Influence of high-altitude on the heart rate and rhythm response to apnea

by

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ABSTRACT

BACKGROUND: Concurrent excitation of the sympathetic and parasympathetic systems occurs at high-altitude (chronic hypoxia), via the carotid chemoreflex. We have demonstrated this autonomic conflict manifests as cardiac arrhythmias during voluntary apnea. We sought to determine the duration of hypoxic exposure at high altitude necessary to unmask cardiac arrhythmias during voluntary apnea. We hypothesized that cardiac arrhythmias during apnea would be increased after 24 hours of high-altitude exposure compared to apnea at low altitude.

METHODS: Measurements of steady state chemoreflex drive (SS-CD), continuous electrocardiogram (ECG; lead II) and SpO_2 (pulse oximetry) were collected in 22 participants (8 females) at low altitude (1045m) and over 8 consecutive days at high-altitude (3800m). Resting SS-CD was quantified as ventilation (L/min) over stimulus index ($P_{ET}CO_2/SpO_2$). Following resting baseline participants performed an end-expiratory apnea to volitional breakpoint. ECG rate and rhythm were evaluated at baseline and during apnea. The nadir heart rate was used to quantify bradycardia and abnormal ECG rhythm was classified based on origin (e.g., sinus node) and rhythm (e.g., arrest). Differences in ventilation, SS-CD, SpO_2, and the heart rate response to apnea on each day at high altitude (Day 1-8) were compared to that at low altitude (Day 0) using a repeated-measures ANOVA design, with a Holm-Sidak post-hoc analysis where main effect of time was significant.

RESULTS: Baseline SpO_2 was lower for all days at high-altitude compared to low altitude ($p<0.01$). On Day 4 and beyond, SS-CD was elevated versus low altitude (Day 4, $p=0.02$; Day 5, $p=0.004$; Day 6, $p=0.003$; Day 7, $p=0.005$; Day 8, $p=0.02$), indicating ventilatory

acclimatization. At high-altitude (all days), baseline resting heart rate was higher compared to low altitude ($p<0.01$). A main effect of time ($p<0.001$) was identified for the change in heart rate during apnea. At low altitude, the average apnea induced decrease in heart rate was -9±15bpm, whereas the average decrease in heart rate at high altitude (all days) was -24±16bpm. Bradycardia became more pronounced with acclimatization, with the greatest drop in heart rate (-31±16bpm) occurring on Day 5. At low altitude 14% (3/22) of participants developed arrhythmias during apnea. At high-altitude, cardiac arrhythmias (e.g., sinus pause and arrest, conduction block) during apnea became most prevalent (>50%) following Day 5. Changes in saturation during apnea and apnea duration were not associated with the magnitude of bradycardia during apnea (saturation, $r= -0.007$ $p=0.92$; apnea duration, $r=0.005$ $p=0.94$). Interestingly, the magnitude of bradycardia was correlated with the incidence (percentage) of arrhythmia per day ($r=0.8$; $p=0.004$), suggesting a similar underlying mechanism.

CONCLUSION AND SIGNIFICANCE: Our data demonstrate that chronic hypoxia is associated with progressive increase in vagal tone throughout acclimatization to altitude, as indicated by augmented bradycardia during apnea and progressively incidence of arrhythmia. Additionally, the increased incidence of arrhythmia following Day 5 at high-altitude suggests that chemoreflex sensitization may play a role in this phenomenon. This study provides insight into cardiac electrophysiology under conditions of heightened stress and has implications as a future functional model for exploring the effects of heightened chemoreflex stress on autonomic control of heart.

PREFACE

This thesis is an original work by Lindsey F Berthelsen. The research conducted for this project received ethics approval from the University of Alberta – Research Ethics Board under the project name: The effects of acclimatization to hypoxia on wakeful and sleeping ECG and heart rhythm (Pro00089322). General ethics for the expedition were approved by the University of Calgary Conjoint Human Research Ethics Board (Protocol REB18-0374) and the Mount Royal University Human Research Ethics Board (Protocol 101879).

The research conducted for this thesis is from a standalone study imbedded within an expedition to Barcroft Station, White Mountain, California USA (3800m) in 2019, as part of an international research expedition led by Professor Richard Wilson and Professor Trevor Day at the University of Calgary and Mount Royal University, respectively.

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data for the work (SVD, ARS, ERVB, JB, SFT, AS, BB, BP, RJAW, NGJ, TAD, CDS), or iii.) drafting the work or revising it critically for important intellectual content (SVD, ARS, ERVB, JB, SFT, AS, BB, BP, RJAW, NGJ, TAD, and CDS). All persons listed have read and approved of the final version of the manuscript.

I would like to thank Dr. Craig Steinback for his mentorship, guidance, and contribution to my research experience, above and beyond this thesis. Thank you to Dr. Sean van Diepen for his mentorship and contribution to this project. Thank you to Andrew Steele and Emily Vanden Berg for their contribution to data acquisition throughout the expedition. Thank you to Dr. Richard Wilson, Dr. Nicholas Jendzjowsky, and Dr. Trevor day for their organization and planning of the expedition. Special thank you to all members of the expedition who helped with and participated in this study.

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LIST OF ABBREVIATIONS

AC Adenylyl Cyclase

ACh Acetylcholine

AUC Area Under the Curve

ANS Autonomic Nervous System

AV Atrioventricular Node

B_2 Adrenergic Receptor B_2 Subtype

Ca^{2+} Calcium ion(s)

CO_2 Carbon Dioxide

CSA Central Sleep Apnea

ECG Electrocardiogram

FiO_2 Fraction of Inspired Oxygen

Gi Inhibitory G Protein Subunit

Gs Stimulatory G Protein Subunit

H^+ Hydrogen ion(s)

HR Heart Rate

HVR Hypoxic Ventilatory Response

K^+ Potassium ion(s)

M_2 Muscarinic Receptor M_2 Subtype

NTS Nucleus Tractus Solitarius

Na^+ Sodium ion(s)

NE Norepinephrine

O_2 Oxygen

OSA Obstructive Sleep Apnea

PAC Premature Atrial Contraction

$PaCO_2$ Partial Pressure of Arterial Carbon Dioxide

PaO_2 Partial Pressure of Arterial Oxygen

$P_{ET}CO_2$ End Tidal Carbon Dioxide

PiO_2 Partial Pressure of Inspired Oxygen

PJC Premature Junctional Contraction

PKA Protein Kinase A

PVC Premature Ventricular Contraction

RPG Respiratory Pattern Generator

ROC Receiver Operator Characteristic Curve

RVLM Rostral Ventrolateral Medulla

SA Sinoatrial

SI Stimulus Index

SNA Sympathetic Nerve Activity

SpO_2 Oxygen Saturation

SS-CD Steady State Chemoreflex Drive

VAH Ventilatory Acclimatization to High Altitude

CHAPTER 1 INTRODUCTION

1.1 Background

The autonomic nervous system (ANS) operates outside of conscious control and primarily functions to maintain homeostasis. The ANS is composed of two branches: the sympathetic ("fight or flight") and the parasympathetic ("rest and digest") systems. These systems are crucial in regulation of cardiovascular responses to perturbations. While these systems are typically characterized as opposites, under conditions of heightened autonomic stress there is a simultaneous and synergistic co-activation of both sympathetic and parasympathetic nervous systems (Paton et al., 2005).

