Vascular Reactivity to Rhythmic Handgrip at Altitude

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

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Abstract

Background: During exercise, there is an increase in sympathetic activity, causing vasoconstriction and reduced blood flow. However, to maintain blood flow and oxygen delivery to the exercising skeletal muscle, a phenomenon known as sympatholysis occurs, which is the reduction in sympathetic vasoconstriction. Evidence suggests that young males and females regulate peripheral blood flow differently in response to stressors, including exercise, but the underlying mechanisms for these differences are unclear. Further, the influence of hypoxia on this relationship is poorly understood. Therefore, the purpose of this thesis was to examine the sympathetic control of the vasculature during rhythmic handgrip exercise and explore any sex-based differences.

Methods: 8 young, healthy participants (4M/4F) were tested at both low (Kelowna, BC; 344m) and high (Barcroft Station, White Mountain; 3800m) altitude during early acclimatization (days 3-11). A larger set of participants were tested at low (n=10; 4M/6F) and high (n=14; 8M/6F) altitude and will be reported separately. Participants performed 3 minutes of rhythmic handgrip exercise at 25% of their maximal voluntary contraction during local infusions of saline, propranolol (beta-blockade), and propranolol plus phentolamine (combined alpha-beta-blockade). Doppler ultrasound was used to examine brachial artery blood flow (FBF) and calculate forearm vascular conductance (FVC).

Results: There was a main effect of condition on FBF (p=0.01), but no effect of altitude exposure (p=0.99) [low altitude (control, 0.18 ± 0.06; beta-blockade, 0.21 ± 0.07; alpha-beta-blockade, 0.26 ± 0.05 mL/min/100mL FAV), high altitude (control, 0.16 ± 0.04; beta-blockade, 0.27 ± 0.06; alpha-beta-blockade, 0.31 ± 0.07 mL/min/100mL FAV)]. Condition had a main effect on the change in FVC (p=0.02), but altitude exposure did not (p=0.50). The change in FVC during

exercise at low altitude was significantly less during alpha-beta-blockade (3 ± 5 a.u.) compared to beta-blockade (14 ± 3 a.u.), but not control (10 ± 7 a.u.). At altitude FVC response during exercise was less during alpha-beta-blockade (5 ± 5 a.u.) compared to control (11 ± 4 a.u.), but not beta-blockade (13 ± 5 a.u.). Sex had an effect on FVC during exercise at high altitude (p=0.02), but not at low altitude (p=0.94). Males showed a greater FVC during exercise at altitude compared to females.

Conclusions: This study demonstrated the importance of alpha-adrenergic receptors in the blood flow response to exercise. It showed that there was no difference in the blood flow response with altitude exposure, suggesting exercise hyperemia is preserved in mild-moderate exercise during early acclimatization. However, males and females may respond differently to an exercise stimulus during high altitude hypoxia exposure; this study suggests males have a greater blood flow response at altitude. Indeed, there may be an interaction between exercise and hypoxia that differentiates how males and females regulate exercising blood flow.

Preface

This thesis is an original work by Lauren E. Maier, none of which has been previously published. This research project received ethics approval from the University of Alberta Human Research Ethics Board, under the project name: "Sex differences in sympathetic vascular reactivity at high altitude", (Pro00096808) on January 4, 2022. This project also received approval for a Health Canada Clinical Trial Application Amendment (PRO00096808 Version 5, NOL control number 264793; Appendix C). It is registered on ClinicalTrials.gov (ID: NCT05525416). This protocol was also approved by the University of British Columbia, as part of an application titled "White Mountain Research Expedition 2022" (H22-01091).

This study was part of an international research expedition in September 2022 to Barcroft Research Station, White Mountain, California, led by Prof. Phil Ainslie (University of British Columbia), Prof. Michael Stembridge (Cardiff Metropolitan University), Prof. Craig Steinback (University of Alberta), and Dr. James Anholm (Loma Linda University).

Prof. Craig Steinback and I worked on the study design, data acquisition, analysis, and interpretation. Emily Vanden Berg, Lydia Simpson, Dr. Jonathan Moore, and Michiel Ewalts were integral to data collection.

This thesis would not have been possible without the support of my supervisor, Prof. Craig Steinback. I would like to thank my committee member, Dr. Christy-Lynn Cooke, who provided helpful advice throughout the project and my external examiner, Dr. Martin MacInnis. Also, I could not have run this project without the contributions and encouragement of my lab mates, colleagues, and fellow graduate students. Finally, I have to thank my family for their unconditional love and unwavering support throughout my education and life.

Acknowledgements

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List of Abbreviations

Ach	Acetylcholine
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
CVLM	Caudal Ventrolateral Medulla
DAG	Diacylglycerol
DBP	Diastolic Blood Pressure
EDHF	Endothelial Hyperpolarizing Factor
E2	Estradiol
FAV	Forearm Volume
FBF	Forearm Blood Flow
FVC	Forearm Vascular Conductance
GPCR	G-Coupled Protein Receptor
HR	Heart Rate
IP_3	Inositol Triphosphate
MAP	Mean Arterial Pressure
MSNA	Muscle Sympathetic Nerve Activity
MVC	Maximal Voluntary Contraction
NA	Nucleus Ambiguous
NE	Norepinephrine
NPY	Neuropeptide Y
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
eNOS	Endothelial Nitric Oxide Synthase
nNOS	Neuronal Nitric Oxide Synthase
NTS	Nucleus of the Solitary Tract
PKA	Protein Kinase A
PKG	Protein Kinase G
pO_2	Partial Pressure of Oxygen
pCO_2	Partial Pressure of Carbon Dioxide
P_{2x}	Purinergic 2X Receptors
P_{2x}	Purinergic 2Y Receptors
RHG	Rhythmic Handgrip Exercise
RPG	Respiratory Pattern Generator
RVLM	Rostral Ventrolateral Medulla
SaO_2	Oxygen Saturation
SA node	Sinoatrial Node
SARs	Stretch Afferent Receptors
SBP	Systolic Blood Pressure
sGC	Soluble Guanylate Cyclase
SNA	Sympathetic Nerve Activity
TPR	Total Peripheral Resistance

VRC   Ventral Respiratory Column
Y_1R Neuropeptide Y_1 Receptors

Chapter 1: Introduction

1.1 Background

During exercise, blood flow is increased to the active skeletal muscles to meet the elevated oxygen demand. This is accomplished through changes to peripheral vascular tone and alterations to cardiac output. With the onset of exercise, higher motor centers in the brain send a signal to activate the cardiovascular system to respond to this stress. Once exercise is initiated, receptors in the active muscle respond to stretch from movement and metabolites produced from exercise, activating the exercise pressor reflex (Amann et al., 2011). An increase in blood pressure with the start of exercise is accommodated by a resetting of the baroreflex to a higher operating point (Raven et al., 2006). Simultaneously, the chemoreceptors respond to changes in the partial pressure of carbon dioxide and oxygen in the blood, increasing sympathetic activity via cardiovascular control centers (Wan et al., 2020). A withdrawal of parasympathetic activity and an increase in sympathetic activity on the sinoatrial (SA) node increases heart rate, while the release of epinephrine that binds to beta-adrenergic receptors on the myocardium increases contractility and an increase in venous return that enhances end-diastolic volume, raises stroke volume and ultimately cardiac output (Gordan et al., 2015). Heightened sympathetic activity to the peripheral vasculature releases neurotransmitters such as norepinephrine, which binds to alpha-adrenergic receptors on the smooth muscle of the blood vessels and triggers vasoconstriction.

Despite global sympathetic hyperactivity and generalized vasoconstriction, the influence is lessened in exercising muscle vasculature. This phenomenon, called sympatholysis, results from increased shear stress of blood flow through the arteries, which triggers the production of nitric oxide, a potent vasodilator. Nitric oxide works in concert with other mechanisms such as

endothelial hyperpolarizing factor (EDHF), prostaglandins, and adenosine to achieve exercise hyperemia under heightened sympathetic activity (Chavoshan et al., 2002; Ozkor et al., 2015; Wilson & Kapoor, 1993). Together, this concert of actions redirects blood flow to the exercising muscle to maintain oxygen and substrate delivery and remove metabolites.

Hypoxia is a significant physiological stressor causing sympathoexcitation (Lundby et al., 2018). Chronic hypoxia is present in a variety of diseases, such as chronic pulmonary obstructive disease, heart failure, and obstructive sleep apnea. Further, millions of individuals live at altitudes above 2500m, considered high altitude, and many more travel to these destinations each year, exposing themselves to altitude-related hypoxia (due to a reduction in the partial pressure of oxygen) (Tremblay & Ainslie, 2021). Despite hypoxemia affecting many populations, it is unclear how it affects adrenergic control of blood flow during exercise. There is contradicting sympathetic vasoconstriction due to sympathetic hyperactivity with vasodilation due to reduced oxygen delivery. This competition between vasoconstrictive and dilatory signals mirrors what is seen during exercise; however, the mechanisms regulating exercise blood flow under hypoxic conditions has not been fully explored.

Despite a similar outcome, evidence supports differences between males and females in the specific physiological responses to exercise (Casey et al., 2014; Hart et al., 2009; Just & DeLorey, 2017); specifically, females appear to have augmented blood flow and vasodilation in response to exercise compared to males. These differences may be due in part to differences in sensitivity of the adrenergic receptors (Fairfax et al., 2013; Hart et al., 2011; Hart et al., 2009; Kneale et al., 2000). Indeed, there may be differences in the contributions of alpha- and beta-adrenergic receptors, which are vasoconstrictive and vasodilatory, respectively. Evidence suggests vasodilatory beta-adrenergic receptors may be upregulated in pre-menopausal females

compared to males (Ferrer et al., 1996; Kneale et al., 2000). Since these differences disappear post-menopause, they may be influenced by sex hormones such as estrogen (Hart et al., 2011; Kneale et al., 2000; Vongpatanasin et al., 2001).

1.2 Research Aim and Hypothesis

The aim of this study was to determine the direct contribution of the adrenergic receptors to the blood flow response to exercise during high-altitude hypoxia exposure. Further, we investigated whether sex-based differences are present in adrenergic control of blood flow during hypoxic exercise. I hypothesized:

1) females would have an augmented vasodilatory response to exercise during hypoxia compared to males, but that this difference would be abolished under beta-adrenergic blockade.

2) both males and females would have a similar blunted blood flow response to exercise under a combined alpha- and beta-blockade.

This hypothesis was centered on evidence from Casey et al. (2014), which suggests females have an enhanced vasodilatory response to forearm exercise, and previous work from our lab which suggests a blunting of the alpha-adrenergic receptors at altitude.

1.3 Significance

This research is important for the understanding of blood flow regulation during exercise under hypoxia. Further, it will contribute to our understanding of sex-based differences in blood flow responses. This is relevant to the millions of people who travel to high altitudes each year, for work or recreation, and the more than 80 million people live above 2500 meters (Tremblay & Ainslie, 2021). Clinically, conditions such as obstructive and central sleep apnea and chronic

obstructive pulmonary disease, in which patients experience chronic hypoxemia, will benefit from a better understanding of these mechanisms. Insight into the direct mechanisms of local blood flow control will shape future research in sex differences at high altitude.

Chapter 2: Literature Review

2.1 Blood Flow Regulation

Global control

Blood flow is regulated through changes in the blood pressure differential across a vascular bed and changes in vascular cross-sectional area (i.e., resistance). The autonomic nervous system is largely responsible for regulating blood pressure and vascular resistance. Blood pressure is tightly regulated throughout the day, responding to acute changes but maintaining a mean pressure within a very narrow range. Mechanistically, sympathetic barosensitive pathways are important in both acute and chronic control of blood pressure (Ishii et al., 2015) and for the regulation of blood flow.

Baroreflex

The baroreflex responds to acute changes in blood pressure via stretch receptors located the aortic arch and carotid sinuses (Kirchheim, 1976). When stretch is detected, these receptors increase afferent firing to the nucleus of the solitary tract (NTS) via the vagus nerve (aortic arch receptors) and the glossopharyngeal nerve (carotid receptors) (Kirchheim, 1976). The excitatory signal is sent to the caudal ventrolateral medulla (CVLM), which then inhibits the rostral ventrolateral medulla (RVLM). The RVLM is considered the origin of sympathetic outflow, and its inhibition results in a decrease in excitatory signals sent to the sympathetic preganglionic neurons in the intermediolateral nucleus. Therefore, a detected rise in pressure results in a subsequent decrease in sympathetic efferent activity to the heart and vasculature, acting to correct blood pressure. Simultaneously, the NTS activates the nucleus ambiguous (NA), which sends parasympathetic efferent signals to reduce heart rate. On the contrary, a decrease in blood

pressure reduces firing of the baroreceptors, causing less activation of the CVLM, thus, not inhibiting the RVLM and increasing efferent sympathetic firing. Further, the NTS will not activate the NA, reducing parasympathetic output to the heart.

The baroreflex operates around a preset blood pressure, referred to as the 'set point' (DiCarlo & Bishop, 2001). However, this operating point can be shifted during prolonged periods of exposure to differing blood pressures (e.g., hypertension), preserving the sensitivity of the baroreflex to respond to acute changes in blood pressure. There is evidence to suggest this occurs during exercise; the baroreflex is reset to a higher point to allow for an increase in blood pressure during exercise (DiCarlo & Bishop, 2001).

Cardiac output

Cardiac output is quantified as the amount of blood ejected from the heart each minute. This is regulated by end-diastolic volume, myocardial contractility, and heart rate. End-diastolic volume is determined by the amount of blood returned to the heart after contraction, which is dependent on blood volume and venous tone, whereas both myocardial contractility and heart rate are controlled by the autonomic nervous system (Gordan et al., 2015). Stressors, such as exercise, cause parasympathetic withdrawal and augment sympathetic activity, increasing heart rate and myocardial contractility, elevating cardiac output and subsequently systemic blood flow.

Vascular Resistance

The other arm of blood flow regulation is through changes to the vascular cross-sectional area, which is altered via vasoconstriction and vasodilation. Resistance to blood flow is determined by smooth muscle tone, which is regulated by a host of mechanisms, including the

autonomic nervous system (Fadel, 2008). Increases in sympathetic activity augment resistance of blood vessels through alpha-adrenergic mediated vasoconstriction, whereas a decrease in sympathetic activity results in decreased resistance through vasodilation. Sympathetic neurotransmitters may also bind to receptors that lead to dilation (discussed below).
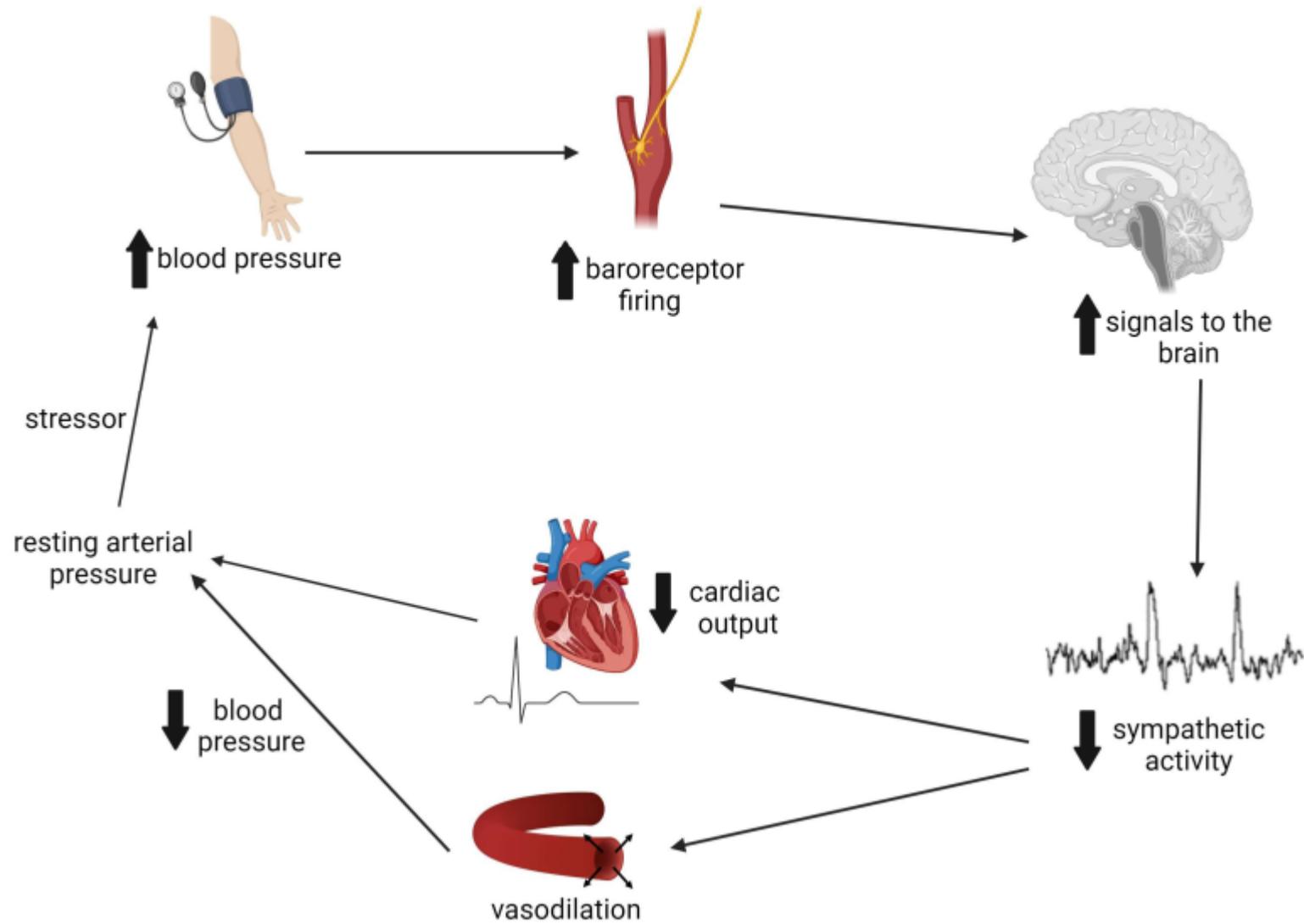
Figure 1. Baroreflex Negative Feedback Loop. When an acute increase in blood pressure is sensed by the mechanoreceptors in the aortic arch and/or carotid bodies, there is an increase in baroreceptor firing. This is relayed to the brainstem, leading to a decrease in efferent sympathetic activity. A decrease in sympathetic activity results in a decrease in heart rate and stroke volume, together decreasing cardiac output from the heart. Simultaneously, reduced sympathetic activity to the peripheral vasculature allows for relaxation of the blood vessels. Together, this reduced blood pressure back to resting levels. Created with BioRender.com

Chemoreflex

The chemoreflex responds to changes in the partial pressure of oxygen, carbon dioxide, and blood pH with chemically-sensitive receptors. There are two types of chemoreceptors, peripheral and central. The peripheral chemoreceptors are located in the carotid and aortic bodies and are more sensitive to changes in pO_2, whereas the central chemoreceptors are located in the brain and respond to changes in pCO_2 indirectly through changes in $[H^+]$.

The chemoreflexes are classically considered ventilatory reflexes for their role in regulation of breathing. However, they also have important cardiovascular influences. When an increase in pCO_2 occurs, central chemoreceptors are activated and relay this to the ventral respiratory column (VRC) in the medulla, via the NTS (Zoccal et al., 2014). Similarly, a decrease in pO_2 triggers a signal from the peripheral chemoreceptors to the NTS, which is relayed to the VRC. The respiratory neurons of the VRC are intertwined with neurons of the RVLM, which generates excitatory sympathetic output via pre-ganglionic sympathetic neurons (Zoccal et al., 2014). Thus, activation of the chemoreflex increases sympathetic activity through the RVLM. This is supported by the presence of respiratory patterns in sympathetic nerve activity, which is important in maintaining sympathetic vasomotor tone (Haselton & Guyenet, 1989).

Simultaneously, increased chemoreceptor firing results in excitation of the respiratory pattern generator (RPG), located in the brainstem, and an increase in breathing (Guyenet, 2014). The RPG increases sympathetic activity; further, with an increase in breathing, there is increased activity of lung stretch afferent receptors (SARs), which act to inhibit parasympathetic activity on the heart (Guyenet, 2014). These responses increase blood pressure and heart rate, therefore increasing blood flow.

The activity of the peripheral chemoreceptors increases dramatically at pO_2 below 70 mmHg; this has been suggested as an inflection point in chemoreflex sensitivity (Kumar &

Prabhakar, 2012). On the contrary, the central chemoreceptors respond to small changes in pCO_2 (Guyenet, 2014). Of importance to this thesis, pCO_2 set point varies during hypoxia and strenuous exercise, when oxygen delivery takes precedence over maintaining pCO_2 (Guyenet, 2014). Thus, pCO_2 tends to drop to lower values in these conditions. This is due to hyperventilation triggered by the hypoxic ventilatory response, a reflex response of the peripheral chemoreceptors to hypoxia exposure (San et al., 2013).

2.2 Post-Synaptic Receptors Responsible for Regulating Vascular Tone

Adrenergic Receptors

Post-synaptic adrenergic receptors regulate blood flow through peripheral vascular resistance by responding to changes in muscle sympathetic nerve activity (Fadel, 2008). Sympathetic nerves release neurotransmitters at the post-synaptic junction that bind to various adrenergic receptors on the vascular smooth muscle. Depending on the neurotransmitter released and the receptor it binds to, there will be differing changes in forearm vascular tone (Figure 2). Post-synaptic α-adrenergic receptors are G-coupled protein receptors (GPCRs) that work through a pathway that increases intracellular calcium concentrations, activating potassium channels and leading to vasoconstriction (Motiejunaite et al., 2021). Conversely, β-adrenergic receptors are GPCRs that activate a secondary messenger cyclic adenosine monophosphate (cAMP), in turn activating protein kinase A, triggering vasodilation (Silva & Zanesco, 2010).
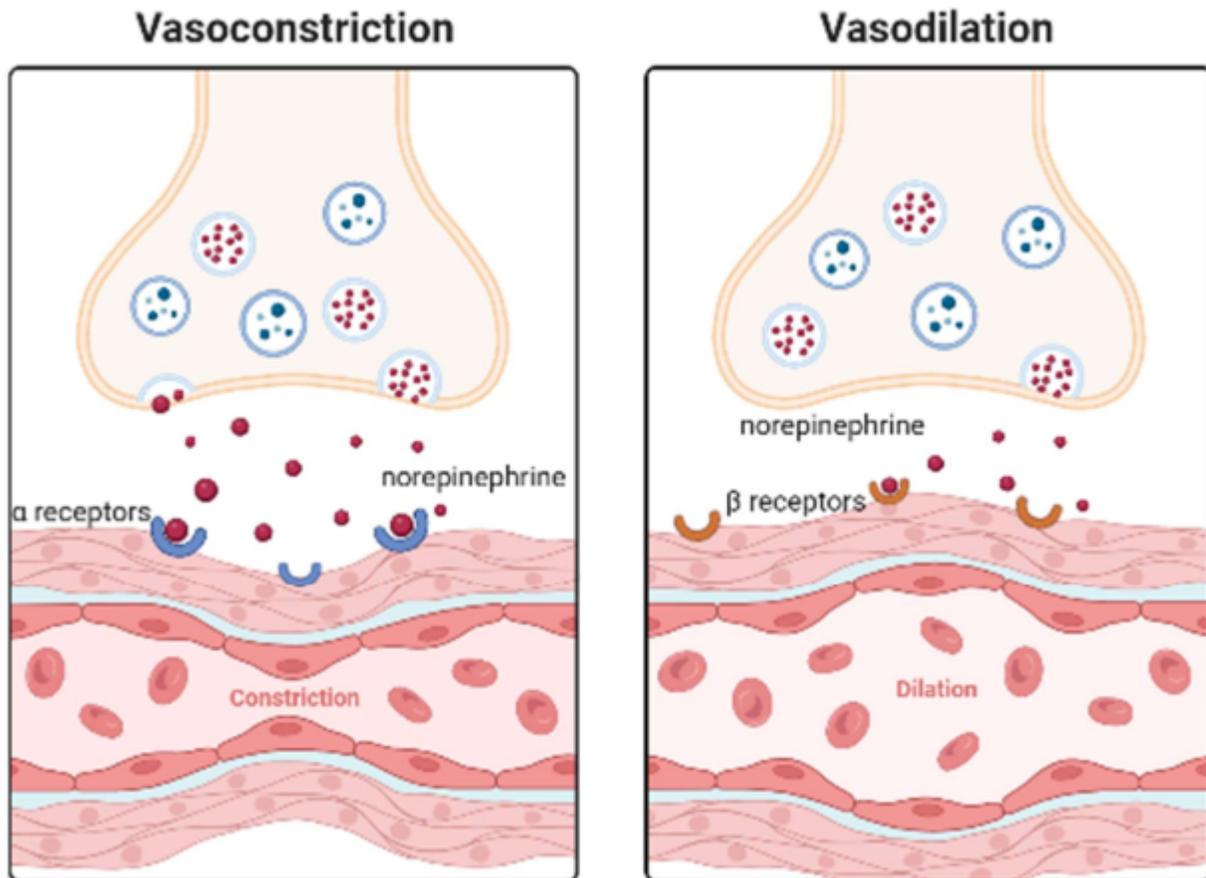
Figure 2. The left-hand panel demonstrates released norepinephrine from the axon terminal binding to post junctional (g-coupled protein) α receptors. This binding results in smooth muscle contraction (i.e., vasoconstriction). The right-hand panel demonstrates the binding of released norepinephrine from the axon terminal to post junctional β receptors. Through a separate pathway, this causes smooth muscle relaxation and vasodilation. The balance between vasoconstriction and vasodilation will determine local vascular cross-sectional area and resistance. Created in BioRender.com

α-Adrenergic Receptors

Different α-receptors act through unique pathways and contribute separately to vascular tone. α_1 receptors will respond to norepinephrine released from the sympathetic nerve terminals or as circulating catecholamines. Blockade of α_1 receptors using prazosin increases blood flow at rest, and abolishes the constrictor response to phenylephrine infusion (an α-receptor agonist), suggesting a significant contribution of these receptors to vascular tone (Dinenno et al., 2002).

