHg) and partial pressure of inspired oxygen (P_iO_2), where F_iO_2 is the fraction of inspired oxygen.

$$P_iO_2 = F_iO_2 * (barometric\ pressure - saturated\ vapour\ pressure\ of\ H_20)$$

With less oxygen available, along with lowered driving pressure, individuals will experience hypoxemia in the form of reduced partial pressure of arterial oxygen (P_aO_2) and peripheral oxygen saturation (S_pO_2) (Beall, 2007). In general, the effects of hypoxemia on human physiology become noticeable at arterial partial pressures of oxygen at or below ~60 mmHg (Marshall, 2015). These physiological changes and adaptations occur in order to preserve the delivery of oxygen to organs and maintain normal function of tissues.

2.3.2 Sympathetic Response to Hypoxia

Also considered a sympathetic stressor, MSNA is elevated during exposure to both acute (Saito et al., 1988) and high altitude hypoxia (Berthelsen et al., 2020; Busch et al., 2020; Hansen & Sander, 2003; Simpson et al., 2019) as a consequence of chemoreflex activation. Despite this augmentation of resting sympathetic activity, MAP is maintained near sea-level values (Berthelsen et al., 2020; Busch et al., 2020; Simpson et al., 2019).

As much of the recent work assessing changes in resting sympathetic activity at high altitude is primarily in males and does not evaluate sex differences (Berthelsen et al., 2020; Busch et al., 2020; Hansen & Sander, 2003; Simpson et al., 2019), it is important to consider the effects that sex has on MSNA when exposed to hypoxia at altitude. Though the magnitude of change is similar in response to hypoxia, females have been shown to have a faster rise MSNA (Jones et al., 1999). However, there does not appear to be a difference between sexes in the magnitude of increases in catecholamines with chronic exposure to high altitude (Mazzeo et al., 1998, 2000).

2.4 Cardiovascular Responses to Hypoxia

Increases in MSNA in response to hypoxic stress has profound impacts on the cardiovascular system. Although sympathetic activity is elevated, blood pressure is maintained near sea-level values in part due to interactions between sympathetic vasoconstriction and hypoxia-induced local vasodilation (Marshall, 2015).

Another contributing factor is the observation that the communication between sympathetic nerve signals and systemic blood pressure (i.e. sympathetic neurovascular transduction) is blunted with both acute (Steele, Skow, et al., 2021) and high-altitude hypoxia (Berthelsen et al., 2020). This points to changes in how the blood vessels respond to sympathetic bursts of activity, suggesting changes in neurotransmitter kinetics or receptor sensitivity may be present.

2.4.1 Acute (Normobaric) Hypoxia

High altitude hypoxia can be simulated in the laboratory at sea level, though this condition is often normobaric and achieved by adjusting the proportion of inspired oxygen. Sympathetic activity increases progressively with hypoxia severity (Rowell et al., 1989). Though MSNA is elevated rapidly upon chemoreflex activation induced by hypoxia (Saito et al., 1988), vasodilation, and therefore a decrease in vascular resistance, is initially observed in the periphery with acute hypoxia (Blitzer et al., 1996), termed hypoxia-induced sympatholysis (Marshall, 2015). Although increases in NO production with hypoxia contribute to relaxation of vascular smooth muscle cells (Blitzer et al., 1996; Casey et al., 2014), a considerable portion of this phenomenon is attributable to increases in β-receptor stimulation (Blauw et al., 1995; Weisbrod et al., 2001). Acute bouts of hypoxia will induce increases in plasma EPI concentration, which

may be a result of increased sympathoadrenal drive (Mazzeo & Reeves, 2003). This mechanism of action on the vascular system is illustrated in **Figure 2**. Conversely, NE concentrations are not observed to increase immediately with acute hypoxia (Rostrup, 1998). This may be a consequence of augmented NE clearance at the synaptic cleft (Leuenberger et al., 1991). Given the greater importance of β-adrenergic receptors in regulating vascular resistance in females, it is not surprising that there is a greater degree of vasodilation observed in females in comparison to males when exposed to acute bouts of hypoxia (Casey et al., 2014).

In addition to vasodilatory influences counteracting sympathetically mediated vasoconstriction in the tonic regulation of basal blood pressure, hypoxia induces changes in the reactivity of vascular smooth muscle in response to sympathetic stressors. Along with resting vascular and sympathetic adaptations to hypoxia, there also is observed changes in vascular reactivity to sympathetically mediated stressors. For example, vasoconstrictive responses to lower body negative pressure (LBNP) and CPT appear attenuated during hypoxia (Heistad & Wheeler, 1970). As vasoconstriction to direct infusions of NE are also lowered, it has been suggested that affinities or sensitivities of adrenergic receptors are responsible for this discrepancy (Heistad & Wheeler, 1970). And as such, may have differential outcomes in males and females. However, there is still uncertainty about the differences between males and females in this reduction of reactivity with hypoxia.

2.4.2 High-Altitude (Hypobaric) Hypoxia (short term acclimatization <10 days)

Sustained high altitude hypoxia exposure differs from the majority of lab-based studies in that it is hypobaric. The decreases in partial pressures of inspired oxygen (P_iO_2) are a function of

increasing elevation, and subsequently result in reductions in oxygen tension throughout the oxygen delivery cascade (Beall, 2007).

Basal levels of sympathetic activity are augmented with high-altitude hypoxia, which is also evidenced by increased circulating catecholamine levels (Hansen & Sander, 2003). This suggests an overall enhanced sympathetic nervous system activation at high altitude. Yet, vascular resistance is preserved, contributing to maintenance of blood pressure (Hansen & Sander, 2003). Since NE clearance does not appear to remain enhanced as it is with acute hypoxia (Calbet, 2003), this change in the balance of MSNA and MAP is possibly through blunting of sympathetic neurovascular transduction, and the degree seems to be inversely proportional to an individual's basal sympathetic activity (Berthelsen et al., 2020).

There are few studies that assess the differences between males and females in the context of high-altitude acclimatization. Many field expedition studies include primarily males, and do not account for sex differences. Though a field study observing hemodynamic changes in females at altitude has been conducted, that particular study failed to directly compare males and females (Zamudio et al., 2001). Therefore, completing the current investigation in both males and females on a field study at high altitude not only provides external validity, but fills a gap in the literature surrounding sex differences at high altitude.

2.5 Summary

Previous studies on neurovascular function at high altitude have been conducted primarily in males, without consideration for differences in sex throughout acclimatization. Given the evidence that suggests females have reliance on differential mechanisms in the neural control of blood pressure, it is important to consider how they may adapt to high altitude hypoxia

in ways that are different from males. This study examined sex differences as an *a priori* research question and contributes to the field of high-altitude adaptation.

Chapter 3: Methods

This study followed the principles set out by Government of Canada Tri-council Policy on Research Ethics Policy Statement (TCPS2) and the Declaration of Helsinki. Ethical approval was obtained through the University of Alberta Health Research Ethics Board – Biomedical Panel (Pro00088122; August 1st, 2019;

Appendix I: Ethics Approval for Human Subjects (University of Alberta)), the University of Calgary Conjoint Human Research Ethics Board (Protocol REB18-0374; Appendix II: University of Calgary Conjoint Health Research Ethics Board (CHREB) Approval) and Health Canada (Protocol # REMO Pro00088122 (Version 4); NOL control number 229503; Appendix III: Health Canada Clinical Trial No Objection Letter (NOL) The project is registered on ClinicalTrials.gov (ID: NCT05001048). This study was part of a research expedition to the Barcroft Field Research Station (3,800m) at White Mountain, California in August 2019. Though participants were involved in other concurrent protocols, each study addressed distinct *a priori* research questions. Researchers ensured adequate time between studies to allow for washout of associated interventions and avoid contamination of results.

Low-altitude testing was conducted in Calgary, AB (1,045m) during the week prior to departure for the expedition. Participants then travelled to Las Vegas, NV, United States by plane, then to the Barcroft Field Research Station (~3,800m) on White Mountain, CA by car (**Figure 4**) over ~5-6 hours for high-altitude testing. Due to time constraints during low-altitude testing, three participants were tested in Edmonton, AB (645m) after at least one month upon return from the expedition.

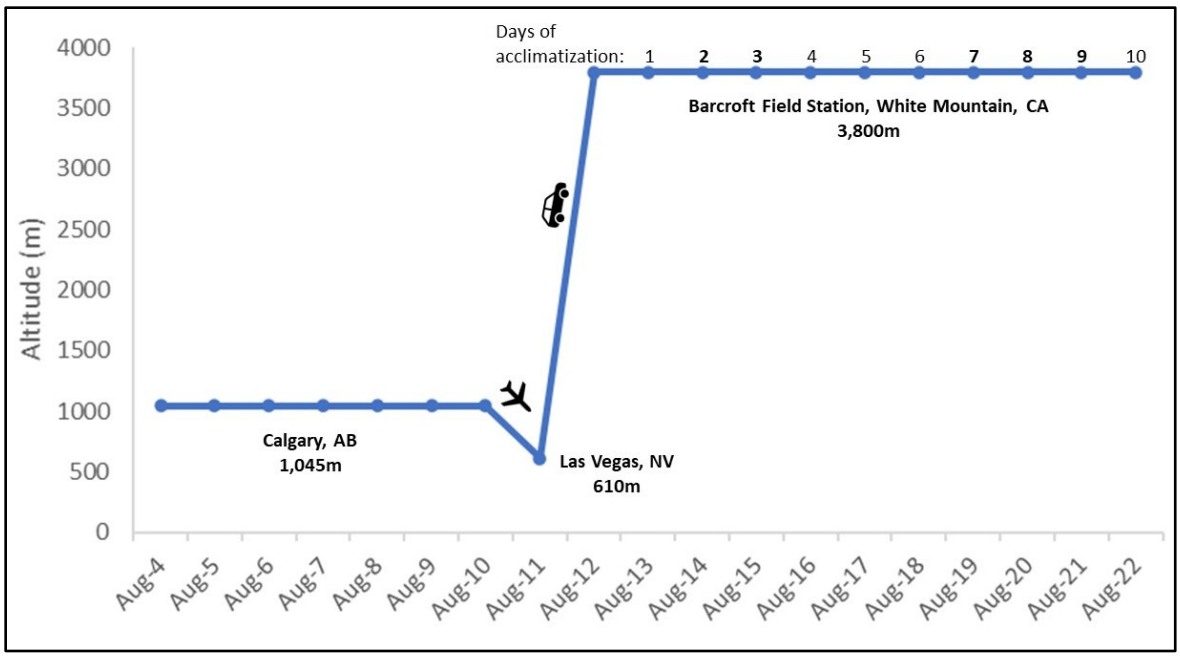

Figure 4. Timeline and ascent profile. **Top panel:** Graphical representation of testing altitudes and timeline. Low-altitude testing was completed during the stay in Calgary, AB (1,045m) (except for three participants, who were tested in Edmonton, AB (645m) following >1 month following decent from high altitude). Participants travelled by plane to Las Vegas, NV where they remained for one night, then travelled by car to the Barcroft Field Station (~3,800m). Early acclimatization testing was completed on day 2 or 3, and late on day 7, 8, or 9. **Bottom panel:** Route and ascent profile from Las Vegas, NV to the Barcroft Field Station (~3,800m) located on White Mountain, CA. Participants travelled by car. Altitude remained relatively consistent for ~340 km, then 2,245m of elevation was gained over approximately two hours.

3.1 Study Population

A convenience sample of n=13 (7 male, 6 female) participants who were members of the expedition were tested. Before the first testing session, participants provided written informed consent (Appendix IV: Consent Form for Participants) and filled out a health history questionnaire (Appendix V: Health History Questionnaire). All participants were non-smokers, and free of known cardiovascular disease. Participants had not been exposed to altitudes of >2,000m for at least two months prior to testing. No participants were taking any medications for relieving altitude sickness (e.g. acetazolamide, dexamethasone). However, 1 participant reported taking prescription anti-anxiety/anti-depression medication (Sertraline, Lorazepam), 2 participants reported taking prescription medication for treating attention deficit hyperactivity disorder (Concerta, Ritalin, Vyvanse, Adderall), 1 participant reported taking medication to treat inflammation/eczema (Prednisone, Dupixent, Alitretinoin), 1 participant reported taking an inhaler for treatment of asthma symptoms (Advair; fluticasone and salmeterol), and 1 participant reported taking antifungal medication (Terbinafire). These medications were kept consistent throughout testing, and therefore were not expected to effect intra-individual variability. Height was measured with a stadiometer and weight was measured with standard calibrated digital scale during pre-expedition testing. Females were confirmed to not be pregnant by a negative urinary pregnancy test prior to the first testing session. All female participants were using hormonal intrauterine devices (IUDs) throughout the testing period (Mirena, n=3; Kyleena, n=1; Jadess, n=1; Liletta, n=1). As such, menstrual cycle phase was not controlled, nor reported due to female participants having irregular or absent menstrual cycles.

3.2 Study Design

Participants were tested at three time-points: 1) a baseline assessment at low-altitude (Calgary AB, 1,045m; or Edmonton AB, 645m); 2) during early acclimatization (day 2-3); and 3) during late acclimatization (day 7-9) at high-altitude (White Mountain CA, 3,800m). During the baseline assessment at low-altitude, participants were tested while breathing room air (i.e. normoxia; $P_{ET}O_2$ ~85mmHg; LOW) and during exposure to acute isocapnic hypoxia ($P_{ET}O_2$ ~48mmHg; $P_{ET}CO_2$ ~37mmHg; ACUTE) equivalent to oxygen conditions at ~3,800m (Steinback & Poulin, 2008). High-altitude assessments were performed breathing room air ($P_{ET}O_2$ ~51mmHg) during early (EARLY) and late (LATE) acclimatization, as well as during exposure to hyperoxia (100% O_2; EARLY-HYP and LATE-HYP, respectively). See **Figure 5** for a detailed protocol schematic, including interventions and measurements.

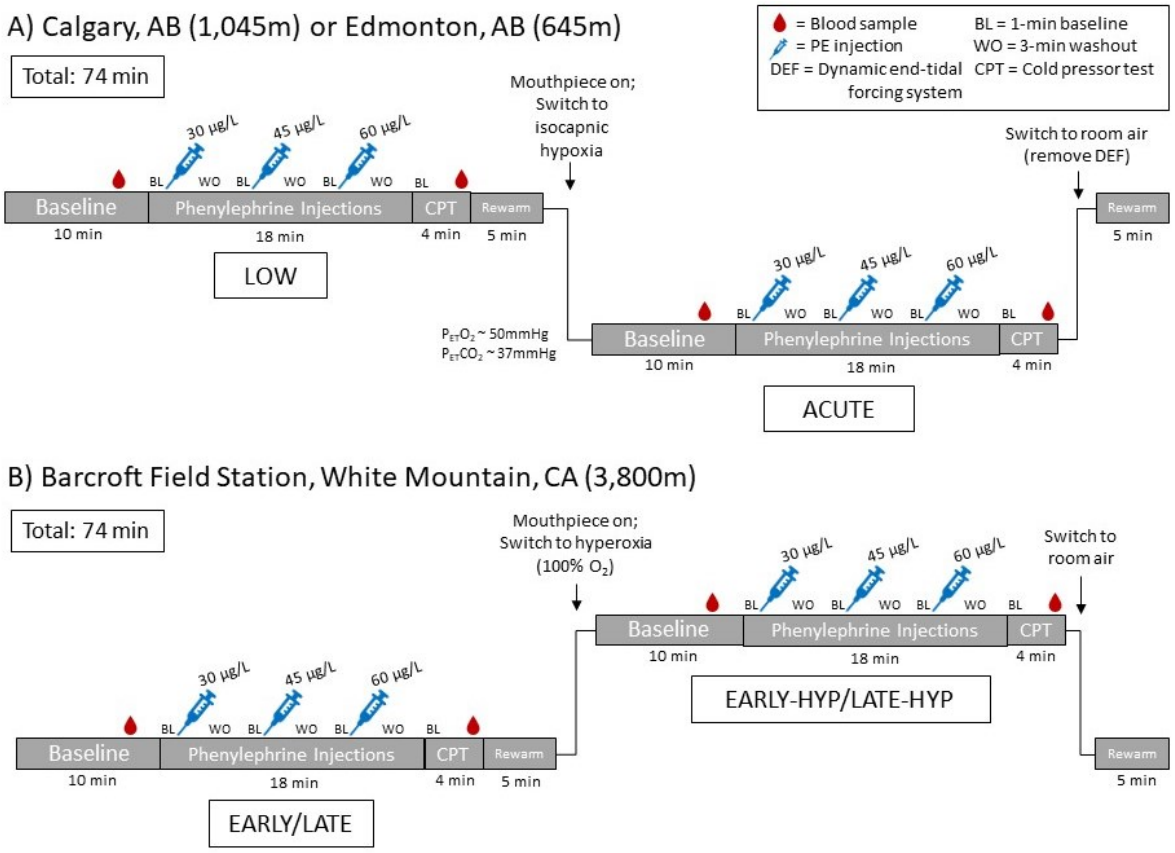

Figure 5. Protocol overview and study design. Each participant was tested at three time points: 1) Baseline testing in Calgary, AB (1,045 m) or Edmonton, AB (645m); 2) Early during high-altitude acclimatization and 3) Late during high-altitude acclimatization at the Barcroft Research Station on White Mountain, CA (3,800m). **A)** The low-altitude assessment involved a 10-minute resting baseline, graded PE injections (indicated by 💉), and CPT under a room air condition ($P_{ET}O_2$ ~85mmHg; LOW), repeated with acute isocapnic hypoxia ($P_{ET}O_2$ ~48mmHg; $P_{ET}CO_2$ ~37mmHg; ACUTE) delivered by a DEF system. **B)** High-altitude assessments involved identical tests under a room air condition ($P_{ET}O_2$ ~51mmHg) at day 2 or 3 (EARLY) and day 7, 8, or 9 (LATE), repeated with hyperoxia (100% O_2; EARLY-HYP and LATE-HYP). Blood samples (indicated by 🩸) for plasma catecholamines were collected during Baseline and during the last minute of CPT. HR, brachial blood pressure, and S_PO_2 were collected continuously throughout the protocol. Ventilation and end-tidal gases were measured immediately preceding the switch to either isocapnic hypoxia or hyperoxia, and throughout the second half of each assessment.

3.3 Reactivity Assessments

3.3.1 Phenylephrine Hydrochloride Injections

In order to determine specific vascular α-adrenergic sensitivity, the drug phenylephrine hydrochloride (PE) was used (Purdy et al., 2019; Simpson et al., 2019). This caused direct stimulation of the α_1-adrenergic receptors independent of endogenous neurotransmitters through sympathetic neural activity. Administration of pharmaceuticals can be an invaluable tool to elucidate the role of specific receptors in vascular regulation and the control of blood pressure. As described previously, the main sympathetic neuroeffector is NE which readily binds to α_1-adrenoreceptors causing vasoconstriction. Though infusion with NE has been utilized previously both systemically and locally in the forearm (Heistad & Wheeler, 1970), it does not allow for targeted application to a preferred receptor, as it also has affinity for α_2-adrenergic receptors which influence NE reuptake and β_2-adrenergic receptors which cause conflicting vasodilation. PE has been used extensively both systemically (intravenous bolus injections) as well as locally in the forearm (graded low-dose infusions). As an α_1-adrenergic receptor agonist, it causes rapid yet transient increases in blood pressure lasting 60-90 seconds (Hart et al., 2010; Rudas et al., 1999) without a preceding rise in sympathetic activity and endogenous NE release. Given its specificity for α_1-adrenergic receptors, PE can be utilized to assess their precise role in blood pressure regulation and vascular reactivity.

A series of PE bolus injections were delivered via an IV catheter in an antecubital vein (see 3.4 Instrumentation and Measurements below). Intravenous delivery of phenylephrine has previously been shown to be safe in humans (Cardenas-Garcia et al., 2015). Three sequential doses (30, 45, and 60 μg/L estimated blood volume) were given, aiming to elicit a ~10, ~20, and ~30mmHg rise in MAP, respectively. The Nadler equation (*Equation 2*; Nadler et al., 1962) was

used to estimate blood volume (L) in order to determine PE dosage. These equations are as follows:

Equation 2. Nadler formulas for estimation of blood volume (L) for a) males and b) females (Nadler et al., 1962).

a) $Males: Blood\ Volume\ (L) = (0.3669\ x\ Height^3) + (0.03219\ x\ Weight) + 0.6041$

b) $Females: Blood\ Volume\ (L) = (0.3561\ x\ Height^3) + (0.03308\ x\ Weight) + 0.1833$

Weight was measured each morning before assessment to account for blood volume changes throughout the testing period at high altitude (Goldfarb-Rumyantzev & Alper, 2014). Each dose of PE was immediately followed by a ~3mL saline flush to clear the IV extension line. Bolus injection rate for the PE and saline flush were ~0.9 mL/s, meaning administration of bolus PE and saline flush took approximately 11 seconds per dose.