Lack of oxygen (i.e., hypoxia) is a significant physiological stress to the body. Exposure to hypoxia is experienced with ascent to altitude as a result of decreasing atmospheric pressure. In response to hypoxia, reflexive increases in ventilation and autonomic nervous system activity occur via activation of the peripheral chemoreceptors in effort to maintain adequate delivery of oxygen to the tissues (Sander, 2016a; Swenson & Bärtsch, 2014). There is evidence to suggest that the peripheral chemoreceptors become sensitized throughout hypoxic exposure (Robbins, 2007; Sander, 2016a; Smith et al., 1986), indicating that they play an important role in the ventilatory and autonomic adjustments that occur throughout acclimatization.

Hypoxia may also be experienced during apnea (breath holding). Apnea activates the peripheral chemoreceptors via arterial hypoxemia, resulting in a reflexive reduction in heart rate and concurrent peripheral vasoconstriction through augmentation of both parasympathetic and sympathetic nervous activity, respectively (Bain et al., 2018). This reflex is known as the diving response, which aims to prioritize blood flow to central organs and conserve oxygen (Foster &

Sheel, 2005). Apnea is a primary feature of certain clinical conditions such as central and obstructive sleep apnea (CSA and OSA, respectively). Chronic peripheral chemoreceptor activation in these conditions has been identified as a contributor to development and progression of disease pathologies (Iturriaga et al., 2017; Narkiewicz et al., 1998), and thus characterizing the cardiovascular effects of chemoreflex activity or sensitivity may be clinically relevant.

We have previously demonstrated that following several days at high altitude (>4000m), voluntary apnea unmasks heightened vagal tone, manifesting as augmented reflex bradycardia and the development of cardiac arrhythmias (Busch et al., 2018; Busch, van Diepen, et al., 2020). We further demonstrated that chemoreflex activation and sensitization is critical for these responses (Busch et al., 2018). Interestingly, apnea during normoxia does not promote these cardiac events (Busch et al., 2018, 2021), nor are they evident during apnea following acute (i.e., <5 hours) hypoxic exposure (Busch et al., 2021). These data suggest that prolonged chemoreflex sensitization (e.g. days) and concurrent elevation in parasympathetic tone at high altitude, as opposed to acute activation (minutes to hours), is critical for the genesis of bradyarrythmias during hypoxic apnea.

1.2 Research Aims and Hypothesis

The aim of this study was to determine whether duration of hypoxic exposure influences the magnitude of bradycardia and the incidence of arrhythmia during apnea at high altitude. We hypothesized that following 24 hours at high-altitude, apnea would result in an augmented bradycardic response and an increased incidence of arrhythmia, as a physiological manifestation of chemoreflex influence on vagal drive. This hypothesis was predicated on results from our

previous studies using apnea at high altitude, as well as previous evidence that i) chemoreflex sensitization occurs progressively over duration of hypoxic exposure (Barnard et al., 1987; Dempsey et al., 2014; Smith et al., 1986; Vizek et al., 1987) and ii) that in the absence of ventilation, chemoreflex stimulation with hypoxia unmasks heightened parasympathetic cardiac tone (Daly & Scott, 1962; Daly et al., 1988; Kato et al., 1988).

1.3 Significance

Identifying whether duration of hypoxic exposure influences the heart rate and rhythm response to apnea has implications for understanding cardiac control under conditions of hypoxia, and may have future implications for individuals living, travelling, or working in hypoxic environments. Additionally, it may provide a better understanding of cardiac regulation in patient populations who are pathologically exposed to chronic hypoxia and exhibit heightened chemoreflex sensitivity. This study also provides a functional model for future research to assess how the autonomic nervous system modulates heart rate and rhythm in different populations. Further, a future determination of the mechanism(s) underlying this phenomenon may contribute to a better understanding of autonomic balance at high-altitude, or in clinical cases of chronic chemoreflex activity.

CHAPTER 2 LITERATURE REVIEW

This section will provide an overview of cardiac electrophysiology, mechanisms of arrhythmia, as well as the ventilatory and cardiovascular responses to perturbations such as hypoxia (acute and chronic) and apnea (i.e., breath holding). Specific focus will be given to how the peripheral chemoreflex regulates the ventilatory and autonomic response to these stimuli. Previous evidence suggests that high altitude (hypobaric hypoxia) may be a "proarrhythmogenic" environment, prompting research into the factors that predispose or contribute to the development of cardiac events at high-altitude. These factors are discussed below, providing context and a brief overview on the current understanding of this phenomenon.

2.1 Basics of Cardiac Electrophysiology

2.1.1 Cardiac action potentials and the cardiac cycle

Cardiac tissue can be differentiated by working cells (atrial and ventricular cardiomyocytes) and specialized pacemaker cells (Becker, 2006; Quinn & Kohl, 2012). Cardiac pacemaker cells exhibit a unique property termed automaticity, meaning they can initiate spontaneous rhythmic electrical excitation (Becker, 2006; Quinn & Kohl, 2012). The pacemaker cells of the heart include the sinoatrial (SA) and the atrioventricular (AV) nodes. The SA node is the primary pacemaker of the heart, and action potentials originating at the sinus node are responsible for the regulation of heart rate and rhythm (i.e., sinus rate). The AV node provides a connection between the atria and the ventricles for coordinated conduction of depolarization through cardiac tissue. AV node intrinsic rate is lower than SA node intrinsic rate, and there is a slight delay as impulses pass through the AV node, which allows for greater ventricular filling prior to ejection (Becker, 2006).