When activated, Gq proteins that are coupled to α_1 receptors activate phospholipase C, producing a secondary messenger that triggers an influx of calcium ions into the intracellular space from the endoplasmic reticulum (Motiejunaite et al., 2021). Simultaneously, protein kinase C contributes to increasing intracellular $[Ca^{2+}]$ by altering membrane ion channels (Motiejunaite et al., 2021). Calcium ion influx enables smooth muscle contraction, causing vasoconstriction (Figure 3).

α_2 receptors also respond to norepinephrine, both as a neurotransmitter and circulating catecholamine. Infusion of an α_2 receptor agonist, clonidine, results in significant vasoconstriction (Kiowski et al., 1983). With blockade of α_1 receptors, the vasoconstrictor response to clonidine did not change; however, with infusion of phentolamine, a non-specific α-adrenergic antagonist (i.e., blockade of both α_1 and α_2 receptors), the vasoconstriction response to clonidine was abolished (Kiowski et al., 1983). The current evidence suggests α_2 receptors contribute approximately 2/3rds of adrenoreceptor-mediated vascular tone (Dinenno et al., 2002).

Contrary to α_1 receptors, α_2 receptors are coupled with inhibitory Gi proteins; activation of α_2 receptors triggers inhibition of adenylyl cyclase (Motiejunaite et al., 2021). Thus, cyclic adenosine monophosphate (cAMP) production from ATP is limited, reducing activation of phosphokinase A by cAMP. The end result is a reduction in activated proteins which normally

act to inhibit smooth muscle contraction and less restriction on efflux of calcium from the sarcoplasmic reticulum (Motiejunaite et al., 2021) (Figure 3).

β Receptors

β-adrenergic receptors are also found on the vascular smooth muscle and contribute to vascular tone through vasodilation. The predominant β receptor found in the peripheral vasculature are β_2 receptors, however evidence suggests existence of β_1 and β_3 receptor subtypes in some arteries as well (Briones et al., 2005; Chruscinski et al., 2001; Dessy et al., 2005). β receptors are most sensitive to epinephrine, followed by norepinephrine (Silva & Zanesco, 2010). As a result, there can be simultaneous activation of α- and β-receptors with norepinephrine, causing opposing actions of the smooth muscle. Eklund and Kaijser (1976) showed a decrease in the vasodilatory response to exercise of more than 50% with infusion of propranolol, a β-adrenergic antagonist. In response to stressors such as exercise, renal sympathetic activity increases, also causing the release of epinephrine from the adrenal medulla into the bloodstream. Epinephrine can bind to β_2 adrenergic receptors and trigger vasodilation in the active tissue (Sarelius & Pohl, 2010).

β_2 receptors are coupled to G-proteins that, when activated, trigger production of cAMP through adenylyl cyclase (Motiejunaite et al., 2021). cAMP works as a catalyst to stimulate protein kinase A, which then phosphorylates downstream proteins involved in regulating smooth muscle tone (Motiejunaite et al., 2021). Concurrently, release of calcium ions from intracellular stores is inhibited by cAMP. This results in relaxation of the vascular smooth muscle (Figure 3).
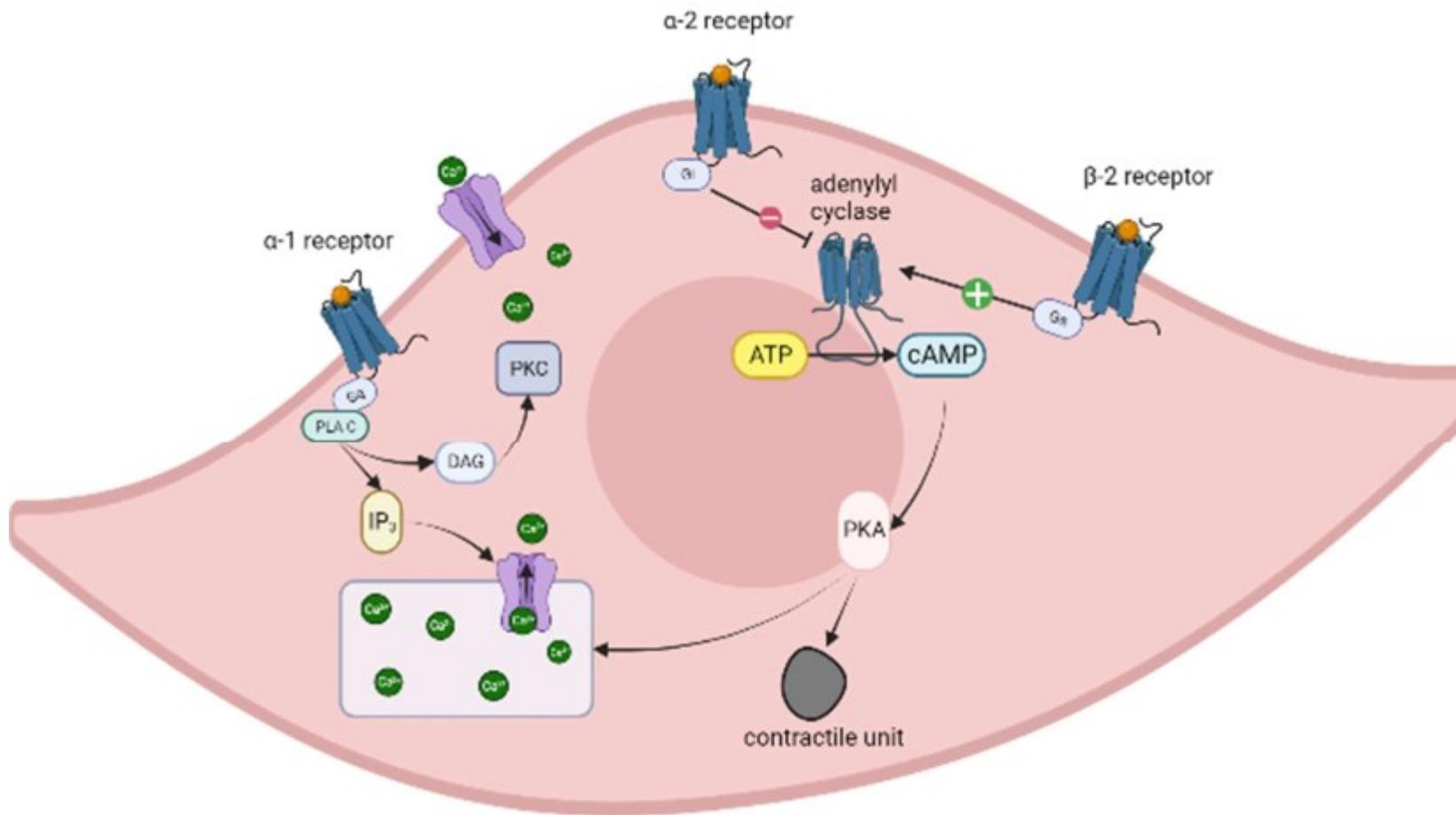
Figure 3. Adrenergic receptors influence on smooth muscle tone in the peripheral vasculature. α_1 receptors are GPCRs that activate phospholipase C, which produces inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 acts as a secondary messenger to increase efflux of Ca^{2+} from the sarcoplasmic reticulum into the cytoplasm. Simultaneously, DAG increases phosphokinase C activity, opening

ion channels in the cell membrane to further increase intracellular [Ca^{2+}], enabling smooth muscle contraction. α_2 receptors are

coupled to inhibitory G-proteins that when activated inhibit adenylyl cyclase. In contrast, β_2 receptors are excitatory GPCRs,

activating adenylyl cyclase. When activated adenylyl cyclase catalyzes the conversion of ATP to cyclic adenosine monophosphate

(cAMP), which triggers excitation of protein kinase A (PKA). PKA simultaneously inhibits the release of Ca^{2+} from intracellular

stores and phosphorylates proteins in the contractile unit that regulate vascular tone, causing relaxation. Created in BioRender.com

Neuropeptide Y Receptors

Although the adrenergic receptors contribute the majority of the regulation of vascular tone, it is important to note that there are other receptors that may contribute to this response as well. Neuropeptide Y (NPY) is a peptide that is co-released with norepinephrine from the sympathetic nerves during periods of high stress, such as exercise (Hodges et al., 2009). NPY Y_1 Receptors (Y_1R) are GPCRs that are located on vascular smooth muscle cells and initiate a cascade of events that results in increased intracellular calcium and contraction of vascular smooth muscle (Hodges et al., 2009). Interestingly, evidence suggests a synergistic effect between NPY and norepinephrine, causing increased vasoconstriction greater than the sum of each individual pathway (Mortensen et al., 2009; Rongen et al., 1994). NPY produces a slow acting and longer duration influence on the vasculature, compared to norepinephrine which is a more rapid onset and then dissipates more quickly (Lundberg & Tatemoto, 1982). NPY acts to enhance vasoconstriction through a positive interaction between NPY Y_1 receptors and α-adrenergic receptors (Hodges et al., 2009). Hodges et al. (2009) proposed that this synergistic effect may occur through secondary messengers, augmenting protein kinase C activation.

Purinergic Receptors

Another family of receptors that contribute to regulating vascular tone are the purinergic receptors (Mortensen et al., 2009). P_{2x} receptors are ligand-gated ion channels that are sensitive to adenosine triphosphate (ATP) (Vulchanova et al., 1996). They are found on the smooth muscle and trigger vasoconstriction by depolarizing the smooth muscle, causing contraction, when ATP binds (Vulchanova et al., 1996).

On the other hand, endothelial P_{2Y} receptors are GPCRs that induce vasodilation by triggering a cascade leading to the release of vasoactive substances such as nitric oxide and prostaglandins (Burnstock, 2007). P_{2Y} receptors are also sensitive to ATP. Blockade of nitric oxide production reduced blood flow during ATP infusion; similarly, blocking production of prostaglandins attenuated the vasodilatory response to ATP (Mortensen et al., 2009). Combined blockade of nitric oxide and prostaglandin production further reduced vasodilation from an ATP infusion than either individually (Mortensen et al., 2009). There are also P_{2Y} receptors located on the smooth muscle, which respond to ATP and cause vasodilation directly (Mortensen et al., 2009).

Further, endothelial P_1 receptors are sensitive to adenosine, which is created when ATP is broken down, and trigger vasodilation (Burnstock, 2007). However, blockade of these receptors found no change in vascular tone, suggesting adenosine does not contribute to ATP-induced vasodilation (Rongen et al., 1994).

2.3 Local Mechanisms of Blood Flow Control

There are multiple mechanisms that contribute to local regulation of blood flow. Although these were not directly assessed as part of this thesis, they are important for considering the complex signaling within the contracting limb to maintain oxygen delivery and control of blood flow.

Nitric Oxide

Nitric oxide plays a significant role in functional sympatholysis. It inhibits sympathetic vasoconstriction in skeletal muscle, both during rest and exercise, thus counteracting adrenergic

control of the vasculature (Chavoshan et al., 2002; Jendzjowsky & DeLorey, 2013). Nitric oxide

is created by nitric oxide synthase (NOS). Both endothelial NOS (eNOS), located on the

endothelium, and neuronal NOS (nNOS), found in the sarcolemma, inhibit sympathetic

vasoconstriction during exercise (Tschakovsky & Joyner, 2008). The main stimulus for eNOS

during exercise is an increase in shear stress. Shear stress acts on the endothelium, activating

mechanosensitive membrane proteins and ultimately the production of nitric oxide (Silva &

Zanesco, 2010). In hypoxic environments, nitrite in the blood can be reduced to nitric oxide as

well (Cosby et al., 2003), increasing bioavailability.

Nitric oxide causes vasodilation by activating soluble guanylate cyclase (sGC) to form

cyclic guanosine monophosphate (cGMP) in the vascular smooth muscle (Tschakovsky &

Joyner, 2008). cGMP then activates protein kinase G (PKG) which activates myosin

phosphatase, releasing calcium from intracellular stores in the smooth muscle and opening

calcium gates to increase uptake into the mitochondria, reducing intracellular calcium levels and

causing relaxation of the muscle and vasodilation (Banik et al., 2014).
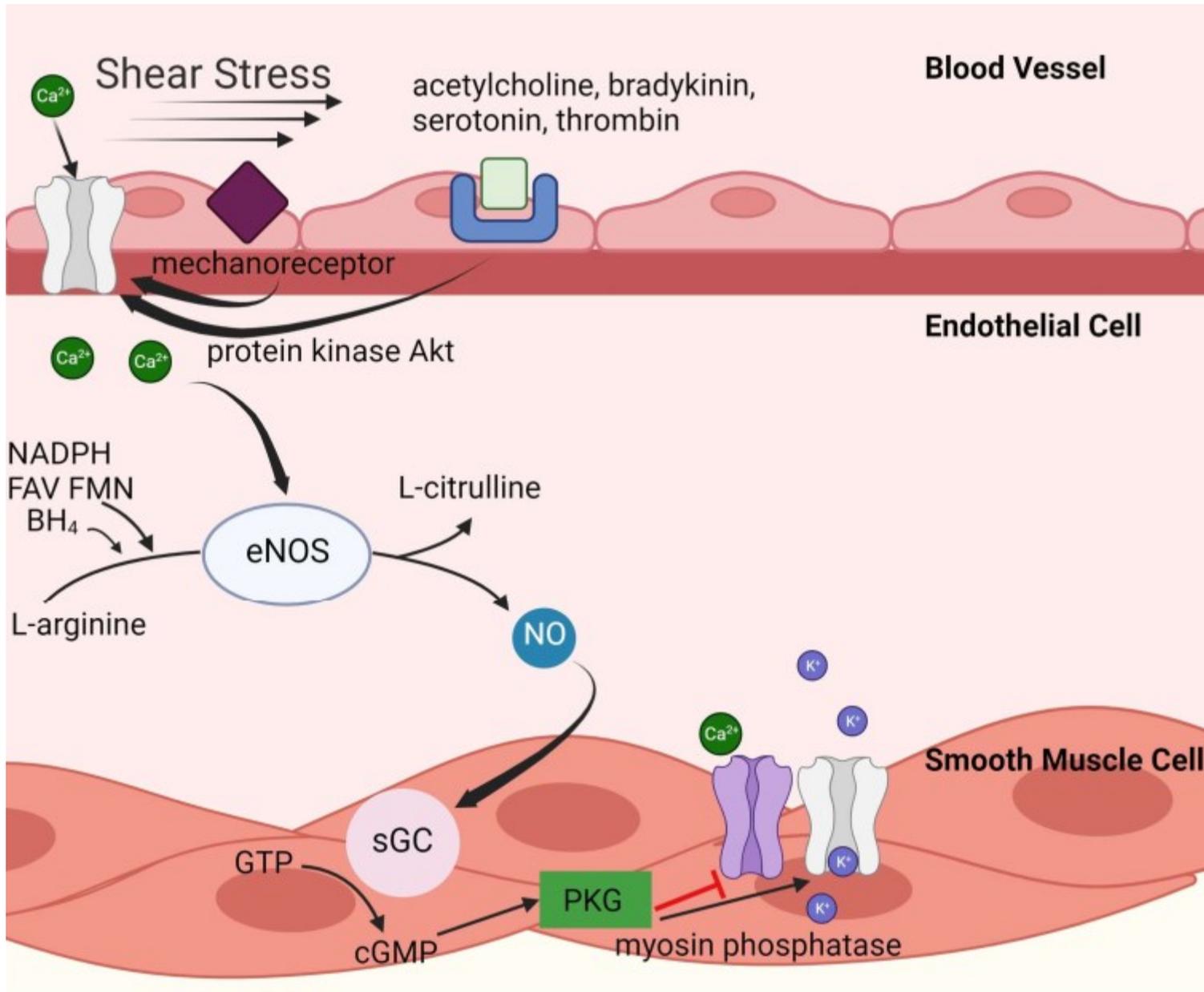
Figure 4. To activate nitric oxide production, shear stress from an increase in blood flow activates mechanoreceptors located on the endothelium, or chemical stimuli within the blood bind to receptors on the endothelium. This increases calcium ion sensitivity, opening Ca^{2+} gates, which activates the endothelial nitric oxide synthase (eNOS) enzyme. L-arginine, in the presence of oxygen and cofactors (NADPH, FAV, FMN, and BH_4), is synthesized by eNOS to produce nitric oxide. Nitric oxide then leaves the endothelium and enters the smooth muscle cell, where it acts on soluble guanylate cyclase (sGC) to form cyclic guanosine monophosphate (cGMP). cGMP activates protein kinase G (PKG) which activates myosin phosphatase. Myosin phosphatase opens potassium ion channels to increase $[K^+]$ in the smooth muscle, and reduces intracellular calcium levels, causing relaxation. Created in BioRender.com

Nitric oxide allows blood flow to be increased during exercise to meet the changing metabolic demands. Nitric oxide has been shown to reduce alpha-adrenergic vasoconstriction in the exercising skeletal muscle (Thomas & Victor, 1998). Further, NOS inhibition increases alpha-adrenergic mediated vasoconstriction during exercise (Patil et al., 1993). This supports the importance of nitric oxide in the blood flow response to exercise.

Endothelial-Dependent Mechanisms

A few other mechanisms that contribute to skeletal muscle blood flow are important to recognize, albeit not being the focus of this study. Endothelial-derived hyperpolarizing factors (EDHF) are other mechanisms that work to offset sympathetic vasoconstriction and contribute to exercising blood flow through vasodilation by activating calcium-dependent potassium channels (Ozkor et al., 2015). Ozkor et al. (2015) found EDHF and nitric oxide to contribute equally to vasodilation during exercise, suggesting EDHF is an important vasodilator for the exercising blood flow response. Prostaglandins are released from the skeletal muscle during exercise, and contribute to exercising hyperemia through vasodilation (Wilson & Kapoor, 1993). Evidence from Boushel et al. (2002) showed a synergistic effect of combined inhibition of prostaglandins and nitric oxide, suggesting there is redundancy in local mechanisms to regulate exercise hyperemia.

Endothelial-Independent Mechanisms

With the initiation of exercise, increases in muscle action potentials results in a release of potassium ions from the skeletal muscle into the interstitial fluid (Rosendal et al., 2004). As a result, smooth muscle cells are hyperpolarized, causing relaxation and vasodilation (Sarelius &

Pohl, 2010). Recent evidence also suggests that the release of potassium ions can enhance endothelium-dependent vasodilatory mechanisms, such as EDHF, supporting the idea of redundancy (Hearon et al., 2019). In addition, the breakdown of ATP results in adenosine, which can interact with the vascular smooth muscle and cause vasodilation (Roseguini et al., 2010). There is evidence supporting a relationship between adenosine levels and blood flow during exercise (Hellsten et al., 1998), whereby blockade of adenosine receptors with theophylline reduced the exercise blood flow response in humans (Rådegran & Calbet, 2001).

2.4 Exercise

Exercise is a global sympathetic stressor, causing an increase in sympathetic efferent activity to the entire body. Sympathetic activity is influenced by multiple reflex pathways engaged during exercise (Figure 5). The following sections explore the activation and interaction of exercise reflexes leading to adrenergic signaling.

Global Control of Blood Flow During Exercise

Central Command

Central command is a proposed feedforward mechanism that descends from higher motor centers in the brain to regulate the cardiovascular response to exercise (Ishii et al., 2016). This descending signal activates the sympathetic nervous system with the onset of exercise to maintain blood pressure and offset the vasodilation and increase in blood flow to the exercising tissue (Boulton et al., 2021). Interestingly, when participants initiate voluntary exercise on their own time, there is prefrontal cortex activation preceding the start of exercise; on the contrary, if the participant starts as soon as a cue is given, the prefrontal activation is not seen until the start of exercise (Ishii et al., 2016). Evidence for the role of central command in the cardiovascular

responses during exercise include attempted exercise with a partial neuromuscular blockade, in which greater central command was required to achieve a certain level of muscular work, and greater responses in heart rate and arterial pressure were observed (Leonard et al., 1985). In terms of central command's role on blood flow regulation, when exercise is initiated voluntarily, there is a greater increase in blood flow than with a cued start (Ishii et al., 2016). This suggests that central command prepares the muscle for the ensuing increase in metabolic demand.

Exercise Pressor Reflex

The exercise pressor reflex is a feedback mechanism that is activated by excitation of mechano- and metaboreceptors in the exercising muscle (Amann et al., 2011). This reflex is activated both by mechanical stretch of the muscle (mechanoreceptors) and by changes in concentrations of metabolites (e.g. hydrogen ions, deprotonated phosphate) in the blood (metaboreceptors). Activation of this reflex signals via type III and IV muscle afferents to increase sympathetic activity within the brainstem. This rise in sympathetic activity enables increases in cardiac output and causes vasoconstriction in the non-exercising limbs, allowing blood flow to increase to the exercising muscle (Amann et al., 2011). Blockade of the type III and IV muscle afferents with fentanyl during dynamic exercise reduced heart rate and blood pressure (Amann et al., 2010), which results in attenuated blood flow to the exercising tissue. This supports the role of the exercise pressor reflex in the blood flow response to dynamic exercise.

Baroreflex

There is an increase in blood pressure with the initiation of exercise that is due to an elevated cardiac output from both augmented sympathetic activity and increased venous return, which raises end-diastolic volume (Gordan et al., 2015). However, instead of the baroreceptors responding to decrease pressure, the operating point of the reflex is 'reset' (Raven et al., 2006). This allows blood flow to be increased to meet the heightened metabolic demand of the exercising muscles.

Chemoreflex

Activation of the chemoreflex during exercise occurs due to increased changes in the partial pressure of oxygen and carbon dioxide in the blood. Further, chemoreceptor sensitivity is enhanced with exercise; exercise has been shown to increase the sympathetic response of the chemoreflex (Seals et al., 1991). This activation of the chemoreflex, increasing sympathetic activity, contributes to the elevation of cardiac output and global vasoconstriction during exercise; thus, augmenting blood flow to the exercising tissue. Interestingly, blockade of the chemoreceptors with dopamine is associated with a decrease in sympathetic activity and an increase in exercising blood flow; therefore, the chemoreceptors contribute to the constraint of exercising blood flow via prevailing SNA (Stickland et al., 2011).
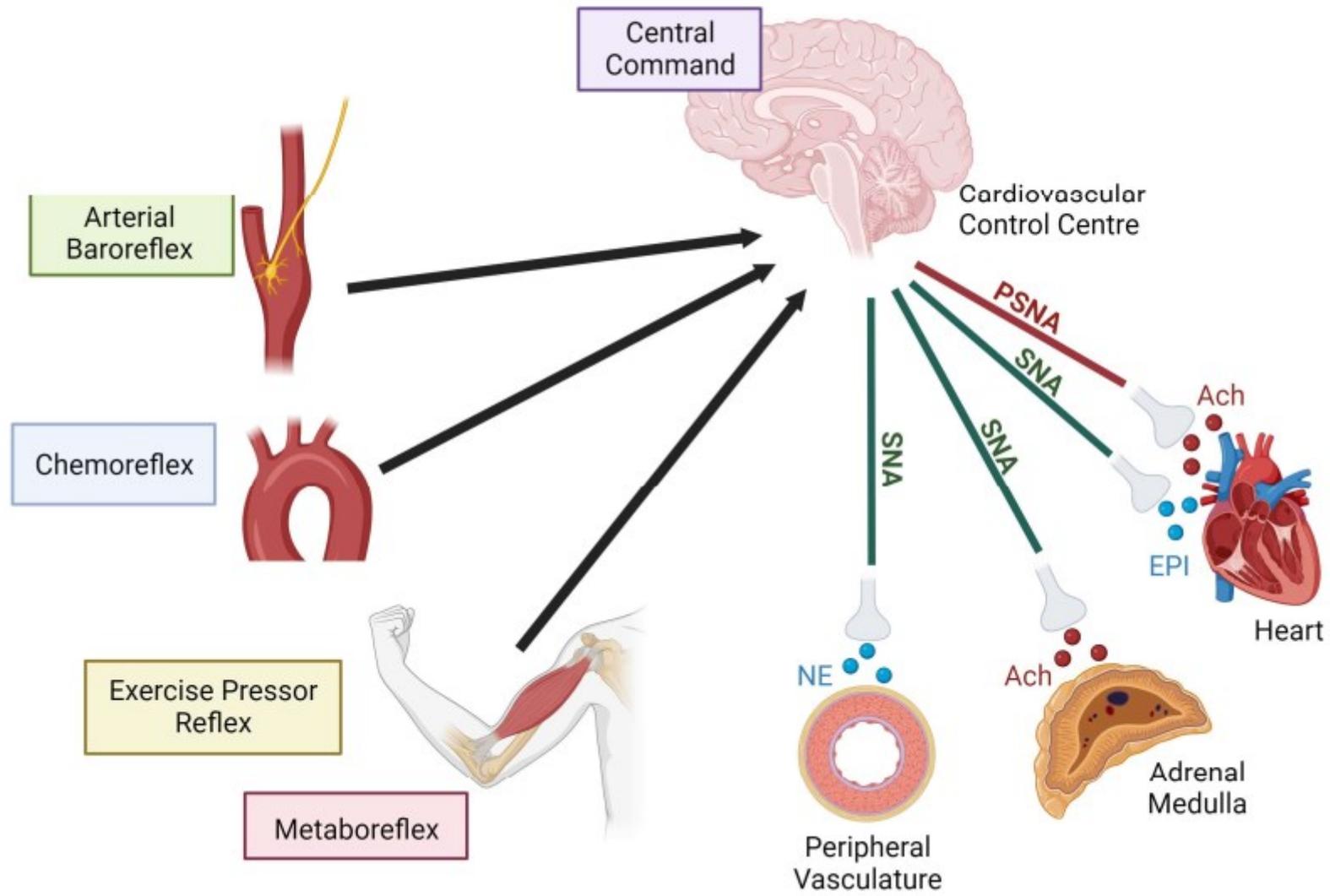
Figure 5. Blood flow during exercise is controlled by a host of mechanisms. With the initiation of exercise, central command activates the sympathetic nervous system to maintain blood pressure and increase blood flow to the exercising tissue. Immediately following initiation, the exercise pressor reflex (which encapsulates the metaboreflex) is activated by stimulation of the mechanoreceptors and metaboreceptors in the exercising tissue, further increasing sympathetic outflow from the brain. The baroreflex is activated in response to stretch of the blood vessels, from changing blood pressure. During exercise, the baroreflex is reset to a higher operating point to allow blood pressure to increase without compromising the sensitivity of the reflex. The chemoreflex are chemically-sensitive receptors that respond to changes in the partial pressure of oxygen and carbon dioxide in the blood. Increases in carbon dioxide during exercise activate this reflex, which increases sympathetic activity from the brainstem. All of these signals are integrated in the cardiovascular control centre in the brain, which regulates autonomic activity. During exercise, parasympathetic activity to the heart (specifically, the SA node), allowing heart rate to increase. Simultaneously, sympathetic activity is increased to: 1) the peripheral vasculature, causing global vasoconstriction; 2) the adrenal medulla, stimulating the release of epinephrine into the blood; and 3) the heart, which increases heart rate and myocardial contractility. Created in BioRender.com

Interactive Effects

Exercise Pressor Reflex and Baroreflex

When activated simultaneously, the exercise pressor reflex and baroreflex interact in an additive nature to increase blood flow to the exercising tissue, albeit through different mechanisms (Kaur et al., 2016). The pressor reflex augments cardiac output by increasing heart rate and contractility, whereas the baroreflex increases vasoconstriction of the non-exercising tissue (Kaur et al., 2016). Nonetheless, these two reflexes work together to meet the heightened metabolic demands of the exercising muscle.