3.3.2 Cold Pressor Test

To assess integrated cardiovascular reactivity to a sympathetic stressor, participants performed a cold pressor test (CPT). This test is a standardized sympathetic stressor and involves the submersion of the hand up to the wrist in ice-cold water (≤4°C) for three minutes (Usselman, Wakefield, et al., 2015; Victor et al., 1987). Measurements were taken during a 1-minute baseline immediately prior to the test, and throughout the 3-minute submersion.

3.3.3 Hypoxia and Hyperoxia

For the ACUTE condition during the low-altitude assessment, acute normobaric isocapnic hypoxia ($P_{ET}O_2$ ~48 mmHg; $P_{ET}CO_2$ ~37 mmHg) equivalent to ~3,800m in elevation

(~64% of the oxygen available at sea level) was delivered to the participant via a custom Dynamic End-tidal Forcing system (DEF; AirForce 5.0, Pneumologix Consulting Ltd., Kelowna BC). The system adjusts the mix of nitrogen, oxygen, and carbon dioxide delivered to the participant on a breath-by-breath basis to reach desired end-tidal values. This allowed for the assessment of the influence of hypoxia per se, independent of acclimatization. Isocapnic hypoxia was chosen to isolate the influence of hypoxia and because cardiovascular responses (i.e. increases MAP and HR) occur immediately on exposure (Steinback & Poulin, 2008). For the EARLY-HYP and LATE-HYP conditions during high-altitude assessments, participants breathed normobaric hyperoxia (100% O_2) from a Douglas bag. This was used to remove both the direct influence of hypoxia as well as silence the chemoreflex to identify any underlying acclimatization.

3.4 Instrumentation and Measurements

As part of standard pre-testing requirements, participants abstained from strenuous exercise, caffeine, and alcohol for at least 12 hours and were fasted for at least 2 hours prior to testing. Participants were placed in a supine position and instrumented (**Figure 6**), followed by at least 10 minutes of rest before starting the protocol. Cardiovascular and respiratory measures were recorded at 1000Hz using a data acquisition system (LabChart Pro, v8.1.16, ADInstruments, New Zealand) and stored for offline analysis.

3.4.1 Intravenous Catheter

A trained phlebotomist placed a 20-gauge intravenous catheter (IV; BD, United States) into an antecubital vein in the participant's arm using sterile technique. The IV was fitted with a three-way stopcock with a port for blood sampling and drug injections.

3.4.2 Cardiovascular Measures

All cardiovascular measures were collected beat-by-beat. Continuous electrocardiogram (ECG; Lead II) traces were used to determine heart rate (bpm). Continuous blood pressure waveforms were collected using photoplethysmography (Finometer Pro, Finapres Medical Systems, Netherlands). This method has been shown to accurately represent arterial blood pressure at rest and during tests that produced a rapid pressor response, including the CPT and PE injections (Parati et al., 1989). Blood pressure waveforms were calibrated using 3 measurements with an automated blood pressure monitor (Omron, Japan), and used to determine beat-by-beat systolic, diastolic, and mean arterial pressure (MAP). Cardiac output (Q; L/min) was determined using the Finometer Modelflow algorithm. Cardiac index (CI; $L \cdot min^{-1} \cdot m^{-2}$) was calculated by normalizing Q to each participant's estimated body surface area (BSA; m^{-2}) calculated using the Mosteller formula (*Equation 3*; Mosteller, 1987).

Equation 3. Formula for calculating a) Cardiac Index (CI; $L \cdot min^{-1} \cdot m^{-2)}$ and b) Body Surface Area (BSA; m^{-2}) using the Mosteller formula (Mosteller, 1987).

$$a)\ CI = \frac{Q}{BSA} \qquad b)\ BSA = \sqrt{\frac{weight\ (kg) \cdot height\ (cm)}{3600}}$$

While resistance and conductance have a mathematically inverse relationship, one measure or the other may be more sensitive in detecting changes depending on blood flow and pressure conditions. For example, total peripheral conductance (TPC; $L \cdot mmHg^{-1} \cdot min^{-1}$) may be more appropriate metric than resistance (TPR; $mmHg \cdot L^{-1} \cdot min^{-1}$) when blood flow changes more than pressure (Lautt, 1989). Further, O'Leary (1991) asserts that blood pressure regulation is better represented by the regional changes in contractile state incorporated in conductance. However, for completeness, both TPR and TPC are reported.

Peripheral blood oxygen saturation (S_pO_2; %) was measured using pulse oximetry (low-altitude: OxiMax N-600x, Nellcor Medtronics, United States; high-altitude: Oximeter Pod, ADInstruments, New Zealand).

3.4.3 Respiratory Measures

Ventilatory measures including respiratory rate (RR; bpm), tidal volume (V_T; L), and minute ventilation (V_E; $L \cdot min^{-1}$) were measured using a pneumotachometer (Hans Rudolph, Inc., United States) heated to body temperature (~37°C). Inspired and expired oxygen and carbon dioxide (%) were measured using a gas analyzer (ADInstruments, New Zealand).

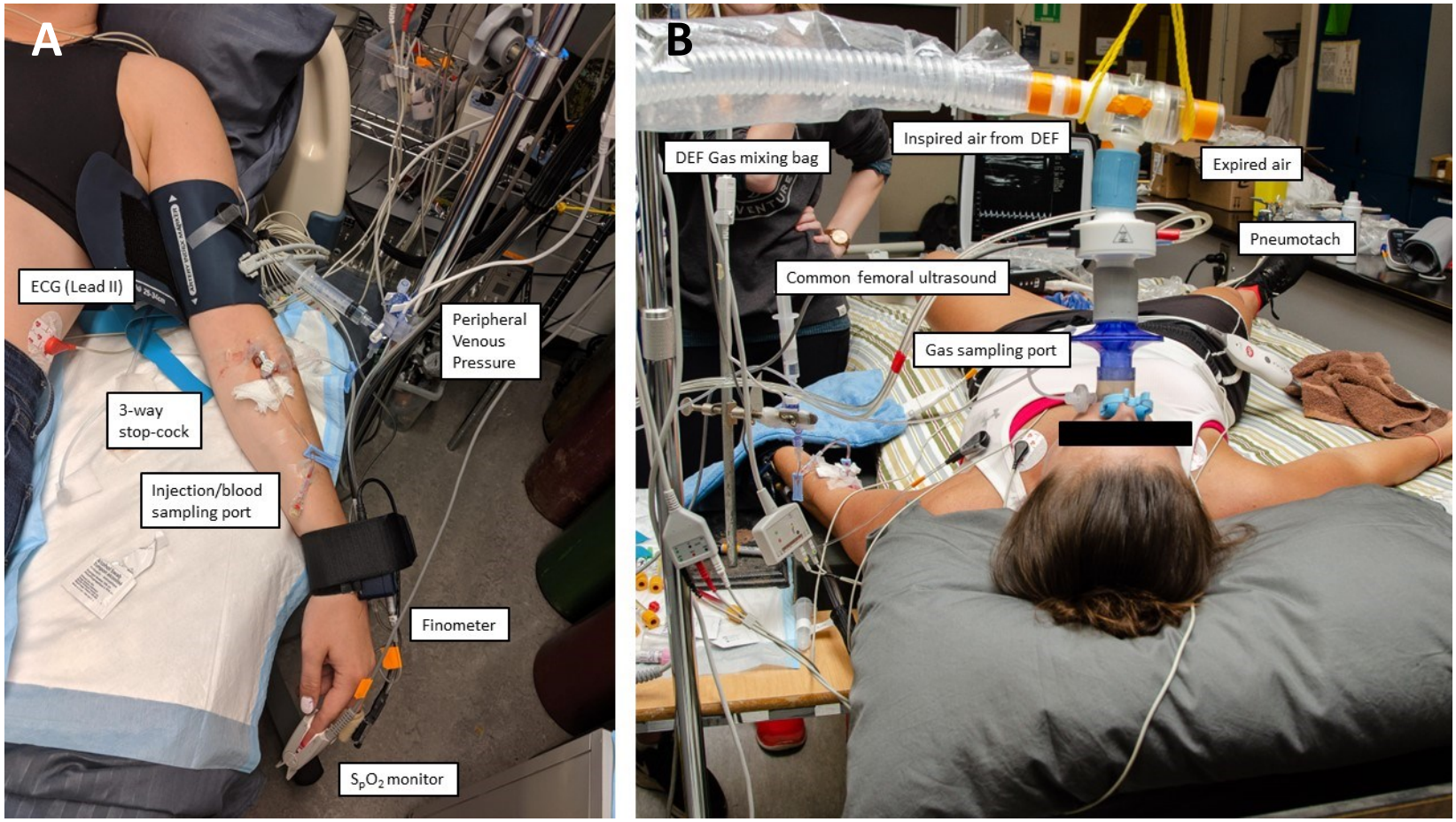

Figure 6. Instrumentation. **A)** Equipment for measurement of cardiovascular variables, including electrocardiogram (ECG; Lead II) for heart rate (bpm) and rhythm, Finometer for beat-by-beat blood pressure (mmHg), and pulse oximeter for monitoring peripheral oxygen saturation (S_PO_2; %). Also shown is an intravenous (IV) catheter inserted into an antecubital vein, fitted with a 3-way stopcock with attachment to the pressure transducer, and a port for bolus drug injections and blood sampling. B) Equipment for measurement of respiratory variables, including a pneumotach for respiratory rate (RR; bpm), tidal volume (V_T; L), and minute ventilation (V_E; L/min), and a gas sampling port for partial pressure of end-tidal oxygen ($P_{ET}O_2$; mmHg) and carbon dioxide ($P_{ET}CO_2$; mmHg). Values obtained from the pneumotach and gas sampler were utilized by the dynamic end-tidal forcing (DEF) system in order to titrate desired end-tidal gas levels.

3.4.4 Plasma Catecholamines

Plasma catecholamine concentrations at rest and in response to CPT were determined using blood plasma samples collected throughout the protocol. Plasma was collected into 6 mL K_2EDTA (dipotassium ethylenediaminetetraacetic acid) tubes (BD Vacutainer, BD, United States). At low-altitude, intravenous blood samples were collected during the 5-minute baselines, CPT baselines, and during the last minute of the CPT. At high altitude, the CPT baseline samples were omitted due to equipment limitations. Samples were immediately refrigerated (4℃), and then spun in a centrifuge (3000rpm, 15 minutes) and plasma was aliquoted into microtubes. For low altitude tests, plasma samples were stored in a -80℃ freezer until analysis. For high-altitude tests, where no ultra-low freezer was available, samples were stored at -18℃ for a maximum of 8 days and analyzed on-site. At this temperature, plasma catecholamines are stable for up to three weeks (Weir et al., 1986). Norepinephrine (NE) and epinephrine (EPI) were quantified using a solid phase enzyme-linked immunosorbent assay (2-CAT ELISA$^{\text{FAST TRACK}}$; LDN REF: BA E-6500; Rocky Mountain Diagnostics, Colorado, United States) according to manufacturer's instructions by a single researcher (ARS).

3.5 Data Analysis

3.5.1 LabChart

All LabChart data files were saved for offline analysis. Prior to analysis, files were blinded by an independent observer (LFB) for time-point, participant identifier, and sex.

Baseline Measurements: Baseline cardiovascular measures during exposure to room air and hypoxia or hyperoxia were taken over five minutes. Baseline respiratory measures breathing

room air were taken over one minute, and over five minutes during hypoxia and hyperoxia, respectively.

α_1-Adrenergic Sensitivity: Responses to PE were analyzed for two minutes following injection. The peak pressor and nadir heart rate responses were selected for analysis, and the change calculated in relation to measures taking during the one-minute period prior to PE injection. α_1-Adrenergic sensitivity was determined as the slope of dose-responses for each individual against the corresponding log-transformed PE dose, as this more accurately captures the linear part of the pharmacological sensitivity sigmoidal curve (Sumner et al., 1982). **Figure 7** demonstrates the process for completing this analysis.

Cold Pressor Test Reactivity: Responses to the CPT were analyzed in rolling one-minute bins starting every 15-seconds, and the minute corresponding to the peak pressor (MAP) and heart rate response was chosen for all further analysis. These peak responses were compared to a one-minute baseline collected immediately prior to each corresponding PE dose and CPT, to ensure any carryover effects of prior interventions did not contaminate the intervention of interest.

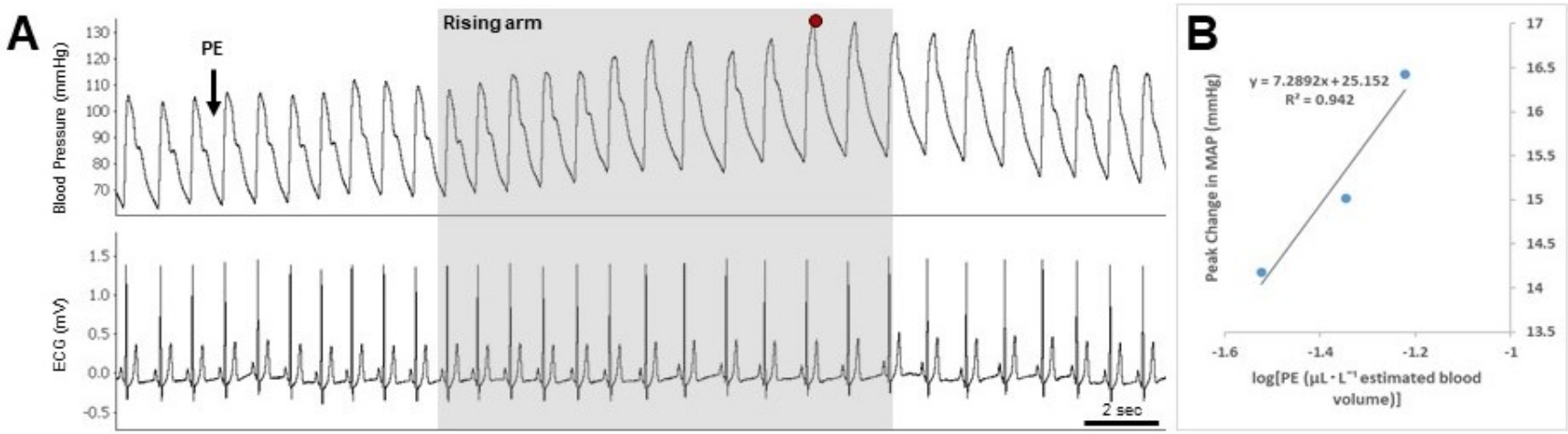

Figure 7. Process for determination of α_1-adrenergic sensitivity slope. A) Following a bolus injection of phenylephrine (PE), **A)** the peak mean arterial blood pressure response (MAP, mmHg; red circle) and corresponding R-R interval (RRI; ms) were assessed during the rising arm of the blood pressure response. Then, values from all three PE doses within each timepoint/condition were regressed against the log transformed PE concentration ($\mu g \cdot L^{-1}$ estimated blood volume). **B)** Slopes were calculated by linear regression, representing the "sensitivity" to the specific α_1 adrenergic agonist.

3.5.2 Statistical Analysis

Group demographics were assessed with unpaired Student T-Tests with equal variance, and baseline data and peak physiological responses to interventions were analyzed using two-way (sex [male, female] x condition [LOW, ACUTE, EARLY, LATE]) repeated measures analysis of variance (ANOVA) or mixed-model ANOVA in the case of missing values (GraphPad Prism Version 9.0.0 for Windows, GraphPad Software, San Diego, CA, United States). The significance level was set at $\alpha=0.05$ for all tests. When significant main effects were identified, pairwise comparisons were made using Holm-Sidak *post hoc* tests. All data are reported as mean ± standard deviation (SD).

Chapter 4: Results

4.1 Participant Demographics

Participant demographics are reported in **Table 1**. A total of 14 participants were enrolled into the study. One participant was withdrawn at the first study visit as researchers were unable to successfully insert the IV. Therefore, data was collected on a total of 13 (7 male, 6 female) participants.

Males and females were not different in age (P=0.5222), but height and weight were both greater in males (P=0.0056 and 0.0057, respectively). Though BMI was not different between groups (P=0.1709), BSA was greater in males (P=0.0024). Therefore, CI was analyzed alongside Q.

Table 1. Participant demographics. Reported data was collected during the initial low altitude assessment.

	Males	**Females**	***P*-value**
Sample Size	n = 7	n = 6	-
Age (years)	25.14 ± 3.44	27.33 ± 7.99	0.5222
Height (cm)	183.71 ± 8.24	169.67 ± 6.15*	**0.0056**
Weight (kg)	82.97 ± 12.20	63.53 ± 7.17*	**0.0057**
BMI (kg/m²)	24.61 ± 3.57	22.09 ± 2.40	0.1709
BSA (m^2)	2.05 ± 0.17	1.73 ± 0.11*	**0.0024**

BMI, body mass index; BSA, body surface area. Values reported as mean ± standard deviation (SD). Comparisons were assessed using unpaired T-tests with equal variance. * = p-value < 0.05 versus males.

4.2 Baseline Characteristics

Baseline characteristics are reported in **Table 2**. Reported values were collected during the 5-minute baseline under each condition. Due to time constraints, one male participant (021) completed only the first (low altitude) assessment (LOW and ACUTE conditions). One participant (005-F) did not complete the late acclimatization assessment beyond the initial (room air) baseline due to complications with the IV insertion. All other participants completed the baseline measurements for all three assessments (low altitude, early and late acclimatization to high altitude). The low-altitude assessments of 10 participants were performed in Calgary, AB (1,050m; atmospheric pressure, 668 ± 2mmHg; $P_{ET}O_2$, ~82mmHg). Due to scheduling limitations, the low-altitude tests of the remaining three participants (004, 009, and 023) were completed in Edmonton, AB (645m; atmospheric pressure, 703 ± 1mmHg; $P_{ET}O_2$, ~93mmHg). However, as most physiological influences of altitude begin to appear at arterial PO_2 <60mmHg (Marshall, 2015), which corresponds to an altitude of ~2,300m, testing location of low-altitude assessments is not expected to impact results. The high-altitude assessments were performed at the Barcroft Station at White Mountain, CA (3,800m) where the atmospheric pressure was significantly lower at 489 ± 1mmHg (P<0.0001). All P-value results from two-way (condition, sex) mixed-model ANOVAs with repeated measures comparing baseline characteristics can be found in supplemental *Table S1* (Appendix VII: Supplemental Data).

Table 2. Participant Baseline Characteristics.