In muscle tissue, action potentials initiate contractile processes through cyclic depolarization/repolarization and release of stored intracellular calcium ions (Ca^{2+}) (Becker, 2006). Within the heart, electrical properties of cardiac myocytes and pacemaker cells differ as a result of differences in ion channel characteristics (Klabunde, 2017). The majority of cardiac muscle tissue exhibits a fast action potential response when cells are depolarized to threshold of -70mV (Klabunde, 2021). The fast action potential response is characterized by an increase in fast sodium ion (Na^{+}) channel conductance (Klabunde, 2021). Cardiac fast action potentials primarily differ from skeletal action potentials through a prolonged plateau phase, mediated by slow inward Ca^{2+} currents, and by an effective refractory period where depolarizing stimuli do not produce a new action potential, preventing tetanus (Klabunde, 2021). This property is cardioprotective, as it prevents constant contraction at higher heart rates and allows for necessary ventricular filling. Cardiac pacemaker cells do not have a true resting membrane potential, but rather generate impulses spontaneously and are largely dependent on Ca^{2+} (rather than fast Na^{+}) conductance. Spontaneous depolarization, or the "pacemaker potential", refers to phase of depolarization that triggers an action potential once a threshold of ~ -40 to -30 mV is reached (Klabunde, 2021b). These differences have implications for function in different regions of the heart as well as for external influences (e.g., autonomic nervous system activity) on cardiac tissue. The cardiac cycle and conduction of depolarization through the heart is summarized below in ***Figure 1***. Hypoxia has been shown to decrease action potential duration and Ca^{2+} release, contributing to a reduction in tension development during cardiac contraction (Allen & Orchard, 1987). This independent influence of hypoxia on ion channels, ion (e.g., Ca^{2+}) concentrations (Allen & Orchard, 1987), cardiac action potentials and contractility may further affect the cardiac response to external drives during hypoxic exposure.

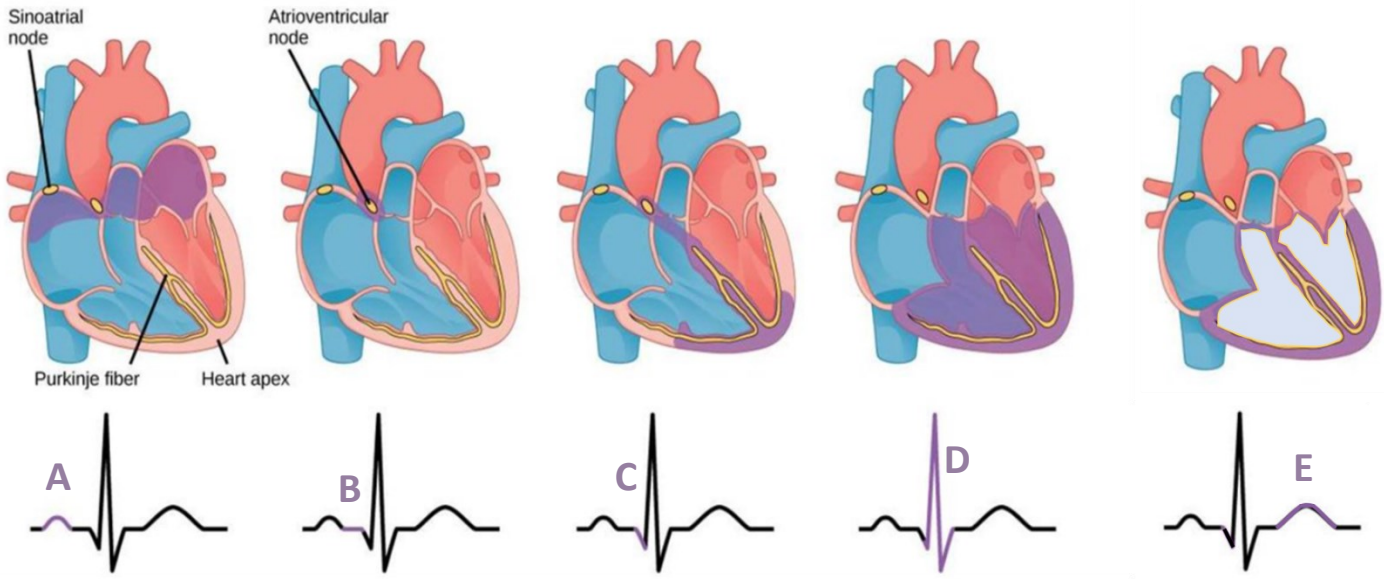

FIGURE 1. Impulse conduction through the heart for one cardiac cycle. An impulse is initiated at the sinoatrial (SA) node and spreads throughout the atria, causing atrial depolarization and contraction (A). The impulse arrives at the atrioventricular (AV) node, which transmits electrical activity from atria to the ventricles (B). The impulse spreads from the AV node through the bundle of His and bundle branches, into extensive Purkinjie fibre network towards the apex of the heart (C). The Purkinjie fibres line the inner ventricular walls, and depolarization spreads through this system and causes ventricular contraction (D). Following contraction, the ventricles relax and enter the phase of ventricular repolarization (E). Image modified from *https://ppt-online.org/279892 (n.d)*

2.1.2 ECG morphology

The ECG carries temporal information about the magnitude and direction of electrical activity in the heart (Shea & Cascino, 2019). Briefly, electrodes are placed on the skin, and the electrodes detect differences in electrical potential between measurement points (Klabunde, 2017). Positioning of electrodes on the skin affects the direction and magnitude (i.e., vectors) shown on an ECG trace; for this review, focus will be given to lead II (***Figure 2***). In lead II, the reference electrode (negative) is placed on the right arm, and the exploring electrode (positive) is placed on the left leg (or left lower abdomen). In this configuration, electrical activity through the heart is directed towards the positive (exploring) electrode, resulting in positive deflections on the ECG trace, since the wave of depolarization is moving towards the positive electrode (Klabunde, 2017). This orientation closely aligns the vector of the ECG with vectors of cardiac depolarization through the SA and AV nodes and left atrium and ventricle, resulting in a clear depiction of ECG waves (P, QRS, T) for optimal identification of cardiac conduction and heart rhythm.

The ECG also provides important information on myocardial contraction; as depolarization spreads through cardiac tissue and contraction occurs, a waveform appears on the ECG. The typical waveform and rhythm of a cardiac cycle measured by ECG in lead II is depicted below in ***Figure 2***. Different intervals and segments between waves also provide an indication of electrical activity in the heart. Notably, the P-R interval, S-T segment, and QT interval give an indication of time intervals between different phases of electrical activity and conduction through cardiac tissue.