Baroreflex and Chemoreflex

The baroreflex and chemoreflex have an important interaction in the response to exercise. Some evidence suggests that during mild intensity exercise, muscle sympathetic nerve activity does not increase as a result of baroreceptor unloading from increased blood volume from the muscle pump (Katayama et al., 2014). However, activating the peripheral chemoreflex with hypoxia reduces the ability of the baroreflex to control efferent sympathetic activity (Katayama et al., 2016). Thus, the chemoreflex has an important role in regulating baroreflex control of sympathetic output (Somers et al., 1991).

Exercise Pressor Reflex and Chemoreflex

Evidence suggests that the combined action of the exercise pressor reflex and the peripheral chemoreflex during exercise potentiates the heart rate and arterial pressure responses, higher than the sum of the individual reflex responses (Wan et al., 2020). Interestingly, with the combination of the exercise pressor reflex and the central chemoreflex, the interaction appears only to be

additive in nature. As a result, exercising blood flow and vascular conductance were restricted more when the peripheral chemoreceptors were activated with the pressor reflex (Wan et al., 2020).

2.5 Sex Differences in Blood Flow Regulation

Charkoudian et al. (2005) suggests that the relationship between sympathetic activation and changes in vascular tone may differ between sexes. In healthy males, total peripheral resistance (TPR) is positively related to MSNA (burst incidence) whereas cardiac output is negatively related to MSNA (Charkoudian et al., 2005); this suggests MSNA is directly related to vascular tone and peripheral blood flow. In contrast, there is no relationship between TPR or cardiac output with MSNA in healthy females (Hart et al., 2009). Further, Hissen et al. (2019) showed a relationship between sympathetic baroreflex sensitivity with resting MSNA and vascular transduction in males, but not females. It appears the mechanisms for blood flow control in females may be different or multi-factorial in comparison to males.

Importantly, the relationship between resting MSNA and prevailing arterial pressure is unclear, due to large interindividual differences in sympathetic activity but relatively similar arterial pressures (Sundlöf & Wallin, 1978). Evidence shows males with high MSNA have lower cardiac output and reduced alpha-adrenergic sensitivity, allowing arterial pressure to be preserved (Charkoudian et al., 2005). This suggests that resting MSNA does not impact blood flow, but changes in sympathetic activity will alter blood flow; however, this may be different between males and females.

Multiple studies have found a greater forearm vascular conductance response to submaximal exercise in females compared to males (Gonzales et al., 2007; Just & DeLorey,

2017; Parker et al., 2007). Thus, there appears to be differences between sexes in blood flow control during exercise. A commonly proposed mechanism underlying these differences is the influence of estrogen on the vasculature.

Estrogen

One possible mechanism for these differences between males and females is the effects of estrogen. There are three forms of estrogen produced in the body: estrone (E1), estradiol (E2), and estriol (E3) (Cui et al., 2013). Estradiol is the most common and potent form in premenopausal females and therefore contributes to the majority of the effect of estrogen on blood flow. Estrogen has a direct role in vasodilation, independent of sympathetic tone, by binding to estrogen receptors and G-protein estrogen receptors on the endothelium (Gilligan et al., 1994; Miller & Duckles, 2008). Estrogen also increases eNOS activation through protein kinase B (Akt), enhancing the vasodilator response with more nitric oxide production (Miller & Duckles, 2008). Evidence supports increased prostacyclin and EDHF production with exposure to estrogen, further increasing vasodilation (Leung & Vanhoutte, 2017; Miller & Duckles, 2008). Finally, estrogen may also upregulate the beta-adrenergic receptors on the vascular smooth muscle, offsetting alpha-adrenergic influences and favouring vasodilation (Ferrer et al., 1996). This may explain why beta-blockade has been previously shown to increase the vasoconstrictor response to norepinephrine infusion in females, equalizing their responses with males (Kneale et al., 2000). Perhaps the strongest evidence of the influence of estrogen on blood flow is the impact of menopause on vascular function in females. Post-menopausal females, whose estrogen levels are comparable to males, have similar blood pressure and flow responses to stressors (Hart et al., 2011). Further, giving post-menopausal females estradiol infusions increases their

vasodilatory response to acetylcholine (Gilligan et al., 1994). Thus, it appears with reduction in

estrogen, there is a change in the vascular response to stressors.
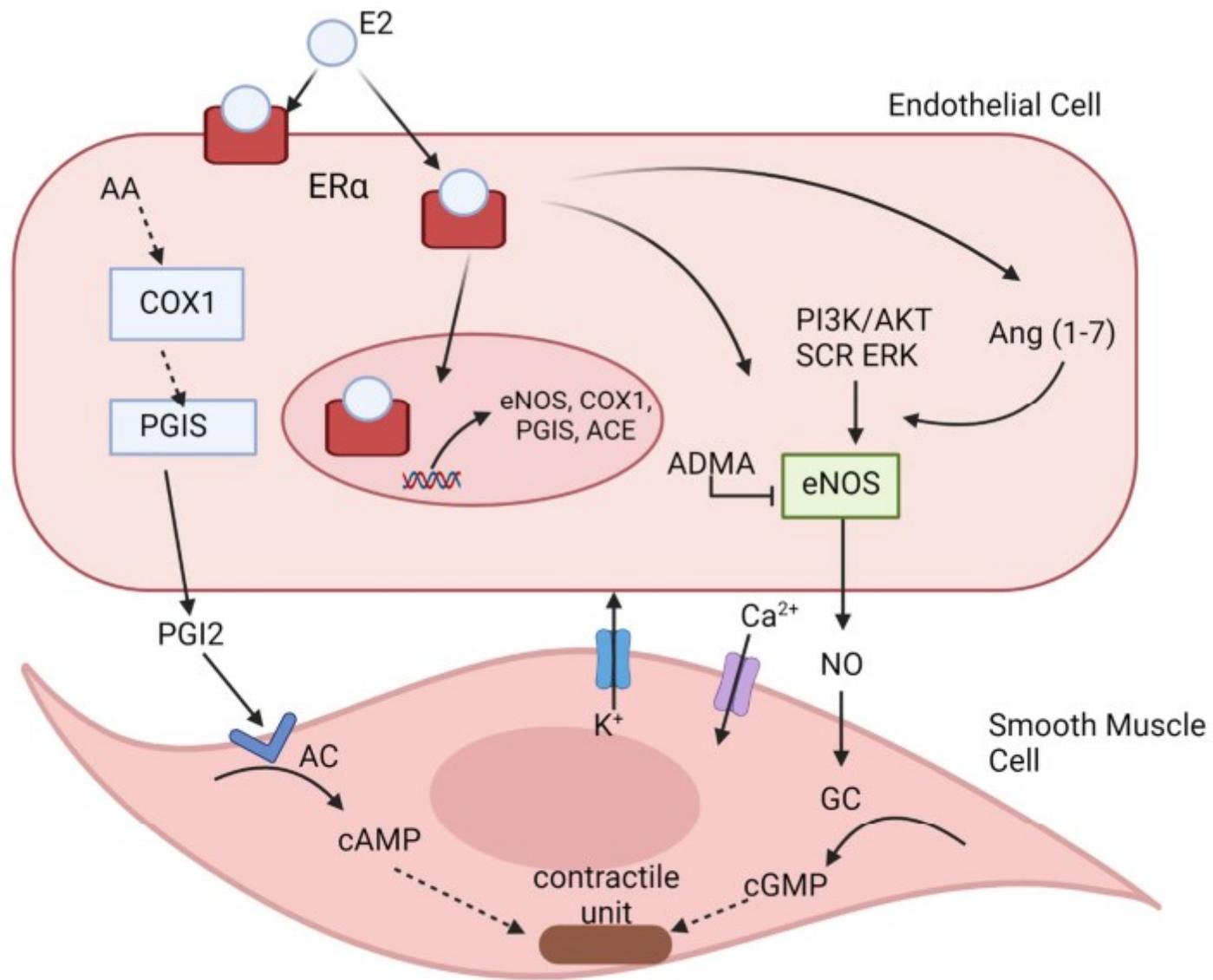
Figure 6. The role of estradiol (E2) on vascular tone. E2 binds to ERα on the endothelium, which causes a genomic response, inducing transcription of specific genes which regulate vasodilatory mechanisms (i.e., eNOS, cyclooxygenase 1, prostacyclin synthase, and angiotensin converting enzymes). Simultaneously, E2 triggers phosphorylation of eNOS through kinase signaling (PI3K/AKT, SCR, ERK) and reduction in asymmetric dimethylarginine (ADMA). Finally, E2 increases production of angiotensin 1-7, which contributes to NO-dependent vasodilation. In result, there is more NO to cause vasodilation through cGMP. E2 acts in a third way by increasing prostacyclin production, which causes relaxation through increased cAMP production. Figure adapted from Novella et al. (2019). Created in BioRender.com

2.6 Hypoxia

During hypoxia, there is a reduction in oxygen availability, which results in hypoxemia, or reduction of oxygen in the tissue. This provides a unique stress on the vasculature because hypoxia is a sympathetic stressor, causing vasoconstriction, but simultaneously increases the demand for oxygen delivery, stimulating a vasodilatory response locally. A primary response to hypoxia is stimulation of the peripheral chemoreceptors by reduced arterial oxygen content, increasing sympathetic activity to increase breathing and blood flow to compensate (Favret & Richalet, 2007). Simultaneously, local mechanisms of vasodilation (e.g., nitric oxide, EDHF) are activated to reduce tissue hypoxia. During exercise under hypoxic conditions, there is a greater vascular conductance response (Rowell et al., 1986). However, evidence suggests that this response is restrained by enhanced sympathetic vasoconstriction, as there is a greater conductance response when the alpha-adrenergic receptors are blocked (Wilkins et al., 2008). Thus, the exercise blood flow response during hypoxia is a balance between increased sympathetic activity and local dilatory signaling. Further, it appears that this compensatory dilation during hypoxic exercise may be attributed to several redundant mechanisms, similar to the mechanisms of sympatholysis in normoxic exercise (Joyner & Casey, 2014).

With prolonged exposure to hypoxia (i.e., high altitude), several physiological adaptations occur through acclimatization to optimize oxygen delivery to the tissues. These adaptations include increased hemoglobin concentration, allowing greater oxygen saturation, enhancing the oxygen carrying capacity of blood (Wagner, 2022). Despite constant sympathetic hyperactivity, there appears to a blunting of the responsiveness of the adrenergic receptors, allowing normalization of blood pressure and cardiac output (Simpson et al., 2021; Sutton et al., 1988). Berthelsen et al. (2020) show blunting of neuro-cardiovascular transduction is related to prevailing sympathetic activity, suggesting higher basal sympathetic activity may be offset by a

greater reduction of sensitivity of the adrenergic receptors, and vice versa. However, even with these adaptations, peak oxygen consumption remains reduced compared to low altitude (Lundby et al., 2008). Further, vasodilation and blood flow are reduced during exercise at altitude (Lundby et al., 2008). Hansen et al. (2022) found this reduction in blood flow and vasodilation to be restrained by adrenergic mechanisms. Interestingly, evidence suggests that muscle mitochondrial respiratory efficiency is improved with acclimatization, reducing metabolic demand (Chicco et al., 2018). Therefore, oxygen delivery during submaximal exercise can be maintained despite sympathetic restraint of blood flow at altitude due to reduced metabolic demand.

Given that the adrenergic receptors are a key piece in regulating blood flow during hypoxic exercise and the evidence for sex differences in adrenergic receptor responsiveness, there is a basis for exploring sex differences during hypoxic exercise. Currently, there is contradictory evidence regarding whether the response to hypoxic stress differs between males and females. Some researchers have found no difference between sexes in vascular reactivity during hypoxia (Miller et al., 2019; Usselman et al., 2015), whereas others have shown a blunted vasoconstriction response in females but not males (Casey et al., 2014; Jacob et al., 2021; Patel et al., 2014). There is very limited research looking at sex differences in the blood flow response to exercise during hypoxia. Casey et al. (2014) found greater relative changes in conductance in females during hypoxic exercise compared to males. However, the underlying mechanisms for these differences are unclear.

2.7 Summary

Previous research investigating mechanisms of blood flow control during hypoxic exercise has been conducted primarily in males, generalizing these findings to all. However, evidence supports differences between males and females in the regulation of blood flow during normoxic exercise. Therefore, this study sought to investigate differences between sexes in the exercising blood flow response and understand if the mechanisms differ with hypoxic exercise.

Chapter 3: Methods

3.1 Research Design

This study was conducted as part of an international research expedition to White Mountain, CA (3800m). Our study addressed a specific *a priori* question; the research was exploratory in nature, due to limited research at altitude and a small convenience sample of participants selected from the expedition team. All participants provided informed consent prior to participation. Ethical approval was received prior to data collection from both the University of Alberta Ethics Board (Pro00096808) and the University of British Columbia (H22-01091). A No Objection Letter was obtained from Health Canada for permission to use the drugs off-label prior to the start of data collection (NOL264793).

Participants were tested at low altitude in Kelowna, BC (340m) prior to the expedition. The altitude component of the study was conducted at Barcroft laboratory in White Mountain, California (Figure 7). Participants travelled to Palm Springs, CA and then to the research station by vehicle (~3800m in 8hrs). At altitude, participants were tested during early acclimatization, between days 3-11 (Figure 8). A total of 16 participants (8F) were included in this study (14 at high altitude). Participants completed a Lake Louise Acute Mountain Sickness Questionnaire on the day of participation. The highest score recorded was a 5 (out of 12), which is classified as mild acute mountain sickness (Roach et al., 2018). No participant experienced other high altitude-related sicknesses (high altitude pulmonary edema, high altitude cerebral edema). Participants were free of any cardiovascular, respiratory, neurological, or metabolic diseases and were non-smokers as determined by self-reported health history questionnaire. However, two participants reported taking medications (Vyvanse and Buproprion). All participants abstained from caffeine, exercise, and alcohol for 12 hours prior to participation. No females were

pregnant (confirmed by a pregnancy test), and all were premenopausal. Two females were taking

oral contraceptives (Lo Loestrin and Yasmin), and four females had intra-uterine devices.

Testing of females was not standardized to menstrual cycle, but menstruation was recorded by

self-reported questionnaire.
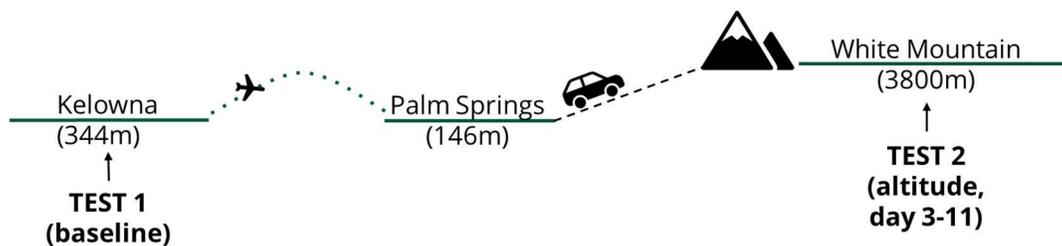
Figure 7. Top: Ascent and acclimatization profile. Participants flew to Palm Springs, CA, then drove 8 hours to Barcroft Station, White Mountain, CA. Participants were tested during early acclimatization to altitude, between days 3-11. Bottom: Picture of Barcroft Station, CA, 3800m. Photo taken by Alex Williams.

3.2 Instrumentation

Participants were tested in a supine position. Brachial artery and venous antecubital catheterization were performed by a medical doctor in the left arm. The brachial artery catheter was then connected to a saline drip at 3mL/hour, which was maintained throughout the test (Figure 8). Heart rate (Electrocardiogram leads II), oxygen saturation (SpO_2; pulse oximetry; ADInstruments, ML320 Oximeter Pod, Pod series, Australia), respiration rate (respiratory belt; ADInstruments), and skin temperature (Thermistor Pod, ADInstruments) were collected continuously throughout the protocol. Blood pressure was collected continuously using a pressure transducer connected to the brachial artery catheter (Edwards VAMP System). Blood pressure was also measured non-invasively on a beat-by-beat basis using finger photoplethysmography (Finometer, Finapres) on the contralateral hand. Peripheral venous pressure was measured using a pressure transducer (Deltran Blood Pressure Transducers) connected by a pressure-rated fluid column to the antecubital venous catheter. Brachial artery diameter and blood flow velocity were measured using Doppler ultrasonography (Terason uSmart 3300, 12L probe) and captured using custom data collection software (J. Lawley, University of Innsbruck). Participants performed 3 maximal voluntary contractions (MVCs) for 5 seconds each on the blockade arm (brachial artery catheter), with minimum 1-minute rest between each. The highest MVC value was then used to determine the target for the exercise bout (25% of MVC).

Figure 8. Experimental set up, Barcroft Research Station, White Mountain. In panel A: bi-lateral brachial artery ultrasonography images are being collected, with arterial and venous pressure (Deltran pressure transducer), blood pressure (Finopres), and heart rate (3-lead ECG) collected continuously. In panel B: a brachial artery catheter connected to 2 ports for saline, propranolol, and phentolamine infusions, measuring arterial pressure, and collecting blood samples. Also shown is a venous catheter for measurements on venous pressure and collecting blood samples.
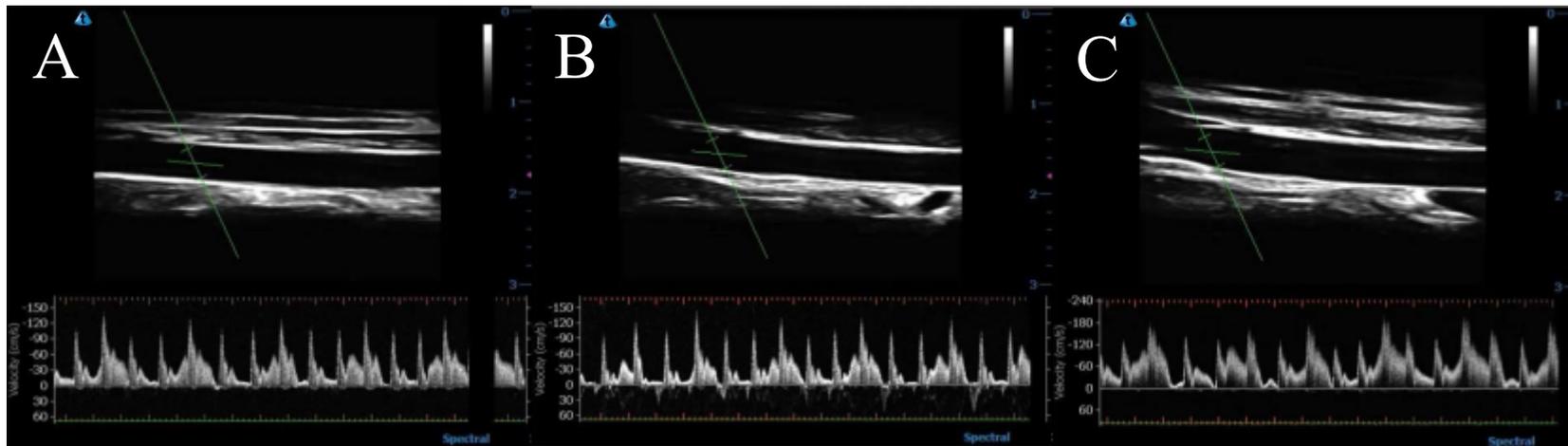
Figure 9. All images are collected during rhythmic handgrip exercise at low altitude in the same participant. Panel A shows brachial artery flow velocity and diameter during the control (saline infusion) condition. Panel B shows the beta-blockade condition, and Panel C shows the alpha-beta-blockade condition.

3.3 Protocol

Following instrumentation, 10 minutes of baseline data was collected with the participant quietly resting in the supine position. Doppler ultrasound images were collected during the last minute, and arterial and venous blood samples was taken to measure partial pressure of oxygen and carbon dioxide, oxygen saturation, hematocrit, hemoglobin, catecholamines, and sex hormones. The first trial was conducted during saline drip only. As this protocol was part of a larger study, a new 2-minute baseline was taken immediately prior to the start of rhythmic handgrip exercise (RHG). Arterial and venous blood samples and doppler ultrasonography were taken in the last minute of baseline. Participants then performed 3 minutes of RHG at 25% MVC at a duty cycle of 2:1. Ultrasound was performed throughout, with the 2nd minute used for analysis (Figure 9). Blood samples were taken during the last minute of RHG.

The second trial occurred during an infusion of propranolol, a beta-adrenergic-antagonist, via brachial artery catheter at a rate of 10 μg/100 mL FAV for 10 minutes (loading dose), then maintained for the remainder of the protocol at 5 μg/100 mL FAV (maintenance dose). These doses were selected based off previous studies that found them to be sufficient blockades local to the forearm (Richards et al., 2017). The same RHG protocol was then repeated under beta-blockade.

The final trial occurred during an infusion of phentolamine, a non-specific alpha-adrenergic-antagonist, via brachial artery catheter at a rate of 10 μg/100 mL FAV for 10 minutes (loading dose), then maintained for the remainder of the protocol at 5 μg/100 mL FAV (maintenance dose). Phentolamine was infused in conjunction with continued infusion of propranolol, ensuring maintenance of the beta-blockade. These doses were selected based off previous studies showing it to be an effective alpha-adrenergic blockade (Hansen et al., 2020).

The same RHG protocol was then repeated under a combined alpha-beta-blockade. Figure 10 shows a detailed visual representation of the protocol.
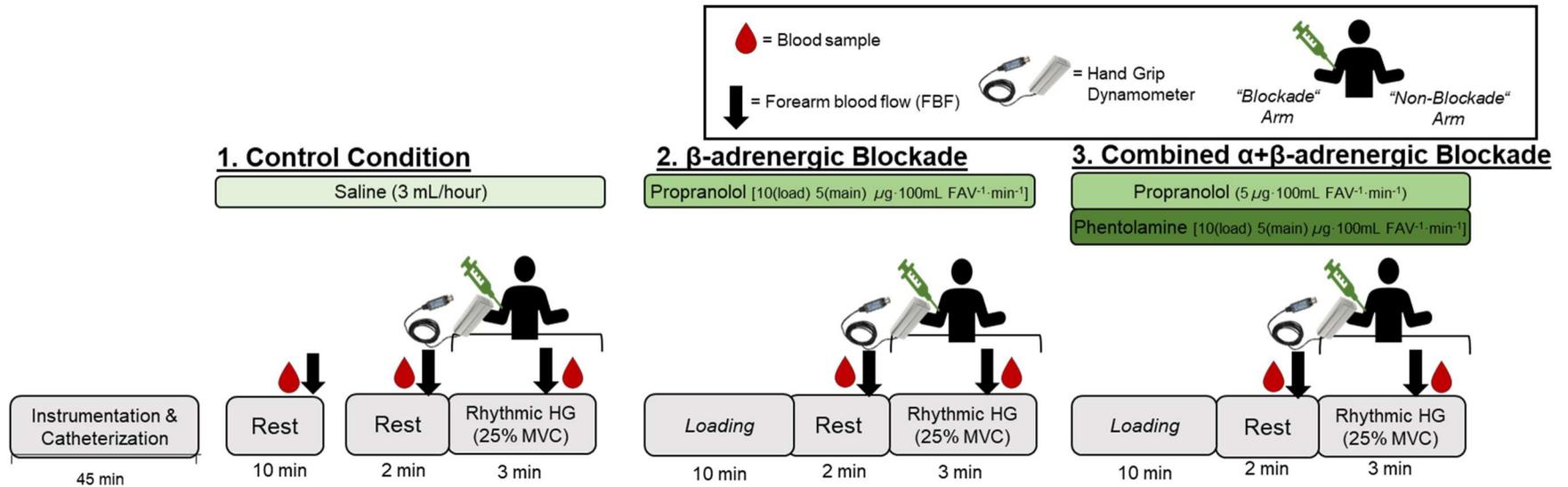
Figure 10. Protocol schematic. Following instrumentation, 10 minutes of baseline data was collected. Under each condition, participants performed a 2-minute baseline immediately preceding 3 minutes of rhythmic handgrip exercise at 25% of maximal voluntary contraction on a duty cycle of 2 seconds on 1 second off. Forearm blood flow was measured continuously throughout baseline and rhythmic handgrip, with the 2nd minute of handgrip exercise being used for analysis. Blood samples were taken in the last minute of baseline and rhythmic handgrip exercise. At the start of each condition, 10 minutes of loading dose is performed to establish an effective blockade, then the maintenance dose is given for the remainder of the protocol to maintain blockade.

3.4 Data Analysis

Baseline cardiovascular measures at low and high altitude were averaged in the last minute of a 10-minute rest. Each bout of rhythmic handgrip exercise had a baseline immediately preceding the exercise. These values were averaged over a one-minute period immediately before initiating exercise. Responses to RHG were averaged in one-minute sections, with the second minute of exercise being used for analysis. The second minute was chosen because in the third minute blood samples were taken, which resulted in the invasive arterial pressure monitoring being turned off to obtain the samples. By the second minute of exercise, participants had achieved steady state exercise.

Statistical Approaches

Group demographics were compared with unpaired Student T-Tests (males vs. females), or paired Student T-Tests (low vs. high altitude). Peak and delta physiological responses to exercise were analyzed using two-way (condition x altitude, or condition x sex) repeated measures analysis of variance (ANOVA) or mixed-model ANOVA in the case of missing values (GraphPad Prism Version 9.0 for Windows, GraphPad Software, San Diego, CA, United States). The significance level was set at $\alpha=0.05$ for all tests. In the cases where significant main effects were identified, pairwise comparisons were made with Holm-Sidak post hoc tests. All data are reported as mean ± standard deviation (SD).