	Low Altitude (≤1050 m)				High Altitude (3800 m)			
	LOW		ACUTE HYPOXIA		EARLY (day 2 or 3)		LATE (day 8, 9, or 10)	
	Males	Females	Males	Females	Males	Females	Males	Females
Sample Size	n = 7	n = 6	n = 7	n = 6	n = 6	n = 6	n = 6	n = 6
CARDIOVASCULAR								
Heart Rate (bpm)	64.53 ± 5.96	71.59 ± 2.64	75.36 ± 9.09	93.71 ± 8.32*†	72.75 ± 10.09	81.09 ± 4.91†‡	71.27 ± 13.36	74.40 ± 10.21‡
R-R Interval (s)	0.94 ± 0.08	0.84 ± 0.03	0.81 ± 0.10	0.64 ± 0.06*†	0.84 ± 0.11	0.74 ± 0.05†‡	0.86 ± 0.13	0.82 ± 0.11‡
Blood Pressure								
Mean (mmHg)	90.45 ± 10.76	81.38 ± 7.43	97.78 ± 10.74	89.18 ± 12.16	94.56 ± 10.19	91.31 ± 8.65†	90.79 ± 10.71	84.69 ± 11.93
Systolic (mmHg)	121.38 ± 10.23	104.9 ± 8.05*	132.36 ± 11.44†	117.90 ± 9.42	120.21 ± 11.52	110.12 ± 6.48	117.76 ± 11.04‡	102.66 ± 12.46
Diastolic (mmHg)	73.65 ± 10.19	66.4 ± 7.37	78.77 ± 10.03	71.76 ± 10.02	80.41 ± 10.06	78.74 ± 7.73†‡	76.30 ± 9.81	72.95 ± 11.32
Q ($L \cdot min^{-1}$)	6.93 ± 1.37	6.22 ± 0.73	8.43 ± 1.66†	8.01 ± 1.22	6.59 ± 1.32	6.32 ± 2.19 (4)	7.03 ± 1.93	6.11 ± 0.89
CI ($L \cdot min^{-1} \cdot m^{-2}$)	3.37 ± 0.54	3.61 ± 0.45	4.10 ± 0.67	4.63 ± 0.54†	3.22 ± 0.51	3.59 ± 1.15 (4)	3.41 ± 0.66	3.56 ± 0.68‡
TPR ($mmHg \cdot L^{-1} \cdot min^{-1}$)	13.47 ± 2.35	13.25 ± 1.38	12.01 ± 2.37	11.44 ± 2.60	14.93 ± 2.86	16.72 ± 8.15 (4)	13.65 ± 3.22	14.23 ± 2.95
TPC ($L \cdot mmHg^{-1} \cdot min^{-1}$)	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	0.07 ± 0.03 (4)	0.08 ± 0.02	0.07 ± 0.02
S_PO_2 (%)	96.78 ± 1.13	98.20 ± 1.35	86.56 ± 3.71†	84.35 ± 2.89†	85.7 ± 2.40†	88.3 ± 1.12†	87.71 ± 1.63†	88.53 ± 1.64†
RESPIRATORY								
V_T (L)	1.10 ± 0.22	0.92 ± 0.25	1.73 ± 0.47	1.70 ± 0.36	1.00 ± 0.20 (3)	0.98 ± 0.29 (5)	1.18 ± 0.27	0.92 ± 0.26 (5)
RR (bpm)	11.03 ± 3.87	14.02 ± 4.03	10.43 ± 1.47	16.90 ± 4.94	15.85 ± 3.74 (3)	14.60 ± 4.65 (5)	12.30 ± 4.19	15.78 ± 6.23 (5)
V_E ($L \cdot min^{-1}$)	11.21 ± 1.89	11.99 ± 1.31	17.23 ± 3.88	29.02 ± 11.36	15.08 ± 1.04 (3)	12.95 ± 1.24 (5)	13.77 ± 3.86	13.76 ± 5.97 (5)
$P_{ET}O_2$ (mmHg)	82.34 ± 5.36	87.33 ± 7.15	48.86 ± 4.25†	47.13 ± 3.21†	50.19 ± 2.79† (3)	49.31 ± 3.89† (5)	51.46 ± 2.27†	51.70 ± 1.33†‡ (5)
$P_{ET}CO_2$ (mmHg)	40.83 ± 5.43	31.45 ± 3.70*	41.08 ± 6.07	33.30 ± 3.09*	29.89 ± 1.54 (3)	25.88 ± 1.55* (5)	28.14 ± 0.67†‡	26.56 ± 0.66*‡ (5)
CATECHOLAMINES								
NE ($pg \cdot mL^{-1}$)	-	-	-	-	317.82 ± 98.38	413.12 ± 209.28	471.39 ± 248.32	439.07 ± 140.59
EPI ($pg \cdot mL^{-1}$)	-	-	-	-	81.27 ± 21.11	54.95 ± 18.68	136.46 ± 66.90§	140.75 ± 20.37§

Values are mean ± standard deviation (n, when different from column total). Q, cardiac output; CI, cardiac index; TPR, total peripheral resistance; TPC, total peripheral conductance; S_PO_2, peripheral blood oxygen saturation; V_T, tidal volume; RR, respiratory rate; V_E, minute ventilation; $P_{ET}O_2$, partial pressure of end-tidal oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; NE, norepinephrine; EPI, epinephrine. Data analyzed with two-way repeated measures ANOVA (catecholamines) or mixed-model ANOVA with repeated measures (all other characteristics). * = $P<0.05$ vs males at same timepoint, † = $P<0.05$ vs LOW, ‡ = $P<0.05$ vs ACUTE, § = $P<0.05$ vs EARLY.

4.2.1 Cardiovascular Measures

Heart rate. Across conditions, heart rate (HR; bpm) was increased and R-R interval (RRI; seconds) was decreased with hypoxia in comparison to LOW (main effect $P<0.0001$ for both). However, this change was not significant in males at any timepoint ($P>0.05$ for all). HR was the highest and RRI the shortest during the ACUTE hypoxia condition in females (LOW, $P=0.0020$ and 0.0002; EARLY, $P=0.0147$ and 0.0090; LATE, $P=0.0246$ and 0.0287, respectively). In females, HR was also elevated and RRI truncated significantly from LOW to EARLY ($P=0.0059$ and 0.0047) but not significantly compared to LATE ($P=0.4791$ and 0.6315), though EARLY and LATE were not different from each other ($P=0.2288$ and 0.1748).

There was a significant main effect of sex on HR and RRI ($P<0.0001$ for both), with females generally displaying higher HR and shorted RRI than males with all conditions. Neither HR nor RRI were significantly different between males and females at LOW, EARLY, or LATE ($P>0.05$ for all). However, with ACUTE, females had a significantly higher HR and lower RRI in comparison to males ($P=0.0119$ and 0.0186, respectively). There was a significant interaction between condition and sex for HR ($P=0.0143$), but not for RRI ($P=0.1078$).

Blood pressure. There was a significant main effect of condition on mean arterial pressure (MAP; mmHg; $P=0.0028$), systolic blood pressure (SBP; mmHg; $P=0.0001$), and diastolic blood pressure (DBP; mmHg; $P=0.0006$). In general, resting blood pressure values had the greatest increase from LOW at ACUTE and EARLY, with values trending back towards LOW values by LATE. MAP increased from LOW with acute and high-altitude hypoxia in both males and females, though this was only significant in females at EARLY ($P=0.0282$; $P>0.05$ for all other comparisons). In males, SBP was elevated at ACUTE vs LOW ($P=0.0309$) but had

returned to LOW-like values by LATE (P=0.8257; ACUTE vs LATE P=0.0371). This trend was similar in females, however none of the comparisons were statistically significant (P>0.05 for all). DBP was increased with all hypoxic conditions in comparison to LOW, with the most marked increase at EARLY in both groups. However, this was found to be significant in females (P=0.0043) but not in males (P=0.4881). In females, DBP under the EARLY condition was also elevated over ACUTE (P=0.0258). Similar to MAP and SBP, DBP trended towards values observed at LOW by LATE (males, P=0.8275; females, P=0.0802).

Though commonly lower in females than in males, there was no statistical difference in resting MAP or DBP between sexes (main effect P=0.2170 and 0.3660, respectively). However, SBP had a significant main effect for sex (P=0.0110). SBP was lower in females compared to males, but this was only significant at LOW (P=0.0309; P>0.05 for all other conditions). There was no interaction effect between condition or sex for any of the blood pressure parameters measured (P>0.05 for all).

Cardiac output. Calculated cardiac output (Q; $L \cdot min^{-1}$) was significantly influenced by condition (main effect P=0.0032). From LOW, Q increased with ACUTE, though this was only significant in males (P=0.0152; females, P=0.1902). Q returned closer to LOW values at EARLY and LATE in both groups (all P>0.05). Cardiac index (CI; $L \cdot min^{-1} \cdot m^{-2}$), which takes into account body size, also had a main effect of condition (P=0.0010). Like Q, values were heighted the most with ACUTE in comparison to LOW, but this was significant in females only (P=0.0265; males, 0.1291). EARLY and LATE CIs were similar to LOW measurements in both groups (P>0.05 for all comparisons). No significant main effect was observed for sex (Q,

P=0.3183; CI, *P*=0.1900), nor was there an interaction effect with condition (Q, P=0.8653; CI, *P*=0.8439).

Total peripheral resistance and conductance. There was no difference in the calculated total peripheral resistance (TPR; $mmHg \cdot L^{-1} \cdot min^{-1}$) measured in either group across conditions (main effect *P*=0.0526). However, calculated total peripheral conductance (TPC; $mmHg \cdot min^{-1} \cdot L^{-1}$) had a significant effect for condition (*P*=0.0186). Though none of the pairwise comparisons were statistically significant (*P*>0.05 for all), compared to LOW, TPC was marginally greater during the ACUTE condition and lower at EARLY. There was no difference between groups for TPR nor TPC (*P*=0.7529 and 0.9854, respectively), and no interaction was found between sex and condition (TPR, *P*=0.7594; TPC, *P*=0.7523).

Peripheral blood oxygen saturation. Though there was no difference between groups (main effect *P*=0.2983), there was a significant main effect of condition (*P*<0.0001) on peripheral oxygen saturation (S_pO_2; %) without an interaction between condition and sex (*P*=0.0551). ACUTE, EARLY, and LATE had significantly lower saturations compared to LOW (males, *P*=0.0041, 0.0004, and 0.0005, respectively; females, *P*=0.0003, <0.0001, and <0.0001, respectively) with no difference between the three hypoxic conditions (*P*>0.05 for all).

4.2.2 Respiratory Measures

Ventilation. A main effect of condition (*P*=0.0008) was observed for tidal volume (V_T; L). Compared to LOW, the largest resting V_T's were measured with ACUTE, but returned to near-LOW values at EARLY and LATE. However, these observed trends were not statistically

significant (all $P>0.05$). Respiratory rate (RR; bpm) did not differ between conditions (main effect $P=0.1447$). However, minute ventilation (V_E; $L \cdot min^{-1}$), which incorporates RR and V_T, was significant for the effect of condition ($P=0.0008$). Though V_E at EARLY and LATE were similar to LOW ($P>0.05$ for all), measurements were increased with ACUTE, though this was not significant in either group (males, $P=0.0636$; females. $P=0.0690$).

There was no difference between the sexes for V_T, RR, and V_E ($P=0.2754$, 0.1069, and 0.1647, respectively), and no interaction between condition and sex for V_T or RR ($P=0.7144$ and 0.0633, respectively). Conversely, an interaction was detected for measurements of V_E ($P=0.0086$); though V_E increase in both males and females from LOW to ACUTE, females displayed a much larger increase in ventilation with this condition.

End-tidal gasses. The partial pressure of end-tidal oxygen ($P_{ET}O_2$; mmHg) had a significant main effect of condition ($P<0.0001$), but no sex or interaction effect ($P>0.05$ for both). In both sexes, $P_{ET}O_2$ was lowered from ~85mmHg at LOW to ~50mmHg with acute hypoxia ($P<0.0001$ for both). The values recorded at ACUTE were similar to those at EARLY and LATE ($P>0.05$ for all), with the exception of females at LATE ($P=0.0378$). However, the mean difference between these two timepoints was only ~3 ± 2mmHg, and no significant difference was detected in $P_{ET}O_2$ between EARLY and LATE in females ($P=0.4386$). Taken together, it is indicated that the end-tidal forcing system delivered an appropriate oxygen stimulus at low altitude to simulate oxygen conditions experienced at high altitude.

Similarly, the partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$; mmHg) changed significantly with condition (main effect $P<0.0001$). $P_{ET}CO_2$ was not different between LOW and ACUTE in either group ($P>0.05$ for both), but this was to be expected as the end-tidal

forcing system controlled for isocapnia under the acute hypoxic condition. However, a marked decreased in $P_{ET}CO_2$ was observed at the EARLY and LATE timepoints in both groups. This decrease was found to be significant at the LATE timepoint in males in comparison to LOW (P=0.0098) and ACUTE (P=0.0149), and females in comparison to ACUTE (P=0.0420) but not significantly to LOW (P=0.1759). The EARLY timepoint was not significantly different from LOW nor ACUTE in either group (P>0.05 for all) but was also found to be similar to LATE values (P>0.05 for both groups).

There was a significant main effect of sex for $P_{ET}CO_2$ (P=0.0069), as well as an interaction effect between sex and condition (P=0.0100). $P_{ET}CO_2$ was measured to be lower in females compared to males at every timepoint (P<0.05 for all), and females appeared to have less of a decrease in $P_{ET}CO_2$ with prolonged high-altitude exposure.

Though $P_{ET}CO_2$ differed between the sexes, hypoxic ventilatory response at low altitude (calculated as $\Delta V_E/\Delta S_PO_2$ with 5-minutes of acute isocapnic hypoxia) was not statistically different between males (0.64 ± 0.47 $L \cdot min^{-1} \cdot \%desaturation^{-1}$) and females (1.15 ± 0.57 $L \cdot min^{-1} \cdot \%desaturation^{-1}$; T-Test, P=0.1063), indicating a similar ventilatory stimulus.

4.2.3 Plasma Catecholamines

Due to equipment failure, plasma catecholamine analysis did not produce viable results for the LOW and ACUTE conditions. Therefore, samples taken only early and late during acclimatization are reported, which includes all participants tested at these timepoints (i.e. 021 is excluded do to lack of testing).

Norepinephrine. The plasma concentration of norepinephrine (NE; pg·mL^{-1}) did not have a significant main effect for condition, sex, or an interaction effect (P>0.05 for all). Though NE appeared to be slightly elevated in both males and females at LATE in comparison to EARLY, this change was not significant (P>0.05 for both).

Epinephrine. Plasma concentration of epinephrine (EPI; pg·mL^{-1}) had a significant main effect of condition (P=0.0004). EPI increased in both males and females (P=0.0161 and 0.0023, respectively) by ~40-80pg·mL^{-1} from EARLY to LATE. EPI was not found to be different between the sexes (main effect, P=0.5334; sex x condition interaction effect, P=0.2836).

4.3 α_1-Adrenergic Sensitivity with Phenylephrine Hydrochloride Injections

No adverse events were observed following the phenylephrine (PE) bolus injections. As described in section

4.2 Baseline Characteristics*,* one participant (021-M) was only tested at the low-altitude timepoint (LOW and ACUTE conditions), but not at EARLY or LATE high-altitude acclimatization timepoints due to time constraints. At LATE, the IV catheter in one participant (005-F) was lost after the first PE dose, and subsequent doses were not delivered as the test was terminated. Other than those described, all other participants received all three PE doses (30, 45, and 60$\mu g \cdot L^{-1}$ estimated blood volume) during the LOW, ACUTE, EARLY, and LATE conditions.

An example trace of a typical biphasic physiological response to the PE bolus injections is demonstrated in **Figure 8**. Blood pressure rose within 10-30 seconds following injection, with a compensatory decrease in heart rate. This led to a subsequent fall in blood pressure and increase in heart rate, before a more sustained blood pressure elevation. Cardiac output (as well as calculated cardiac index, and total peripheral resistance and conductance) as determined by the ModelFlow algorithm mirrored the temporal changes of heart rate, with an initial decrease and recovery, followed by a more sustained decrease in values. S_pO_2 was consistent throughout the evaluation period.

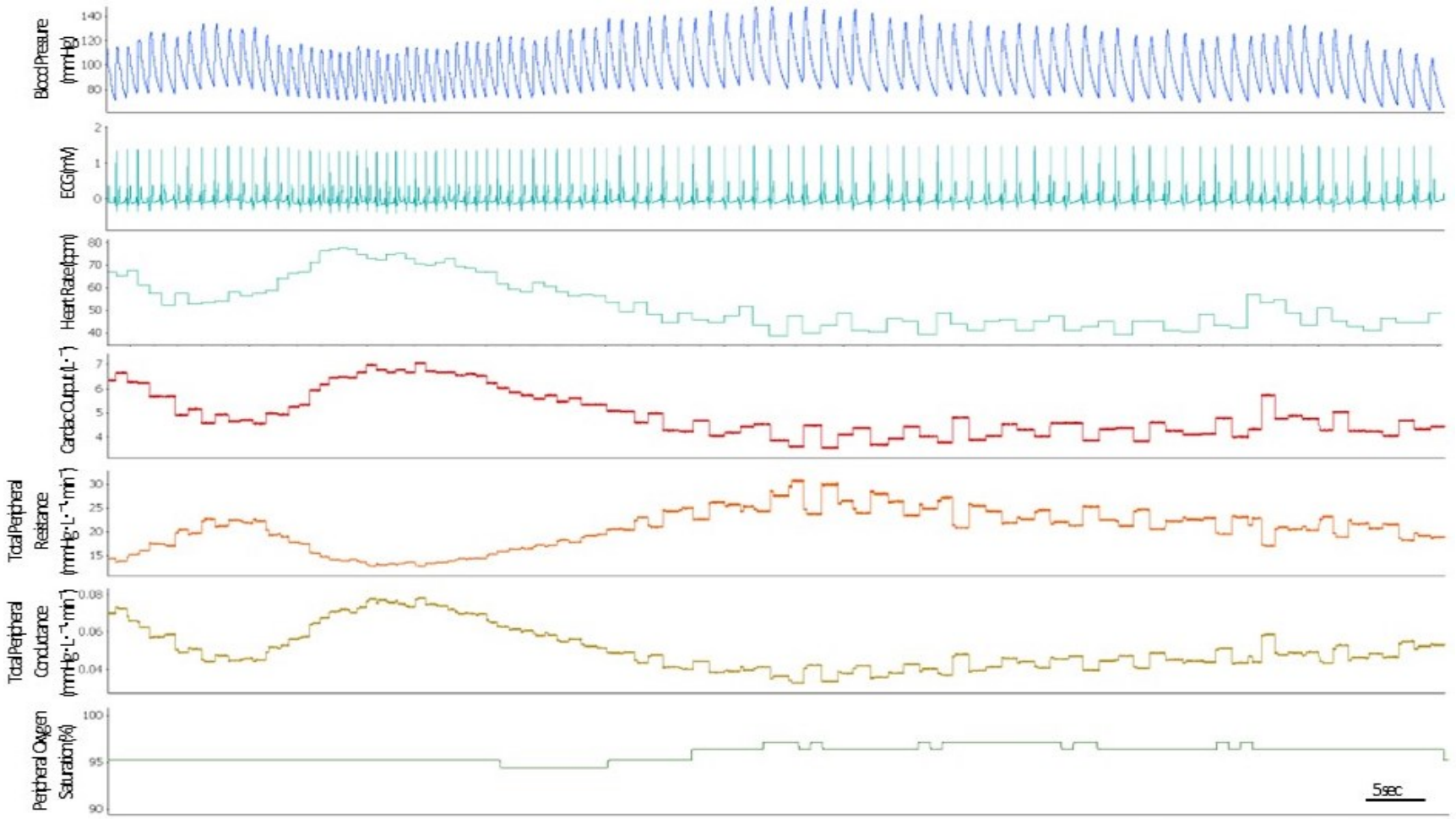

Figure 8. Raw tracings of cardiovascular responses to bolus injection of phenylephrine (PE; α_1-adrenergic receptor agonist). Beginning of timescale represents point at which PE bolus dose was delivered. All injections were followed by a 2-3mL flush of normal saline, and variables were assessed for 2-minutes which encompassed the biphasic pressor response.

MAP responses to individual PE doses in males and females across conditions are depicted in **Figure 9**. With the LOW condition, the 30 and 45μg·L^{-1} doses were significantly lower than the 60μg·L^{-1} dose (P<0.05 for both). With ACUTE, the 30μg·L^{-1} dose was significantly lower than the 60 μg·L^{-1} dose (P=0.0054), but the 45 μg·L^{-1} dose was not (P=0.0952). There were no significant differences in responses between males and females (main effect, P=0.6700), and nor was there an interaction effect (P=0.5282). There was a significant main effect of PE dose across conditions (P<0.0001). Overall, the MAP increase in response to PE appeared attenuated at high altitude at both the EARLY and LATE timepoints in comparison to low altitude (LOW and ACUTE). This difference was significant with the 60μg·L^{-1} PE dose at EARLY, and all three doses at LATE (P<0.05 for all) in comparison with the same dose at LOW. Additionally, the 30μg·L^{-1} dose at EARLY, and the 30 and 45μg·L^{-1} doses at LATE were significantly lower than the corresponding doses during the ACUTE condition (P<0.05 for all).

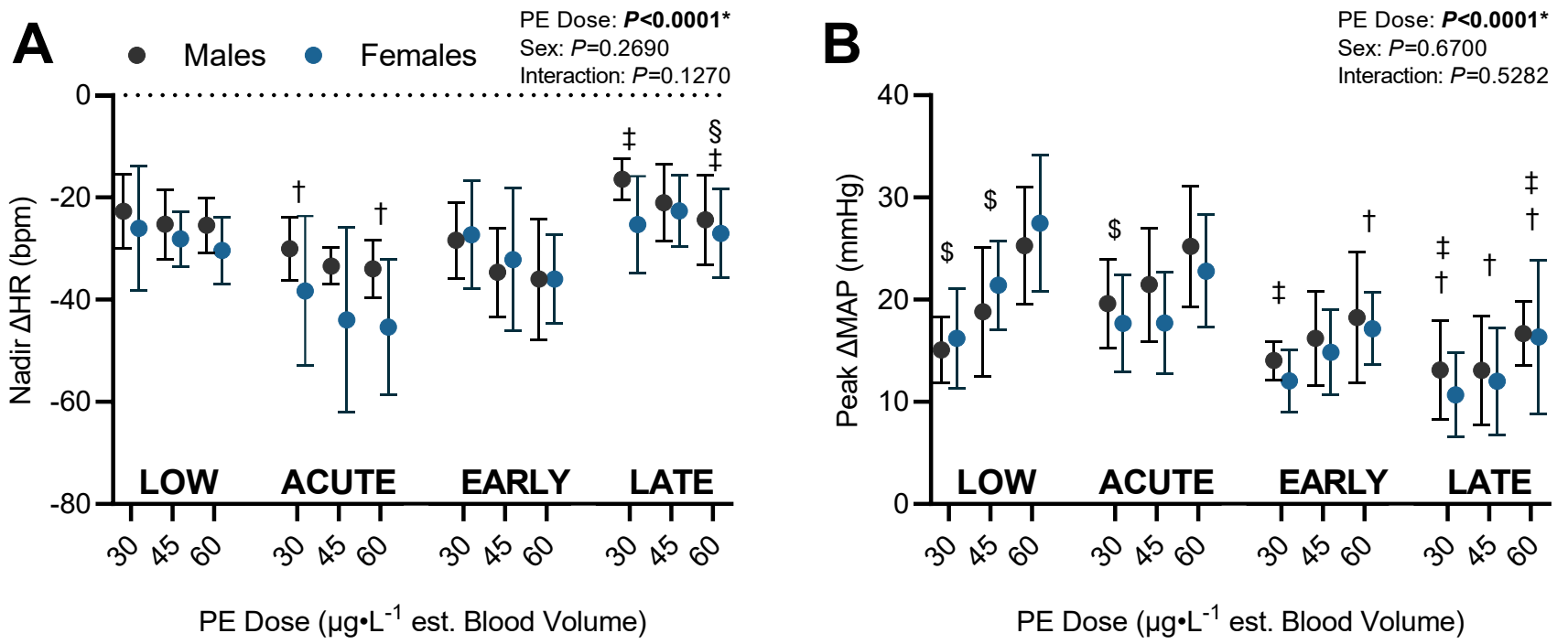

Figure 9. Absolute **A)** nadir heart rate (HR; bpm) and **B)** peak mean arterial pressure (MAP; mmHg) response to sequential doses of phenylephrine (PE; 30, 45, and 60µg·L^{-1} estimated blood volume) during each condition in males (grey) and females (blue). Absolute and percent change MAP responses to PE appeared were attenuated with hypoxia, particularly during EARLY and LATE acclimatization (main effect $P<0.0001$ for both). There was no significant effect of sex on absolute or percent change MAP response to PE ($P=0.6700$; $P=0.7592$, respectively). Data was analyzed using a two-way (PE dose x sex) mixed-model ANOVA. Values are means ± standard deviation. † = $P<0.05$ vs LOW at same PE dose; ‡ = $P<0.05$ vs ACUTE at same dose; $ = $P<0.05$ vs 60µg·L^{-1} estimated blood volume within same condition.