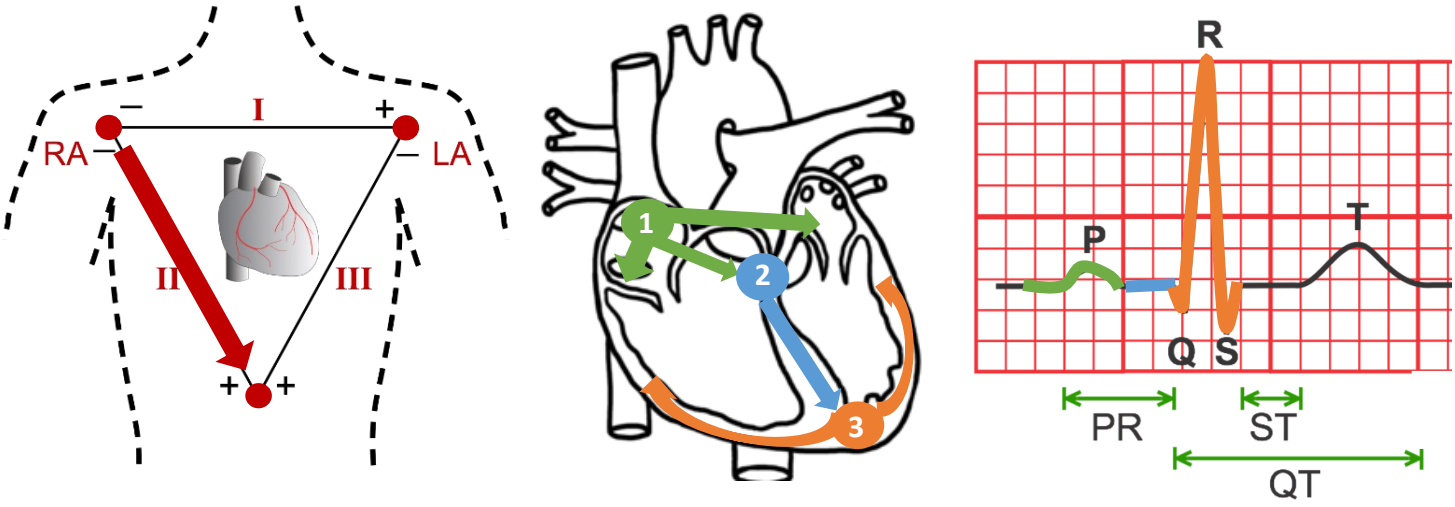

FIGURE 2. **Positioning of ECG electrodes in lead II, impulse conduction and typical ECG trace obtained in lead II.** In lead II, the negative (reference) electrode is placed on the right arm, and the positive (exploring) electrode is placed on the left limb. Since the wave of depolarization travels towards the positive electrode, this configuration results in positive deflections on the ECG trace (*pictured on the right*). In the ECG trace, the P wave represents depolarization of the atria (green); the QRS complex represents ventricular depolarization and contraction (orange); the T wave represents ventricular repolarization. The pause between P-R wave (P-R interval) reflects conduction through the AV node (blue). The S-T segment is the time interval between the end of ventricular contraction (S) and initiation of ventricular repolarization (T wave), the time at which both ventricles are completely depolarized (Klabunde, 2019). The QT interval is the duration from the beginning of ventricular contraction (Q) to the end of ventricular relaxation (T), and therefore is roughly representative of the duration of an average ventricular action potential (Klabunde, 2019). The QT interval varies greatly with changes in heart rate, and is shortened for a higher heart rate and longer for a lower heart rate (Klabunde, 2019). Image on left from Klabunde 2017, image on right from Klabunde 2019.

2.1.3 ECG abnormalities: common arrhythmias

Cardiac arrhythmias may result from altered cardiac cell depolarization or electrical conduction (Perry & Illsley, 1986). Arrhythmias can be classified based on origin and rhythm. This review will focus on common types of ectopy and arrhythmias originating at the SA and AV nodes.

Ectopy refers to an impulse being initiated somewhere in the heart other than the SA node. The origin or location where the impulse is generated is the ectopic foci. If SA node pacemaking fails, other abnormal pacemaker sites within atria, AV junction, or ventricles will usually exhibit automaticity (Klabunde, 2021) and assume pacemaking ability at their own intrinsic rate (Dubin, 2000). Ectopic pacemaking follows a sequential order based on the intrinsic rates of different sites with pacemaking potential, whereby the foci that assumes pacemaking ability suppresses all lower (slower) automaticity foci (overdrive suppression) (Dubin, 2000). If SA node pacemaking fails, atrial ectopic foci within the atrial conduction system will assume pacemaking ability; then, junctional foci (at AV junction) will assume pacemaking in absence of stimuli from atria; ventricular foci (within Purkinjie fibre network) will assume pacemaking in the absence of pacemaking stimuli from above, with ventricular escape as the final step if no impulse is initiated within the Purkinjie fibres (Dubin, 2000). Premature ectopic beats arise when an ectopic foci spontaneously fires an action potential (Dubin, 2000). Ectopic beats are common in most people and are generally benign. Some common examples of premature ectopic beats are demonstrated below in ***Figure 3***.

A pause in SA node pacing may result in ectopic beats and or ectopic rhythm, where the above mentioned ectopic foci (atria, junctional, and ventricular) may assume pacemaking and cause escape beat(s) or escape rhythm in the absence of sinus pacing (Dubin, 2000). In the case

of sinus arrest, where SA node essentially stops all pacemaking activity, these redundancies in other areas of the heart are protective and can maintain cardiac contraction (Dubin, 2000). With sinus pause, there is a transient (e.g. 1 cycle) pause in SA pacemaking, which may or may not cause an escape beat before resumption of normal sinus rhythm (Dubin, 2000). An example of sinus block is shown below in ***Figure 4***.

Atrioventricular (conduction) block is a slowing or elimination of conduction between the atria and ventricles. There are 3 main types of conduction block. First degree conduction block is seen on the ECG as a gradually prolonged P-R interval, indicating a slower (delayed) AV node conduction. Second degree AV block can be further divided into type types: Mobitz I (Wenckebach) and Mobitz II. In Mobitz I, there is a gradual lengthening of P-R interval followed by a dropped beat (missing QRS), indicating a failure of impulse conduction between atria (p wave) and ventricles (no QRS indicates no ventricular depolarization) (Dubin, 2000). In Mobitz II, the P-R interval remains constant with intermittent dropped beat(s), appearing on the ECG as an 'outnumbering' of P waves to QRS complexes, often seen in a 2:1 or 3:1 ratio (Dubin, 2000). Complete AV block (third degree block) is the most severe and is characterized by total failure of conduction between the atria and ventricles. Third degree AV block appears on the ECG as electrical dissociation between P waves and QRS complexes, resulting from atrial depolarization failing to pass to the ventricles, and so ventricular rhythm is maintained by an ectopic focus below the AV junction (Dubin, 2000). Examples of types of AV block are shown below in ***Figure 5***.