Chapter 4: Results

4.1 Participant Demographics

Participant demographics and baseline characteristics are reported in Table 1 and 2. A total of 16 participants took part in this study. However, 2 participants were lost to follow up at altitude due to 1) unsuccessful insertion of arterial catheter, and 2) the participant unable to take part in the altitude portion of the expedition. Therefore, these results will contain data on 14 participants who completed the altitude portion of the study (Table 1). The 2 participants lost to follow up at altitude will be presented in the low-altitude data only (Table 3). Although six additional participants completed the altitude portion first, completing the low altitude portion following at least 2 months of deacclimatization, these data have not been analyzed due to technical issues. Therefore, this thesis contains data on 8 participants with repeated measures (Table 2) and data on 10 participants at low altitude (Table 1).

Table 1. Participant Demographics.

	Low Altitude	High Altitude
Number of Participants (M/F)	10 (4/6)	14 (8/6)
Age (years)	27.7 ± 2.8	27.4 ± 4.4
BMI (kg/m^2)	22.7 ± 2.3	23.3 ± 2.9
Heart Rate (bpm)	58.1 ± 7.2	73.3 ± 15.7
Mean Arterial Pressure (mmHg)	90.2 ± 8.3	97.3 ± 6.4 (13)
Systolic Arterial Blood Pressure (mmHg)	136.4 ± 12.3	151.1 ±11.7 (13)
Diastolic Arterial Blood Pressure (mmHg)	69.1 ± 6.6	74.0 ± 5.2 (13)
SaO$_2$ (%)	97.6 ± 0.6	88.8 ± 2.9

BMI, body mass index; bpm, beats per minute; SaO$_2$, arterial oxygen saturation. Data are presented as mean ± SD. Data were successfully collected in both locations in 8 participants, represented in Table 2. n=10 at low altitude and n=14 at high altitude unless indicated in parentheses. Characteristics were compared with unpaired Student T-Tests.

Table 2. Participant Demographics of Repeated Measures.

	Low Altitude	High Altitude
Number of Participants (M/F)	8 (4/4)	
Age (years)	27.4 ± 4.4	
BMI (kg/m^2)	23.3 ± 2.9	
Heart Rate (bpm)	56.4 ± 6.4	73.9 ± 14.0 *
Mean Arterial Pressure (mmHg)	92.0 ± 8.3	97.9 ± 8.1 (7) *
Systolic Arterial Blood Pressure (mmHg)	138.4 ± 13.0	150.4 ±12.3 (7) *
Diastolic Arterial Blood Pressure (mmHg)	70.4 ± 6.8	74.8 ± 6.6 (7)
SaO$_2$ (%)	97.6 ± 0.5	88 ± 3.3 *

BMI, body mass index; bpm, beats per minute; SaO$_2$, arterial oxygen saturation. Data are presented as mean ± SD. * represents $p<0.05$ compared to low altitude. n=8 unless indicated in parentheses. Characteristics were compared with paired Student T-Tests.

Table 3. Participant Demographics Divided by Sex.

| | Low Altitude | | High Altitude | |
	Males	Females	Males	Females
Sample size (n)	4	6	8	6
Age (years)	27.8 ± 3.8	27.8 ± 2.9	27.8 ± 3.8	27.8 ± 2.9
BMI (kg/m^2)	23.7 ± 2.3	22.0 ± 2.2	23.0 ± 1.8	23.6 ± 4.0
Heart rate (bpm)	54.8 ± 7.6	60.3 ± 6.6	70.3 ± 14.6	78.0 ± 1.0
Mean Arterial Pressure (mmHg)	89.9 ± 10.0	90.4 ± 8.0	97.3 ± 5.6 (7)	94.9 ± 9.4
SaO_2 (%)	97.4 ± 0.6	97.7 ± 0.6	88.4 ± 3.3	89.3 ± 2.7

BMI, body mass index; bpm, beats per minute; SaO_2, arterial oxygen saturation. Data are presented as mean $\pm$ SD. There were no differences between males and females in any baseline characteristics at either low or high altitude. n=8 males at high altitude unless indicated in parentheses. Characteristics were compared with unpaired Student T-Tests.

4.2 Baseline Cardiovascular Measures

Heart Rate. Altitude exposure significantly increased resting heart rate (56.4 ± 6.4 vs. 73.9 ± 14.0 bpm; p=0.004). There were no differences between males and females at either low altitude (54.8 ± 7.6 vs. 60.3 ± 6.6 bpm, p=0.26) or high altitude (70.3 ± 14.6 vs. 78.0 ± 16.0 bpm, p=0.37).

Blood Pressure. At altitude, mean arterial pressure was higher (92.0 ± 8.3 vs. 97.9 ± 8.1 mmHg, p=0.008), driven by augmented systolic (138.4 ± 13.0 vs. 150.4 ± 12.3 mmHg, p=0.014), but not diastolic (70.4 ± 6.8 vs. 74.8 ± 6.6 mmHg, p=0.061) blood pressure. There were no differences between males and females at low altitude in MAP (p=0.94), SBP (p=0.55), or DBP (p=0.85). Similarly, no sex differences were present at high altitude (MAP, p=0.59; SBP, p=0.06; DBP, p=0.75). Venous pressure was not different between altitudes (p=0.27), nor between sexes (low altitude, p=0.17; high altitude, p=0.93).

Forearm Blood Flow. Normalized basal forearm blood flow was not different between low and high altitude (0.0622 ± 0.038 vs. 0.0731 ± 0.034 mL/min/100mL FAV, p=0.54). There was no difference in basal blood flow between sexes at low altitude (p=0.18) or high altitude (p=0.38).

Forearm Vascular Conductance. There was no difference between low and high altitude in conductance when normalized to forearm volume (p=0.59). Sex had no effect on conductance at low altitude (p=0.14) or high altitude (p=0.32).

Blood Samples. As expected, altitude exposure resulted in a significantly decreased arterial oxygen saturation (p<0.001), partial pressure of oxygen (p<0.001), and partial pressure of carbon dioxide (p<0.001). Total hemoglobin was not different between low and high altitude (p=0.26). There were no differences between males and females at low altitude in SaO_2 (p=0.56), pO_2 (p=0.43), pCO_2 (p=0.18) or at high altitude (SaO_2, p=0.61; pO_2, p=0.16; pCO_2, p=0.06).

However, total hemoglobin was higher in males at both low altitude ($p=0.013$) and high altitude ($p=0.046$). Hematocrit was not different between low and high altitude ($p=0.28$), but it was higher in males at low altitude ($p=0.015$) and high altitude ($p=0.048$).

Table 4. Baseline Cardiovascular Characteristics at Low and High Altitude.

	Low Altitude	High Altitude
Number of Participants (M/F)	8 (4/4)	
Cardiovascular Measures		
Heart Rate (bpm)	56.4 ± 6.4	73.9 ± 14.0 *
Mean Arterial Pressure (mmHg)	92.0 ± 8.3	97.9 ± 8.1 (7) *
Systolic Arterial Blood Pressure (mmHg)	138.4 ± 13.0	150.4 ±12.3 (7) *
Diastolic Arterial Blood Pressure (mmHg)	70.4 ± 6.8	74.8 ± 6.6 (7)
Venous Pressure (mmHg)	9.4 ± 4.7 (6)	12.4 ± 4.9 (7)
Forearm Blood Flow (mL/min/100mL FAV)	0.0622 ± 0.038	0.0731 ± 0.034 (7)
Brachial Artery Diameter (mm)	3.9 ± 1.2	3.6 ± 0.7
Brachial Artery Resistance (mmHg/mL/min)	2.3 ± 1.2	1.7 ± 1.0 (6)
Brachial Artery Conductance (mL/min/mmHg/100mL FAV)	6.7 ± 3.7	7.7 ± 3.4 (6)
Blood Samples		
SaO_2 (%)	97.6 ± 0.5	88 ± 3.3 *
pO_2 (mmHg)	91.0 ± 5.2	50.7 ± 6.4 *
pCO_2 (mmHg)	39.4 ± 1.9	27.3 ± 2.0 *
Hemoglobin (g/dL)	13.1 ± 0.8	13.4 ± 1.1
Hematocrit (%)	40.2 ± 2.4	41.1 ± 3.5

Only those data from repeated measures are presented. Data presented as mean ± SD. * represents $p < 0.05$ compared to low altitude. n=8 unless indicated in parentheses. Characteristics were compared with paired Student T-Tests.

Table 5. Baseline Cardiovascular Characteristics at Low Altitude Divided by Sex.

	Males	Females
Number of Participants	4	6
Cardiovascular Measures		
Heart Rate (bpm)	54.8 ± 7.6	60.3 ± 6.6
Mean Arterial Pressure (mmHg)	89.9 ± 10.0	90.4 ± 8.0
Systolic Arterial Blood Pressure (mmHg)	139.5 ± 18.3	134.3 ± 7.5
Diastolic Arterial Blood Pressure (mmHg)	68.6 ± 8.7	69.5 ± 5.7
Venous Pressure (mmHg)	12.0 ± 2.2 (3)	7.6 ± 4.4 (5)
Forearm Blood Flow (mL/min/100mL FAV)	0.0829 ± 0.047	0.0492 ± 0.018 (5)
Brachial Artery Diameter (mm)	4.4 ± 1.7	3.4 ± 0.3 (5)
Brachial Artery Resistance (mmHg/mL/min)	1.2 ± 0.4	3.0 ± 1.0 (5) *
Brachial Artery Conductance (mL/min/mmHg/100mL FAV)	9.0 ± 4.2	5.4 ± 2.3 (5)
Blood Samples		
SaO_2 (%)	97.4 ± 0.6	97.7 ± 0.6
pO_2 (mmHg)	89.2 ± 6.4	93.2 ± 8.1
pCO_2 (mmHg)	40.3 ± 1.7	38.3 ± 2.3
Hemoglobin (g/dL)	13.6 ± 0.8	12.4 ± 0.4 *
Hematocrit (%)	41.7 ± 2.6	37.9 ± 1.3*

Data presented as mean ± SD. * represents $p<0.05$ compared to males. n=8 males and n=6 females unless indicated in parentheses. Characteristics were compared with unpaired Student T-Tests.

Table 6. Baseline Cardiovascular Characteristics at High Altitude Divided by Sex.

	Males	Females
Number of Participants	8	6
Cardiovascular Measures		
Heart Rate (bpm)	70.3 ± 14.6	78.0 ± 16.0
Mean Arterial Pressure (mmHg)	97.3 ± 5.6 (7)	94.9 ± 9.4
Systolic Arterial Blood Pressure (mmHg)	156.2 ± 12.4 (7)	144.6 ± 5.5
Diastolic Arterial Blood Pressure (mmHg)	73.4 ± 4.8 (7)	72.1 ± 8.6
Venous Pressure (mmHg)	10.8 ± 6.9 (7)	10.5 ± 3.1 (5)
Forearm Blood Flow (mL/min/100mL FAV)	0.0753 ± 0.034 (5)	0.0561 ± 0.032 (5)
Brachial Artery Diameter (mm)	3.9 ± 0.4 (6)	2.7 ± 0.4 (5) *
Brachial Artery Resistance (mmHg/mL/min)	1.3 ± 0.4 (4)	3.0 ± 2.2 (5)
Brachial Artery Conductance (mL/min/mmHg/100mL FAV)	8.2 ± 3.2 (4)	5.9 ± 3.3 (5)
Blood Samples		
SaO_2 (%)	88.4 ± 3.3	89.3 ± 2.7
pO_2 (mmHg)	51.2 ± 6.6	56.0 ± 4.9
pCO_2 (mmHg)	27.9 ± 1.4	26.3 ± 1.2
Hemoglobin (g/dL)	14.2 ± 0.7	13.1 ± 1.2 *
Hematocrit (%)	43.5 ± 2.2	40.1 ± 3.5*

Data presented as mean $\pm$ SD. * represents $p < 0.05$ compared to males. n=8 males and n=6 females unless indicated in parentheses. Characteristics were compared with unpaired Student T-Tests.

4.3 Responses to Exercise

Altitude Exposure

In this section, only data collected in 8 participants with repeated measures will be presented (Table 2).

Heart Rate. The blockade condition had a main effect on the absolute heart rate response to exercise (p=0.001); heart rate was lower during the alpha-beta-blockade compared to the control condition at low altitude (p=0.023), and lower during both the beta-blockade (p=0.006) and alpha-beta-blockade (p=0.001) compared to the control condition at high altitude. As expected, altitude exposure also had a main effect on the absolute heart rate response to exercise (p= 0.006). The heart rate response to exercise was significantly lower at low altitude in all three conditions (control, p=0.002; beta-blockade, p=0.004; alpha-beta-blockade, p=0.004). There was no interaction between condition and altitude exposure (p=0.594). However, the changes from baseline to exercise were not different between conditions (main effect, p=0.224). Further, there was no main effect of altitude exposure on the change in heart rate from baseline to exercise (p=0.882).

Blood Pressure. There was no effect of condition (main effect, p=0.885) or altitude exposure (main effect, p=0.758) on mean arterial pressure during exercise. Similarly, condition or altitude exposure had no main effect of the change in mean arterial pressure from rest to exercise (condition, p=0.789; altitude, p=0.063). Systolic blood pressure was not different between conditions (p=0.984) or altitudes (p=0.204) during exercise; however, condition had a main effect on the change in systolic pressure from rest to exercise (p=0.045). Alpha-beta-blockade increased systolic blood pressure during exercise at low altitude compared to the control condition (p=0.029). Condition or altitude exposure had no main effect on diastolic blood pressure during exercise (condition, p=0.924; altitude, p=0.081), or the change from rest to

exercise (condition, p=0.352; altitude, p=0.598). Finally, venous pressure during exercise was not affected by condition (p=0.065) or altitude exposure (p=0.077). In addition, there was no main effect of condition (p=0.379) or altitude exposure (p=0.979) on the change in venous pressure from rest to exercise.

Forearm Blood Flow. As predicted, blockade condition had a main effect on normalized forearm blood flow during handgrip exercise (p=0.011). Alpha-beta-blockade increased forearm blood flow during exercise compared to both control and beta-blockade conditions at low altitude (control, p=0.008; beta-blockade, p=0.028). At high altitude, forearm blood flow was lower in the control condition during exercise compared to beta-blockade (p=0.047) and alpha-beta-blockade (p=0.001). Surprisingly, FBF during exercise was not different between beta-blockade and alpha-beta-blockade conditions (p=0.083). Further, altitude had no main effect on blood flow during exercise (p=0.993). Similar to the absolute FBF during exercise, the blockade condition had a main effect on the change from rest to exercise (p=0.006). However, there were no individual differences at low altitude between conditions (control vs. beta-blockade, p=0.292; control vs. alpha-beta-blockade, p=0.292; beta-blockade vs. alpha-beta-blockade, p=0.110). At high altitude, alpha-beta-blockade was different from control (p=0.022) and beta-blockade (p=0.020) conditions, but the control and beta-blockade conditions were not different from each other (p=0.060). Unexpectedly, there was no main effect of altitude exposure on the change in FBF from rest to exercise (p=0.824).

Forearm Vascular Conductance. The blockade condition had a significant main effect on forearm vascular conductance during exercise (p=0.007). Alpha-beta-blockade increased FVC both at low altitude (p=0.030) and high altitude (p=0.020) during exercise compared to control. Opposite to expected, altitude did not influence FVC during exercise (p=0.756). Similarly, the

change in FVC from rest to exercise was affected by blockade condition (p=0.019). At low altitude, the alpha-beta-blockade condition was lower compared to the beta-blockade (p=0.043), but the control condition was not different from beta- (p=0.234) or alpha-beta- (p=0.234) blockades. At high altitude, alpha-beta-blockade reduced the change in FVC compared to control (p=0.044), but beta-blockade was not different than control (p=0.398) or alpha-beta-blockade (p=0.140) conditions. Altitude exposure did not have an influence on the change in FVC (p=0.501).

Arterial-Venous Oxygen Difference. There was a main effect of blockade on a-v O_2 difference during rhythmic handgrip (p=0.017). At low altitude, alpha-beta blockade significantly reduced the oxygen difference between arterial and venous blood during exercise compared to beta-blockade (p=0.004), but not compared to control (p=0.078). As expected, there was a main effect of altitude exposure on a-v O_2 difference during handgrip (p<0.001). Altitude exposure significantly reduced a-v O_2 difference during control (p<0.001), beta-blockade (p<0.001), and alpha-beta-blockade (p<0.001) conditions.

Table 7. Responses to Exercise in Each Condition Divided by Altitude Exposure.

	Low Altitude			High Altitude			Main effect of condition	Main effect of altitude	Interaction
	Control	Beta-blockade	Alpha-beta-blockade	Control	Beta-blockade	Alpha-beta-blockade			
Heart Rate (bpm)	62.9 ± 9.6	58.7 ± 8.6	57.6 ± 8.3 *	$81.6 \pm 16.9^{\sigma}$	76.0 ± 16.9 (7) *σ	74.8 ± 18.8 (7) *σ	**0.001**	**0.006**	0.594
Mean Arterial Pressure (mmHg)	103.3 ± 8.6	102.2 ± 5.5 (6)	103.8 ± 5.9	102.0 ± 7.0	105.8 ± 12.8 (5)	103.4 ± 5.0 (6)	0.885	0.758	0.583
Systolic Blood Pressure (mmHg)	134.7 ± 12.8	133.5 ± 9.4 (6)	133.7 ± 8.9	128.4 ± 9.5	133.3 ± 11.1 (5)	129.0 ± 12.2 (6)	0.984	0.204	0.617
Diastolic Blood Pressure (mmHg)	80.9 ± 6.7	79.9 ± 4.4 (6)	81.5 ± 5.2	83.1 ± 7.5	87.2 ± 14.9 (5)	84.3 ± 4.2 (6)	0.924	0.081	0.422
Venous Pressure (mmHg)	14.0 ± 5.6 (5)	9.6 ± 4.6 (7)	9.8 ± 4.8 (7)	16.1 ± 6.0	15.4 ± 7.0 (7)	14.4 ± 6.2 (6)	0.065	0.077	0.433
Forearm Blood Flow (mL/min/100mL FAV)	0.18 ± 0.06	0.21 ± 0.07	0.26 ± 0.05 *†	0.16 ± 0.04	0.27 ± 0.06 (6) *	0.31 ± 0.07 (7) *	**0.011**	0.993	0.369
Forearm Vascular Conductance (mL/min/mmHg/100mL FAV)	17.2 ± 5.6	20.9 ± 4.2 (6)	25.0 ± 5.8 *	16.3 ± 5.1	23.9 ± 2.8 (4)	32.6 ± 2.0 (6) *	**0.007**	0.756	0.887
Arterial-Venous Oxygen Difference (mmHg)	70.1 ± 4.1	74.7 ± 10.0	62.2 ± 7.9 (6) †	30.0 ± 6.8 σ	30.5 ± 7.5 (4) σ	25.1 ± 10.7 (5) σ	**0.017**	**<0.001**	0.372

Only data in participants with repeated measures is presented (n=8). n=8 unless indicated in parentheses.* Represents $p<0.05$

compared to control; † represents $p<0.05$ compared to beta-blockade; σ represents $p<0.05$ compared to same condition at low altitude.

Reponses were analyzed using a two-way (condition x altitude) repeated measures mixed-model ANOVA (GraphPad Prism Version

9).

Table 8. Changes from Baseline to Exercise in Each Condition Divided by Altitude Exposure.

	Low Altitude			High Altitude					
	Control	Beta-blockade	Alpha-beta-blockade	Control	Beta-blockade	Alpha-beta-blockade	Main effect of condition	Main effect of altitude	Interaction
Heart Rate (Δbpm)	9.0 ± 4.7	6.3 ± 2.5	1.3 ± 6.4	6.7 ± 3.7	6.1 ± 3.6	6.5 ± 2.1	0.224	0.882	0.103
Mean Arterial Pressure (ΔmmHg)	8.7 ± 4.6	8.1 ± 2.2 (6)	7.8 ± 4.0	3.5 ± 7.9	4.9 ± 8.4 (5)	4.2 ± 3.8 (6)	0.789	0.063	0.734
Systolic Blood Pressure (ΔmmHg)	-6.8 ± 8.4	-3.5 ± 10.2 (6)	5.3 ± 6.3 *	-18.2 ± 22.9	-8.7 ± 9.1 (5)	-3.3 ± 11.4 (6)	**0.045**	0.188	0.527
Diastolic Blood Pressure (ΔmmHg)	8.6 ± 4.1	7.8 ± 2.2 (6)	6.6 ± 3.8	7.6 ± 5.1	7.2 ± 11.2 (5)	5.1 ± 1.5 (6)	0.352	0.598	0.911
Venous Pressure (ΔmmHg)	1.5 ± 4.3 (6)	2.2 ± 1.8 (7)	1.2 ± 3.7	2.2 ± 2.3	2.3 ± 2.0 (7)	0.4 ± 1.2 (6)	0.379	0.979	0.779
Forearm Blood Flow (ΔmL/min/100mL FAV)	0.11 ± 0.06	0.14 ± 0.05	0.05 ± 0.04	0.11 ± 0.03	00.17 ± 0.07 (6)	0.06 ± 0.05 (7) *†	**0.006**	0.824	0.416
Forearm Vascular Conductance (ΔmL /min/mmHg/100mL FAV)	10.1 ± 6.5	13.9 ± 3.4 (6)	3.1 ± 5.1 †	11.0 ± 3.8	13.0 ± 4.8 (4)	4.7 ± 5.3 (6) *	**0.019**	0.501	0.768

Only data in participants with repeated measures is presented (n=8). n=8 unless indicated in parentheses.* represents p<0.05 compared to control; † represents p<0.05 compared to beta-blockade. Reponses were analyzed using a two-way (condition x altitude) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).
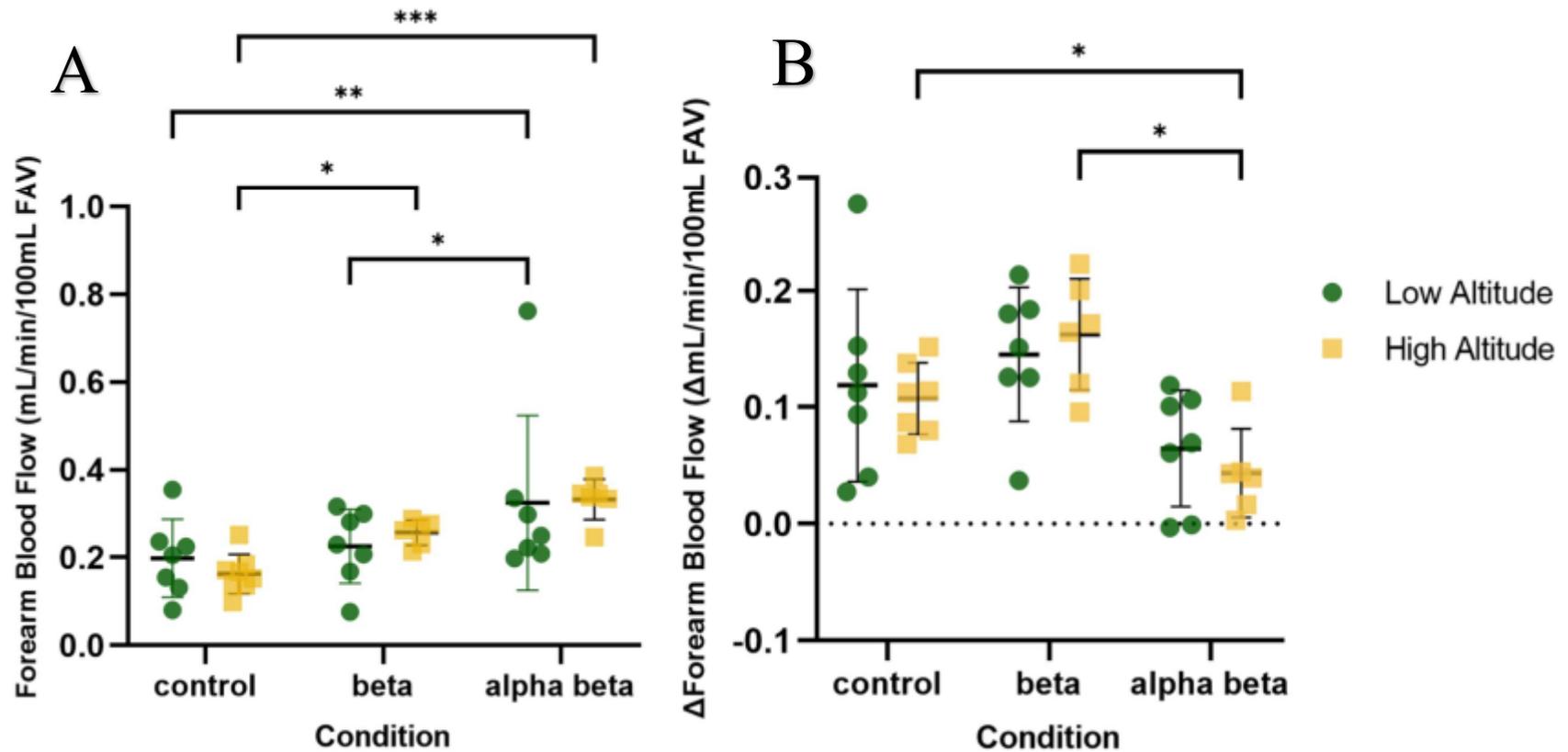
Figure 11. Panel A: absolute forearm blood flow during rhythmic handgrip exercise under each blockade condition at low and high altitude (n=8; beta-blockade at high altitude n=6; alpha-beta-blockade at high altitude n=7). There was a main effect of condition (p=0.011), but no main effect of altitude exposure (p=0.993). Alpha-beta blockade significantly increased forearm blood flow at low altitude compared to control (p=0.008) and beta-blockade (p=0.028). At high altitude, exercising blood flow was higher in the beta-blockade (p=0.047) and alpha-beta-blockade (p=0.001) compared to the control condition. Panel B: the change in forearm blood flow