Figure 10 and **Figure 11** show the PE sensitivity slopes in males and females under each condition. As outlined in *3.5 Data Analysis*, PE sensitivity slopes were determined as the slope of the physiological change with PE as a function of the log transformed PE dose concentration.

Neither HR nor RRI sensitivity to PE were changed with condition (main effect, both $P>0.05$). However, measures of blood pressure PE sensitivity appeared to be attenuated with hypoxic conditions in comparison to LOW. There was a significant effect of condition on MAP, SBP, and DBP sensitivity ($P=0.0027$, $P=0.0046$, and $P=0.0144$, respectively). Both acute and high-altitude hypoxia at early and late timepoints had blunted sensitivity in comparison to low altitude. In particular, females had significantly lower SBP sensitivity slopes during the ACUTE condition in comparison to LOW ($P=0.0131$). S_pO_2 sensitivity did not change with PE administration or between sexes (both $P>0.05$). Sensitivity slopes for CO and CI were not different across conditions (main effects $P>0.05$ for both). Though TPR was not impacted by condition (P=0.5054), attenuation of TPC was observed to have a decreased sensitivity to PE administration with hypoxia ($P=0.0445$).

Further, there was no difference between males and females, and no interaction between condition and sex for any variable measured (main effect, all $P>0.05$).

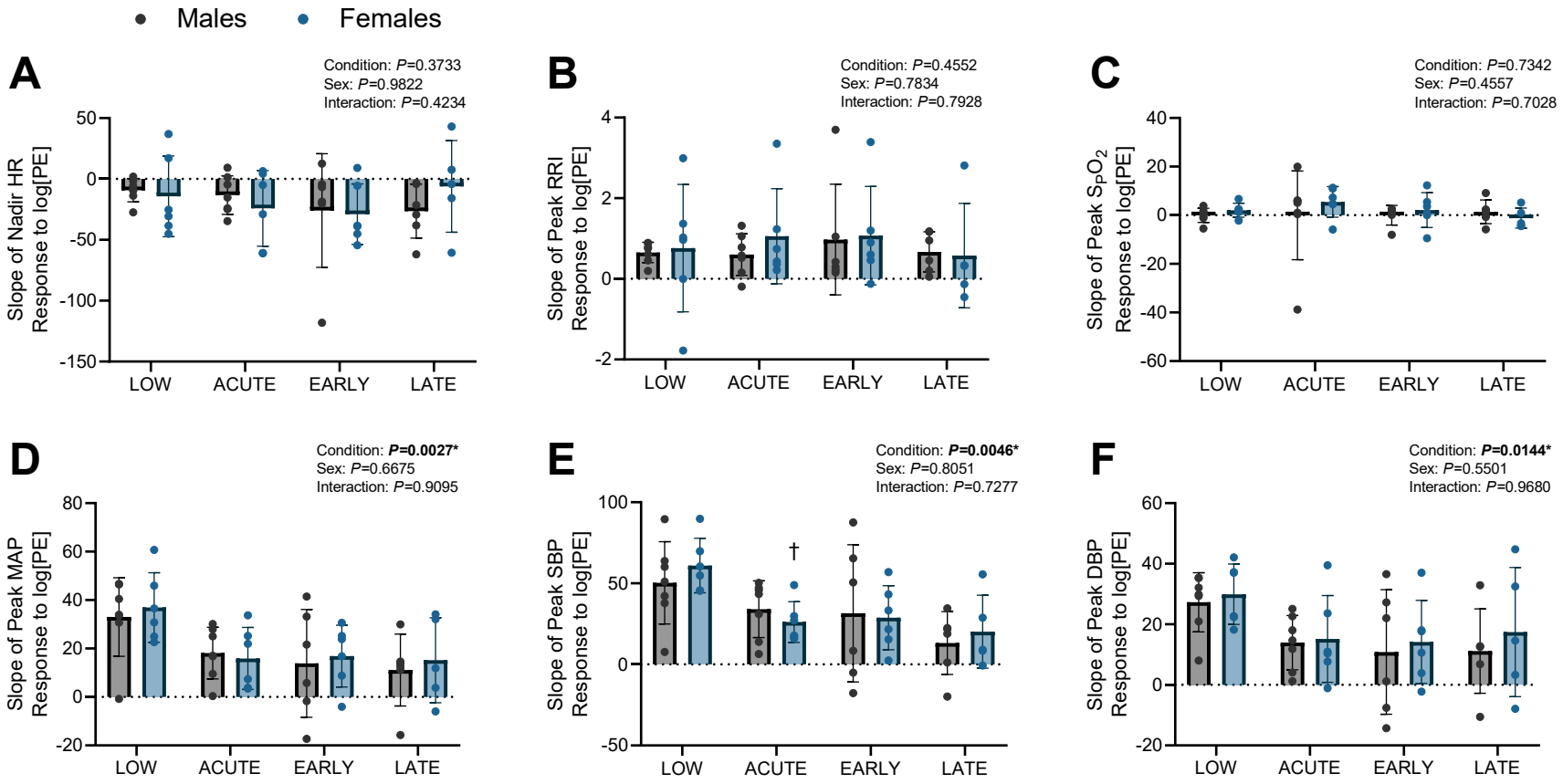

Figure 10. Absolute α_1-adrenergic sensitivity of **A)** heart rate (HR; bpm), **B)** R-R interval (RRI; s), **C)** peripheral oxygen saturation (S_pO_2; %), **D)** mean arterial pressure (MAP; mmHg), **E)** systolic blood pressure (SBP; mmHg), and **F)** diastolic blood pressure (DBP; mmHg). Following identification of peak and nadir responses to sequential bolus injections of phenylephrine (PE), sensitivity slopes were determined by plotting the change from baseline with each log transformed dose (30, 45, and 60µg·L^{-1} est. blood volume). Data were analyzed with a two-way repeated measures mixed model ANOVA. Values are reported as means ± standard deviation. † = $P<0.05$ vs LOW.

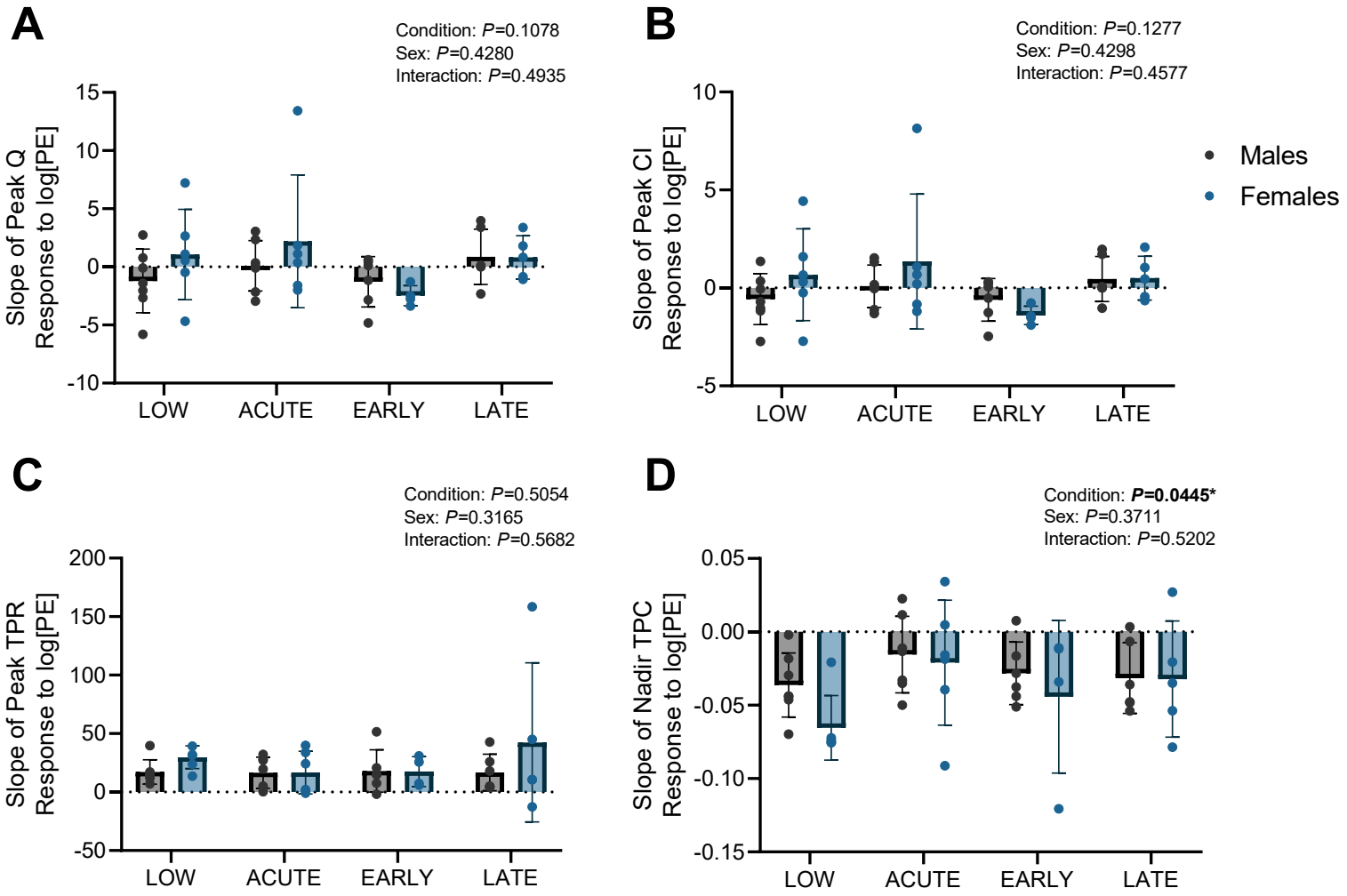

Figure 11. Absolute α_1-adrenergic sensitivity of variables calculated by ModelFlow algorithm (Finometer Pro, The Netherlands) from heart rate and blood pressure data. **A)** cardiac output (Q; $L \cdot min^{-1}$), **B)** cardiac index (CI; $L \cdot min^{-1} \cdot m^{-2}$), **C)** total peripheral resistance (TPR; $mmHg \cdot L^{-1} \cdot min^{-1}$), and **D)** total peripheral conductance (TPC; $L \cdot mmHg^{-1} \cdot min^{-1}$). Following identification of peak and nadir responses to sequential bolus injections of phenylephrine (PE), sensitivity slopes were determined by plotting the change from baseline with each log transformed dose concentration (30, 45, and 60$\mu g \cdot L^{-1}$ est. blood volume). Data were analyzed with a two-way repeated measures mixed model ANOVA. Values are reported as means ± standard deviation.

4.4 Cold Pressor Test

At low altitude, all participants completed the CPT with room air (LOW), and 12 of 13 completed the acute hypoxia condition (ACUTE). Due to unrelated equipment complications, one female (003) with ACUTE and one male (013) with EARLY-HYP did not perform the CPT. At the late acclimatization timepoint, one female (005) did not perform the CPT with either the room air or hyperoxia condition as the IV catheter was not able to be inserted by researchers. One other female (016) only completed part of the CPT during the ACUTE condition; approximately halfway through, the participant's oxygen saturation fell below the safety cut-off (70%) and the test was terminated. All other participants completed the full 3-minutes of the cold pressor test (CPT) at each timepoint.

An example trace of a typical physiological response during CPT is depicted in **Figure 12**. Following the submersion of the hand in cold water, blood pressure and HR increased, and remained markedly elevated for the duration of the test. Calculated Q and TPR also increased, while TPC decreased. S_pO_2 either remained unchanged or became slightly elevated depending on the condition (explained below).

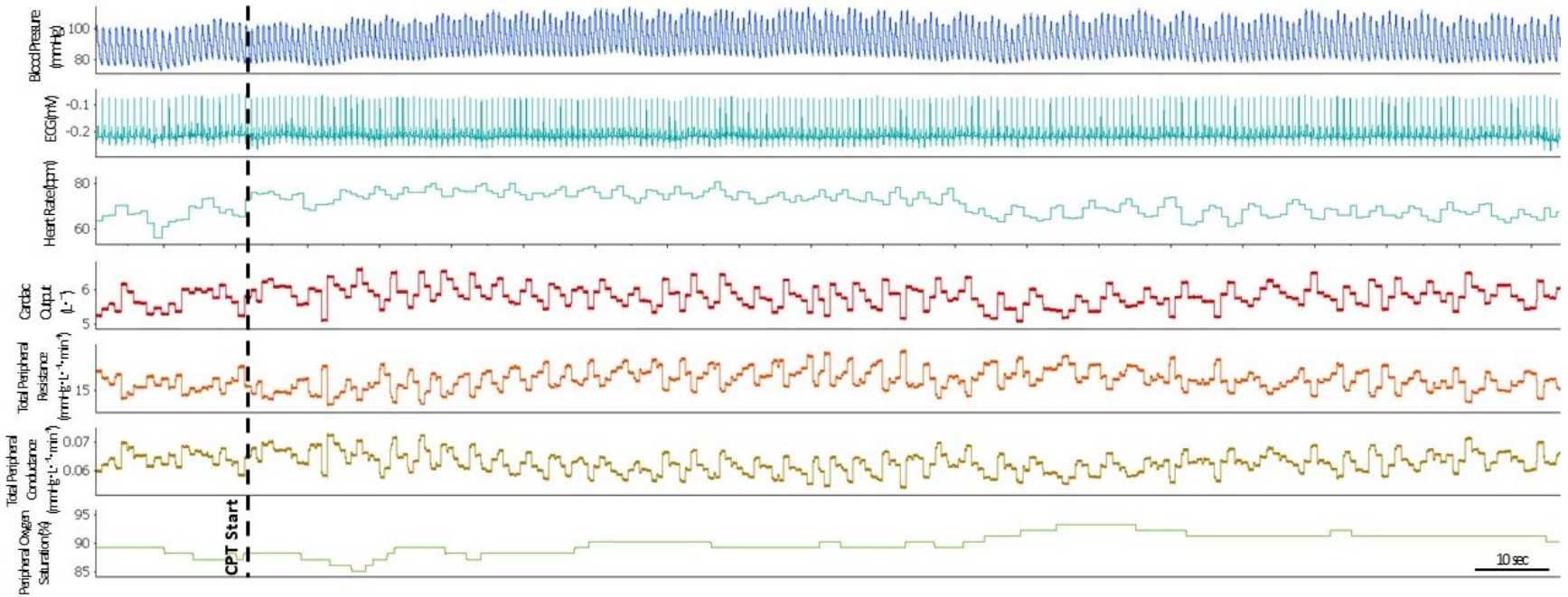

Figure 12. Raw tracings of cardiovascular responses to cold pressor test (CPT). Dashed line represents start of CPT. Continuous variables were assessed throughout the 3-minute CPT, and compared with measurements during the 1-minute baseline immediately prior to the test. In general, mean arterial pressure (MAP; mmHg), heart rate (HR, bpm), cardiac output ($L \cdot min^{-1}$), and total peripheral resistance (TPR; $mmHg \cdot L^{-1} \cdot min^{-1}$) increased in reaction to the stressor, and total peripheral conductance (TPC; $L \cdot mmHg^{-1} \cdot min^{-1}$) fell. Peripheral oxygen saturation (S_pO_2; %) remained consistent during the low-altitude assessment (LOW and ACUTE) but increased in comparison to baseline at high altitude (EARLY and LATE; as pictured). It is likely that this is due to individuals not being fully saturated during these timepoints (as they were at LOW), and because partial pressures of end-tidal oxygen ($P_{ET}O_2$; mmHg) were not dynamically controlled as they were during ACUTE.

The average minute-by-minute responses of MAP and HR to CPT are represented in **Figure 13**. HR increased from baseline during the CPT during all conditions with a significant main effect for time ($P<0.05$ for all). While this increase was significant across most (if not all) of the averaged bins in males, it was only significantly increased during minute one of CPT in females at LOW ($P=0.0293$). Under all other conditions, HR in females was not elevated significantly above baseline values throughout ($P<0.05$ for all). There was no main effect of sex at LOW, EARLY, or LATE ($P<0.05$ for all), but there was during ACUTE ($P=0.0045$). However, baseline HR was significantly higher in females ($P=0.0460$), and there was no interaction effect between time and sex across the CPT ($P=0.9659$).

In comparison to baseline, MAP also increased with all conditions (main effect $P<0.05$ for all). This increase was significant throughout all bins in males across all conditions ($P<0.05$ for all). There was a significant influence of sex with the room air (LOW) and acute hypoxic (ACUTE) conditions at low altitude on the MAP response ($P<0.0001$ for both), as well as an interaction effect with time across the CPT ($P=0.0275$ and 0.0301, respectively). With both LOW and ACUTE females appeared to have a more modest increase in MAP (particularly during minutes 2 and 3), with no timepoint significantly elevated above baseline ($P>0.05$ for all). However, both the sex and interaction effects disappear at both high altitude timepoints (main effect $P>0.05$ for all). The pattern of MAP response to CPT in females appears to follow those of the male's responses at EARLY and LATE, with sustained increases above baseline values.

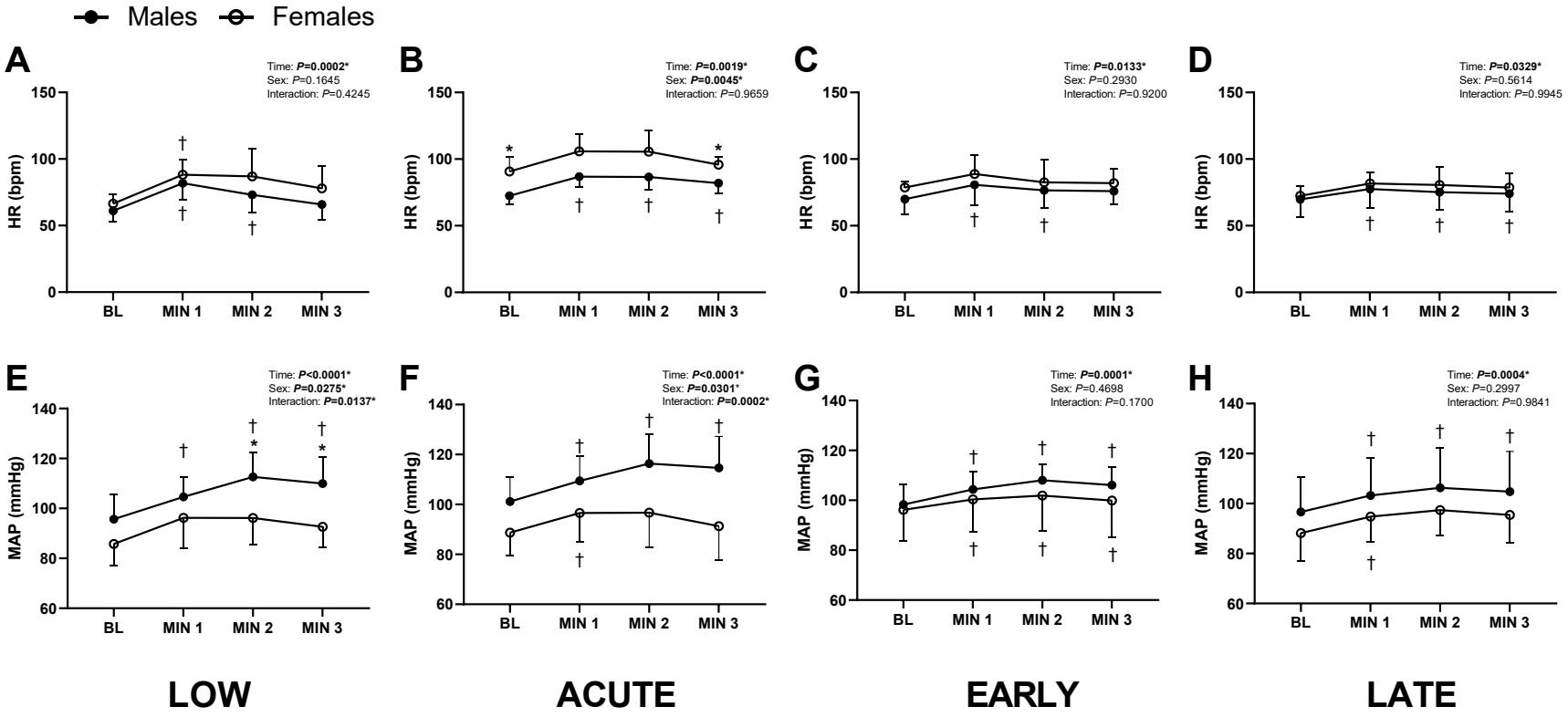

Figure 13. Absolute minute-by-minute responses to the cold pressor test (CPT) in heart rate (HR, bpm; top) and mean arterial pressure (MAP, mmHg; bottom) at low altitude (LOW; **A** and **E**), during exposure to acute isocapnic hypoxia (ACUTE; **B** and **F**), and following 2-3 days (EARLY; **C** and **G**) and 7-9 days (LATE; **D** and **H**) of acclimatization to high altitude at White Mountain, CA (3,800m) in males (closed circles) and females (open circles). Responses were averaged over one-minute bins. Data points represent means ± standard deviation and were analyzed using with a two-way repeated measures mixed-model ANOVA. For *post hoc* comparisons, each minute of the CPT was compared to baseline values. * = $P<0.05$ males vs females; † = $P<0.05$ vs baseline.