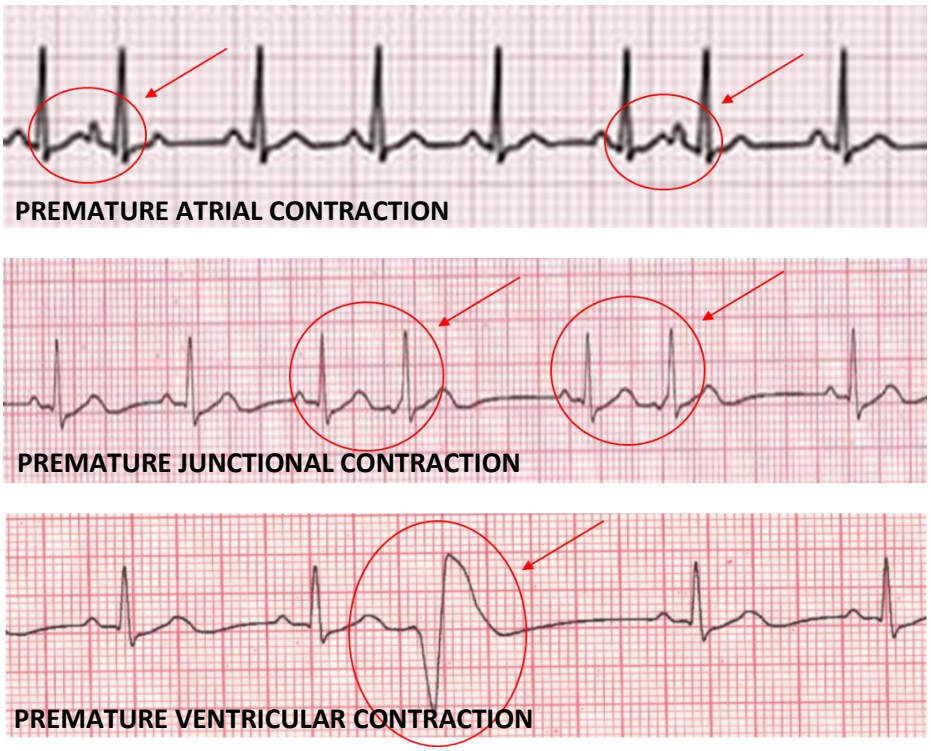

FIGURE 3. Examples of ectopy. Premature atrial contraction (top panel): atrial contraction is premature, exhibits different morphology to previous P wave, and premature beat is followed by a compensatory pause. Premature junctional contraction (middle panel): beat is premature (based on proceeding R-R interval) and absence of P wave since the impulse is initiated below the atria. Premature ventricular contraction (bottom panel): QRS widened and change in morphology with compensatory pause before initiation of next sinus beat. Arrhythmias within the ECG trace are indicated by red circles and red arrows. All images retrieved from https://thephysiologist.org/study-materials/ectopic-beats-palpitations/

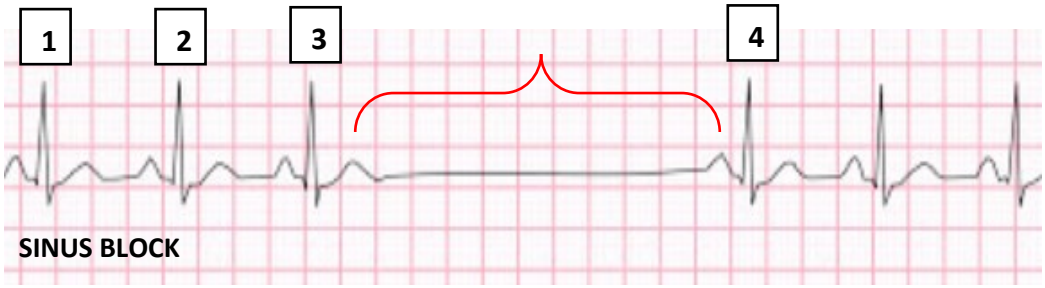

FIGURE 4. Sinus block. Sinus block occurring between third and fourth R wave pictured on ECG trace, exhibiting a characteristic pause following T wave before initiation of next heartbeat. Notably, the p wave shape is the same before and after the sinus pause, indicating they are both of sinus origin (and so resumption of rhythm in this case is not a result of escape beat or ectopic pacemaker). Image from *https://ecg-educator.blogspot.com/2016_11_13_archive.html*

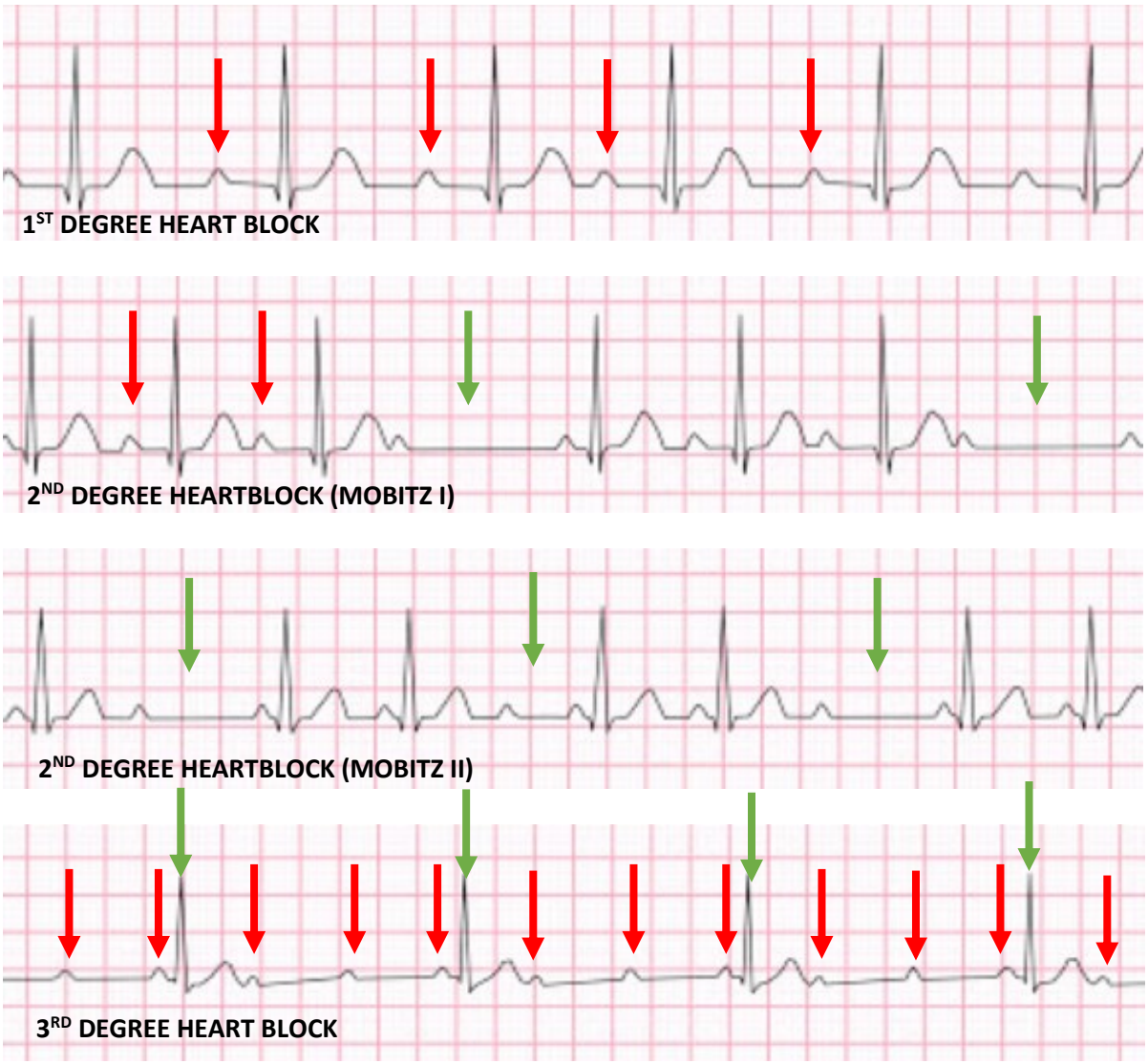

FIGURE 5. Atrioventricular (AV) Block. First degree AV block (top panel), where gradual increase in P-R interval is demonstrated by red arrows. Mobitz I (second panel), with gradual lengthening of P-R interval (red arrows) and dropped beat (missing QRS; green arrow). Mobitz II (third panel), or 2:1 AV block, shows impulse conduction failure between atria to ventricles (missing QRS) by green arrows. Third degree AV block (bottom panel), where atria and ventricles are beating in an uncoordinated manner (atrial contraction, red arrows; ventricular