normalized to forearm volume from rest to exercise under each blockade condition at low and high altitude (n=8; beta-blockade at high altitude n=6; alpha-beta-blockade at high altitude n=7). There was a main effect of condition (p=0.006), but no effect of altitude exposure (main effect, p=0.824). The change in blood flow during exercise was significantly less during alpha-beta-blockade compared to control (p=0.022) and beta-blockade (p=0.020) conditions at high altitude. Reponses were analyzed using a two-way (condition x altitude) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).
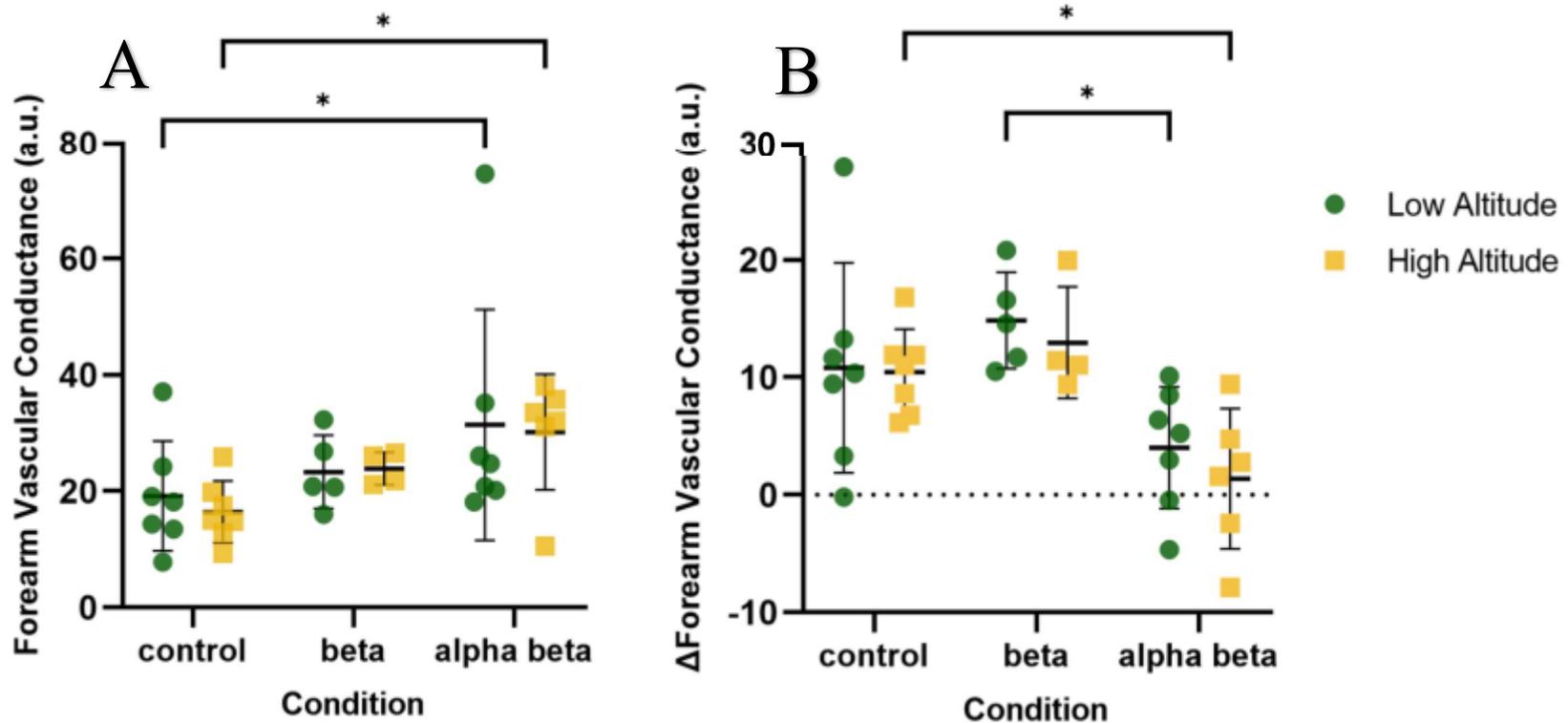
Figure 12. Panel A: forearm vascular conductance during rhythmic handgrip exercise across conditions and altitude exposures (n=8; beta-blockade at low altitude, n=6; beta-blockade at high altitude, n=4; alpha-beta-blockade at high altitude, n= 6). There was a main effect of condition (p=0.007), but no effect of altitude (main effect, p=0.756). Alpha-beta-blockade increased exercising conductance compared to control both at low (p=0.030) and high altitude (p=0.020). Panel B: the change in forearm vascular conductance from rest to exercise across conditions at low and high altitude (n=8; beta-blockade at low altitude, n=6; beta-blockade at high altitude, n=4; alpha-beta-blockade at high altitude, n= 6). There was a main effect of blockade condition (p=0.019), but not altitude (main effect,

p=0.501). At low altitude, alpha-beta-blockade reduced the change in conductance during exercise compared to beta-blockade (p=0.043). At high altitude, alpha-beta-blockade reduced the change in conductance during exercise compared to control (p=0.044). Reponses were analyzed using a two-way (condition x altitude) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).

Comparing Sexes at Low Altitude

In this section, data presented includes 12 participants at low altitude (Table 3).

Heart Rate. At low altitude, there was a main effect of condition on heart rate during handgrip exercise (p=0.019). There were no differences in females between conditions (control vs. beta-blockade, p=0.399; control vs. alpha-beta-blockade, p=0.057; beta-blockade vs. alpha-beta-blockade, p=0.399); however, in males, the control condition showed a higher heart rate during exercise than beta-blockade (p=0.016) or alpha-beta-blockade (p=0.020). There was no difference between beta- and alpha-beta-blockade in male's heart rate during exercise at low altitude (p=0.856). Similarly, the change in heart rate from rest to exercise was influenced by condition at low altitude (main effect, p=0.031). However, there were no significant differences between conditions for either females or males. Sex did not have an effect on either the absolute heart rate during exercise (main effect, p=0.119) and the change in heart rate from rest to exercise (main effect, p=0.907) at low altitude.

Blood Pressure. There were no main effects of condition or sex on mean arterial pressure during exercise at low altitude (condition, p=0.187; sex, p=0.254). Similarly, the change in MAP to exercise at low altitude was not influenced by condition (main effect, p=0.790) or sex (main effect, p=0.146). In agreeance, no main effects of condition or sex on systolic and diastolic pressures during exercise were found (SBP condition, p=0.806; SBP sex, p=0.128; DBP condition p=0.237; DBP sex, p=0.394). The change in DBP from rest to exercise was not influenced by condition (main effect, p=0.588) or sex (main effect, p=0.233); however, there was a main effect of condition on the change in SBP at low altitude (p=0.001). In males, there was an increase in SBP during exercise during alpha-beta-blockade compared to the control condition (p=0.003). There was no effect of sex on the change in SBP to exercise at low altitude (main effect, p=0.711). There was no effect of condition or sex on the venous pressure during exercise

at low altitude (main effect; condition, p=0.083; sex, p=0.566), or on the change in venous pressure from rest to exercise (main effect; condition, p=0.598; sex, p=0.600).

Forearm Blood Flow. At low altitude, there was no main effect of condition or sex on normalized forearm blood flow during exercise (condition, p=0.083; sex, p=0.229). On the contrary, there was a main effect of condition of the change in FBF from rest to exercise (p=0.022). The alpha-beta-blockade reduced the change in FBF compared to the beta-blockade condition (p=0.027) but was not different from the control condition (p=0.133). The change in FBF was not different during beta-blockade compared to control (p=0.133). Further, there was no main effect of sex on the change in FBF at low altitude (p=0.581).

Forearm Vascular Conductance. There was a main effect of condition on forearm vascular conductance during exercise at low altitude (p=0.038). However, there were no differences between any individual conditions (control vs. beta, p=0.097; control vs. alpha-beta, p=0.068; beta vs. alpha-beta, p=0.409). Sex had no influence on FVC during exercise (main effect, p=0.943). Similarly, blockade condition had a main effect on the change in FVC from rest to exercise (p=0.017). The alpha-beta-blockade reduced the change in FVC compared to beta-blockade (p=0.026). However, there was no difference between control and beta conditions (p=0.059) or control and alpha-beta conditions (p=0.059). Sex had no influence on the change in FVC at low altitude (main effect, p=0.219).

Arterial-Venous Oxygen Difference. There was a main effect of blockade condition on a-v O_2 difference during rhythmic handgrip at low altitude (p=0.006). Post-hoc analysis revealed no individual differences between conditions within sexes, but alpha-beta-blockade significantly reduced a-v O_2 difference compared to control (p=0.028) and beta-blockade (p=0.028) conditions when sexes are pooled. Sex had no influence on O_2 difference during handgrip at low altitude (main effect, p=0.691).

Table 9. Responses to rhythmic handgrip exercise at low altitude divided by sex.

	Control		Beta-blockade		Alpha-beta-blockade		Main effect of condition	Main effect of sex	Interaction
	Males	Females	Males	Females	Males	Females			
Heart Rate (bpm)	60.6 ± 8.4	67.8 ± 9.8	54.4 ± 9.7*	64.6 ± 5.5	54.1 ± 6.6*	62.6 ± 8.5 (5)	**0.019**	0.119	0.497
Mean Arterial Pressure (mmHg)	104.6 ± 10.3	97.3 ± 9.7	104.4 ± 6.0 (3)	94.8 ± 8.2 (5)	105.1 ± 8.1	100.5 ± 5.4 (5)	0.187	0.254	0.873
Systolic Blood Pressure (mmHg)	135.9 ± 13.7	126.8 ± 15.2	139.8 ± 4.5 (3)	123.3 ± 8.6 (5)	138.3 ± 9.4	126.3 ± 8.4 (5)	0.806	0.128	0.540
Diastolic Blood Pressure (mmHg)	81.8 ± 8.5	76.4 ± 7.3	80.4 ± 5.8 (3)	74.8 ± 7.1 (5)	81.7 ± 7.2	79.9 ± 4.2 (5)	0.237	0.394	0.608
Venous Pressure (mmHg)	12.1 ± 6.7 (3)	15.5 ± 6.8 (5)	11.9 ± 2.4	8.7 ± 6.2 (4)	12.6 ± 2.2	9.9 ± 8.8 (5)	0.083	0.566	0.056
Forearm Blood Flow (mL/min/100mL FAV)	0.19 ± 0.05	0.18 ± 0.07 (5)	0.22 ± 0.07	0.21 ± 0.08 (5)	0.29 ± 0.06	0.22 ± 0.02 (4)	0.540	0.083	0.229
Forearm Vascular Conductance (mL/min/mmHg/100 mL FAV)	18.4 ± 4.3	18.4 ± 8.2 (5)	18.4 ± 3.9 (3)	25.1 ± 4.2 (4)	28.3 ± 6.9	21.8 ± 2.1 (4)	**0.038**	0.943	0.161
Arterial-Venous Oxygen Difference (mmHg)	68.7 ± 5.3	71.8 ± 2.9	70.4 ±5.5	71.8 ± 6.4	62.3 ± 7.3 (4)	62.0 ± 12.4 (2)	**0.006**	0.691	0.706

Data presented are n=4 males and n=6 females unless indicated in parentheses. * represents p<0.05 compared to control; † represents

p<0.05 compared to beta-blockade. Reponses were analyzed using a two-way (condition x sex) repeated measures mixed-model

ANOVA (GraphPad Prism Version 9).

Table 10. Changes from Baseline to Exercise at Low Altitude Divided by Sex.

	Control		Beta-blockade		Alpha-beta-blockade				
	Males	Females	Males	Females	Males	Females	Main effect of condition	Main effect of sex	Interaction
Heart Rate (Δbpm)	9.6 ± 5.9	7.7 ± 3.4	5.8 ± 3.5	6.5 ± 1.3	6.2 ± 3.7	6.9 ± 3.8 (5)	**0.031**	0.907	0.352
Mean Arterial Pressure (ΔmmHg)	10.0 ± 5.3	6.2 ± 3.7	7.8 ± 2.3 (3)	5.5 ± 4.4 (5)	9.8 ± 1.7	6.4 ± 4.3 (5)	0.790	0.146	0.842
Systolic Blood Pressure (ΔmmHg)	-9.3 ± 8.6	-7.3 ± 8.2	-4.7 ± 8.7 (3)	-6.5 ± 11.2 (5)	7.1 ± 6.0*	3.4 ± 6.0 (5)	**0.001**	0.711	0.468
Diastolic Blood Pressure (ΔmmHg)	9.8 ± 5.3	6.4 ± 2.9	6.9 ± 1.0 (3)	5.8 ± 4.5 (5)	8.1 ± 2.2	5.7 ± 4.4 (5)	0.588	0.233	0.782
Venous Pressure (ΔmmHg)	2.5 ± 2.7 (3)	4.2 ± 3.2 (3)	3.1 ± 1.6	1.3 ± 1.1 (4)	3.1 ± 2.0	1.1 ± 6.0 (4)	0.598	0.600	0.388
Forearm Blood Flow (ΔmL/min/100mL FAV)	0.10 ± 0.05	0.13 ± 0.07 (5)	0.14 ± 0.04	0.15 ± 0.07 (5)	0.05 ± 0.05	0.05 ± 0.05 (4)	**0.022**	0.581	0.726
Forearm Vascular Conductance (ΔmL/min/mmHg/100mL FAV)	8.4 ± 6.1	11.1 ± 6.4 (5)	11.8 ± 3.7 (3)	18.6 ± 5.4 (4)	2.6 ± 5.8	3.6 ± 5.1 (4)	**0.017**	0.219	0.664

Data presented are n=4 males and n=6 females unless indicated in parentheses. * represents $p<0.05$ compared to control. Reponses were analyzed using a two-way (condition x sex) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).

Figure 13. Panel A: absolute normalized forearm blood flow during rhythmic handgrip across each condition in males and females at low altitude (n=9; 4M/5F; alpha-beta-blockade in females, n=4). There were no main effects of condition (p=0.083) or sex (p=0.229) on forearm blood flow. Panel B: the change in forearm blood flow from rest to handgrip exercise across conditions in males and females at low altitude (n=9, 4M/5F; alpha-beta-blockade in females, n=4). There was a main effect of condition on the change in forearm blood flow (p=0.022), but no effect of sex (main effect, p=0.581). Reponses were analyzed using a two-way (condition x sex) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).

Figure 14. Panel A: forearm vascular conductance during rhythmic handgrip across each blockade condition in males and females at low altitude (n=9, 4M/5F; beta-blockade in males, n=3; beta-blockade in females, n=4; alpha-beta-blockade in females, n=4). There was a main effect of condition (p=0.038), but no effect of sex (p=0.943). Panel B: the change in forearm vascular conductance from rest to exercise across blockade conditions at low altitude in males and females (n=9, 4M/5F; n=3; beta-blockade in females, n=4; alpha-beta-blockade in females, n=4). There was a main effect of condition (p=0.017), but not sex (main effect, p=0.219). Reponses were analyzed using a two-way (condition x sex) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).

Comparing Sexes at High Altitude

In this section, data presented includes 14 participants at high altitude (Table 3).

Heart Rate. At high altitude, there was a main effect of condition on heart rate during exercise (p=0.009) but no effect of sex (main effect, p=0.305). There were no differences in females' HR during exercise between conditions, but males had a higher HR during the control condition compared to beta-blockade (p=0.017). However, there were no effects of condition or sex on the change in HR from rest to exercise at high altitude (main effect; condition, p=0.089; sex, p=0.995).

Blood Pressure. At high altitude, there was no main effect of condition on MAP during exercise (p=0.484). However, sex influenced MAP during exercise (p=0.049). MAP is higher in males during the beta-blockade (p=0.048), but not the control (p=0.829) and alpha-beta-blockade (p=0.174) conditions. The change in MAP from rest to exercise was not influenced by condition (main effect, p=0.609) or sex (main effect, p=0.986). Both systolic and diastolic pressures during exercise were not affected by condition (main effect; SBP, p=0.306; DBP, p=0.459) or sex (main effect; SBP, p=0.171; DBP, p=0.142). However, there was a significant interaction between condition and sex on SBP during exercise (p=0.019). There was no effect of condition on the change is SBP from rest to exercise at high altitude (main effect, p=0.071), but there was an effect of sex (main effect, p=0.031). Further, there was a significant interaction between condition and sex on the change in SBP (p=0.046). There is a greater reduction in SBP in males in the control compared to the alpha-beta-blockade condition (p=0.013). Further, there is greater reduction in SBP during exercise in males compared to females in the control condition at high altitude (p=0.019). Condition nor sex influenced the change in DBP to exercise (main effect; condition, p=0.740; sex, p=0.305). There was no main effect of condition or sex on venous

pressure during exercise (condition, p=0.151; sex, p=0.713) or the change in venous pressure from rest to exercise (condition, p=0.141; sex, p=0.454) at high altitude.

Forearm Blood Flow. At high altitude, there was a main effect of condition on normalized FBF during exercise (p<0.001) as well as a main effect of sex (p=0.008). In females, FBF was increased during alpha-beta-blockade compared to control (p=0.031), but not compared to beta-blockade (p=0.110). In males, FBF was significantly higher in the alpha-beta-blockade compared to control (p=0.002) and beta-blockade (p=0.022) conditions. Despite a main effect, there are no individual differences between males and females in control (p=0.105), beta-blockade (p=0.145), and alpha-beta-blockade (p=0.105) conditions. There is also a main effect of condition on the change in FBF from rest to exercise (p=0.014), but no effect of sex (p=0.066). The alpha-beta-blockade reduced the change in FBF at high altitude compared to beta-blockade (p=0.024), but not control (p=0.057). The control condition was not different from the beta-blockade condition in the change in FBF (p=0.057).

Forearm Vascular Conductance. At high altitude, there was a main effect of condition (p=0.001) and sex (p=0.016) on FVC during exercise. In males, alpha-beta-blockade elevated FVC during exercise compared to control (p=0.008) and beta-blockade (p<0.001) conditions. There were no differences between sexes in FVC during exercise in control (p=0.073), beta-blockade (p=0.274), and alpha-beta-blockade (p=0.274) conditions. There was a main effect of condition (p=0.033) but not sex (p=0.144) on the change in FVC from rest to exercise at high altitude. In males, alpha-beta-blockade reduced the change in FVC compared to the control (p=0.014) but not compared to the beta-blockade (p=0.084) condition. There were no differences for females between conditions in the change in FVC. Similarly, there were no differences between sexes for any individual condition in the change in FVC at high altitude.

Arterial-Venous Oxygen Difference. There was no main effect of blockade condition on a-v O_2 difference during rhythmic handgrip exercise at altitude (p=0.184). Further, sex had no influence on a-v O_2 difference (main effect, p=0.625).

Table 11. Responses to Rhythmic Handgrip Exercise at High Altitude Divided by Sex.

	Control		Beta-blockade		Alpha-beta-blockade		Main effect of condition	Main effect of sex	Interaction
	Males	Females	Males	Females	Males	Females			
Heart Rate (bpm)	73.5 ± 15.5	82.5 ± 17.4	68.0 ± 15.7	79.0 ± 13.3 (5)	68.7 ± 17.3	77.8 ± 13.4 (5)	**0.009**	0.305	0.811
Mean Arterial Pressure (mmHg)	99.5 ± 5.4	100.4 ± 9.0	109.4 ± 10.3 (6)	94.7 ± 4.1 (4) $^\sigma$	106.6 ± 6.2 (7)	98.0 ± 7.0 (4)	0.484	**0.049**	**0.029**
Systolic Blood Pressure (mmHg)	128.0 ± 14.4	133.7 ± 11.4	147.3 ± 8.6 (6)	127.3 ± 11.8 (4)	136.8 ± 14.9 (7)	125.4 ± 9.9 (4)	0.306	0.171	**0.019**
Diastolic Blood Pressure (mmHg)	80.0 ± 7.4	79.4 ± 9.4	88.0 ± 13.3 (6)	75.3 ± 6.3 (4)	85.6 ± 4.3 (7)	78.4 ± 7.7 (4)	0.459	0.142	0.120
Venous Pressure (mmHg)	18.3 ± 5.5 (6)	12.9 ± 3.4 (5)	13.1 ± 8.3 (7)	12.4 ± 3.7 (4)	12.3 ± 6.6 (7)	12.2 ± 3.5 (3)	0.151	0.713	0.143
Forearm Blood Flow (mL/min/100mL FAV)	0.18 ± 0.05	0.11 ± 0.05	0.24 ± 0.08	0.15 ± 0.08 (4)	0.32 ± 0.05 (7) *†	0.23 ± 0.07 (5) *	**<0.001**	**0.008**	0.716
Forearm Vascular Conductance (mL/min/mmHg/100mL FAV)	17.8 ± 5.1	11.0 ± 4.7	20.0 ± 5.4 (6)	13.4 ± 7.3 (3)	30.5 ± 5.7 (7) *†	23.0 ± 7.7 (4)	**0.001**	**0.016**	0.950
Arterial-Venous Oxygen Difference (mmHg)	29.6 ± 8.6 (7)	31.7 ± 8.0 (5)	32.6 ± 7.9 (5)	32.2 ± 18.0 (2)	27.1 ± 9.1 (6)	26.8 ± 15.5 (2)	0.184	0.625	0.855

Data presented are n=8 males and n=6 females unless indicated in parentheses.* represents p<0.05 compared to control; † represents

p<0.05 compared to beta-blockade; σ represents p<0.05 compared to males in the same condition. Reponses were analyzed using a

two-way (condition x sex) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).

Table 12. Changes from Baseline to Exercise at High Altitude Divided by Sex.

	Control		Beta-blockade		Alpha-beta-blockade		Main effect of condition	Main effect of sex	Interaction
	Males	Females	Males	Females	Males	Females			
Heart Rate (Δbpm)	5.7 ± 2.5	5.5 ± 5.0	4.0 ± 2.7	4.9 ± 4.7 (5)	7.9 ± 4.0	6.1 ± 3.0 (5)	0.089	0.995	0.474
Mean Arterial Pressure (ΔmmHg)	-0.2 ± 8.1	5.3 ± 3.9	4.5 ± 7.1 (6)	0.3 ± 3.3 (4)	4.9 ± 3.5 (7)	3.5 ± 1.5 (4)	0.609	0.986	0.102
Systolic Blood Pressure (ΔmmHg)	-25.8 ± 17.6	-2.2 ± 6.4$^\sigma$	-9.1 ± 8.8 (6)	-4.6 ± 5.9 (4)	-3.5 ± 10.1 (7)	-1.4 ± 4.5 (4)	0.071	**0.031**	**0.046**
Diastolic Blood Pressure (ΔmmHg)	4.3 ± 6.7	5.7 ± 3.3	6.4 ± 10.0 (6)	0.6 ± 3.0 (4)	5.4 ± 1.1 (7)	3.3 ± 1.0 (4)	0.740	0.305	0.316
Venous Pressure (ΔmmHg)	2.9 ± 3.3 (6)	1.9 ± 2.5 (5)	1.3 ± 1.2 (7)	2.9 ± 2.0 (4)	0.01 ± 0.6 (7)	1.1 ± 1.4 (3)	0.141	0.454	0.228
Forearm Blood Flow (ΔmL/min/100mL FAV)	0.11 ± 0.03	0.07 ± 0.05	0.15 ± 0.07	0.10 ± 0.05 (4)	0.05 ± 0.05 (7)	0.05 ± 0.05 (5)	**0.014**	0.066	0.399
Forearm Vascular Conductance (ΔmL/min/mmHg/100mL FAV)	11.2 ± 3.2	6.5 ± 4.3	12.3 ± 3.8 (6)	7.4 ± 1.8 (3)	3.8 ± 5.3 (7) *	5.6 ± 4.8 (4)	**0.033**	0.144	0.126