As outlined in the research methods (*Section 3.5 Data Analysis*), the maximum physiological response from baseline was identified using one-minute rolling averages (starting every 15 seconds) and expressed as a change from baseline measurements taken immediately prior to the CPT. These absolute cardiovascular changes are represented in **Figure 14** and **Figure 15**.

Maximum HR and RRI responses to CPT were attenuated with hypoxia (main effect $P<0.0001$ for both). It's possible this is due to a ceiling effect, as baseline HR was elevated with hypoxia at rest. However, baseline HR was highest at ACUTE, and had returned to near LOW values by LATE, so this is not likely to be the driving factor. There was no impact of sex on HR and RRI responses to CPT, nor was there an interaction effect ($P>0.05$ for all). S_pO_2 was affected by condition (main effect $P=0.0269$), increasing during the CPT at EARLY and LATE. These increases are consistent with there being an increase in ventilation during CPT (Stone et al., 2019). No changes were observed during LOW or ACUTE, as at LOW participant's were fully saturated at rest, and the ACUTE condition end-tidal gasses were controlled with the DEF system.

MAP, SBP, and DBP maximal responses were reduced following EARLY and LATE acclimatization to high-altitude hypoxia (main effect $P<0.05$ for all). Though there was not a significant effect of sex on blood pressure (main effect $P<0.05$ for all), females exhibited a lower blood pressure response to CPT at all timepoints except for LATE, where responses became similar to males.

Changes in Q and CI with CPT appeared lowered with hypoxia exposure, though this was not significant ($P=0.0524$ and 0.0738, respectively). CPT responses in TPR and TPC were

unaffected by condition (P=0.7048 and 0.4613, respectively). Q, CI, TPR, and TPC were unaffected by sex (P>0.05 for all).

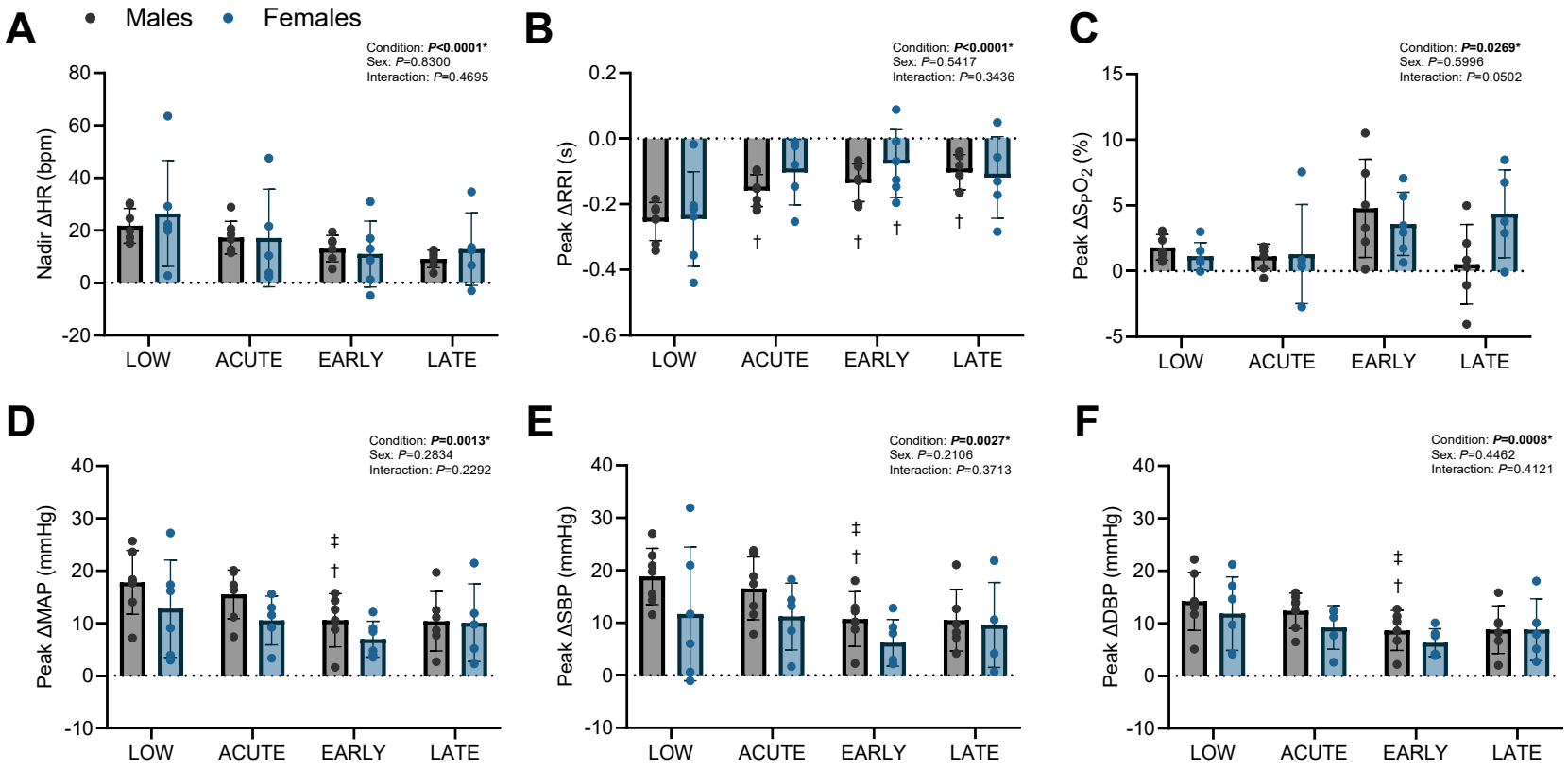

Figure 14. Absolute peak responses to the cold pressor test (CPT) for **A)** heart rate (HR; bpm), **B)** R-R interval (RRI; s), **C)** peripheral oxygen saturation (S_pO_2; %), **D)** mean arterial pressure (MAP; mmHg), **E)** systolic blood pressure (SBP; mmHg), and **F)** diastolic blood pressure (DBP; mmHg). Using 1-minute rolling averages starting every 15 seconds, the maximal peak and nadir responses to CPT determined. Data were analyzed with a two-way repeated measures mixed model ANOVA. Values are reported as means ± standard deviation. † = $P<0.05$ vs LOW; ‡ = $P<0.05$ vs ACUTE.

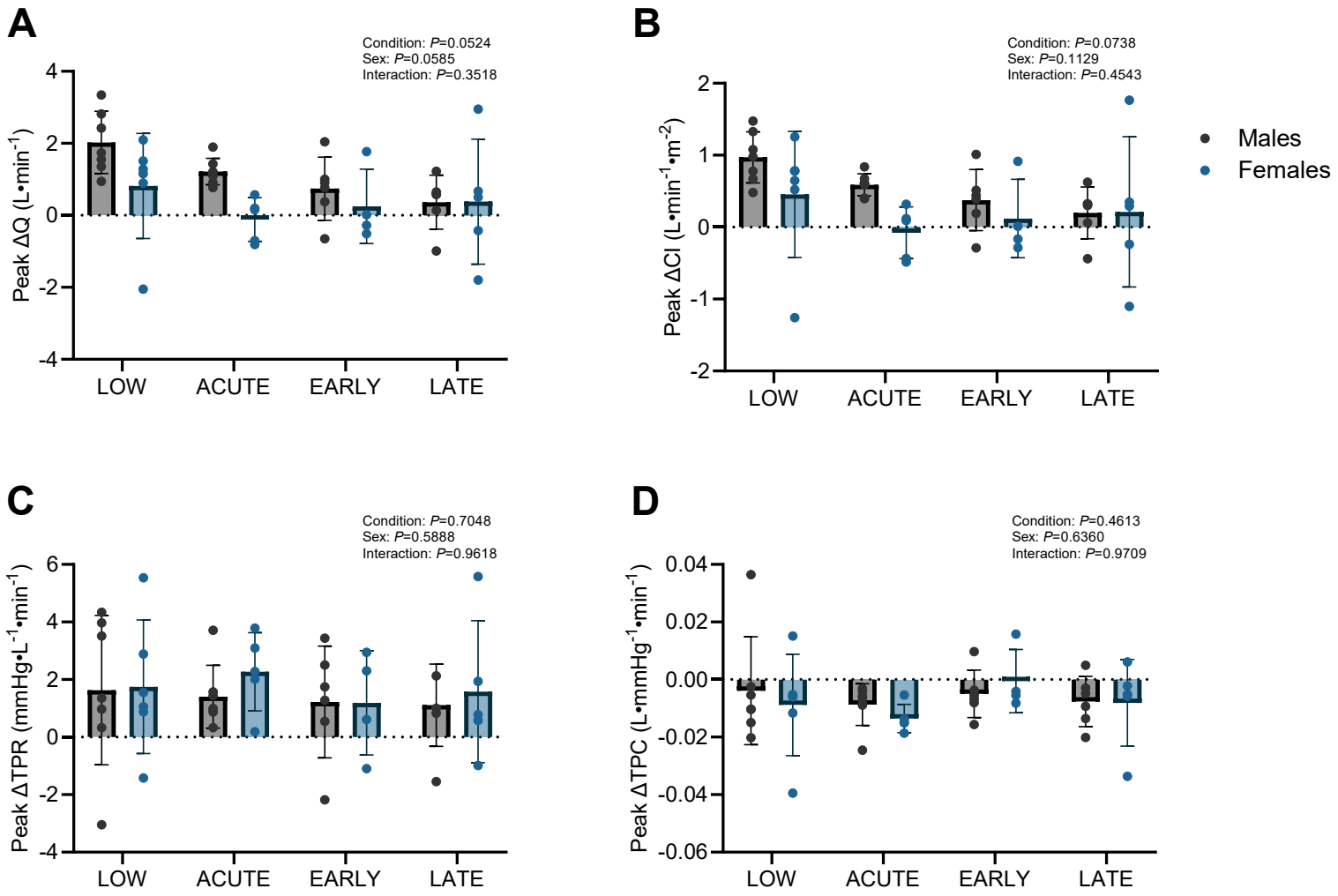

Figure 15. Absolute responses to the cold pressor test (CPT) of variables calculated by ModelFlow algorithm (Finometer Pro, The Netherlands) from heart rate and blood pressure data. **A)** cardiac output (Q; $L \cdot min^{-1}$), **B)** cardiac index (CI; $L \cdot min^{-1} \cdot m^{-2}$), **C)** total peripheral resistance (TPR; $mmHg \cdot L^{-1} \cdot min^{-1}$), and **D)** total peripheral conductance (TPC; $L \cdot mmHg^{-1} \cdot min^{-1}$). Using 1-minute rolling averages starting every 15 seconds, the maximal peak and nadir responses to CPT determined. Data were analyzed with a two-way repeated measures mixed model ANOVA. Values are reported as means ± standard deviation.

4.5 Hyperoxia Rescue Condition

Finally, to assess sympathoexcitatory and compensatory adaptation to high altitude, participants repeated each assessment while breathing 100% oxygen (hyperoxia) at both EARLY and LATE timepoints during acclimatization. **Figure 16** depicts resting MAP, HR, and plasma catecholamine levels at EARLY and LATE while exposed to room air as well as hyperoxia (EARLY-HYP and LATE-HYP).

4.5.1 Baseline Parameters

Sex did not influence any of the variables highlighted in **Figure 16**. (main effect $P>0.05$ for all). Additionally, these were compared to LOW resting values (hatched lines). Resting MAP was significantly affected by the timepoint (main effect $P=0.0319$), with pressures elevated at EARLY, but decreasing by LATE. The hyperoxic condition did not adjust pressures from the room air condition at either timepoint ($P=0.2217$). In particular, resting MAP was significantly higher in comparison to LOW in females at EARLY ($P=0.0048$), yet was not rescued by the hyperoxia condition (LOW vs EARLY-HYP; $P=0.0333$).

Conversely, HR was lowered by the hyperoxia condition (main effect $P<0.0001$). In fact, though HR was augmented at high altitude compared to LOW, it tended to be reduced below low altitude levels with the hyperoxic conditions. but was unaltered by the duration spent at altitude (main effect $P=0.5965$). In addition, there was a significant interaction effect between condition and timepoint ($P=0.0051$); it appeared that hyperoxia attenuated HR more at the EARLY timepoint than at LATE.

As explained in *4.2 Baseline Characteristics,* plasma catecholamine concentrations were not available for consideration for the LOW timepoint. Sex did not impact NE nor EPI ($P>0.05$

for both). Both NE and EPI increased over time at high altitude (P=0.0120 and 0.0003, respectively). NE did not have a main effect for condition (room air vs hyperoxia; P=0.2675), however, there was a significant interaction effect between condition and timepoint (P=0.0491). Plasma concentration of NE appeared to decrease with hyperoxia at EARLY, but not at LATE. In contrast, EPI was unaffected by hyperoxia at both timepoints, with no significant effect of condition (P=0.9468).

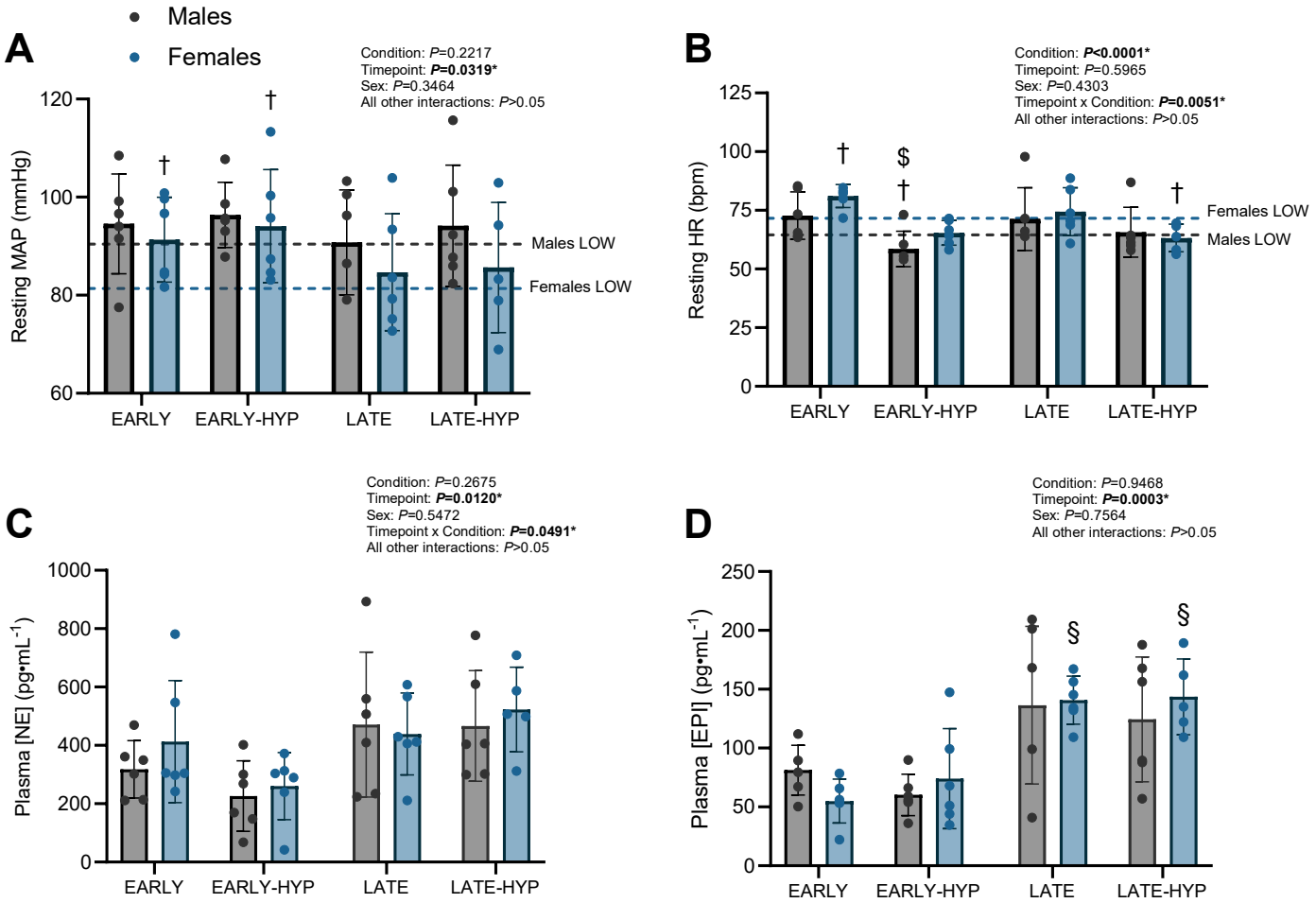

Figure 16. Resting measures during the hyperoxia rescue condition. Resting A) mean arterial pressure (MAP; mmHg), B) heart rate (HR; bpm), and plasma C) norepinephrine (NE) and D) epinephrine (EPI; pg·mL^{-1}) concentrations with early and late acclimatization to high altitude (3,800m), as well as during a 100% oxygen hyperoxia condition at each timepoint. Data was analyzed with a three-way mixed model ANOVA (timepoint [EARLY or LATE] x condition [room air or hyperoxia] x sex [male or female]) with repeated measures. Comparisons with LOW timepoint were performed with planned T-tests using an experiment wise error rate of α'=0.0421. † = P<0.05 vs LOW; $ = P<0.05 vs hyperoxia condition at same timepoint; § = P<0.05 vs EARLY under same condition.

4.5.2 α_1-Adrenergic Sensitivity Slope

MAP and HR sensitivity slopes in response to PE during the hyperoxia trials at EARLY and LATE are reported in **Figure 17**. There was no significant main effects for condition, timepoint, or sex for either MAP or HR (P>0.05 for all). In comparison to LOW, MAP sensitivity slopes were considerably smaller. HR slopes were found to be similar to those at LOW.