contraction, green arrows). All images from *https://ecg-educator.blogspot.com/2016_11_14_archive.html*

2.2 Autonomic innervation of the Heart

In addition to intrinsic pacemaking properties of the heart, the autonomic nervous system continuously modulates cardiac conduction (dromotropic effects), heart rate and rhythm (chronotropic effects), and contractility (inotropic effects). This influence is largely concentrated at the SA and AV nodes where the two branches of the ANS exert opposing effects, with parasympathetic promoting a decrease in heart rate, and sympathetic promoting an increase in heart rate. These changes in heart rate are accomplished through direct influence of ANS activity on the discharge frequency of pacemaker cells (Martins & Zipes, 1980; Shen & Zipes, 2014). Additionally, postganglionic sympathetic nerves innervate the ventricular myocardium, concomitantly increasing contractility in situations of high sympathetic activity.

Increases in parasympathetic activity increase the release of acetylcholine (ACh) from the vagus nerve, which binds to muscarinic cholinergic receptors (primarily M_2) at the SA and AV node, decreasing discharge frequency of these pacemaker cells and slowing heart rate (Black et al., 2019). Acetylcholine binding at M_2 receptors initiates an inhibitory effect on adenylyl cyclase via inhibitory subunits of the G protein (Gi), resulting in a decrease in cAMP production (***Figure 7***) (Harvey, 2012). Decreased production of cAMP leads to hyperpolarization of ionic Na^{2+} and K^{+} pacemaker channels, reducing the rate of spontaneous depolarization and slowing heart rate (Harvey, 2012). The net effect of an increase in cardiovagal activity are slowed sinus rate and decreased AV node conduction. An increase in sympathetic activity results in release of norepinephrine (NE) from postganglionic sympathetic terminals. Norepinephrine binds to B-receptors at pacemaker cells, increasing discharge frequency, and at the ventricular myocardium, resulting in a greater force of contraction (Black et al., 2019). B-receptor activation in cardiac tissue results in activation of adenylyl cyclase via excitatory subunits of the G protein (Gs),

leading to an increase in cAMP production and subsequent shift in voltage of pacemaker channels in a depolarizing direction (***Figure 7***) (Harvey, 2012). In pacemaking cells, ion channel regulation is predominantly mediated through cAMP activation of protein kinase A (PKA) dependent phosphorylation, which includes phosphorylation of L-type Ca^{2+} channels and voltage dependent Na^{+} channels, affecting cardiac muscle contraction, propagation of electrical impulses, and cardiac action potential duration (Harvey & Belevych, 2003). At rest, vagal tone typically predominates; with an increase in physiological stress (for example, during exercise), vagal activity is withdrawn, and sympathetic activity is increased (Olshansky et al., 2008). Autonomic innervation of the heart is shown below in ***Figure 6.*** It should also be mentioned that the intrinsic cardiac nervous system, where complex centers within cardiac ganglia process and integrate information from parasympathetic efferent neurons, afferent neurons, intra and inter ganglionic neurons, and sympathetic postganglionic neurons (Gray et al., 2004) may affect heart rate and rhythm. However, this review will focus specifically on cardiac modulation by parasympathetic and sympathetic activity.