Data presented are n=8 males and n=6 females unless indicated in parentheses. * represents $p < 0.05$ compared to control; σ represents $p < 0.05$ compared to males in the same condition. Reponses were analyzed using a two-way (condition x sex) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).
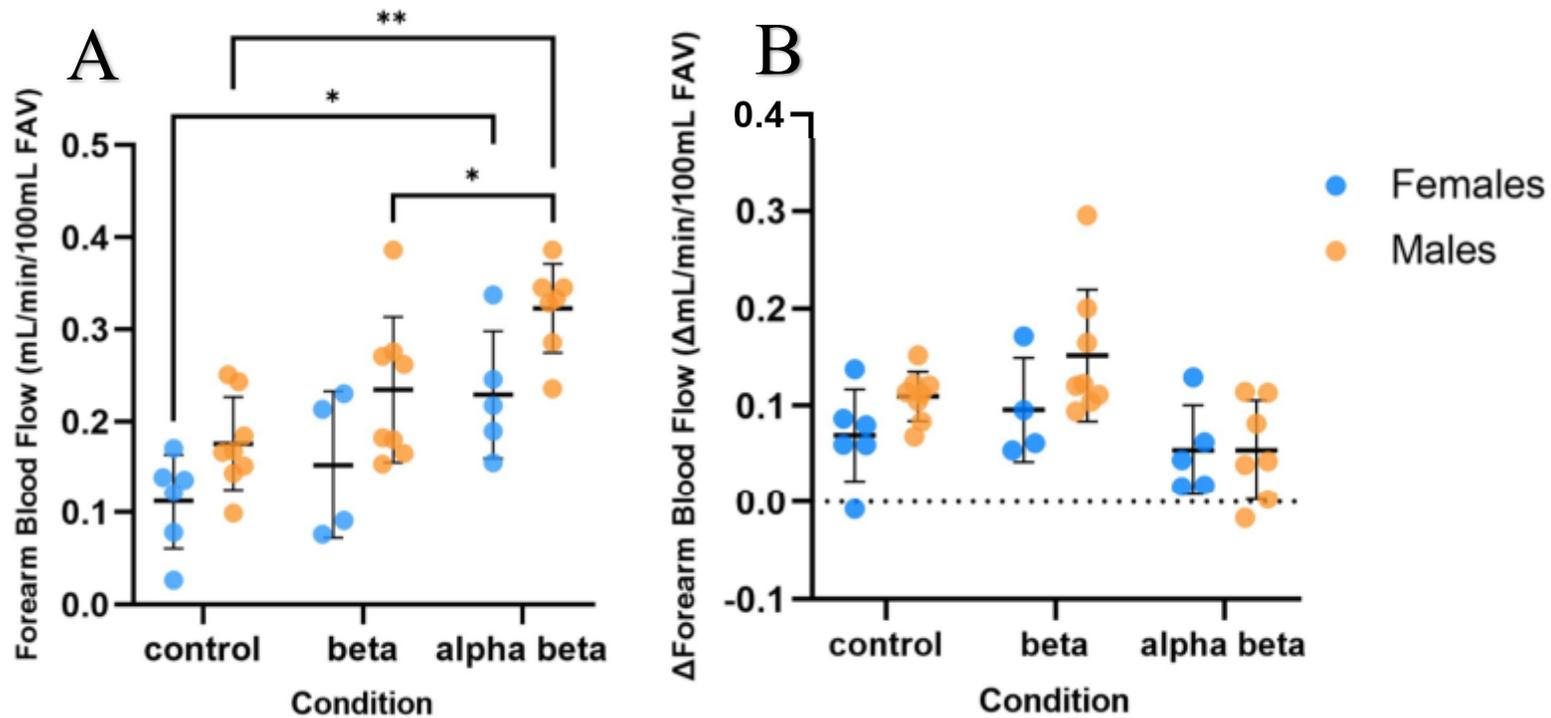
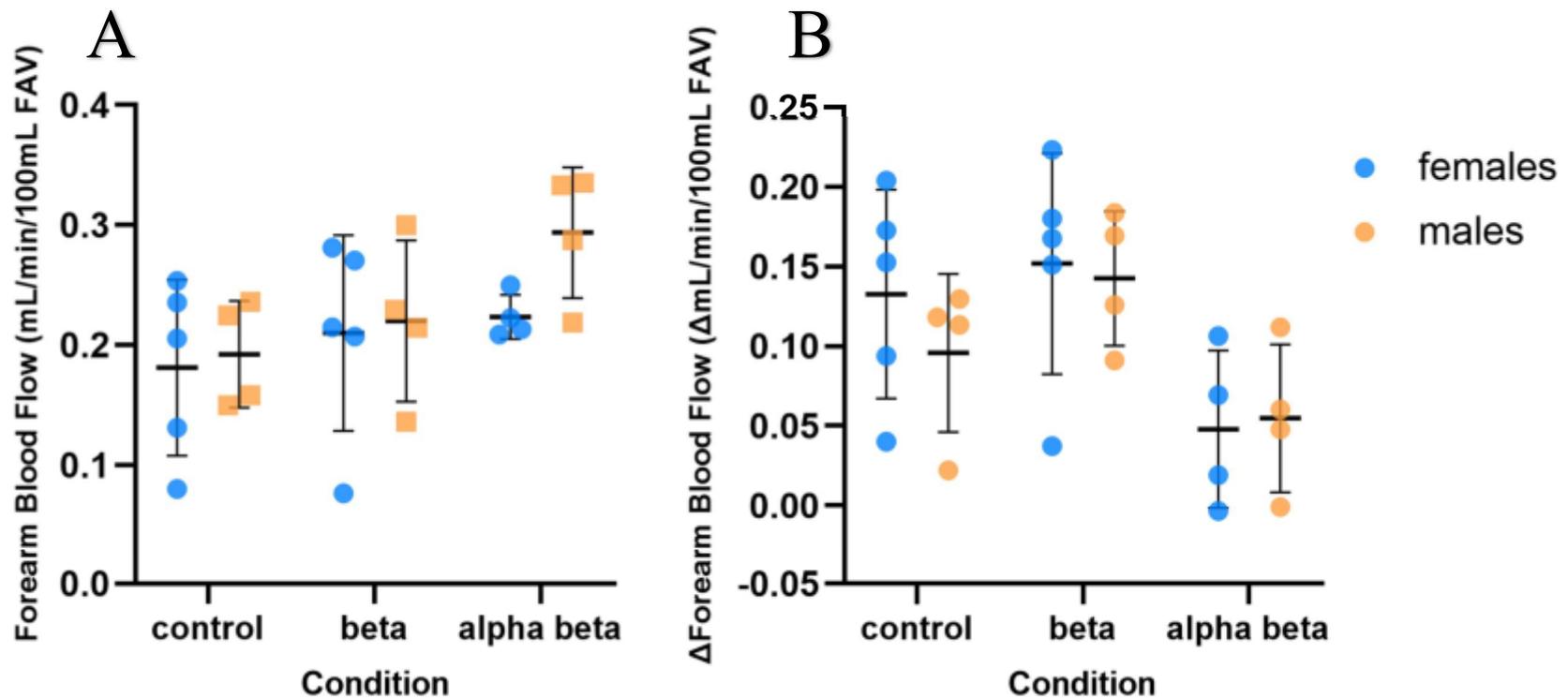Figure 15. Panel A: forearm blood flow normalized to forearm volume during rhythmic handgrip across blockade conditions at high altitude in males and females (n=14, 8M/6F; except beta-blockade in females n=4; alpha-beta-blockade in males, n=7; alpha-beta-blockade in females n=5). There was a main effect of both condition (p<0.001) and sex (main effect, p=0.008), but no interaction between condition and sex (p=0.716). Alpha-beta-blockade increased exercising blood flow compared to control in males (p=0.002) and females (p=0.031); alpha-beta-blockade also increased blood flow compared to beta-blockade in males (p=0.022). Panel B: the change in forearm blood flow from baseline to exercise across blockade conditions in males and females at high altitude (n=14,

8M/6F; except beta-blockade in females n=4; alpha-beta-blockade in males, n=7; alpha-beta-blockade in females n=5). There was a main effect of condition (p= 0.014), but not sex (main effect, p=0.066). Reponses were analyzed using a two-way (condition x sex) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).
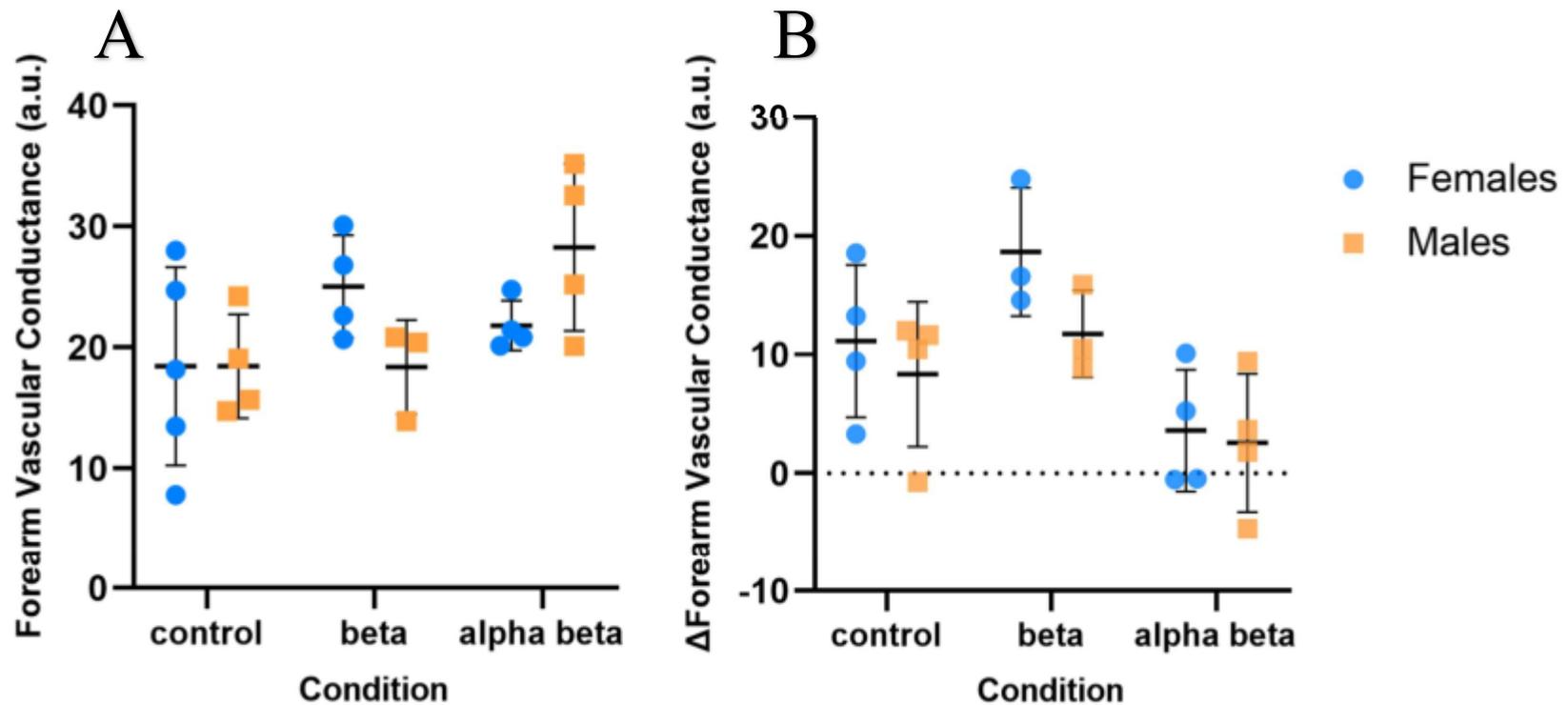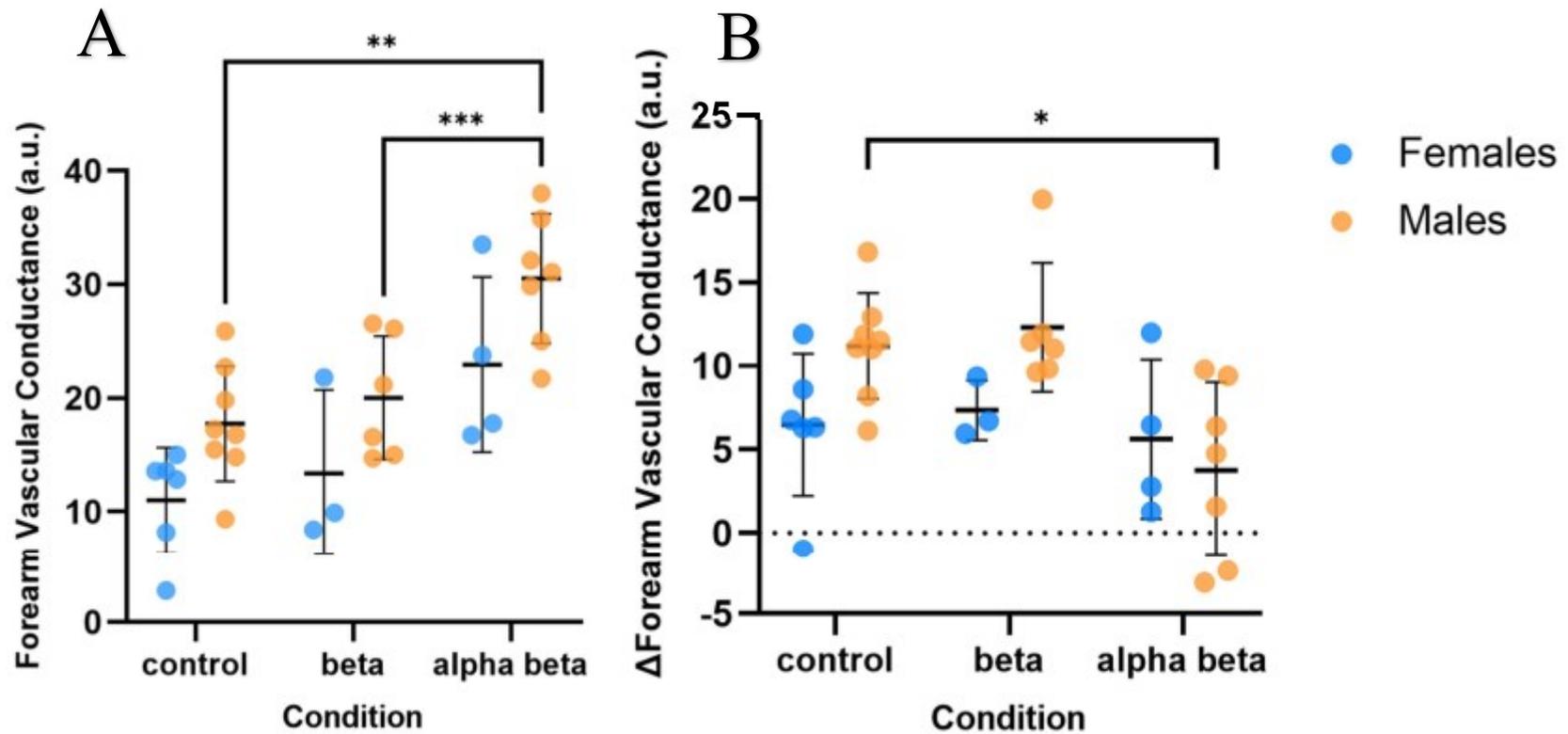
Figure 16. Panel A: forearm vascular conductance during exercise across blockade conditions comparing males and females at high altitude (n=14, 8M/4F; except beta-blockade in males, n=6; beta-blockade in females, n=3; alpha-beta-blockade in males, n=7; alpha-beta-blockade in females, n=4). There was a main effect of both condition (p=0.001) and sex (p=0.016) on forearm vascular conductance, but no interaction between condition and sex (p=0.950). In males, alpha-beta-blockade increased exercising conductance compared to control (p=0.008) and beta-blockade (p<0.001) conditions. Panel B: the change in forearm vascular conductance from

baseline to exercise across conditions at high altitude in males and females (n=14, 8M/4F; except beta-blockade in males, n=6; beta-blockade in females, n=3; alpha-beta-blockade in males, n=7; alpha-beta-blockade in females, n=4). There was a main effect of condition (p=0.033), but no main effect of sex (p=0.144) and no interaction between condition and sex (p=0.126) on the change in forearm vascular conductance. Alpha-beta-blockade reduced the change in conductance during exercise compared to the control condition in males only (p=0.014). Reponses were analyzed using a two-way (condition x sex) repeated measures mixed-model ANOVA (GraphPad Prism Version 9).

Chapter 5: Discussion

In this thesis, I aimed to investigate sympathetic control of the peripheral vasculature in response to exercise and hypoxia stressors. Further, I explored sex differences in the local control of the vasculature in response to these stressors. This study demonstrated that the adrenergic receptors contribute to the blood flow response to exercise. In addition, there were no differences in the contributions of these receptors to exercise hyperemia between low and high altitude. However, during high altitude hypoxia males and females appear to respond differently to an exercise stimulus; specifically, males showed a greater blood flow response to exercise at high altitude. Together, this data suggests that blood flow regulation during combined exercise and hypoxia stimuli is multi-factorial and complex. There may be an interaction between these stressors that differentiates how males and females regulate exercising blood flow.

5.1 Response to Altitude

The adaptations of the cardiovascular system during altitude exposure have been well described in the literature (Naeije, 2010). Immediately following exposure onset, cardiac output is increased, driven by a rise in heart rate, to match the heightened oxygen demand in the tissues (Klausen, 1966). Following initial exposure, cardiac output is returned to normoxic levels; however, heart rate remains elevated, so this reduction is achieved through attenuated stroke volume (Vogel & Harris, 1967). These transient changes are most prevalent over the first 48 hours in a high-altitude environment (Klausen, 1966). Therefore, as our data was collected between days 3-11 of altitude exposure, cardiac output was likely reduced from the initial increase upon altitude exposure. Our participants had significantly higher resting heart rate at altitude (Table 2) but resting forearm blood flow was not different compared to low altitude

(Table 4), supporting an underlying reduction in stroke volume and attenuation of cardiac output. Reduced stroke volume is likely due to a decrease in plasma volume that is associated with early acclimatization, lowering total blood volume (Robach et al., 2002). Further, evidence supports this reduction in cardiac output is enabled by an increase in oxygen extraction at the tissue; thus, oxygen delivery is always maintained (Naeije, 2010). Our data shows reduced arterial saturation and partial pressure of oxygen at altitude (Table 4). With this attenuated oxygen delivery, extraction and/or utilization would have to be improved if oxygen delivery was maintained; however, we can only speculate as we did not measure tissue oxygenation. Measured a-v O_2 difference indicates reduced oxygen extraction from the blood at altitude during handgrip, supporting improvements in oxygen utilization to meet oxygen demand (Table 7).

In this study, there was a significant increase in both mean arterial and systolic blood pressure at altitude (Table 4), likely due to augmented sympathetic activity during hypoxia (Simpson et al., 2021). In this scenario, the baroreflex maintains its sensitivity but is reset to accommodate sympathetic hyperactivity, which is likely offset by vascular control mechanisms. Due to heightened sympathetic activity, there is also blunting of neurovascular transduction to maintain blood pressure, which could be due to reduced endogenous release or binding of neurotransmitters resulting from diminished receptor sensitivity (Berthelsen et al., 2020). Our observations of no differences in brachial artery diameter or resistance with altitude exposure (Table 4) is likely due to this blunting of neurovascular transduction offsetting sympathetic hyperactivity.

Hypoxia exposure influences physiological responses to exercise as well. With any workload, cardiac output is increased; however, sympathetic hyperactivity may constrain blood flow and oxygen delivery, resulting in reduced peak oxygen consumption (VO_{2max}), workload,

and heart rate (Hartley et al., 1967; Mazzeo et al., 1995a; Vogel et al., 1974). Our data suggests no difference between low and high altitude in forearm blood flow or forearm vascular conductance at rest (Table 4) or during exercise (Tables 7 and 8). This contrasts with our understanding of autonomic control of the vasculature during hypoxia; sympathetic hyperactivity increases adrenergic tone of the vasculature, which should restrict blood flow. However, Simpson et al. (2023) suggests adrenergic sensitivity is blunted with chronic hypoxia exposure in males; clearly, there is a balance between sympathetic hyperactivity and reduced adrenergic sensitivity. It is possible that the autonomic system has a noticeable influence on maximal exercise responses but is accommodated for by other mechanisms at submaximal intensities.

The current results argue that during submaximal exercise, blood flow is maintained (Table 7) in the absence of changes in [Hb] and hematocrit at altitude. Therefore, we can interpret that a) the muscle is "over-perfused" at submaximal intensities and oxygenation is not challenged by moderate altitude exposure; or b) that oxygen extraction and/or utilization is augmented to meet demand. Previous evidence indicates blood flow during submaximal exercise may actually be restrained at altitude (Calbet, 2003; Hansen et al., 2022; Hansen & Sander, 2003; Lundby et al., 2008) but maintains oxygen delivery through enhanced muscle mitochondrial efficiency and increased oxygen carrying capacity (i.e., increased [Hb]) (Chicco et al., 2018). With prolonged exposure, there is an increase in erythropoiesis which further enhances hematocrit and may contribute to changes in blood flow regulation and oxygen delivery (Bogaard et al., 2002).

5.2 Adrenergic Responsiveness

Our data supports that the adrenergic receptors play an important role in blood flow regulation during exercise, regardless of altitude exposure (Tables 9-12). This is in agreeance with a large body of literature, illustrating the role of the adrenergic receptors in regulation of vascular resistance in response to changes in sympathetic activity (Dinenno et al., 2002; Eklund & Kaijser, 1976; Fadel, 2008; Kiowski et al., 1983).

At low altitude, our data shows a main effect of blockade condition on both absolute forearm vascular conductance and the change in FVC response to exercise (Tables 9 and 10). Alpha-beta-blockade significantly reduced the change in conductance during exercise compared to beta-blockade alone (Table 10). Although not statistically different, absolute conductance during exercise appeared elevated and the change in conductance appeared reduced during the alpha-beta-blockade compared to the control and beta-blockade condition (Table 9). On the contrary, beta-blockade was not different from control in either instance. This contradicts existing literature that indicates an important role of beta-receptors in modulating the blood flow response to sympathetic stressors (Eklund & Kaijser, 1976; Kneale et al., 2000; Samora et al., 2019; Silva & Zanesco, 2010). However, Cooper et al. (2021) found that beta-blockade did not alter sympathetic vasoconstriction in the contracting muscle in rats. It is important to note that muscle contraction was induced by external stimulation of the triceps surae muscles, so any potential influence of central command would not be present. Nonetheless, these results aligned with our findings and suggest the beta-adrenergic receptors respond differently in the exercising limb compared to non-exercising peripheral vasculature. Thus, it appears that of the adrenergic receptors, alpha receptors are most influenced during sympatholysis.

At high altitude, sympathetic activity is chronically elevated, which results in increased alpha-adrenergic stimulation from higher concentrations of circulating norepinephrine (Calbet, 2003; Hansen & Sander, 2003). Indeed, basal vascular resistance is modestly higher at altitude (Hansen et al., 2022; Tymko et al., 2020). However, the influence of sympathetic signaling on peripheral blood vessels is offset by the blunting of neurovascular transduction and possibly reducing the sensitivity of the alpha-adrenergic receptors (Berthelsen et al., 2020; Simpson et al., 2021). Current evidence suggests that both basal and exercising blood flow are reduced at altitude, likely due to augmented alpha-adrenergic signaling (Calbet, 2003; Hansen et al., 2022; Lundby et al., 2008). However, our results found no effect of altitude on the increase in forearm blood flow or forearm vascular conductance during exercise (Tables 7 and 8). There are a few possible explanations for this.

First, previous work has shown with increasing hypoxia severity, there are corresponding increases in sympathetic activity and blunting of alpha-adrenergic receptors (Fisher et al., 2018; Simpson et al., 2021). Since our study was conducted at a more moderate altitude of 3800m, compared to other studies showing restrained blood flow which were conducted above 4500m (Calbet et al., 2003; Hansen et al., 2022; Lundby et al., 2008), it is possible less vascular restraint was occurring in our participants. Further, if alpha-adrenergic receptor sensitivity was preserved in our participants due to a lower hypoxic stimulus, the withdrawal of sympathetic vasoconstriction during exercise may have a greater effect than studies at higher altitudes (i.e., similar, not blunted, sympatholysis compared to low altitude).

Second, studies utilizing acute hypoxia exposures show increased basal blood flow and vascular conductance (Casey et al., 2014; Jacob et al., 2021), whereas data from altitude suggest that following longer duration acclimatization, resting blood flow and vascular conductance are

reduced compared to basal flow at sea level (Hansen et al., 2022; Tymko et al., 2020). These studies were performed after at least 11 days acclimatization; thus, as our study falls within the period of early acclimatization, this may account for the lack of differences in blood flow and conductance. Indeed, we know the initial increase in cardiac output is attenuated within the first 48 hours (Klausen, 1966), but plasma catecholamines and sympathetic nerve activity do not reach peak values until days 4-7 (Mazzeo et al., 1995b; Mazzeo et al., 1998). Therefore, it is likely that changes in resting blood flow and vascular conductance are occurring until catecholamines and sympathetic nerve activity plateau. Further, if changes in resting vascular regulation are occurring, surely changes in reactivity will be present as well. Thus, it is important to consider the effect of the length of hypoxia exposure on the exercise response.

Third, Hansen et al. (2022) found that despite restrained blood flow and conductance during exercise at altitude, oxygen delivery and consumption was maintained. This is likely achieved by increased [Hb], hematocrit, and improved skeletal muscle respiratory efficiency (Bogaard et al., 2002; Hansen et al., 2022). However, our data showed no difference in [Hb] or hematocrit between low and high altitude. Therefore, it is possible that if oxygen delivery and utilization mechanisms had not improved as a result of altitude exposure, blood flow and vascular conductance must remain similar to low altitude conditions in order to meet oxygen demands. Additional evidence suggests that oxygen consumption may actually be reduced at altitude for the same exercise workload (Braun, 2008; Hansen et al., 2022; Jacobs et al., 2013; Jacobs et al., 2012). This may occur due to improved mitochondrial efficiency (Braun, 2008; Jacobs et al., 2013; Jacobs et al., 2012), enhanced ATP availability matched with demand, or improved metabolite homeostasis and regulation (Hoppeler et al., 2003). However, since our data is during early acclimatization (days 3-11), it is likely these adaptations have not yet occurred,

resulting in a greater reliance on blood flow to meet energy demands. A-V O_2 differences were reduced from sea level values at high altitude in our participants (Table 7). Yet, no changes in [Hb] or hematocrit suggest that oxygen delivery and extraction have not yet acclimatized; but, there may be an improvement in oxygen utilization to meet oxygen demands.

Lastly, our study is the first to include female participants when examining adrenergic control of sympatholysis at altitude. Ample evidence supports sex differences in regulation of blood flow and vascular conductance (Casey et al., 2014; Hart et al., 2011; Hart et al., 2009; Kneale et al., 2000; Samora et al., 2019; Usselman et al., 2015). Specifically, females have a reduced vasoconstrictor response to noradrenaline infusions (Kneale et al., 2000) and handgrip exercise and post-exercise circulatory occlusion (Samora et al., 2019), that disappears when given a beta-adrenergic antagonist. Therefore, it is possible that males have restrained blood flow in response to exercise at altitude, as found in other studies (Calbet et al., 2003; Hansen et al., 2022; Tymko et al., 2020). Conversely, females could have a different response and may be driving the lack of differences found between low and high altitude in our study. The following section will explore sex differences in our dataset.

5.3 Sex Differences

In contrast to the body of literature suggesting females have a greater vascular conductance response to exercise in normoxia (Gonzales et al., 2007; Just & DeLorey, 2017; Parker et al., 2007; Rogers & Sheriff, 2004), our study found no significant differences between males and females in forearm blood flow or vascular conductance responses to exercise at low altitude (Tables 9 and 10). One important difference between our study and the previous literature is the exercise intensity. We used a mild-intensity stimulus, while other studies have

performed exercise to exhaustion (Gonzales et al., 2007; Parker et al., 2007), full body treadmill exercise (Rogers & Sheriff, 2004), or nerve stimulation producing high-intensity contractions (Just & DeLorey, 2017). It is possible that a higher intensity exercise stimulus than was utilized here is needed to elicit sex differences in the reactive hyperemia response. On the other hand, Limberg et al. (2010) showed differences in alpha-mediated vasoconstriction between males and females at lower (15% MVC) but not higher (30% MVC) exercise intensities, dependent on menstrual phase. Specifically, alpha-adrenergic vasoconstriction during low intensity exercise appeared to be attenuated during the early luteal phase, when estrogen and progesterone levels are elevated (Limberg et al., 2010). However, they suggested that there are no sex-specific differences in the sympatholytic response to exercise (Limberg et al., 2010).