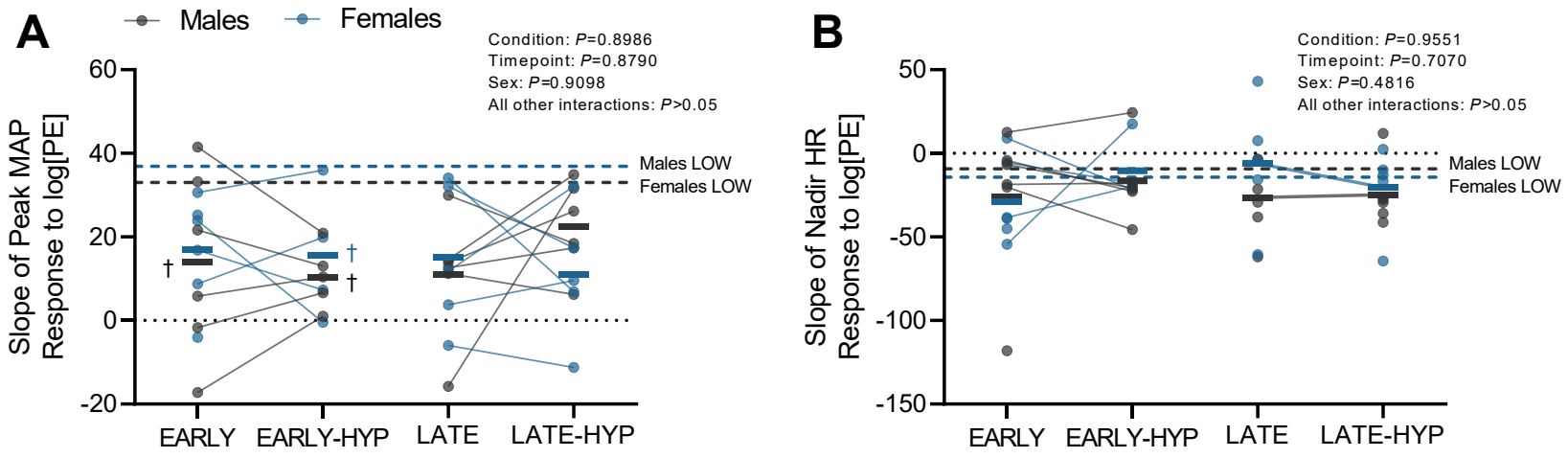

Figure 17. α_1- sensitivity slopes for hyperoxia rescue condition. **A)** Mean arterial pressure (MAP; mmHg) and **B)** heart rate (HR; bpm) at early and late acclimatization to high altitude (3,800m), as well as during a 100% oxygen hyperoxia condition at each timepoint. Data was analyzed with a three-way mixed model ANOVA (timepoint [EARLY or LATE] x condition [room air or hyperoxia] x sex [male or female]) with repeated measures. Comparisons with LOW timepoint were performed with planned T-tests using an experiment wise error rate of α'=0.0421. † (black) = P<0.05 vs LOW in males; † (blue) = P<0.05 vs LOW in females.

4.5.3 Cold Pressor Test

Responses to CPT during room air and hyperoxia trials at high altitude are reported in **Figure 18**. With CPT, MAP was unaffected by both condition (room air vs hyperoxia; P=0.5990) and timepoint (EARLY vs LATE; P=0.2253) at high altitude. In comparison to LOW, males demonstrated attenuated MAP responses to CPT at both EARLY (P=0.0002) and LATE (P=0.0171) and were not brought back to low-like responses with hyperoxia at either timepoint (P= 0.0048 and 0.0058, respectively). Females also exhibited reduced MAP responses with room air and hyperoxia at EARLY (P=0.06400 and 0.05860, respectively) and LATE (P=0.2821 and 0.2271, respectively) compared to LOW, though none of these relationships were significant.

At high altitude, HR was not significantly affected by hyperoxia nor duration at altitude (main effect, P=0.4714 and 0.0801, respectively). However, a reduced HR response was observed in males and females at both timepoints during acclimatization. Change in HR was also lower with the hyperoxia condition at EARLY and LATE in males (P<0.0164 and 0.0040, respectively) but not significantly in females (P=0.0954 and 0.0513, respectively).

The increase in NE during CPT was elevated with hyperoxia in comparison to room air (main effect, P=0.0350). However, absolute concentrations of NE during hyperoxia at EARLY (males, 358.65±106.89pg·mol^{-1}; females, 372.91±169.86pg·mol^{-1}) were similar to those during the room air condition (males, 328.59±104.94pg·mol^{-1}; females, 324.03±106.89pg·mol^{-1}), and similarly absolute [NE] at LATE-HYP (males, 517.99±209.04pg·mol^{-1}; females, 705.54±227.60pg·mol^{-1}) was not different from LATE (males, 507.94±251.99pg·mol^{-1}; females, 531.25±209.04pg·mol^{-1}; main effect P=0.1526). However, absolute values were higher with later acclimatization compared to early (main effect P=0.0222). As depicted in **Figure 16**, resting plasma NE decreased during the hyperoxia condition, particularly during the EARLY timepoint.

Therefore, it appears that NE increases to a similar concentration with sympathoexcitation under hyperoxic conditions (regardless of baseline levels being reduced), and overall absolute baseline and CPT response concentrations are increased with later acclimatization. Additionally, within the same condition, the change in NE concentrations during the CPT from baseline are similar at EARLY and LATE.

Changes in EPI with CPT were not different between hyperoxia and room air (main effect, P=0.4839), indicating the increases above baseline values was similar between the two. However, there was a significant main effect of time on EPI responses (P=0.0240), with augmented EPI increases at LATE. Not only are baseline levels and the absolute concentration of EPI higher during CPT at LATE, but the change in [EPI] with CPT at late is greater than at EARLY (main effect for time, P=0.0240).

Sex did not influence any of the parameters with relation to the hyperoxic trials, nor did it interact with timepoint or condition (P>0.05 for all).

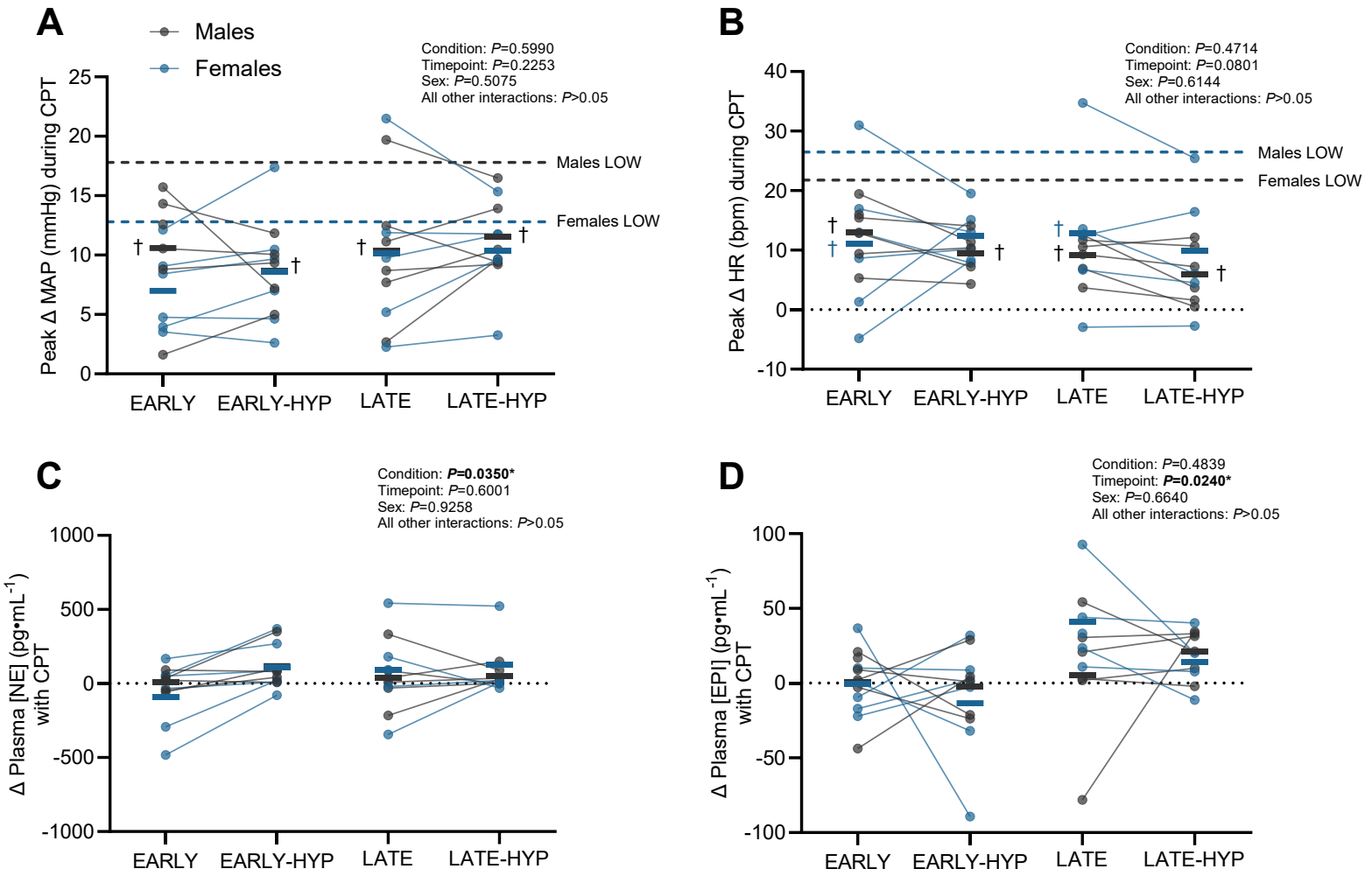

Figure 18. Peak CPT responses during hyperoxia rescue condition. **A)** Mean arterial pressure (MAP; mmHg) and **B)** heart rate (HR; bpm), and changes in plasma concentration of **C)** norepinephrine (NE) and D) epinephrine (EPI; pg·mL^{-1}) at early and late acclimatization to high altitude (3,800m), as well as during a 100% oxygen hyperoxia condition at each timepoint. Data was analyzed with a three-way mixed model ANOVA (timepoint [EARLY or LATE] x condition [room air or hyperoxia] x sex [male or female]) with repeated measures. Comparisons with LOW timepoint were performed with planned T-tests using an experiment wise error rate of α'=0.0421. † (black) = $P<0.05$ vs LOW in males; † (blue) = $P<0.05$ vs LOW in females.

4.6 Post hoc *Sample Size Calculations and Effect Sizes*

Effect size, power, and *post hoc* sample size calculations were performed for MAP α_1-adrenergic sensitivity slopes and maximal CPT response as well as BRS slope *post hoc* to assess the reliability of the statistical analyses (G*Power, Version 3.1.9.7, Germany) and are reported in **Table 3**. Total sample size calculations were determined using an α error probability = 0.05, and power (1 - β error probability) = 0.80. According to Cohen Cohen (1977), for F-tests (ANOVA) a small effect size = 0.10, a medium effect size = 0.25, and a large effect size = 0.40.

Given the large effect size, high power, and small required n for the main effect of condition, it's likely that we did not falsely reject the null hypothesis, and that α_1-adrenergic sensitivity is attenuated with high altitude hypoxia. Given the results reported in **Table 3**, it is also likely that sex is not a determinant factor in this high-altitude adaptation.

Though MAP responses to CPT had a large effect size for condition, this analysis was slightly under powered. There was a high power for the effect of sex, and a large effect size, but we did not reach the required sample size. However, the interaction between condition and sex had a medium effect size, and the number of participants in the study did not reach the calculated *a priori* required n. It is possible that we did not have enough participants enrolled in this study in order to reach significance for a sex and interaction effect due to smaller effect sizes.

Table 3. G*Power output for effect size, power, and calculated *a priori* sample size. Total sample size calculations were determined using an α error probability = 0.05, and power (1 - β error probability) = 0.80. Mean arterial pressure (MAP; mmHg) was selected as the response variable for α_1-Sensitivity slope and max CPT response. Required n reported represents the total sample size; i.e. 2x the number of participants in each group (males, females).

Main Effect	Effect Size	Power	Required n
α_1-Sensitivity Slope			
Condition	0.9721	1.0000	4
Sex	0.1081	0.0736	422
Interaction	0.1445	0.1664	80
Max CPT Response			
Condition	0.7906	0.9999412	6
Sex	0.5514	0.6300278	20
Interaction	0.2933	0.5265188	24

Chapter 5: Discussion

In this study, we aimed to determine the influence of sex on vascular responsiveness to sympathetic activation with acute hypoxia and following short-term acclimatization to high altitude. We demonstrated that α_1-adrenoreceptor sensitivity and sympathetically mediated pressor responses were reduced with hypoxia. However, following ~7 days at high altitude, the pattern of pressor response in females converged with that of males. Together, these data indicate that males and females experience similar attenuation of α_1-adrenergically mediated vascular reactivity with hypoxia. Yet, a separate mechanism may be responsible for differential vascular adaptation between the sexes during chronic hypoxia exposure at high altitude. This study adds evidence to the growing body of literature relating to the influence of sex in relation to high altitude acclimatization.

5.1 Attenuated Adrenergic Sensitivity with Hypoxia

Our first objective was to analyze the specific role of α_1-adrenergic receptors in vascular adaptation to high altitude. To assess this, we administered increasing doses of the α_1-adrenoreceptor agonist phenylephrine hydrochloride (30, 45, and 60$\mu g \cdot L^{-1}$ estimated blood volume) and determined a "sensitivity slope" by regressing the physiological changes from baseline against the log-transformed dose concentration. We found that hypoxia exposure was significantly related to attenuation of α_1-specific sensitivity regardless of sex. Further, we found that reversing hypoxia using hyperoxia at high altitude did not rescue α_1-adrenoreceptor sensitivity back to previously measured sea-level responses, indicating that the observed blunting is an adaptive process rather than a response to hypoxemia per se.

It is well established that chronic exposure to an agonist can cause receptor desensitization. In rats, elevated sympathetic activity induced by chronic intermittent hypoxia causes attenuation of arterial vascular reactivity to adrenergic agonists (Phillips et al., 2006; Silva & Schreihofer, 2011). Further, *in vitro* cell studies indicate that treatment with norepinephrine results in an impaired ability for α_1-adrenergic receptors to enact their associated signalling cascade through phosphorylation of the receptor itself, leading to adrenoreceptor desensitization (Leeb-Lundberg et al., 1987). Previous studies in rats and dogs have demonstrated blunting of α-adrenergic sensitivity with chronic hypoxia exposure, suggesting that this mechanism may be applicable to humans as well (Doyle & Walker, 1991). Given that basal sympathetic activity (and therefore NE release) is elevated with exposure to high altitude, it is possible that α_1-adrenoreceptors undergo desensitization, manifesting as an attenuated responses to phenylephrine. Though we did not directly measure resting sympathetic nerve activity in this study, there is substantial evidence (from our lab and others) that sympathetic nervous activity is elevated at altitude (Berthelsen et al., 2020; Busch et al., 2020; Hansen & Sander, 2003; Simpson et al., 2019). Further, plasma catecholamine concentrations (NE and EPI) measured during early and late acclimatization typically are elevated in comparison to sea level (Mazzeo et al., 1998), however we are unable to draw direct conclusions on this point as we did not obtain a low-altitude measurement.

An inverse relationship between SNA and neurovascular transduction has been observed in a number of studies (Silva & Schreihofer, 2011; Wallin & Nerhed, 1982). This supports that persistently higher sympathetic signalling may result in a downregulation of neurotransmitter-receptor communication. Our lab has also demonstrated that compared to low altitude, sympathetic neurovascular transduction is reduced with high altitude exposure in healthy

individuals, and is related to heightened prevailing MSNA (Berthelsen et al., 2020). Here, we also demonstrated that hyperoxia did not rescue α_1-adrenergic reactivity to non-hypoxemic responsiveness. Together, data from this study and previous evidence indicates that augmented basal MSNA is a primary mechanism driving a lesser neurovascular transduction at altitude, not the effects of hypoxia on the vasculature itself.

In addition to a desensitization of adrenergic receptors with chronic hypoxia when NE levels are elevated, we also observed reduced PE sensitivity slopes during acute hypoxia exposure. This is also in keeping with recent work from our lab demonstrating reduced sympathetic transduction during hypoxia (Steele, Skow, et al., 2021). However, the previously discussed down-regulation of receptor sensitization with chronically elevated SNA is unlikely in this scenario. It has been established that NE concentrations are not significantly elevated during short-term acute hypoxia exposure (Mazzeo et al., 1995), which may be due to altered NE reuptake and washout at the synaptic cleft (Leuenberger et al., 1991). Rather than a blunting of α_1-receptor sensitivity within the short timeframe participants were hypoxemic, elevated washout may also play a role in the reduced PE sensitivity. However, individualized absolute responses to PE doses were significantly altered with hypoxic condition. Notably, with ACUTE, increases in MAP to the lowest doses of PE appeared to be augmented in comparison to LOW. This may be in part due to the susceptibility of individual pressor responses to a single vasoactive pharmacological dose being heavily influenced by baroreflex buffering (Jordan et al., 2002). In other words, a greater MAP response to a specific PE dose may indicate an impaired ability of the baroreflex to buffer the pressor response (Jordan et al., 2002). Indeed, acute hypoxia has been shown to blunt baroreflex sensitivity (Kronsbein et al., 2020). However, this greater pressor response was only evident at the lower doses of PE, and after considering the dose response, a

blunted slope was apparent. It should also be noted that maintained α-adrenergic receptor sensitivity has been previously observed during acute hypoxia, albeit in response endogenous release of NE stimulated with tyramine (Dinenno et al., 2003) . This also reinforces the utility of our approach using a PE sensitivity slope as our measure of α_1-adrenergic receptor sensitivity, rather than the pressor response to a single dose of PE.

5.2 Influence of Sex on High Altitude Acclimatization

Here, we demonstrated no sex differences in basal α_1-adrenergic sensitivity, and similar reductions in sensitivity in males and females with altitude exposure. We also show that peak CPT responses are not significantly different between males and females, regardless of condition. However, when considering the entirety of the response during the CPT exposure, females exhibit an overall attenuated MAP response during the CPT at low altitude, including during an acute hypoxic trial. Following short-term acclimatization to 3,800m (2-9 days), they present with a MAP response pattern on par with that of males. This suggests that there may be a compensatory process that is upregulated in females at altitude, or a mechanism that usually augments vasodilation that also undergoes blunting at altitude that disproportionally affects females.

As previously discussed, a possibility for the reductions in α_1-adrenergic sensitivity with hypoxia is receptor desensitization secondary to increased sympathetic activity. Therefore, it stands to reason that β_2-adrenoreceptors may experience desensitization as well, reducing their capacity to induce vasodilatory mechanisms within the vasculature. Upon exposure to high altitude hypoxia, there is excitation of the sympathoadrenal system, eliciting increases in plasma EPI concentrations. EPI increases quickly upon hypoxemia, and remains elevated for several

days (Mazzeo & Reeves, 2003). This is thought to be a protective mechanism, as β_2-adrenergic stimulation (and subsequent NO release) offsets sympathetically mediated vasoconstriction (Marshall, 2015). However, there is also evidence that higher levels of EPI at rest are associated with increased vascular resistance (Zamudio et al., 2001). A possible explanation is that the β_2-adrenergic receptors are becoming desensitized, which may be a function of the increased circulating epinephrine. For example, pre-treatment with isoproterenol (a β-adrenergic receptor agonist) in rat aortas induced desensitization of β_2-adrenoreceptors, and reduced their ability to elicit vasodilation (Hayes et al., 1986; Schutzer et al., 2006; Vleeming et al., 1990). More applicably to high-altitude travel, β_2-adrenergic reactivity has been shown to be attenuated following 7-days of epinephrine infusion (Tsujimoto & Hoffman, 1985). Thus, it is likely that β-adrenergic receptors are similarly desensitized during chronic sympathetic activation at altitude.

It is widely supported that females exhibit augmented β-adrenergic support of blood pressure regulation in comparison to males (Hart, Charkoudian, et al., 2009; Barry J. Kneale et al., 2000). Given this dependence on paradoxical vasodilation with sympathetic activation in females, it is possible the pattern of vascular reactivity to sympathoexcitation elicited similar responses to males following high altitude acclimatization due to a loss of this compensatory mechanism. Since there is a discrepancy on the time course associated with elevations in the catecholamines norepinephrine and epinephrine (Mazzeo & Reeves, 2003; Rostrup, 1998), it is possible that desensitization of α- and β-adrenoreceptors occurs along different time courses, but this has not been established. Therefore, we can expect that β_2-adrenergic receptor desensitization may occur alongside α_1 receptors, though this may occur with differing temporal manifestations. This also may be a mechanistic explanation for the differences observed in males and females and point to potential differences in adaptation to high altitude.

5.3 Other Potential Receptors and Neuroeffectors

In this study, we have demonstrated attenuation of α_1-adrenergic sensitivity with hypoxia exposure. However, other adrenergic mechanisms also undergo desensitization with prolonged agonist exposure. For example, α_2-adrenoreceptos have been shown to be downregulated with high altitude exposure (Fischetti et al., 2000). Therefore, it is important to consider other signalling processes that might also be affected.

As discussed previously, there are several other neurotransmitters and receptors that are involved in the signalling cascade from increased sympathetic activity to vasoconstriction of vessels. For example, NPY is released from the synaptic cleft during depolarization of sympathetic neurons. Since it is preferentially released with high frequency burst activity (Kennedy et al., 1997), it is an important molecule to consider when elucidating vascular mechanisms during the cold pressor test. It is also important to consider when examining sex differences, as NPY Y1 receptor activity in rats is greater in males than it is in females (Jackson et al., 2005). This may play a role in the greater vasoconstrictive response observed in males throughout the CPT. Though we have demonstrated reduced receptor sensitivity in relation to α_1-adrenergic mechanisms, Y1 receptors appear to maintain sensitivity with hypoxia exposure (Coney & Marshall, 2007). Therefore, these receptors are important to consider in relation to sex differences but may not influence attenuated vascular responses with hypoxia.