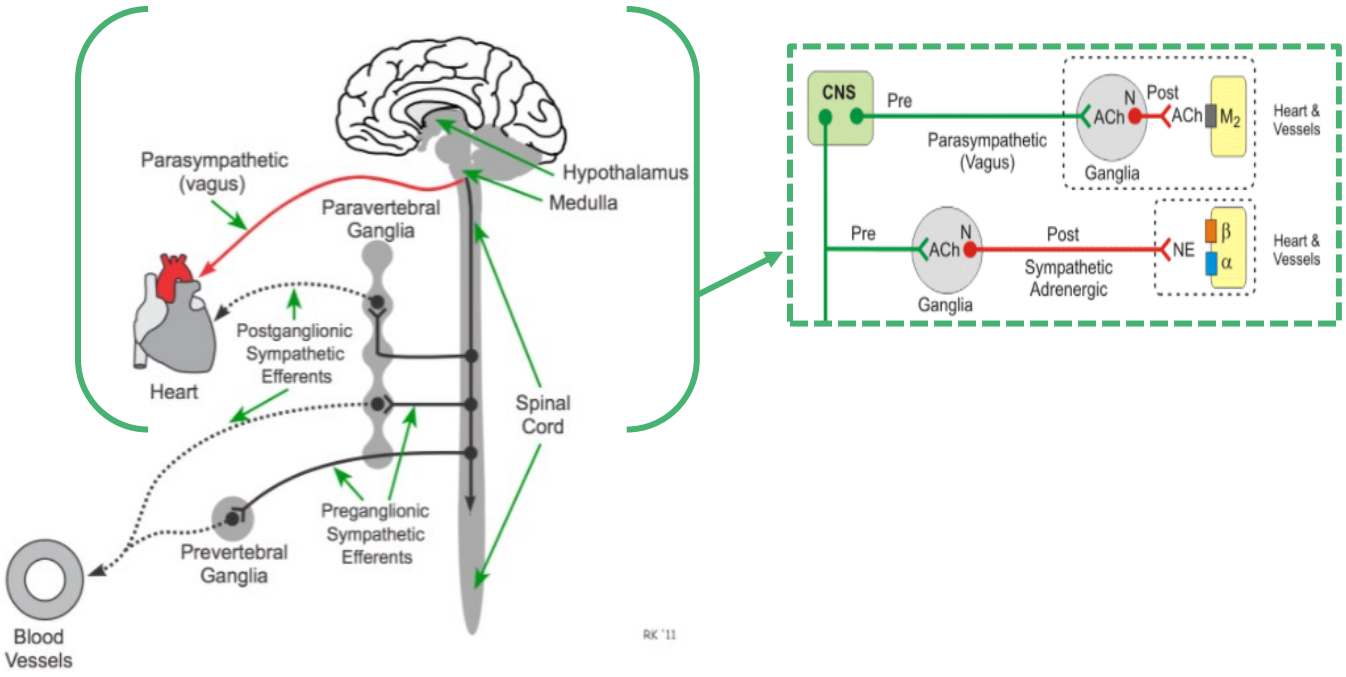

FIGURE 6. Autonomic innervation of the heart. Parasympathetic efferent activity arises from vagal nuclei (nucleus ambiguus) within the brainstem (medulla) (Petko & Tadi, 2019). Efferent activity is carried from the brainstem via cranial nerve 10 (vague nerve). The vagus nerve releases ACh which binds to muscarinic cholinergic receptors at both the SA and AV node, decreasing discharge frequency and thus decreasing heart rate (negative chronotropic effect). Sympathetic efferent activity to the heart originates from the medulla and travels down the spinal cord. Preganglionic sympathetic fibres originate in the thoracic region (T1- T4) of the spinal cord and synapse in paravertebral (sympathetic chain) ganglia, releasing ACh which binds to nicotinic cholinergic receptors on post ganglionic synapse. Post ganglionic adrenergic sympathetic fibres travel from paravertebral ganglia to the heart, where they release NE. NE binds to B-adrenergic receptors at SA node, AV node and ventricular myocardium. Increases in sympathetic activity has positive chronotropic (increase heart rate) and positive inotropic (increase contractility)

effects. Image adapted from *Cardiovascular Pharmacology Concepts – Autonomic Ganglia; https://www.cvpharmacology.com/autonomic_ganglia; Richard E Klabunde, 2010.*

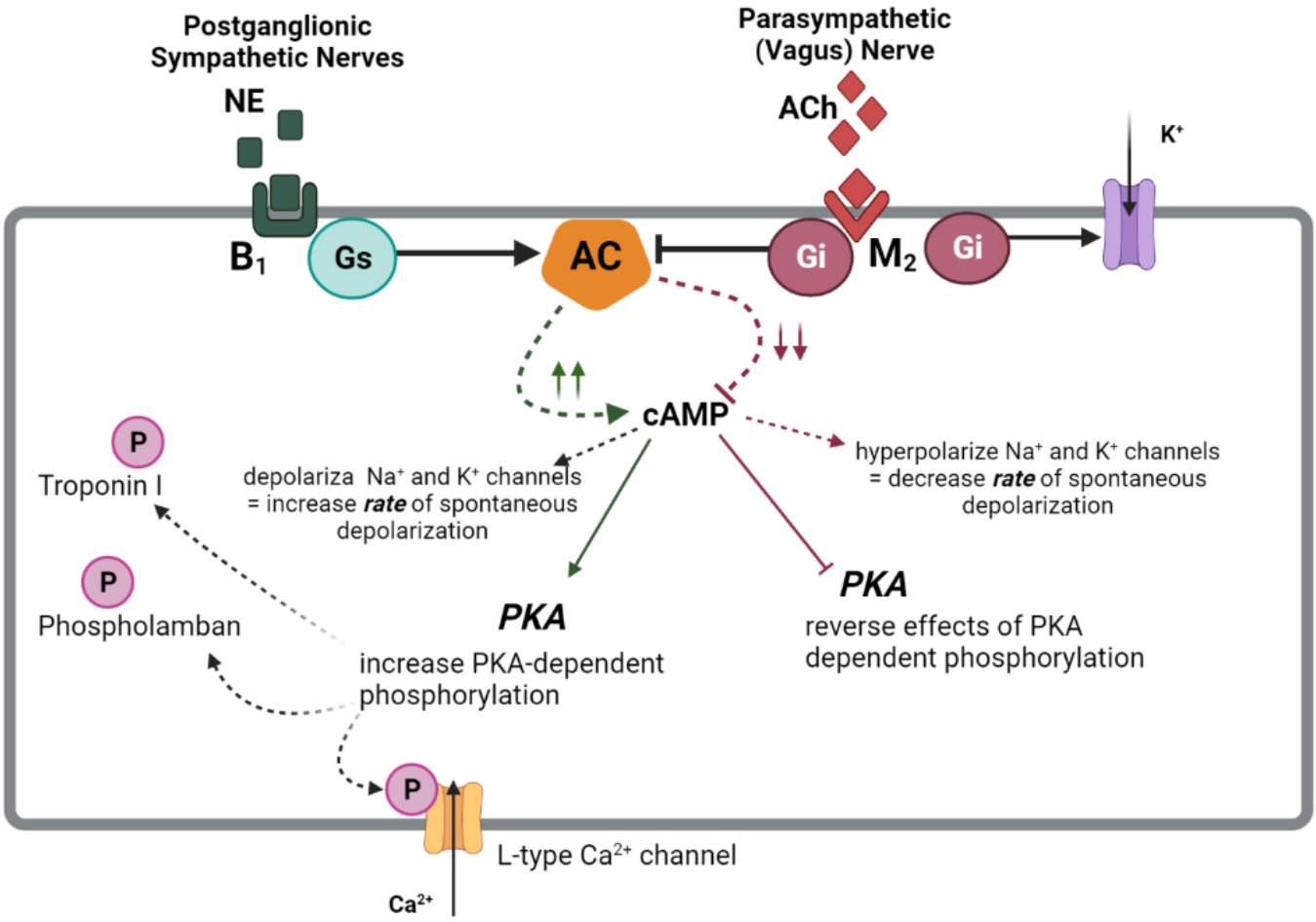

FIGURE 7. Acetylcholine and norepinephrine mechanism of action at cardiac tissue.

Acetylcholine (Ach) is released from the vagus nerve and binds at muscarinic (M_2) receptor. Acetylcholine binding activates the inhibitory subunit of the G protein (Gi), activating potassium (K+) channels and inhibiting adenylyl cyclase (AC). Inhibition of AC decreases cAMP production, resulting in hyperpolarization of pacemaker Na^+ and K^+ channels, decreasing the rate of sponatenous depolarization. Inhibition of cAMP also decreases the activity of protein kinase A (PKA), reducing the effects of PKA-dependent phosphorylation (P) on troponin I, phospholamban, and L-type Ca^{2+} channels. Norepinephrine is released from postganglionic sympathetic nerve terminals and binds at B receptors (primarily B_1), activating the stimulatory

subunit of G protein (Gs). Gs increases the activity of adenylyl cyclase and cAMP. Increased cAMP activity increases the rate of spontaneous depolarization at pacemaker Na^+ and K^+ channels. cAMP also increases PKA, leading to greater PKA-dependent phosphorylation of troponin I, phospholamban, and L-type Ca^{2+} channels.