Our data showed a main effect of sex on forearm vascular conductance during exercise at high (but not low) altitude, with females exhibiting lower conductance (Table 11). It is possible there is an interaction between hypoxia and exercise that elicits a sex difference that is not present in either condition individually. However, previous evidence suggests females may have a greater vasodilatory response during acute hypoxia exposure compared to males (Casey et al., 2014; Jacob et al., 2021). Studies at altitude suggest that following acclimatization, resting blood flow and vascular conductance are reduced in males (Hansen et al., 2022; Tymko et al., 2020).

Contrary to our hypothesis, forearm blood flow and vascular conductance were not augmented during exercise in females compared to males under the control condition. In fact, we found no interaction between condition and sex on exercising forearm blood flow or vascular conductance at low or high altitude (Tables 9-12). This suggests that the adrenergic receptors may not contribute to sex differences in sympatholysis at altitude. Our data align with a new interpretation of the influence of sex on the adrenergic receptors. Although prior evidence

supports differences between males and females in sympathetic vasoconstriction in non-exercising skeletal muscle (Casey et al., 2014; Hart et al., 2011; Hart et al., 2009), our data, coupled with other published work, supports no sex differences in the role of the adrenergic receptors in sympatholysis (Jendzjowsky & DeLorey, 2013; Just & DeLorey, 2017; Limberg et al., 2010). More research is needed to understand the role of sex/sex hormones in the vascular response to exercise.

5.4 Other Potential Effectors

Despite previous evidence that supports an important role of beta-adrenergic receptors in vascular reactivity (Hart et al., 2009; Jacob et al., 2021; Kneale et al., 2000; Samora et al., 2019; Silva & Zanesco, 2010), we did not find any differences between beta-blockade and control conditions in forearm blood flow or vascular conductance in response to exercise. Any main effects of condition appear to be driven by alpha-adrenergic receptors. Pellinger and Halliwill (2007) showed a similar lack of difference in vascular conductance during exercise with and without beta-blockade. Clearly, other mechanisms are key for reducing sympathetic vasoconstriction in exercising skeletal muscle.

Neuropeptide Y and ATP are two nonadrenergic mechanisms that are released from sympathetic nerve terminals that contribute to the sympathetic vasoconstrictor response (Pernow et al., 1989; Shoemaker et al., 2015). Buckwalter et al. (2003) found that similar to but independent of alpha-adrenergic receptors, ATP activation of purinergic 2X receptors is attenuated in exercising vasculature, reducing vasoconstriction. This was independent of nitric oxide production, supporting a role for P_{2X} receptors in vasoconstriction during exercise. Similarly, neuropeptide Y is released with norepinephrine and causes vasoconstriction

(Buckwalter et al., 2004). During exercise, this response is blunted; there was no change in neuropeptide Y Y_1 receptor vasoconstriction during exercise with nitric oxide synthase blockade (Buckwalter et al., 2004), suggesting NPY Y_1 receptors contribute to vascular tone during exercise independent of nitric oxide production. These two mechanisms may in part explain the increase in blood flow and vascular conductance we observed during exercise under the combined alpha-beta-blockade; if nonadrenergic mechanisms contribute to sympathetic vasoconstriction, attenuation of these will increase blood flow during exercise. Thus, adrenergic receptors are important to but not the sole mechanism for sympatholysis.

Within the exercising limb, nitric oxide is released to counteract adrenergic vasoconstriction. Its production and release is triggered by shear stress, or an increase in blood flow, which activates endothelial nitric oxide synthase (Silva & Zanesco, 2010). Secondly, nitric oxide synthases can be activated by chemical stimuli that are released during exercise (Nosarev et al., 2014). Interestingly, Just and DeLorey (2017) suggest that nitric oxide-mediated sympatholysis is modulated by sex. Blockade of nitric oxide synthase normalized sympatholysis between sexes, whereas with no blockade females had augmented sympatholysis. Indeed, animal models have shown higher levels of endothelial and neuronal nitric oxide synthase in females (Ishihara et al., 2002; Laughlin et al., 2003). Fadel et al. (2004) found impaired sympatholysis in postmenopausal females, but following one month of estradiol replacement, sympatholysis was restored compared to premenopausal females. Further, ovariectomy augmented sympathetic vasoconstriction compared to ovary-intact female rats (Fadel et al., 2003). It appears levels of estrogen may influence nitric oxide-mediated blunting of vasoconstriction; the exact mechanisms that contribute to this enhanced sympatholysis are unclear. Nitric oxide is a possible explanation

for the main effect of sex we found in the current study, which was not related to adrenergic receptors.

Further, evidence from studies utilizing nitric oxide synthase blockades suggest nitric oxide is more important for sympatholysis in females; blockade of NOS in males did not change the degree of sympatholysis (Dinenno & Joyner, 2003; Jendzjowsky & Delorey, 2013; Just & DeLorey, 2017). On the contrary, others suggest nitric oxide is important for restricting alpha-adrenergic vasoconstriction during exercise (Patil et al., 1993; Thomas & Victor, 1998). Jendzjowsky and Delorey (2013) showed the level of nitric oxide-mediated sympatholysis is enhanced following exercise training; thus, level of fitness likely alters the contribution of nitric oxide. Our participants were healthy and active, all meeting Canada's physical activity guidelines at a minimum. We can speculate that they would have enhanced nitric oxide-mediated sympatholysis compared to non-active individuals. In addition, hypoxemia can stimulate the reduction of nitrite in the blood to nitric oxide (Cosby et al., 2003), further contributing to the attenuation of sympathetic vasoconstriction during exercise. It appears unlikely that there would be no nitric-oxide mediated sympatholysis occurring in our participants during exercise at altitude. Figures 11-16 show that even with complete adrenergic receptor blockade, there is still an increase in blood flow and vascular conductance during exercise.

Likely, there are redundant mechanisms that contribute to blunting of sympathetic vasoconstriction during exercise. Redundancy is an important physiological characteristic; it allows for the exercise hyperemic response to be achieved in multiple ways, preserving oxygen delivery in many conditions. Boushel et al. (2002) found combined inhibition of nitric oxide synthase and prostaglandins reduced blood flow up to 50% during exercise. Others suggest that nitric oxide may inhibit endothelial derived hyperpolarizing factor (Bauersachs et al., 1996;

Schildmeyer & Bryan, 2002). Further, evidence supports interactions between nitric oxide, adenosine, and potassium (Casey et al., 2013; Dua et al., 2009). Lamb and Murrant (2015) showed that potassium has an inhibitory influence on nitric oxide and adenosine, which can be abolished by inhibiting potassium channels and sodium/potassium adenosine triphosphatase. This is important evidence for redundancy in local vasodilatory mechanisms. These redundant mechanisms are illustrated in our data as the increase in blood flow and conductance during exercise under the combined alpha-beta-blockade. However, more research is needed to understand the interplay of these mechanisms with hypoxic exercise and the influence of sex and sex hormones on blood flow regulation.

5.5 Methodological Considerations

As is the nature of all expedition and field research, we were unable to control many variables. While at altitude, two participants tested positive for COVID-19 soon after participation. It is possible they had already contracted it when they participated in our study, or that other participants had COVID-19 but tested negative. Due to the large list of symptoms and effects of COVID-19, some of which are still not understood, it is possible that the physiological responses to altitude and exercise would be altered. However, we did not identify either of these participants as outliers, so their data was included in the results. Further, we tried to normalize testing conditions as much as possible between low and high altitude, but factors such as room temperature were different between locations (colder at altitude).

One major caveat to this study was our inability to control for menstrual phase or contraceptive use. D'Urzo et al. (2018) showed that endothelial function (assessed through flow-mediated dilation) in response to handgrip exercise did not differ between phases of the same

menstrual cycle. In contrast, large intra-individual variability between cycles may cause fluctuations in flow-mediated dilation (Liu et al., 2021). At a group level, menstrual phase or cycle did not impact endothelial function, but there was a high degree of variability. Further work is needed to understand the role of sex hormones on vascular function.

It is well known that circulating catecholamine concentrations change with altitude exposure and acclimatization (Mazzeo et al., 1991; Mazzeo et al., 1995a; Mazzeo et al., 1998). Mazzeo et al. (1994) found that epinephrine levels were elevated from initial altitude exposure and remained elevated to day 20. Norepinephrine levels increased with altitude exposure until peak concentrations between days 6 and 7, remaining elevated until the end of altitude exposure in this study (day 20) (Mazzeo et al., 1994). At sea level, exercise does not increase circulating plasma epinephrine levels (Mazzeo et al., 1991). With both acute and chronic altitude exposure, epinephrine levels are increased at rest and progressively during moderate cycling exercise, but not different between acute and chronic exposure (Mazzeo et al., 1991). During 15 minutes of moderate cycling exercise, acute altitude exposure did not change plasma norepinephrine concentrations (Mazzeo et al., 1991). On the contrary, following 3 weeks of altitude exposure, norepinephrine concentrations were higher at rest and during exercise (Mazzeo et al., 1991). Further, with a vast body of evidence supporting differences between males and females in adrenergic reactivity (Casey et al., 2014; Charkoudian et al., 2005; Fairfax et al., 2013; Hart et al., 2011; Hart et al., 2009; Miller et al., 2019; Miller & Duckles, 2008; Parker et al., 2007; Patel et al., 2014; Usselman et al., 2015), it is important to consider the influence of sex/sex hormones on catecholamine levels at rest and during exercise. Mazzeo et al. (1998) suggest that menstrual phase does not alter sympathoadrenal responses to altitude; plasma and urinary catecholamines were not different between follicular and luteal phases. Moreover, Mazzeo and colleagues found

similar catecholamine responses to both acute and chronic altitude exposure in males and females, suggesting sex does not influence circulating catecholamine concentrations (Mazzeo et al., 1995a; Mazzeo et al., 1998).

Surprisingly, we saw differences in the heart rate response to exercise between conditions (Tables 7, 9, 10). It appears with the beta-blockade, the heart rate response was reduced compared to control condition, both at low and high altitude. Despite using small doses of propranolol, there may have been small systemic effects of the beta-antagonist (i.e., blocking cardiac beta-adrenergic receptors, attenuating heart rate increases with exercise). Further, this could act to alter the blood flow response; a decrease in heart rate independent of any changes in stroke volume would reduce cardiac output; thus, attenuating blood flow. Similarly, condition had a main effect on the change in systolic blood pressure with exercise (Tables 8 and 10). Alpha-beta-blockade resulted in a greater increase in SBP at low altitude, which may have influenced the change in vascular conductance. It is possible that due to the alpha-adrenergic blockade, there was less vasodilation with exercise, thus causing systolic blood pressure to increase. However, since mean arterial and diastolic blood pressure were not different between conditions, the influence on vascular conductance is likely minimal.

Chapter 6: Conclusion

6.1 Main Findings

In summary, our findings show a complex, multi-factorial picture of the control of the exercising vasculature between males and females at altitude. Adrenergic receptor mechanisms were found consistently to be important for reducing sympathetic vasoconstriction both at low and high altitude. Combined blockade of both alpha- and beta-adrenergic receptors significantly reduced the change in blood flow and vascular conductance to exercise. However, beta-blockade showed no difference in blood flow or vascular conductance during exercise, suggesting beta-adrenergic receptors are not necessary for functional sympatholysis. Altitude exposure had no effect on blood flow or vascular conductance; thus, it is possible that the blood flow response to light- to moderate-intensity exercise is preserved at altitude. Further, since this study was conducted during early acclimatization, there may be changes in the vascular response to exercise as acclimatization occurs. Specifically, improvements in oxygen extraction and utilization in the tissue that decrease oxygen consumption may result in reduced blood flow and conductance to the same exercise stimulus at altitude (Braun, 2008; Hoppeler et al., 2003; Jacobs et al., 2013; Jacobs et al., 2012).

Contrary to our predictions, differences between males and females in the sympatholytic response may be explained by mechanisms other than those involving adrenergic receptors. Differences between sexes were observed only at high altitude, suggesting a potential interaction between altitude exposure and the mechanism for these sex differences. No interaction between blockade condition and sex supports that the adrenergic receptors are not the primary mechanism for differences between males and females. Despite evidence that suggests beta-adrenergic receptors are responsible for sex differences in vascular reactivity (Casey et al., 2014; Hart et al.,

2011; Hart et al., 2009), our data suggest a limited role for the beta receptors in the local exercise response. Moreover, blood flow is regulated by different mechanisms in the exercising vs. non-exercising skeletal muscle. A possible explanation is found in investigating local mechanisms of blood flow control; specifically, nitric oxide. Evidence proposes estrogen levels augment sympatholysis in females through nitric oxide (Fadel et al., 2004; Jendzjowsky & DeLorey, 2013; Just & DeLorey, 2017). Future work is required to investigate the interaction of hypoxia exposure and nitric oxide-mediated sympatholysis in males and females.

6.2 Perspectives and Future Directions

These results provide important implications for people travelling to and living at high altitude. High altitude pulmonary edema (HAPE) is a significant risk when travelling to altitude; when untreated, HAPE can have mortality rates of up to 50% (Nieto Estrada et al., 2017). One risk factor for HAPE is increased pulmonary vascular reactivity; thus, if we can improve the understanding of how the vasculature is controlled at altitude, this may provide important insights into HAPE risk and prevention. Further, we collected plasma samples to analyze catecholamine levels at rest and during exercise; however, these have not been analyzed yet. These results will add to our understanding and show a more complete picture of sympathetic control of the vasculature at altitude.

Further, there is extremely limited research including females in investigations of physiology at altitude. Despite evidence that females regulate their vasculature differently (Casey et al., 2014; Charkoudian et al., 2005; Hart et al., 2011; Hart et al., 2009), our current understanding of the influence of hypoxia and altitude on vascular reactivity comes mostly from investigations in males. Further work exploring the role of sex hormones in the exercise response

under hypoxic conditions is needed. We collected serum samples in our participants to measure

sex hormones. However, these samples have not been processed, so we are unable to comment

on any effects or differences in hormones. This may lead to better insights into how we should

approach treating pre- and post-menopausal females and expand our understanding of

populations living with chronic hypoxia.

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Appendix A: University of Alberta Health Research Ethics Board Approval

Re-Approval Form

Date:	January 04, 2022
Principal Investigator:	Craig Steinback
Study ID:	Pro00096808
Study Title:	Sex differences in sympathetic vascular reactivity at high altitude
Approval Expiry Date:	January 3, 2023
Sponsor/Funding Agency:	NSERC - Natural Sciences And Engineering Research Council

The Health Research Ethics Board - Biomedical Panel has reviewed the renewal request and file for this project and found it to be acceptable within the limitations of human experimentation.

The re-approval for the study as presented is valid for another year. It may be extended following completion of the annual renewal request. Beginning 30 days prior to expiration, you will receive notices that the study is about to expire. Once the study has expired you will have to resubmit. Any proposed changes to the study must be submitted to the HREB for approval prior to implementation.

All study-related documents should be retained so as to be available to the HREB on request. They should be kept for the duration of the project and for at least five years following study completion. In the case of clinical trials approved under Division 5 of the Food and Drug regulations of Health Canada, study records must be retained for 25 years.

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.

Approval by the REB does not 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, Covenant Health, community organizations, school boards) are obtained, before the research begins.

Sincerely,

Ann Moore, REB Specialist on behalf of

S.K.M. Kimber, MD, FRCPC
Chair, HREB Biomedical

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

Appendix B: University of British Columbia Ethics Approval

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

ETHICS CERTIFICATE OF FULL BOARD APPROVAL

PRINCIPAL INVESTIGATOR:	INSTITUTION / DEPARTMENT:	UBC CREB NUMBER:
Philip Ainslie	UBC/UBCO Health & Social Development/UBCO Health and Exercise Sciences	H22-01091

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:	
Institution	**Site**
UBC	Okanagan

Other locations where the research will be conducted:
Barcroft Station, affiliated with University of California Las Angeles (UCLA), White Mountain, California, USA

CO-INVESTIGATOR(S):
Andrew Steele
Liisa Wainman
Alexandra Williams
Christina Bruce
Jennifer Duffy
Mypinder Singh Sekhon
Hannah Caldwell
Joshua Tremblay
Jared B. Baylis
Connor Howe

SPONSORING AGENCIES:
- Michael Smith Health Research BC - "The effect of temperature on brain bioenergetic stress in hypoxia"
- UBC Unrestricted Research Funds - "Unrestricted Research Account"
- Wilderness Medical Society - "The impact of pentoxifylline on the physiological response to hypoxia and its potential therapeutic use for altitude-related illnesses: a series of double-blinded, placebo-controlled studies"

PROJECT TITLE:
White Mountain Research Expedition 2022

THE CURRENT UBC CREB APPROVAL FOR THIS STUDY EXPIRES: April 26, 2023

The full UBC Clinical Research Ethics Board has reviewed the above described research project, including associated documentation noted below, and finds the research project acceptable on ethical grounds for research involving human subjects and hereby grants approval.

This approval applies to research ethics issues only. The approval does not obligate an institution or any of its departments to proceed with activation of the study. The Principal Investigator for the study is responsible for identifying and ensuring that resource impacts from this study on any institution are properly negotiated, and that other institutional policies are followed. The REB assumes that investigators and the coordinating office of all trials continuously review new information for findings that indicate a change should be made to the protocol, consent documents or conduct of the trial and that such changes will be brought to the attention of the REB in a timely manner.

REB FULL BOARD MEETING REVIEW DATE: April 26, 2022	
DOCUMENTS INCLUDED IN THIS APPROVAL:	**DATE DOCUMENTS APPROVED:**

Document Name	Version	Date
Protocol:		
Proposal Document	2	May 12, 2022
Consent Forms:		
Consent Form	3	June 1, 2022
Investigator Brochures:		
Inhaled nitric oxide	1	June 1, 2006
Pentoxifylline	1	July 10, 2012
Phentolamine	1	September 1, 2012
Dopamine	1	November 3, 2016
Ascorbic acid	1	October 1, 2013
Glycopyrrolate	1	June 23, 2016
Sodium Nitroprusside	1	July 19, 2018
Norepinephrine	1	December 20, 2018
Fentanyl	1	October 21, 2019
Propranolol	1	June 19, 2013
Caffeine	1	October 30, 2018
Phenylephrine hydrochloride	1	December 1, 2012
Questionnaire, Questionnaire Cover Letter, Tests:		
Durham Caffeine Inventory	2	January 1, 2008
Groningen Sleep Scale	2	January 1, 1988
Caffeine questionnaire	1	April 11, 2022
7-day food log	2	May 12, 2022
Health screening questionnaire	1	April 11, 2022
Profile of mood states (POMS) questionnaire	2	January 1, 1993
Exercising intensity questionnaire	2	January 1, 2000
Epworth Sleep Scale	2	January 1, 1997
Stanford Sleep Scale	2	January 1, 1972
Lake Louise AMS questionnaire	1	January 1, 2018
Other Documents:		
Health screening data collection form	3	May 10, 2022
Dr Glen Foster Peer Review	1	April 7, 2022

June 1, 2022

CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.

2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.

3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent

form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

Approval of the Clinical Research Ethics Board by:
Dr. Stephen Hoption Cann,
Chair

Appendix C: Health Canada No Objection Letter

Health Canada   Santé Canada

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

27 August 2020

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

Your file   Votre référence
HC6-24-c241172
Our file   Notre référence

No Objection Letter RE: Protocol # PRO00096808 (Version 4)

Dear Dr. Craig Steinback:

I am pleased to inform you that the information and material to support your Clinical Trial Application for **NEO-SYNEPHRINE/ LEVOPHED/ NIPRIDE/ PROPRANOLOL**, control number **241172**, received on August 4, 2020, 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: https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/health-canada-clinical-trials-database.htm

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,
This document has been signed electronically using the Health Canada docuBridge system.

Larissa Lefebvre
Acting Manager, Submission Management Division
Office of Clinical Trials

LL/mw

Canada

Appendix D: Consent Form for Participants

UNIVERSITY OF ALBERTA

Faculty of Kinesiology, Sport, and Recreation

1-052 Li Ka Shing Center
Edmonton, AB, Canada
T6G2H9

Tel: 780.493.5553

PARTICIPANT CONSENT FORM

Title of Research Study: Sex differences in sympathetic vascular reactivity at high altitude

Principal Investigator: Dr. Craig Steinback, PhD

Research Coordinators: Emily Vanden Berg, BSc
Lauren Maier, BKin
Rayna Sharma, BSc
Lindsey Berthelsen, MSc

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.

COVID-19 Information

The COVID-19 pandemic and physical distancing measures have changed how we do research. Because of the need to keep participants and researchers safe, we have adjusted our practice to reduce the risk of transmission of the virus. The changes we have made are detailed throughout this consent form.

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 a healthy lowlander (live below 2500 meters). All lowlanders will be taking part of our high altitude research expedition. 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. Our findings will help us to understand differences between men and women, especially those living at and 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. The sympathetic nervous system 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 nervous system 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 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 looking at the ways in which your body regulates your blood pressure. We will do this by giving you small doses of safe drugs that are commonly used in hospitals. They will cause your blood pressure to go up or down briefly. This will tell us more about the responsiveness of your blood vessels. We will also be using two tests that cause your sympathetic nervous system to be activated. The first is a cold stress test, where you will put your hand in ice cold water. The second is a handgrip exercise test. After squeezing the handgrip device for a short time we will inflate a blood pressure cuff around your arm for a short time to trap the chemicals your muscles made during the exercise in your forearm. Both of these tests will activate your SNS and raise your blood pressure. After all tests, your blood pressure will return to normal. Looking at the way you respond to these tests will tell us more about how the sympathetic nervous system causes changes in blood pressure.

What will happen in the study?

If you meet the criteria for this study, you will be asked to be tested two times. Lowlander participants will do Part A and Part B during baseline testing (School of Health and Exercise Sciences, University of British Columbia – Okanagan, Kelowna, BC; and/or Neurovascular Health Lab, University of Alberta, Edmonton, Alberta) on two separate days. The location of the lab is at the University of British Columbia – Okanagan in the ARTs building, and at the University of Alberta room 4-269 in the Van Vliet Centre building on 87 Ave and 114 St NW. It is accessible by city transit and near two train stations (University or Health Sciences/Jubilee). Both parts will be repeated once at high altitude (Barcroft Station, CA).

COVID-19 Risk Mitigation Procedures

Before you arrive at the laboratory, the researcher will sanitize all equipment that will be used in your test, as well as surfaces (door handles, tables, sinks, etc.). Your researcher will also be wearing personal protective equipment (PPE), including a face mask and a face shield, and will regularly sanitize their hands.

When you arrive at the laboratory, you will be asked the following questions:

1. Are you experiencing any of the following: severe difficulty breathing (e.g., struggling for each breath, speaking in single words), severe chest pain, having a very hard time waking up, feeling confused or lost consciousness?
2. Are you experiencing any of the following: shortness of breath at rest, inability to lie down because of difficulty breathing, chronic health conditions that you are having difficulty managing because of your current respiratory illness?
3. In the past 10 days, have you experienced any of the following: fever, new onset of cough or worsening of chronic cough, new or worsening shortness of breath, new or worsening difficulty breathing, sore throat or runny nose?
4. Do you have any of the following: chills, painful swallowing, stuffy nose, headache, muscle or joint ache, feeling unwell, fatigue or severe exhaustion, nausea, vomiting, diarrhea or unexplained loss of appetite, loss of sense of smell or taste, or conjunctivitis (pink eye)?
5. In the past 14 days, did you return from travel outside of Canada, or did you have close contact with someone who is confirmed as having COVID-19?

If your answer is 'yes' to any of these questions, we will ask to reschedule your visit. If you answer 'no' to these questions, the researcher will invite you to enter the laboratory and ask you to sanitize your hands using hand-sanitizer (provided). Then, you will be asked to wear a disposable facemask (provided) throughout the 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 161mL (<11 tbsp, or ~1/3rd of a blood donation) throughout each test.
    2. Delivery of the study drugs (phenylephrine, sodium nitroprusside).
    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 electrocardiogram (ECG) stickers will be used to monitor your heart rate constantly throughout the experiment. Each set has three stickers. Two stickers go 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 your elbow and/or leg 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.

- We will measure the activity of your sympathetic nervous system. Once all the above equipment has been put in place, we will set-up the equipment required to measure **how** your blood pressure is controlled. This is different to measuring your blood pressure (which is done using a cuff on your upper arm). Your blood pressure is controlled by your nervous system, which is the bodily system that includes your brain, spinal cord and nerves. In order to record your nervous system signals, we place two very small needles, similar to acupuncture needles, in the lower part of your leg. A trained researcher will insert the first of these needles just under your skin below the knee. The same trained researcher will then insert the second needle into a nerve located just below the knee. From this second needle, we will listen for an audio signal that allows us to record your nervous system activity.

  Finding a useable nervous system activity signal may take some time. We will not do this in any one spot for more than ten minutes and we will not search for an appropriate signal for longer than 45 minutes in total. These are standard practices following current recommendations. Once the nerve recording is obtained, we will ask you to sit very still.

  If we do not obtain a useable signal after 45 minutes, we will remove both needles and begin the testing without this measurement. In addition, if you feel uncomfortable with this procedure, for whatever reason, you may stop at any time. We can always continue the rest of the test without this measurement.

Part B only:
- We will place a catheter into an artery in your arm. This will be done by a trained medical doctor. This will be used to infuse a small amount of fast acting drugs (norepinephrine, phenylephrine, phentolamine and propranolol) into. Since the dose is so small, you should not feel the effects of these drugs. We will also connect a device to measure the blood pressure inside of your artery. This device is filled with fluid (saline), and the movement of the fluid allows us to measure the pressure in your artery. It will not move into or out of your catheter as the pressure is very low.

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 finger clip, oxygen monitor, 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 Part A:
Following instrumentation, we will get you to lay still for 10 minutes and relax. This will allow us to obtain measurements of normal (baseline) values for each measure we are recording. Then we will perform several tests:
1) Modified oxford protocol: This will involve a sodium nitroprusside injection followed by a phenylephrine hydrochloride injection. First, we will inject a dose of sodium nitroprusside into the IV line in your arm. The drug will decrease your blood pressure slightly by causing your blood vessels to relax, without deactivating your sympathetic nervous system. The dose is designed to decrease your blood pressure by 15 mmHg. After 60-90s we will inject a dose of phenylephrine hydrochloride into the IV line in your arm. The dose is designed to increase you blood pressure by 15mmHg. The drugs will wash out of your system within 10 minutes, and your blood pressure will return to normal.