There are several local and circulating factors that may influence the transduction of sympathetic nerve signals into changes in vascular resistance. For example, angiotensin II elicits vasoconstriction through a G-protein coupled receptor mediated pathway in the vascular smooth muscle (Saris et al., 2000). Angiotensin II is formed through the actions of renin, which are

released in response to low blood pressure or signalling through sympathetic innervation of the kidney (Hong et al., 2016). Endothelin, an extremely potent vasoconstrictor, is produced in the endothelium and acts on the smooth muscle cells (Yanagisawa et al., 1988). Endothelin has been shown to contribute to increased blood pressure (Cardillo et al., 1999), and also increases with physiological stressors such as hypoxia (Kourembanas et al., 1991). Likewise, competing vasodilatory factors may also dampen basal sympathetic transduction, such as nitric oxide or endothelium derived hyperpolarizing factors (Ozkor et al., 2011). Furthermore, sex differences have been demonstrated in the vascular influences of angiotensin II, endothelin, and NO, which may contribute to differential blood pressure regulation in males and females (B J Kneale et al., 1997; J. A. Miller et al., 1999; Stauffer et al., 2010). In summary, there are many other non-adrenergic effectors that may alter the direct translation of bursts of sympathetic activity into constriction of blood vessels that should be considered.

5.4 Methodological Considerations

Plasma catecholamines. One notable limitation of this study was that resting plasma catecholamine concentrations at the low altitude timepoint (LOW, ACUTE) were unable to be processed. However, according to similar studies, it is probable that resting concentrations of plasma EPI would be elevated at ACUTE from LOW, but NE would not be significantly higher (Mazzeo & Reeves, 2003).

Ventilation. We also did not measure ventilation during the LOW, EARLY, and LATE conditions. In terms of the PE sensitivity responses, we do not expect this to be a confounding factor as respiratory parameters have not been shown to be a factor in PE responsiveness (Kronsbein et al., 2020). However, ventilation is linked to sympathetic outflow (Guyenet, 2014)

and has also been shown to increase fairly dramatically during the cold pressor test (Stone et al., 2019). Nonetheless, as there are no differences between the ventilatory response to CPT in males and females, we do not expect this to impact results (Stone et al., 2019).

Menstrual cycle. Another limitation of this study was that menstrual cycle was not controlled for, nor was cycle phase reported due to the absence of menstruation in most of the female participants (all of which were using hormonal intrauterine devices). We also did not measure serum concentrations of steroid sex hormones, which has been suggested as an important method for confirming cycle phase (along with calendar tracking and luteinizing hormone surge testing) (de Jonge et al., 2019; Schaumberg et al., 2017). Additionally, as many as 30% of physically active females have been found to have ovulatory disturbances (Schaumberg et al., 2017). Due to the nature of expedition research, it would have been near impossible to standardize menstrual cycle phase, even with cycle phase confirmation with serum estrogen and progesterone concentrations. It has been demonstrated previously that PE responses are unchanged throughout the menstrual cycle, suggesting that α_1-adrenergic specific sensitivity as determined in this study would be unaffected (Minson et al., 2000). However, since estrogen has direct regulatory effects on β-adrenoreceptors (Machuki et al., 2018), this is an important caveat to consider.

5.5 Implications

Our study was conducted in low altitude dwellers that ascended to high altitude and has direct implications for in population for short sojourns at high elevations. There are also a number of populations that are indigenous to high altitudes, including the Nepalese Sherpa, Andean Quechua, and Ethiopian highlanders. Though these populations have developed genetic adaptations to living at high elevations, we have previously demonstrated that their

neurovascular transduction is blunted to a similar degree as lowlanders at high altitude, and have suggested that this reduced transduction is a function of resting levels of MSNA (Berthelsen et al., 2020). This may be a mechanistic explanation for the observation that high altitude indigenous populations also experience hypertension disproportionately to lowlanders, and that it may in part be due to chronically increased SNA (Narvaez-Guerra et al., 2018).

The results of this study may also have utility in vascular changes with aging. It has been demonstrated that MSNA increases with age, and disproportionally so in females post menopause so that they become aligned with males (Keir et al., 2019). This signifies another state where MSNA is elevated, thereby potentially having desensitizing effects on sympathetically mediated receptors. It has been suggested that β_2-adrenergic receptors become less sensitive with aging (Lakatta, 1987), and that this may be as a result of higher levels of circulating catecholamines as a consequence of increased MSNA (Petrie et al., 2000). Since females display increased sensitivity of β-adrenergic receptors, this proposes an important consideration for mechanisms of aging and receptor desensitization, and how this may impact health and disease.

Given the previous discussion surrounding the cause of adrenergic receptor desensitization being the increased sympathetic outflow that occurs with hypoxia, rather than as a consequence of hypoxia itself, our findings may be relevant to other conditions with chronically elevated levels of sympathetic activity. For example, conditions such as chronic obstructive pulmonary disease (COPD) present with high sympathetic nervous activity (Grote et al., 2000; Steele, Berthelsen, et al., 2021). Individuals with this pathology also exhibit impaired sympathetic neurovascular transduction (Steele, Berthelsen, et al., 2021). This may partially be as a result of adrenergic receptor desensitization, as evidenced by reduced vascular reactivity

(Heistad et al., 1972). This is further supported by the demonstration that both α- and β-adrenoreceptors are less sensitive in individuals with COPD (Grote et al., 2000). Since there are differences in the reliance males and females have on adrenergic mechanisms in the maintenance of blood pressure, our results may be relevant to pathologies that are hallmarked by augmented sympathetic activity.

Chapter 6: Conclusion

6.1 Main Findings

Our results indicate that vascular reactivity is attenuated with high altitude hypoxia exposure, and this in part may be due to a reduction in α_1-adrenergic receptor sensitivity. We also discussed how the desensitization of α_1-adrenoreceptors may be elicited by a sustained elevation in sympathetic (and therefore norepinephrine) stimulation rather than the effects of hypoxia per se. This effect appears to be consistent in males and females. Additionally, a hyperoxia "rescue" condition did not correct responses to those observed at sea-level, indicating an adaptive process that is not an acute reaction to hypoxemia.

In response to sympathoexcitation elicited by the cold pressor test, females exhibited a lesser pressor response. However, following exposure hypoxia at high altitude, the difference between males and females appeared to converge. Since α_1-adrenergic sensitivity was attenuated to a similar degree in males and females, this suggests an additional potent mechanism whereby females lose their ability to present with paradoxical vasodilation in response to stress. Given their greater reliance on β_2-adrenergic mechanisms, it is possible that desensitization of β_2-adrenoreceptors is driving this process.

6.2 Future Directions

Further studies should focus on specific mechanistic approaches to elucidate the roles of various adrenergic receptors in blood pressure regulation in both males and females. Using microneurography to measure muscle sympathetic nerve activity with concurrent arterial blood flow would also allow for concurrent analyses relating to sympathetic neurovascular

transduction. It would also be of interest to utilize infusions of adrenergic pharmacologics localized to the forearm (e.g. Dinenno et al., 2002), rather than systemic bolus injections as we did in this study. This would allow us to avoid the systemic compensatory mechanisms (i.e. the baroreflex), and focus on the individual role of targeted receptors. Here, we postulate the differences observed between males and females may be in part due to differences in activity of β_2-adrenergic receptors. Applying a β-antagonist such as propranolol in conjunction with sympathoexcitation (either endogenously through sympathoexcitatory tests or by applying an agonist such as norepinephrine) would allow us to specifically investigate β_2-adrenoreceptor sensitivity, which are suggested to be of greater importance in females.

Along with a more targeted approach to elucidate the actions of adrenergic receptors, it would be of interest to quantify circulating levels of sex hormones in both males and females at the time of testing. Since sex hormones appear to influence β-adrenergic activity, characterizing hormone concentrations in both sexes could allow us to gain a better understanding on their role in vascular regulation.

Finally, since our results indicate tonic exposure to high levels of catecholamines may be responsible for the desensitization of adrenergic receptors, it may be of interest to test a later timepoint than was achieved in this study, when the temporal pattern of plasma EPI concentration begins to diverge from NE and fall back towards sea-level values.

6.3 Conclusion

In conclusion, we have demonstrated that specific α_1-receptor sensitivity is reduced with high altitude hypoxia in both males and females. This may be due to adrenoreceptor desensitization as a result of chronically elevated levels of catecholamines as a result of

augmented resting sympathetic nervous activity. This study adds to the growing body of literature on sex differences and emphasizes specific directions for future research on the way males and females adapt to hypoxia.

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

Approval Form

Date:	August 02, 2019
Principal Investigator:	Craig Steinback
Study ID:	Pro00088122
Study Title:	Sex differences in sympathetic activity and vascular reactivity during acute and chronic hypoxia.
Approval Expiry Date:	August 1, 2020
Date of Informed Consent:	Approval Date / Approved Document 8/2/2019 Optional Biobanking Consent (LAB) CLEAN 8/2/2019 Consent Form (ALTITUDE) CLEAN 8/2/2019 Consent Form (LAB) CLEAN
Funding/Sponsor:	NSERC - Natural Sciences And Engineering Research Council

Thank you for submitting the above study to the Health Research Ethics Board - Biomedical Panel, which was reviewed at the June 12, 2019 meeting. All issues arising from the meeting have been addressed. The study is now approved. The following documentation forms part of this approval:

- Protocol Overview (Undated);
- Prescribing Information for Phenylephrine Hydrochloride Injection USP 1% (11/99);
- Main Consent Form Version 5 (27 Jul 2019);
- Main Consent Form Version 5 (27 Jul 2019);
- Optional Biobanking Consent Form Version 3 (02 Jul 2019);
- Letter of Initial Contact (Undated);
- Health History Questionnaire Version 2 (01 May 2019);
- Recruitment Poster with tabs;
- Recruitment Poster social media.

We acknowledge receipt of the Health Canada No Objection Letter re: Protocol #REMOPro00088122 Version 4 (30 Jul 2019).

The Health Research Ethics Board assessed all matters required by section 50(1)(a) of the Health Information Act. Subject consent for access to identifiable health information is required for the research described in the ethics application, and appropriate procedures for such consent have been approved by the HREB - Biomedical Panel. In order to comply with the Health Information Act, a copy of the approval form is being sent to the Office of the Information and Privacy Commissioner.

Any proposed changes to the study must be submitted to the REB for approval prior to implementation.

A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date (August 1, 2020), you will have to re-submit an ethics application.

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

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

Sincerely,

Donald W. Morrish, MD, PhD, FRCPC
Associate Chair, Health Research Ethics Board – Biomedical Panel

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

Appendix II: University of Calgary Conjoint Health Research Ethics Board (CHREB) Approval

CERTIFICATION OF INSTITUTIONAL ETHICS APPROVAL

The Conjoint Health Research Ethics Board (CHREB), University of Calgary has reviewed and approved the following research protocol:

Ethics ID:	REB18-0374
Principal Investigator:	Richard James Alfred Wilson
Co-Investigator(s):	There are no items to display
Student Co-Investigator(s):	Brittney Herrington
Study Title:	Integrative human physiological responses and acclimatization to high altitude.
Sponsor:	Natural Sciences and Engineering Research Council

Effective: Tuesday, June 4, 2019 **Expires:** Thursday, June 4, 2020

This application was reviewed and approved by the Conjoint Health Research Ethics Board at its meeting on May 2, 2019.

The following documents have been approved for use:

- Pre-screening information/ Participant ID assignment, 2, May 12, 2019
- UC CHREB CONSENT FORM WHITE MOUNTAIN 2019 - FINAL, 4, May 23, 2019
- AMS scoring system, 1, March 31, 2019
- Daily Measures Participant Data Sheet, 1, March 31, 2019
- Sleep log (for study #2), 1, March 31, 2019
- White Mountain Barcroft Lab Expedition Project Proposals For CHREB Application - FINAL , 4, May 23, 2019
- Budget, 2, June 4, 2019
- participant numbers, 1, May 12, 2019

The CHREB is constituted and operates in accordance with the current version of the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans* (TCPS); International Conference on Harmonization E6: Good Clinical Practice Guidelines (ICH-GCP); Part C, Division 5 of the Food and Drug regulations, Part 4 of the Natural Health Product Regulations and the Medical Device Regulations of Health Canada; Alberta's Health Information Act, RSA 2000 cH-5; and US Federal Regulations 45 CFR part 46, 21 CFR part 50 and 56.

You and your co-investigators are not members of the CHREB and did not participate in review or voting on this study.

Restrictions:

This Certification is subject to the following conditions:

1. Approval is granted only for the research and purposes described in the application.
2. Any modification to the approved research must be submitted to the CHREB for approval.
3. An annual application for renewal of ethics certification must be submitted and approved by the above expiry date.
4. A closure request must be sent to the CHREB when the research is complete or terminated.

Approval by the REB does not necessarily constitute authorization to initiate the conduct of this research. The Principal Investigator is responsible for ensuring required approvals from other involved organizations (e.g., Alberta Health Services, community organizations, school boards) are obtained.

Approved By:	**Date:**
Stacey A. Page, PhD, Chair , CHREB	Tuesday, June 4, 2019

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

Appendix III: Health Canada Clinical Trial No Objection Letter (NOL)

Health Canada / Santé Canada

Therapeutic Products Directorate
5th Floor, Holland Cross, Tower B
Address Locator # 3105A
OTTAWA, Ontario
K1A 0K9

JUL 30 2019

Dr. Craig Steinback
Assistant Professor
Governors of The University of Alberta
1-059A Li Ka Shing Center For Research
11203-87 Ave. NW
EDMONTON, Ontario
T6G 2H5
(780) 492-5553

Your file / Votre référence
HC6-24-c229503
Our file / Notre référence

No Objection Letter RE: Protocol # REMO Pro00088122 (Version 4)

Dear Dr. Steinback:

I am pleased to inform you that the information and material to support your Clinical Trial Application for **PHENYLEPHRINE**, control number **229503**, received on July 16, 2019, have been reviewed and we have no objection to your proposed study. I would remind you of the necessity of complying with the *Food and Drug Regulations*, Division 5, in the sale of this product for clinical testing. In addition, the regulations impose record keeping responsibilities on those conducting clinical trials. You are also reminded that all clinical trials should be conducted in compliance with the Therapeutic Products Directorate's *Guideline for Good Clinical Practice*.

Please note that Health Canada has implemented electronic reporting of adverse drug reactions and is currently in pilots with some sponsors. Those sponsors who have an established electronic connection with Canada Vigilance Production stream should submit their reports using the distribution rules provided to them by Health Canada, and reporting to multiple directorates is no longer required. For the sponsors who have not yet established this connection, they should continue submitting their reports to the applicable directorate by fax or by courier. The following website provides further clarification on Health Canada's adverse drug reactions reporting requirements for clinical trials:
http://www.hc-sc.gc.ca/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/guide-ld/ich/efficac/e2a_pre_notice_avis-eng.pdf

Consistent with Health Canada's Notice - *Registration and Disclosure of Clinical Trial Information* of November 30, 2007, sponsors are encouraged to register their clinical trials within 21 days of the trial's onset, using a publicly available registry that conforms with international standards for registries such as: Clinicaltrials.gov (www.clinicaltrials.gov); Current Controlled Trials (www.controlled-trials.com).

Should you have any questions concerning this letter, please contact the Office of Clinical Trials (613) 941-2132.

Yours sincerely,

Maurica Maher, MD, MSc., FRCPC
Clinical Manager
Office of Clinical Trials

MM/km

Canada

Appendix IV: Consent Form for Participants

PARTICIPANT CONSENT FORM

Title of Research Study: **Sex differences in sympathetic activity and vascular reactivity during acute hypoxia.**

Principal Investigator: **Dr. Craig Steinback, PhD**

Research Coordinators: **Emily Vanden Berg, BSc**
Andrew Steele, BSc (Kin)
Lindsey Berthelsen, B.H.K.

The purpose of this letter is to provide you with the information you need to make an informed decision as to whether you wish to take part in our study. Before you make a decision one of the researchers will go over this form with you. Please ask questions if you feel anything needs to be made clearer. You will be given a copy of this form for your records.

Why am I being asked to take part in this research study?
You are being asked to participate in this research study because you are healthy. The aim of our study is to assess whether there is a difference between men and women in how their blood vessels respond when breathing a lower than normal amount of oxygen. We are also interested in the effect that different levels of sex hormones have. Our findings will help us to understand differences between men and women, especially to those travelling to high altitudes, where oxygen is less available.

What is the reason for doing the study?
At high altitudes you are exposed to a lower than normal amount of oxygen. The body responds to try and keep enough oxygen going to its tissues. One of these responses is an increase in activity in the sympathetic nervous system (SNS). The SNS is responsible for your "fight or flight" response. This system typically acts to constrict blood vessels, also known as vasoconstriction, and increase your blood pressure.

Research has shown that men and women control their blood pressure in different ways. For example, women tend to have lower blood pressure than men. Women also seem to have less vasoconstriction when their SNS is stressed. It is also possible that the female sex hormone estrogen causes relaxation or dilation of the blood vessels. Whether sex differences exist during acclimatization to high altitude remains unknown. As such, the purpose of this study is to determine if women have less vasoconstriction than men during brief periods of low oxygen. This study could have important implications for women's health and in the field of high altitude travel.

We will be using two tests to cause short term constriction of the blood vessels and increases in blood pressure. The tests will cause your blood pressure to go up a similar amount it does when you exercise for 10-15 minutes and will return to normal after the test. The first test we will use is the drug phenylephrine. It will cause your blood pressure to go up a smaller amount than it does when you exercise. The reason we are using this test is to increase your blood pressure without activating the sympathetic nervous system first. The second test is a cold pressor test where you will put your hand in ice-cold water. This is to activate the sympathetic nervous system and cause your blood vessels to constrict. Looking at the difference between these two tests will tell us more about how the SNS causes changes in blood pressure.

Am I eligible to take part in this study?

You have already been pre-screened for general criteria making you eligible for this study. Following giving your consent, we will provide you with a questionnaire designed to gather more information on your current and previous health. If any current or previous health concerns are identified which exclude you from participating, we will tell you and the testing session will be cancelled.

What will happen in the study?

If you meet the criteria for this study, you will be asked to be tested three times. Once during baseline testing at the Integrative Physiology Lab (B126) in Calgary, AB (1,045m), and twice during a stay at the Barcroft Station on White Mountain, CA (3,800m). The location of the lab in Calgary is in room B126 at Mount Royal University 4826 Mt Royal Gate SW. It is accessible by city transit. You will need to visit the lab one time. You will also be tested two times during your stay on White Mountain: once early (day 2 or 3) and late (day 9 or 10). We will need you to stay for a total of 1-2 hours total per test.

Equipment:

- We will place an intravenous (IV) line in a vein on the inside of one of your elbows. It will also have a three-way valve attached. This will allow us to do three things:
    1. Take blood samples. In total we will take about 72mL (<5 tbsp, or ~1/7th of a blood donation) throughout the test.
    2. Deliver injections of the study drug.
    3. Connect a device to measure the blood pressure inside of your vein. This device is filled with fluid (saline), and the movement of the fluid allows us to measure the pressure in your veins. It will not move into or out of your IV as the pressure is very low.

- Two sets of small electrocardiogram (ECG) stickers will be used to monitor your heart rate constantly throughout the experiment. Each set has three stickers. Two stickers goes on your left shoulder, two on the right shoulder, and two on your left side.

- An arm cuff will be placed around one arm for taking blood pressure in the same way that your doctor would. In addition, a small finger cuff will be placed on the middle finger of the same arm. This finger cuff will allow us to measure your blood pressure during every heartbeat.

- A small clip will be placed on the index finger of one hand to measure the amount of oxygen in your blood.

- An ultrasound probe will be used on the inside of one of your elbows to measure changes in your artery.

- A mouthpiece (similar to one used while snorkeling) and nose clip to allow us to measure the rate and depth that you breathe as well as the amount of oxygen and carbon dioxide you breathe in and out.

Once equipment is set up, we will turn on the blood pressure finger cuff, oxygen monitor, and ask you to breathe through the mouthpiece. The face mask, finger clip, and ECG leads must be worn throughout the entire duration of the experiment. If the equipment becomes uncomfortable during any part of the protocol, an investigator will help readjust the equipment.

Protocol:
Following instrumentation, we will get you to lay still for 10 minutes and relax. This will allow us to obtain measurements of normal values (baseline) for each measure we are recording. Then we will perform two tests while you breathe room air:

1) Phenylephrine hydrochloride injections (3 total). We will inject three separate doses of phenylephrine into the IV line in your arm. This will be done by a trained researcher who has experience with this type of injection under the guidance of a physician (Dr. Peter Ondrus). The drug will increase your blood pressure slightly by causing your blood vessels to constrict, without activating your sympathetic nervous system at the same time. The doses are designed to increase your blood pressure by 10, 20, and 30mmHg. These doses will be based on your estimated blood volume, which we will calculate from your height and weight. The drug will wash out of your system between 2-3 minutes, and your blood pressure will return to normal.
2) Cold pressor test (CPT). During this test you will be asked to place your hand in a bowl of iced water up to your wrist for up to 3 minutes. We will encourage you to keep your hand in the ice water for the full 3 minutes, by if this is too uncomfortable for you, you can remove your hand from the bowl whenever you wish. Once you remove your hand from the bowl, we will give you a heading pad or a hot water bottle to rewarm your hand.

After the room air tests, the air you are breathing through the mouthpiece is switched. The gas composition will vary depending on the testing location.

- During baseline testing in Calgary, AB, the air will be switched to a gas composition that mimics being at an altitude of about 4,500m. You will feel like you need to breathe more, but this is natural. After 10 minutes of breathing this gas composition we will repeat the same phenylephrine injections and cold pressor test. The mouthpiece will then be removed, and you will be able to rewarm your hand while breathing room air.
- During early (day 2 or 3) and late (day 9 or 10) testing at White Mountain, CA, the air will be switched to a gas composition that mimics your own gas values measured during baseline testing in Calgary. After 10 minutes of breathing this gas composition we will repeat the same phenylephrine injections and cold pressor test. The mouthpiece will then be removed, and you will be able to rewarm your hand while breathing room air.

What will I be asked to do while I am in the study?
You will be asked to not eat anything for 12 hours (overnight) before coming into the lab. We ask you not have any caffeine the morning of your test or alcohol the night before. Finally, please do not go to the gym or do any physical activity more than normal walking / stair climbing the morning of your visit.

You will be most comfortable if you wear loose fitting shorts and a tank top or short sleeve top that is loose fitting (males may go topless if they wish). We ask that persons with long hair tie it up. Once in the lab, it is important that you are comfortable and relaxed and tell us if anything is wrong or uncomfortable.

We will go over all procedures in advance and we can address any questions or concerns you might have. We will take one blood sample when you first come into the lab and take eight additional blood samples throughout the procedure to determine certain hormone levels (i.e. progesterone, estrogen, testosterone) and nervous system signals (catecholamines such as norepinephrine). We will take less than one tablespoon of blood for each sample. After the fasted blood sample is taken, we will provide you with a standard breakfast. If you have any dietary restrictions these will be accommodated. After eating, you will then lay comfortably on a hospital bed and we will begin to put on the equipment required to do the study.

Women: During your recruitment visit, you will be given a calendar and a home ovulation test kit. We ask that you fill out past menstrual cycle data (such as the day you start your period) and track your current cycle on the calendar. We ask that you also use the ovulation test kit every day from now until the end of the White Mountain trip. Do these tests at the same time every day. When you have a positive test result, mark it on your calendar hand out.

What are the benefits to me?
You are not expected to benefit directly from being in this research study.

What are the risks and discomforts?

Reduced Oxygen: Breathing air with a reduced amount of oxygen may cause you to breathe deeper and/or faster. This is a normal response and we encourage you to breathe however you feel most comfortable. In some individuals, breathing deeper or faster may cause a sensation of breathlessness or claustrophobia. If at any point you feel uncomfortable and do not wish to continue, we can switch the gas back to room air. This should immediately relieve any discomfort. Reduced oxygen, much lower than used in the current study, may cause dizziness or loss of consciousness. We will be monitoring the amount of oxygen in your blood throughout the study and can terminate the study at any point if your oxygen should drop below what is considered acceptable. We also have oxygen on hand to breathe if needed, which will return the amount of oxygen in your blood back to normal very quickly.

Acute Mountain Sickness (AMS): While breathing a lower than normal level of oxygen you may develop symptoms of acute mountain sickness (AMS). Acute mountain sickness may occur in some people (about 1 in 4) who ascend rapidly to an altitude greater than 2,500m. Because

of the rapid drop in oxygen in the air you're breathing in, you may experience AMS. Symptoms of AMS include headache, dizziness, peripheral paresthesia (i.e. tingling or numbness in the arms or legs) and breathlessness. If this happens, you will be disconnected from the gas circuit right away and the facemask will be removed. If your symptoms go away, you may choose to either resume or end the test. There are no lasting effects of AMS, and symptoms resolve upon "descent" from altitude - in this case breathing normal room air.

Phenylephrine Injections: This drug will cause your blood vessels to constrict and your blood pressure to go up slightly. The increase in blood pressure is similar to how much it would go up if you were to exercise for about 10-15 minutes. It may cause you to feel a slight tingling in your fingertips. You also may feel your heart rate slow down and/or a heart-pounding sensation. These are completely normal and will go away within 3 minutes, when the drug washes out of your system. There is also a possibility of you having an adverse reaction to the drug, such as an allergy (i.e. redness in the area the drug was given) or rarely, a heart arrythmia. There is a possible risk of you fainting during these changes in blood pressure. Your heart rate and blood pressure will be monitored constantly throughout the test. The test will be stopped if you feel light-headed, nauseated, or experience any other adverse sign or symptom, and the researchers will help you accordingly.

Blood Samples: With any procedure using needles, there is minimal risk of infection. The researcher who takes your blood samples will be trained to do so and will ensure that they follow all procedures that reduce the risk of infection (e.g. handwashing, wearing gloves, sterile techniques, single use equipment). Placing the IV in your arm may result in some discomfort, minimal pain upon insertion, swelling, redness, or bruising. Sterile technique will be used and only a certified technician will take blood samples. Only a certified phlebotomist will insert the IV into your arm. Pressure will be applied when the IV is taken out to avoid bruising. We must disclose that we are unable to give results of blood tests at the time of investigation.

Cold Pressor Test: There are no risks associated with exposure to ice water for the duration used in this study (3 min). Cold stress is a familiar experience to most people (handling frozen foods, making snowballs, etc.). This exposure is meant to cause a minimal amount of discomfort (stress). This may be experienced as a sharp sensation on the skin, tingling, or numbness. You are encouraged to keep your hand in the ice water for the full 3 minutes, but this is voluntary, and you may remove your hand at any point. Any discomfort or sensations will go away quickly once your hand is removed from the ice water and a heating pad will be used to rewarm your hand.

***Ultrasound*:** The ultrasound used to image your heart and blood vessels has no known risks involved with it. You will be provided with a hospital gown to wear during the heart scan and a trained female sonographer will complete the scan.

Blood Pressure Monitors: The finger and/or arm cuff for measuring blood pressure may cause some discomfort including numbness, tingling, or discoloration (bruising) in the finger or arm. These will return to normal soon after the cuff is removed.

Abnormal findings: Within this study, we take many different measurements that tell us about your heart and blood vessels. It is possible, but rare, that we may identify abnormalities that

require further consultation from a medical professional. If any abnormal findings are identified during your participation in the study, we will provide you with full details, contact your chosen medical professional (e.g. your family doctor or obstetrician) and, with your permission, refer you to a responsible medical doctor.

If you experience any abnormal and ongoing problems as a result of any of the study procedures, we ask that you inform the researchers immediately. We will ensure that you receive necessary medical treatment, at no additional cost to you. Again, we will provide you with full details of the study and our measurements, contact your chosen medical professional (e.g. your family doctor) and refer you to a responsible medical doctor. If you suffer any ongoing problems, please call either Dr. Craig Steinback at 780-492-5553. Should you need urgent medical care, please go to the hospital.

Other: If we find out anything new during the course of this research which may change your willingness to be in the study, we will tell you about these findings.

Do I have to take part in this study?
Being in this study is your choice. If you decide to be in this study, you can change your mind and stop being in the study at any time, and it will in no way affect the care or treatment you are entitled to.

Can my participation in the study end early?
You are free to withdraw from this study at any time for any reason. You can do this by contacting the investigators. If after participating in the study you wish to remove your information or blood samples from the study, you have until December 31, 2022 to do so. After this time all information will be used. We may request that you withdraw from the study during the protocol if we are at all worried about your general health (i.e. high blood pressure, irregular heart rhythm etc.). We will notify you of our reason should this occur.

Will I be paid to be in the research?
You will not be paid for participation in this study, nor should you incur any expenses related to this study.

Will my information be kept private?
During the study we will be collecting health data about you. We will do everything we can to make sure that this data is kept private. No data relating to this study that includes your name will be released outside of the researcher's office or published by the researchers. Sometimes, by law, we may have to release your information with your name so we cannot guarantee absolute privacy. However, we will make every legal effort to make sure that your health information is kept private.

The researchers will ask you questions about your personal health. Any personal health information that you share with us will only be what is needed for the study.

During research studies it is important that the data we get is accurate. For this reason your health data, including your name, may be looked at by people from the University of Alberta, University of Alberta auditors and members of the Research Ethics Board and Health Canada.

By signing this consent form you are giving permission for the study staff to collect, use and disclose information about you from your personal health records as described above.

After the study is done, we will still need to securely store your health data that was collected as part of the study. We keep data stored for 25 years after the end of the study.

If you leave the study, we will not collect new health information about you, but we will need to keep the data that we have already collected.

Any data collected will be kept in a locked cabinet. Digital data will be stored in a password protected and encrypted computer. Only study investigators have access to these data. Your name will be excluded. We will only use the data collected for research purposes. Any research data published as a result of this study will be presented as group data and will not identify you as a participant. Study data and your blood samples (your name excluded) will be kept indefinitely.

Your blood samples collected for this study will be kept until analysis for tests described above. If any blood samples are left over after analysis, they will be stored for up to 25 years and may be used by other members of our research group for future research examining metabolic cardiovascular health. These will be broader analyses on sympathetic neurotransmitters across conditions pooled with other studies from our lab. Left-over samples will not be labeled with your name and will be stored securely in the Physical Activity and Diabetes Laboratory (1-052 Li Ka Shing Center) or the Neurovascular Health Lab (4-269 VVC) at the University of Alberta. You can ask for your left-over samples to be destroyed at any time. However, since your personal information will be destroyed after 25 years, we will no longer be able to match the sample to you after this time.

What happens if I am injured because of this research?
If you become ill or injured as a result of being in this study, you will receive necessary medical treatment, at no additional cost to you. By signing this consent form you are not releasing the investigator(s), institution(s) and/or sponsor(s) from their legal and professional responsibilities. If you suffer a research-related injury, please call Dr. Craig Steinback at 780-492-5553. Should you need urgent medical care, please go to the hospital.

What if I have questions?
If you have any questions about the research now or later, please contact Dr. Craig Steinback at 780-492-5553. If you have any questions regarding your rights as a research participant, you may contact the Health Research Ethics Board at 780-492-2615. This office is independent of the investigators.

This study is funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

Title of Study: Sex differences in sympathetic activity and vascular reactivity during acute hypoxia.
Principal Investigator: Dr. Craig Steinback. PhD **Phone Number: 780-492-5553**
Research Coordinators: Emily Vanden Berg, BSc; Andrew Steele, BSc (Kin); Lindsey Berthelsen, B.H.K.

	Yes	No
Do you understand that you have been asked to be in a research study?	☐	☐
Have you read and received a copy of the attached Information Sheet?	☐	☐
Do you understand the benefits and risks involved in taking part in this research study?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Do you understand that you are free to leave the study at any time, without having to give a reason and without affecting your future medical care?	☐	☐
Has the issue of confidentiality been explained to you?	☐	☐
Do you understand who will have access to your records, including personally identifiable health information?	☐	☐
Do you want the investigator(s) to inform your family doctor that you are participating in this research study? If so, give his/her name _____	☐	☐

Who explained this study to you? _____

I agree to take part in this study:

Signature of Research Participant: _____

(Printed Name) _____

Date: _____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of Investigator or Designee: _____

Date: _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH PARTICIPANT

Appendix V: Health History Questionnaire

STUDY: Sex differences in sympathetic activity and vascular reactivity during acute hypoxia.
SUBJECT ID #:
RESEARCHER INITIALS: **DATE:**

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

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

ALL PARTICIPANTS

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

_____

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

_____

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

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

_____

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

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

	Yes	No
Do you smoke?	☐	☐

(If yes, how many cigarettes per day?)

_____

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

_____

	Yes	No
Do you use cannabis?	☐	☐

	Yes	No
(If yes, do you smoke it?)	☐	☐

(If yes, how many times per week?)

_____

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

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

_____

_____

	Yes	No
Are you allergic to sulfites?	☐	☐

	Yes	No
Do you have any other allergies? (If yes, please list/explain)	☐	☐

_____

_____

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

_____

_____

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

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

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

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

DEFINITIONS:

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

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

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

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

- μ often
- μ sometimes
- μ never/rarely

WOMEN

Please check **any and all** that apply

	Yes	No
Are you post-menopausal? (If not, how long since the first day of your last period?)	☐	☐
Are you on hormone replacement therapy?	☐	☐
Are you currently using oral contraceptives? (If yes, what is the type/brand?)	☐	☐
Are you currently using other hormonal contraceptives? (i.e. hormonal IUD) (If yes, what is the type/brand?)	☐	☐
Are you pregnant? (If yes, how many weeks?)	☐	☐
Have you been pregnant previously? (If yes, please indicate the number of previous pregnancies. Were there any complications, including pregnancy related hypertension, gestational diabetes, or pre-eclampsia?)	☐	☐

Appendix VI: Sample "Day-Of" Data Sheet and Protocol Checklist

Date: _____ Participant ID: _____

Day-of Participant Information Form

Study Title: Sex differences in sympathetic activity and vascular reactivity during acute and chronic hypoxia

Sex: _____ D.O.B: _____ (DD/MM/YYYY) Age: _____ years

Height: _____ cm Weight: _____ kg BMI: _____ kg/m^2

If female:
Hormonal contraceptive use: Y / N If yes, type: OC / IUD / Other: _____

Day 1 of last menstrual cycle: _____

Menstrual phase: High / Low phase (OC) OR EF / ML phase (no OC)

Test day: ☐ Baseline (Calgary) ☐ Early (day ____) ☐ Late (day ____)

Time of data collection: _____ AM / PM

Atmospheric pressure: _____ mmHg Temperature: _____ °C

Name of file: _____

Consent form signed: Y / N

Time since last meal: _____ Size of last meal: small / medium / large Gum: Y / N

Have you abstained from caffeine for the past 12 hours? Y / N If no, how long: _____

Have you abstained from alcohol for the past 12 hours? Y / N If no, how long: _____

Have you abstained from exercise for the past 12 hours? Y / N If no, how long: _____

Bloods taken: Y /N Fasted: Y / N Date: _____
Box #: _____

Lake Louise Mountain Sickness Score:

Headache
0—None at all
1—A mild headache
2—Moderate headache
3—Severe headache, incapacitating

Gastrointestinal symptoms
0—Good appetite
1—Poor appetite or nausea
2—Moderate nausea or vomiting
3—Severe nausea and vomiting, incapacitating

Fatigue and/or weakness
0—Not tired or weak
1—Mild fatigue/weakness
2—Moderate fatigue/weakness
3—Severe fatigue/weakness, incapacitating

Dizziness/light-headedness
0—No dizziness/light-headedness
1—Mild dizziness/light-headedness
2—Moderate dizziness/light-headedness
3—Severe dizziness/light-headedness, incapacitating

AMS Clinical Functional Score
Overall, if you had AMS symptoms, how did they affect your activities?
0—Not at all
1—Symptoms present, but did not force any change in activity or itinerary
2—My symptoms forced me to stop the ascent or to go down on my own power
3—Had to be evacuated to a lower altitude

TOTAL: _____

Medications:

Have you taken any medications in the last 24 hours? Y / N

If yes, please list:

Medication	Time	Dose

Participant ID: _____

TEAM:

Computer: _____
DEF: _____
Blood Draws/PE: _____
Ultrasound: _____
Aliquoting: _____
Floater: _____

	MEASUREMENT (CM)
Sternum - Finger	
Sternum - Femoral	
Transducer – Heart height	

	1	2	3
Manual Blood Pressure			

RTF: _____ _____

Phenylephrine:

Phenylephrine concentration: _____ µg/mL

Saline bag #: _____ Mixed on: _____ (date)

Estimated blood volume: _____ L

Dose #1: _____ mL Dose (mL) * [PE] (µg/mL) = _____ µg / est. blood vol. (L) = **30 µg/L**

Dose #2: _____ mL Dose (mL) * [PE] (µg/mL) = _____ µg / est. blood vol. (L) = **45 µg/L**

Dose #3: _____ mL Dose (mL) * [PE] (µg/mL) = _____ µg / est. blood vol. (L) = **60 µg/L**

NOTES:

1. NORMOXIA (ROOM AIR) (24 – 27 min + 5-10 min DEF/rewarming)

☐ **Baseline (5 min)**
- ☐ Blood sample ☐ Ultrasound

$P_{ET}O_2$: _____ torr $P_{ET}CO_2$: _____ torr

S_pO_2: _____ % BP: _____ mmHg

☐ **Phenylephrine Injections (15 – 18 min)**

☐ PE #1 (_____ mL)
- ☐ Baseline (1 min)
  - ☐ Ultrasound
- ☐ PE (1 – 2 min)
  - ☐ Ultrasound
- ☐ Washout (3 min)

☐ PE #2 (_____ mL)
- ☐ Baseline (1 min)
  - ☐ Ultrasound
- ☐ PE (1 – 2 min)
  - ☐ Ultrasound
- ☐ Washout (3 min)

☐ PE #3 (_____ mL)
- ☐ Baseline (1 min)
  - ☐ Ultrasound
- ☐ PE (1 – 2 min)
  - ☐ Ultrasound
- ☐ Washout (3 min)

☐ **Cold Pressor Test (4 min)**
- ☐ Baseline (1 min)
  - ☐ Blood sample ☐ Ultrasound
- ☐ CPT (3 min)
  - ☐ Blood sample ☐ Ultrasound

☐ **Attach DEF (room air) (≥3 min)**

☐ Rewarm hand (5-10 min)

2. HYPOXIA ($P_{ET}O_2$~ 45 torr; $P_{ET}CO_2$ ~ 40 torr) OR HYPEROXIA (24-27 min + 5-10 min rewarm)

Baseline values (in Calgary):

$P_{ET}O_2$: _____ torr $P_{ET}CO_2$: _____ torr S_pO_2: _____ %

☐ ****Switch to hypoxia/hyperoxia****

☐ **Hyperoxia reached**

☐ **Baseline (5 min)**
- ☐ Blood sample ☐ Ultrasound

$P_{ET}O_2$: _____ torr $P_{ET}CO_2$: _____ torr S_pO_2: _____ %

☐ **Phenylephrine Injections (15 – 18 min)**

☐ PE #1 (_____ mL)
- ☐ Baseline (1 min)
  - ☐ Ultrasound
- ☐ PE (1 – 2 min)
  - ☐ Ultrasound
- ☐ Washout (3 min)

☐ PE #2 (_____ mL)
- ☐ Baseline (1 min)
  - ☐ Ultrasound
- ☐ PE (1 – 2 min)
  - ☐ Ultrasound
- ☐ Washout (3 min)

☐ PE #3 (_____ mL)
- ☐ Baseline (1 min)
  - ☐ Ultrasound
- ☐ PE (1 – 2 min)
  - ☐ Ultrasound
- ☐ Washout (3 min)

☐ **Cold Pressor Test (4 min)**
- ☐ Baseline (1 min)
  - ☐ Blood sample ☐ Ultrasound
- ☐ CPT (3 min)
  - ☐ Blood sample ☐ Ultrasound

☐ ****Switch to room air****

☐ Rewarm hand (5-10 min)

Appendix VII: Supplemental Data

Table S1: Main effects of statistical analysis of baseline characteristics. Parameters were analyzed with two-way repeated measures ANOVA (catecholamines) or mixed-model ANOVA with repeated measures (all other measures). Significant P-values ($\alpha<0.05$) are bolded.

	P-Value (CONDITION)	P-Value (SEX)	P-Value (INTERACTION)
CARDIOVASCULAR			
Heart Rate (bpm)	**<0.0001**	**0.0401**	**0.0143**
R-R Interval (s)	**<0.0001**	**0.0357**	0.1078
Brachial Blood Pressure			
Mean (mmHg)	**0.0028**	0.2170	0.5151
Systolic (mmHg)	**0.0001**	**0.0110**	0.7013
Diastolic (mmHg)	**0.0006**	0.3660	0.2688
Cardiac Output ($L \cdot min^{-1}$)	**0.0032**	0.3183	0.8653
Cardiac Index ($L \cdot min^{-1} \cdot m^{-2}$)	**0.0010**	0.1900	0.8439
Total Peripheral Resistance ($mmHg \cdot L^{-1} \cdot min^{-1}$)	0.0526	0.7529	0.7594
Total Peripheral Conductance ($L \cdot mmHg^{-1} \cdot min^{-1}$)	**0.0186**	0.9854	0.7523
Peripheral Blood Oxygen Saturation (%)	**<0.0001**	0.2983	0.0551
RESPIRATORY			
Tidal Volume (L)	**0.0008**	0.2754	0.7144
Respiratory Rate (bpm)	0.1447	0.1069	0.0633
Minute Ventilation ($L \cdot min^{-1}$)	**0.0008**	0.1647	**0.0086**
Partial Pressure of End-tidal Oxygen (mmHg)	**<0.0001**	0.7645	0.1695
Partial Pressure of End-Tidal Carbon Dioxide (mmHg)	**<0.0001**	**0.0069**	**0.0100**
PLASMA CATECHOLAMINES			
Norepinephrine ($pg \cdot mL^{-1}$)	0.1738	0.7234	0.3225
Epinephrine ($pg \cdot mL^{-1}$)	**0.0004**	0.5334	0.2836