Protocol Part B:
Following instrumentation, we will get you to lay still for 10 minutes and relax. This will allow us to obtain measurements of normal (baseline) values for each measure we are recording. Then we will perform the following tests:
1) Phenylephrine infusions (3 total). We will infuse three different doses of phenylephrine into the arterial line in your arm. This allows for the drug to act locally. The infusions will cause the blood vessels in your arm to constrict.
2) Norepinephrine infusions (3 total). We will infuse three different doses of norepinephrine into the arterial line in your arm. This allows for the drug to act locally. The infusions will cause the blood vessels in your arm to constrict.
3) Isometric handgrip and post-exercise circulatory occlusion. During this test you will be asked to squeeze a device at 30% of your maximum grip strength for 2 minutes. At the end of the handgrip, we will inflate a blood pressure cuff on your arm for 3 minutes. This will keep the chemicals your muscles made during the exercise in your arm. The cuff will then be deflated and your blood flow will return to normal.
4) Rhythmic handgrip. During this test you will be asked to squeeze a device at 25% of your maximum grip strength for 3 minutes. with a duty cycle of 2-second contraction, 1-second relaxation as determined by a metronome.
5) Cold pressor test. 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.

6) Then, we will perform an infusion of the drug propranolol. We will infuse this drug through the arterial line in your arm. This drug will also act locally, so you shouldn't feel it. We will then record another baseline (with you resting quietly), and then repeat the first four tests.

7) Next, we will perform an infusion of the drug phentolamine. We will infuse this drug through the arterial line in your arm, at the same time as the propranolol infusion. This will also act locally, so you shouldn't feel any effects. We will then record another baseline and repeat the first four tests.

All drug infusions will be done by a trained researcher who has experience with this type of injection under the guidance of a physician (Dr. Sean van Diepan). The size of the doses will be based on your body size.

What will I be asked to do while I am in the study?

You will be asked to not eat anything for 2 hours 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. You will then lay comfortably on a hospital bed and we will begin to put on the equipment required to do the study.

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?

COVID-19: Until current COVID-19 physical distancing restrictions are lifted, the following steps will be taken to reduce the risk of transmission of the virus:

- Before you come to the laboratory, you will be asked a series of screening questions. These follow Alberta Health Services COVID-19 screening procedure. Your researchers will also complete the same screening procedure before entering the laboratory.
- When you enter the laboratory, you will be asked to sanitize your hands and wear a disposable facemask throughout your visit. These will be provided. Your researcher will also be wearing a face mask and face shield, and will regularly wash and sanitize their hands.
- Only two researchers will be within 2 meters of you during your visit. These researchers will be wearing full PPE. Other researchers will be present in the laboratory, but will remain greater than 2 meters away from you and will also wear a face mask and regularly sanitize their hands and surfaces.
- All study materials will be handled with gloved hands and any materials that we give to you will be cleaned with an anti-viral wipe (e.g. Lysol wipes).
- All equipment and surfaces (e.g. door handles, tables, sinks) will be sanitized prior to your arrival at the laboratory.
- Our laboratory is working at low in-person capacity. That means only researchers who are directly involved in the research study will be in the laboratory. Therefore your exposure to other people will be kept at a minimum.

- When inside the laboratory, we ask that you bring as few personal items as possible. We will provide you with a locker to store belongings. We encourage you to bring your own water bottle to the testing.
- After we finish each test, all equipment and surfaces are thoroughly sanitized to reduce the risk of transmission to others.

If there are changes to the risks related to the COVID-19 virus (i.e. changes to recommendations from the national or provincial government), we will update you so that you can reconsider your participation in the study.

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. 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. Symptoms of AMS include headache, dizziness, peripheral paresthesia (i.e. tingling or numbness in the arms or legs) and breathlessness. 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.

Nerve Activity Recording: As we search for a suitable location to record nervous system activity you may feel odd or new sensations. This may include changes in temperature, tingling, mild cramping, or pressure in your lower leg. These are all normal and may help us to know if we are recording from the right location within the nerve. These sensations occur only briefly and go away quickly if searching is paused or if the needle is pulled back from the spot that caused the sensation. You will be asked to tell us if you feel any of these signals. Between one and four people out of 100 may find these sensations intolerable, if that is the case for you, we will stop searching for your nervous system activity.

Following the experiment, there is a risk that you may experience lasting effects of the procedure (up to two weeks). Between 1 and 5 out of 100 people report mild tenderness, numbness, increased sensitivity, and redness around the location of the needles. In rare circumstances (less than 3 out of 1000), there is also the risk of persistent muscle weakness in your lower leg, known as foot drop. This can last as long as 6 months or may even lead to permanent disability. Foot drop can alter how you walk (dragging of foot or toes) and can result in numbness or tingling of the lower leg. Treatment for foot drop includes orthoses (foot brace) and/or physical therapy. To minimize the risk of any persistent effect, we limit the amount of time for which we search for a suitable signal (10 min in one spot and no more than 45 min in total). This is within the current recommendations for this technique. We also recommend that you do not take part in any physical activity greater than walking in the 24 hours after the protocol is complete to help reduce any inflammation that may result.

With any procedure using needles, there is a minimal risk of infection; we follow all appropriate steps to sterilize both the skin and the needles to help minimize this risk. This includes washing hands thoroughly, using alcohol wipes on the skin where the needles will be inserted, and sterilizing the multi-use needles using the same technique used for surgical equipment (autoclaving).

Study Drugs:

Phenylephrine: 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.

Sodium Nitroprusside: This drug causes your blood vessels to dilate and your blood pressure to go down slightly. You may feel slightly lightheaded or nauseas due to this decrease in blood pressure. You will remain laying down to reduce the risk of low blood pressure. The effects of this drug should wash out within 10 minutes.

Propranolol: You may feel faint and potentially nauseas due to an expected decrease in blood pressure. The most common side-effect symptoms include mild diuresis (increased urine production and therefore need to urinate), a small decrease in blood pressure, or feelings of dizziness and sleepiness. You will remain laying down to reduce the risk of low blood pressure. The small dose of the drug we were using in this study means that any side effects will be very short lasting (2-3 hours). Because we are using propranolol locally in your arm, it is very unlikely you will feel the effects of the drug in the rest of your body.

Norepinephrine: This drug will cause your blood vessels to constrict. Because we are using such a small amount of norepinephrine locally in your arm, it is very unlikely you will feel the effects of the drug in the rest of your body.

Phentolamine: This drug may result in a reduction of blood pressure during the test. You may feel faint or potentially nauseous. You will remain laying down to reduce any risk of low blood pressure. The small dose of the drug we were using in this study means that any side effects will be very short lasting (2-3 hours). Because we are using phentolamine locally in your arm, it is very unlikely you will feel the effects of the drug in the rest of your body.

There is also a possibility of you having an adverse reaction to any of the drugs, 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.

Brachial artery catheterization: Placement of a needle into your blood vessel might be slightly painful. However, we will give you a numbing agent so you won't feel anything. While the catheter is in place, you should not feel much discomfort. If you do, please tell our doctor. There is a very small risk (less than 1 out of 1000) that the artery can become damaged, but we do our best to avoid this small risk by using highly sterile and proven techniques and employing staff who are highly experienced.

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.

Handgrip Exercise and Occlusion: The handgrip exercise should cause no risk to you, but you may feel a little stiff after. You may have minor soreness in your arm for the next few days. The blood flow occlusion may feel uncomfortable but should not feel painful and can be stopped at any time if needed. While unlikely, slight bruising may occur on your arm where the cuff was placed.

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 blood vessels has no known risks involved with it.

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 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 one year from the date of your test 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?
Lowlanders (i.e. as part of the research expedition), you will not be paid for participation in this study, nor should you incur any expenses related to this study.

Privacy and Confidentiality
During this study we will be collecting information (or "study data") about you. We will use the data to help answer research questions and we will share (or "disclose") your information with others such as the study sponsor and other researchers. Your study data may also be shared with government

departments involved in the approval of drugs for sale in a country. These departments are often called "regulatory authorities". An example of a regulatory authority is Health Canada.

Below we describe in more detail how your data will be collected, stored, used and disclosed.

What data will we be collecting?
During this study we will be collecting data about you. Examples of the types of data we may collect includes your name, where you live, your ethnic background, your date of birth, your age, your health conditions, your health history, your medications and results of tests or procedures that you may have had. We will only look for and collect the information that we need do the research. We will get this information by asking you questions and doing the tests outlined in this form.

How will the study data be stored?
The study data we collect which will include your name will be securely stored by the study doctor during and after the study. We will also put a copy of this consent form in your clinical record, so that doctors you see in the future will know you were in the study. In Canada, the law says we have to keep the study data stored for at least 25 years after the end of the study.

The study doctor will not release your name to anyone unless the law says that they have to.

How will the study data be used?
Your study data will be coded (with a number) so that it no longer contains your name, address or anything else that could identify you. Only your study doctor will be able to link your coded study data to you. Your coded study data will be sent to the Sponsor. This coded study data will be kept by the Sponsor in a secure manner and will be used now and in the future to
- learn more about how the study drug (and possibly similar drugs) works and how safe it is;
- learn more about how to treat your disease and other similar diseases;
- apply for get approval to sell the study drug in Canada or other countries;

This coded study data may also be shared with people who work with the Sponsor and with regulatory authorities. The Sponsor and/or the people they work with may be located outside of Canada, in countries that do not have the same privacy laws as in Canada. However, because nothing that is sent to the Sponsor will contain your name, no one who uses this information in the future will be able to know it came from you. The risk to your privacy, then, should be very small.

When the study is done, the Sponsor may place your coded study data into a secure database. The coded data may then be used to answer other research questions in the future. Only researchers who have the training and experience to do the research (also known as "qualified researchers") will be allowed to use the data. {State whether or not data will be anonymized (i.e. No way to link back to individual ever) or de-identified (link remains at study site) and state how long it will be stored for future use})

Who will be able to look at my health data?
During research studies it is important that the data we get is accurate. Therefore, your study data and original medical records may also be looked at by people from: the study sponsor, the University of Alberta auditors and members of the Research Ethics Board, Health Canada, and/or other foreign regulatory authorities.

By signing this consent form you are saying it is ok for the study doctor/staff to collect, use and disclose information from your medical records and your study data as described above.

If you would like to see the study data collected about you, please ask the study doctor. You will be able to look at the study data about you and you can ask for any mistakes to be corrected. The study doctor may not be able to show you your study data right away and you may have to wait until the study is completed or another time in the future before you can see your study data.

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.

What if I have questions?
If you have any questions about the research now or later, or if you experience any adverse effects, or think that you have suffered a research related injury 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).

UNIVERSITY OF ALBERTA

Faculty of Kinesiology, Sport, and Recreation

1-052 Li Ka Shing Center   Tel: 780.493.5553
Edmonton, AB, Canada
T6G2H9

Title of Study: Sex differences in blood pressure regulation at high altitude.
Principal Investigator: Dr. Craig Steinback, PhD   Phone Number: 780-492-5553
Research Coordinators: Emily Vanden Berg, BSc; Lauren Maier, BKin; Rayna Sharma, BSc;
Lindsey Berthelsen, MSc

	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 E: Sample "Day Of" Data Collection Sheet

Date: _____   Participant ID: _____

Day-of Participant Information Form

Study Title: Sex differences in sympathetic vascular reactivity at high altitude – PART B

Sex: _____   D.O.B: _____   Age: _____ years

Height: _____cm   Weight: _____kg   BMI: _____kg/m²   FAV: _____mL

> **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 (Edmonton/Kelowna)   ☐ Altitude (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   Lake Louise Mountain Sickness Score: _____

Medications:
Have you taken any medications in the last 24 hours?   Y / N
If yes, please list:

Medication	Time	Dose (e.g. mg)

<u>TEAM:</u>
Computer: _____
IV/Art. catheter: _____
Blood Draw: _____
Ultrasound: _____
Aliquoting: _____

	MEASUREMENT (CM)
Sternum - Femoral	
Transducer – catheter	

	1	2	3
Manual Blood Pressure			

RTF: _____▁_____

Propranolol:
Propranolol concentration: _____ µg/mL   Bag #: _____   Date <u>mixed:</u>_____

Loading dose: _____ mL/min   Maintenance dose: _____ mL/min

Norepinephrine:
Norepinephrine concentration: _____ µg/mL Bag #: _____   Date <u>mixed:</u>_____

Dose #1: _____mL/min   Dose #2: _____mL/min   Dose #3: _____ mL/min

Phentolamine:
Phentolamine concentration: _____ µg/mL   Bag #: _____   Date <u>mixed:</u>_____

Loading dose: _____ mL/min   Maintenance dose: _____ mL/min

Phenylephrine:
Phenylephrine concentration: _____ µg/mL Bag #: _____   Date <u>mixed:</u>_____

Dose #1: _____mL/min   Dose #2: _____mL/min   Dose #3: _____ mL/min

Ultrasound Videos:

Recording path: _____   Date: _____

(YYYY-MM-DD)

Video Start Times (on recording laptop) (HH-MM-SS):

	Control	Alpha Blockade (PR)
Baseline		

NE Infusions		
CPT		

□ Video files renamed?

Isometric Handgrip Protocol:

MVC: _____N   □ MVC set to 100% in LabChart   Handedness: R / L

....

Rhythmic Handgrip Protocol:

MVC: _____N   □ MVC set to 100% in LabChart   Handedness: R / L

....

Cold pressor test protocol:

Temp of water: _____°C

NOTES:

Appendix F: Health History Questionnaire

Health History Questionnaire

Please complete the survey below.

Thank you!

Participant study ID: [initials]

Date of Birth

_____

Sex: ○ Male
        ○ Female

Height (cm):

_____
(Self-reported)

Weight (kg):

_____
(Self-reported)

BMI (kg/m^2):

_____
(Self-reported)

Ethnic background (check all that apply):

☐ American Indian, Alaska Native, First Nations, Inuit, and/or Métis
☐ Black, African American, or African Canadian
☐ Caucasian
☐ East Asian
☐ Hispanic or Latino
☐ Native Hawaiian or Other Pacific Islander
☐ South Asian
☐ Unknown
☐ Other
☐ Prefer not to say

Please specify "other":

_____

Have you been to altitudes of ≥2000m in the last 1 month?

○ Yes
○ No

Please select all that apply to your personal history:
- ☐ Stroke
- ☐ Hypertension
- ☐ Heart Attack
- ☐ Heart Mummur
- ☐ Blood Clots
- ☐ Other cardiovascular disorders
- ☐ Type 1 Diabetes
- ☐ Type 2 Diabetes
- ☐ Obesity
- ☐ Other metabolic disorders
- ☐ Asthma
- ☐ Sleep Apnea
- ☐ COPD
- ☐ Other respiratory/breathing disorders
- ☐ Alzheimers
- ☐ Cognitive Impairment
- ☐ Parkinsons
- ☐ ALS
- ☐ Seizures
- ☐ Other neurological disorders

Please specify "other cardiovascular disorders":

_____

Please specify "other metabolic disorders":

_____

Please specify "other respiratory/breathing
disorders":

_____

Please specify "other neurological disorders":

_____

Please select all that apply to your family history
(parents, siblings, and grandparents):
- ☐ Stroke
- ☐ Hypertension
- ☐ Heart Attack
- ☐ Heart Mummur
- ☐ Blood Clots
- ☐ Other cardiovascular disorders
- ☐ Type 1 Diabetes
- ☐ Type 2 Diabetes
- ☐ Obesity
- ☐ Other metabolic disorders
- ☐ Asthma
- ☐ Sleep Apnea
- ☐ COPD
- ☐ Other respiratory/breathing disorders
- ☐ Alzheimers
- ☐ Cognitive Impairment
- ☐ Parkinsons
- ☐ ALS
- ☐ Seizures
- ☐ Other neurological disorders

Please specify "other cardiovascular disorders":

_____

Please specify "other metabolic disorders":

_____

Please specify "other respiratory/breathing disorders":

_____

Please specify "other neurological disorders":

_____

Have you had COVID-19 in the past 1 year?

○ Yes
○ No

If yes, how long ago?

_____

Do you have any lingering symptoms?

_____

Any other major surgery, illness, or injury not listed above? Please specify.

_____

Were you born premature (before 37 weeks)?

○ Yes
○ No

Do you smoke currently?

○ Yes
○ No

How many cigarettes per day?

_____

Have you quit after smoking previously?

○ Yes
○ No

If you quit smoking, how long since your last cigarette?

_____

Do you vape or use e-cigarettes?

○ Yes
○ No

How often do you vape or use e-cigarettes?

_____

Do you use cannabis?

○ Yes
○ No

Do you smoke cannabis?

○ Yes
○ No

How many times per week do you smoke cannabis?

_____

Have you ever fainted before?

○ Yes
○ No

Please explain your previous fainting episodes.

_____

Please list any current medications you are taking.

_____

Are you taking any of the following medications?

☐ Tri-cyclic antidepressants
☐ Other antidepressants
☐ Monoamine oxidase inhibitors
☐ Beta-blockers
☐ Blood pressure medications
☐ Anti-arrhythmic medications
☐ Catecholamine depleting drugs
☐ Migraine medications

Please specify medication type and dosage:

_____

Are you allergic to sulfites?

○ Yes
○ No

Please list any other allergies you have.

_____

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
☐ Two meals per day
☐ Three meals per day
☐ Snack(s) every day
☐ Special diet or other nutrition plan
☐ Trying to follow Canada's Food Guide to Healthy Eating

Please specify when you eat your meals/snacks, and what any special diets/nutrition plans you are on involve.

_____

What types of physical activity have you performed in the past month?

_____

How often do you do each type of physical activity listed above (times/week)?

_____

What is the average duration you perform each type of physical activity listed above (minutes)?

_____

What intensity do you perform physical activity at?

☐ light
☐ moderate
☐ strenuous
(select all that apply)

Where do you perform each type of physical activity listed above? (e.g. outside, gym, etc)

_____

REDCap®

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

○ often
○ sometimes
○ never/rarely

Females only:

Are you post-menopausal?

○ Yes
○ No

When was the first day of your last period?

_____

Are you on hormone replacement therapy?

○ Yes
○ No

Are you currently using oral contraceptives?

○ Yes
○ No

What type/brand of oral contraceptives?

_____

Are you currently using other hormonal contraceptives (i.e. hormonal IUD)?

○ Yes
○ No

What type of hormonal contraceptives?

_____

Are you pregnant?

○ Yes
○ No

How many weeks are you (weeks+day)?

_____

Have you ever been pregnant previously?

○ Yes
○ No

How many times have you been pregnant?

_____

When was your last pregnancy?

_____

Were there any complications, including pregnancy related hypertension, gestational diabetes, or pre-eclampsia?

_____

Appendix G: BioRender Publication Usage Rights

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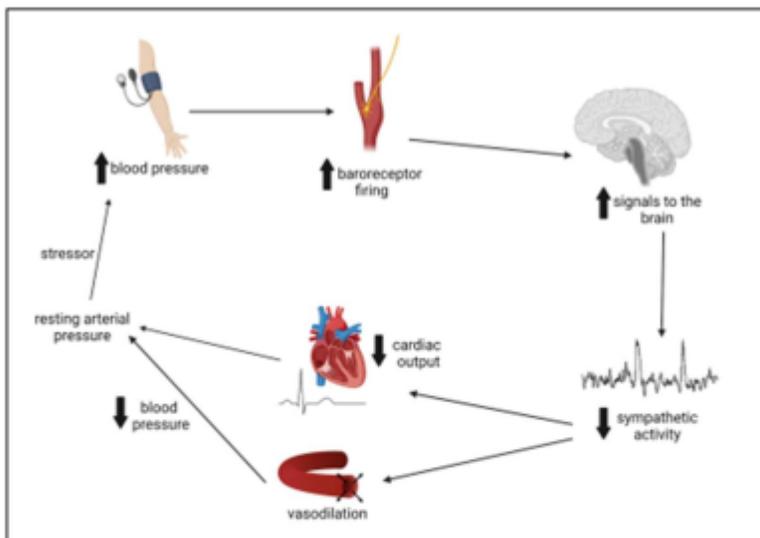
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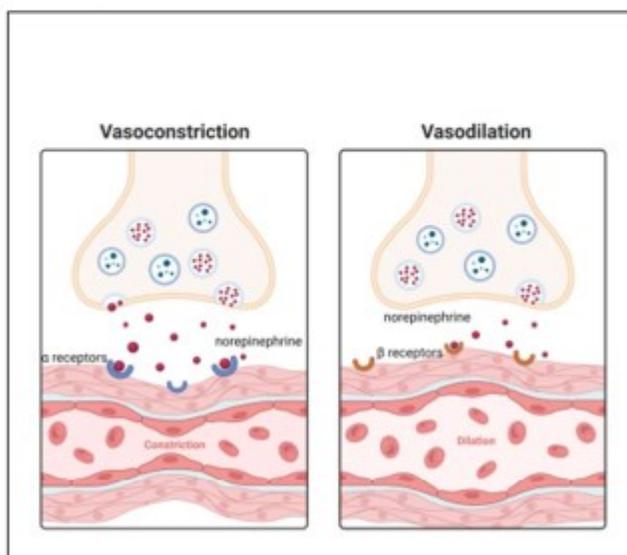
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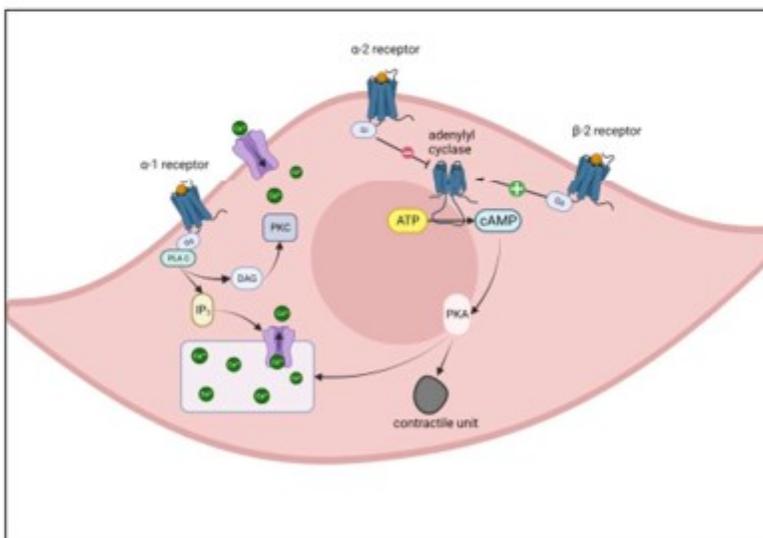
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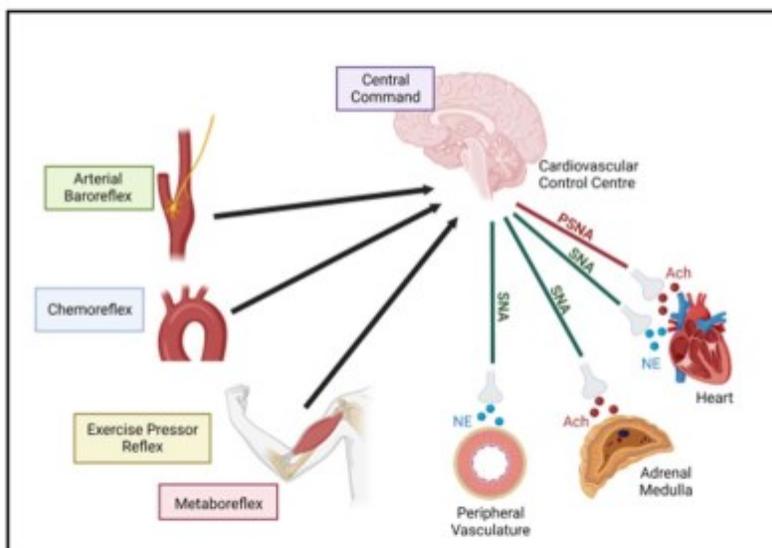
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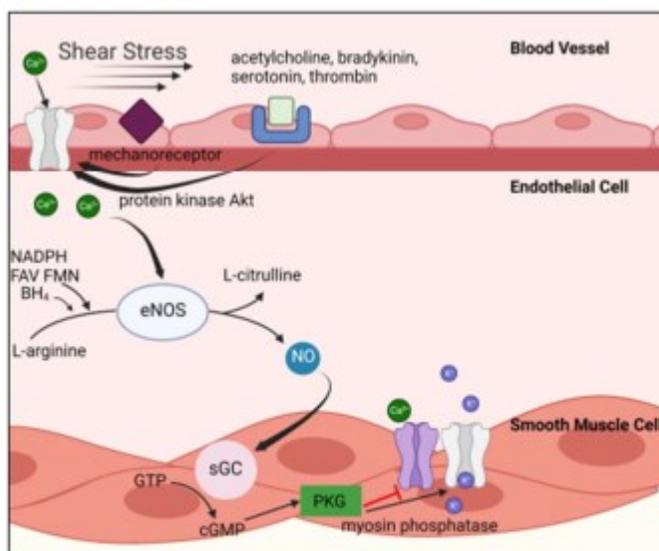
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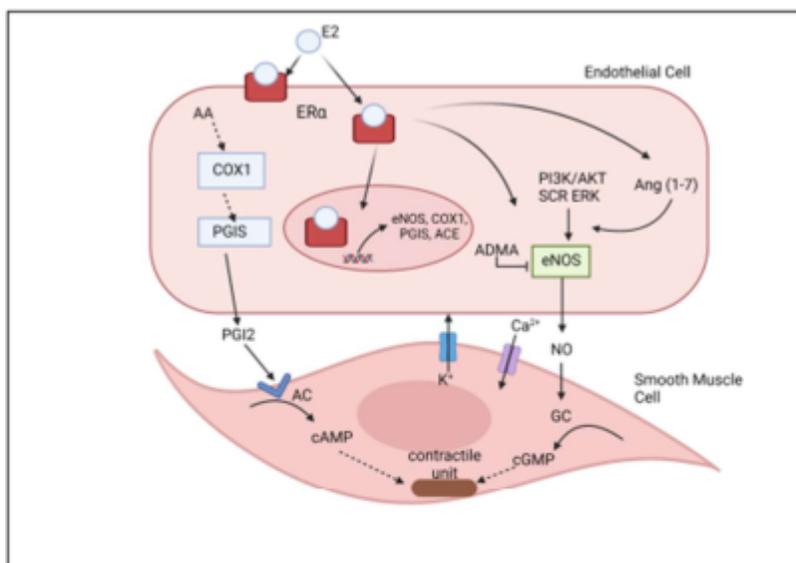
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