2.2.1. Reflex control of autonomic activity and heart rate

Afferent activity in one branch of the ANS typically results in reflexive efferent responses in both sympathetic and parasympathetic divisions (Swenson & Bärtsch, 2014). Trigeminal nerve stimulation, chemoreceptor and baroreflex activation, and or pulmonary afferent feedback are among some of the reflex changes that modify ANS outflow, resulting in differential effects on heart rate and contractility (Chapleau & Sabharwal, 2011). For example, a rise in arterial blood pressure activates mechanical sensitive baroreceptors, evoking a reflexive increase in cardiovagal activity and decreasing heart rate (Chapleau & Sabharwal, 2011). Contrarily, a fall in blood pressure would cause increases in sympathetic activity and inhibit cardiac vagal activity (via the arterial baroreflex), resulting in an increase in heart rate and contractility (Chapleau & Sabharwal, 2011).

Heart rate also varies in phase with the respiratory cycle (i.e., respiratory sinus rhythm), where heart rate increases during inspiration and decreases during expiration, which is functional to optimize ventilation perfusion in the lungs and maintain alveolar to arterial O_2 gradient (Neff et al., 2003). Mechanistically, the interaction between cardiac and respiratory centers are not fully understood, and both systems are characterized by their own rhythms which are generated by different neural centers in medulla (Perry et al., 2019). So, respiratory patterns cannot fully explain heart rate in all scenarios.

Further, there is an apparent coupling between the respiratory cycle, lung stretch and autonomic activity (Macefield & Gunnar Wallin, 1995; Macefield & Wallin, 1995; Seals et al., 1990). Inflation of the lungs (activation of pulmonary stretch receptors) causes reflex inhibition of sympathetic nerve activity (SNA), reduction in vascular resistance, and increases in heart rate (Chapleau & Sabharwal, 2011; Macefield & Gunnar Wallin, 1995). Notably, this does not appear

to be baroreflex mediated, likely because of the temporal delay of SNA to peak heart rate and blood pressure responses (Macefield & Gunnar Wallin, 1995). Ventilation (lung stretch) has been shown to suppress both sympathetic and parasympathetic activity (Kato et al., 1988; Macefield & Gunnar Wallin, 1995; Seals et al., 1990; Steinback et al., 2010).

The chemoreceptors detect changes in circulating chemical stimuli (e.g., O_2, CO_2, H^+) and modify autonomic outflow and ventilation accordingly. Peripheral arterial chemoreceptors respond primary to changes in oxygen, initiating marked increases in ventilation and autonomic activity in situations where oxygen is reduced (i.e., hypoxia). The direct cardiac effect of peripheral chemoreflex stimulation with hypoxia is bradycardia (de Burgh Daly, 1997; Kato et al., 1988). However, this is masked by concurrent hyperventilation, resulting in phasic tachycardia mainly as a response to increased lung stretch afferent activity (Bain et al., 2018; Kato et al., 1988). The effects of chemoreflex activation will be reviewed in depth in Sections 2.4 and 2.5.

2.3 Introduction to Apnea

2.3.1 The Diving Response

The diving response is a physiological reflex that is engaged to defend against hypoxia during periods of apnea (Foster & Sheel, 2005). This response is characterized by vagally mediated bradycardia and peripheral vasoconstriction, and thus relies on integration of both sympathetic and parasympathetic activity (Bain et al., 2018; Foster & Sheel, 2005). Functionally, this response serves to conserve oxygen and direct blood flow centrally (Foster & Sheel, 2005). The bradycardic response to apnea is also critical in prolonging breath hold duration by dramatically reducing myocardial O_2 consumption (Bain et al., 2018; Hoiland et al., 2017), which is important because the heart typically has high metabolic rate (Paton et al., 2005). A greater degree of bradycardia has been associated with a slower rate of oxygen desaturation during apnea (Hoiland et al., 2017) supporting the physiological role of bradycardia in conserving oxygen in response to apnea.

Initial bradycardia during apnea is proposed to occur from removal of pulmonary stretch afferent input and is strengthened through trigeminal nerve stimulation (Bain et al., 2018; Costalat et al., 2020; Foster & Sheel, 2005). The extent of bradycardia during apnea is dependent on several mechanisms, including presence or absence of facial cooling (trigeminal nerve stimulation), level of hypoxia reached (chemoreflex), and mean arterial pressure (baroreflex) (Bain et al., 2018). However, notable differences exist in the engagement of these mechanisms between individuals and particularly between those who are trained (e.g., breath hold divers) versus untrained, as the cardiovascular response to apnea seems to be largely dependent on apnea duration and degree of desaturation (Heusser et al., 2009).

2.3.2. Apnea and incidence of cardiac arrhythmias

Previous studies have identified cardiac arrhythmias towards the end of apnea in trained breath hold divers (Costalat et al., 2020; Hansel et al., 2009). In these studies, cardiac responses to apnea were dependent on duration (Costalat et al., 2020; Hansel et al., 2009) and arterial oxygen saturation reached (Costalat et al., 2020), indicating that this may be dependent on individual ability to perform maximal apnea. However, cardiac arrhythmias have been recorded during relatively short breath holds during cold water immersion (Shattock & Tipton, 2012). This phenomenon has been attributed to a dual activation of both sympathetic (cold shock response) and parasympathetic (diving reflex) drives, where these opposite signals may result in "autonomic conflict" at the heart and consequent cardiac arrhythmias (Shattock & Tipton, 2012). Arrhythmias have also been linked to instances of apnea experienced pathologically (e.g., in obstructive sleep apnea) and at high altitude (Busch et al., 2018; Busch et al., 2020).

2.3.3 Apnea in Pathologies

Clinically, cardiac rhythm disturbances during apnea are relevant to conditions such as obstructive and central sleep apnea syndromes (OSA and CSA, respectively), where apnea is both pathologic and a primary characteristic feature. These patients are chronically exposed to periods of intermittent hypoxia during sleep, which has been linked to pathologic elevations in both autonomic activity and chemoreflex drive (Mansukhani et al., 2014; Prabhakar, 2016). There have been several reported accounts of OSA-related cardiac arrhythmias during sleep (Geovanini & Lorenzi-Filho, 2018; Javaheri et al., 2017; Leung, 2009; May et al., 2017; Zwillich et al., 1982). The mechanism(s) underlying these cardiac events are not totally understood, but may be attributable to overnight surges/and or imbalances in autonomic activity, intrathoracic pressure swings, and or arterial hypoxemia (May et al., 2017).

2.4 Introduction to Hypobaric Hypoxia

The body requires oxygen for basic physiological function. Thus, exposure to hypoxia either pathologically (disease states) or environmentally (high altitude) represents a significant homeostatic challenge. Ascent to high altitude results in a decrease in barometric pressure as a function of increasing altitude. This drop in barometric pressure drives a reduction in the partial pressure of inspired oxygen (PiO_2; *equation 1*), leading to systemic hypoxia.

Equation 1. $PiO_2 = FiO_2$ x (barometric pressure – saturated vapour pressure of H_2O)

Where PiO_2 is partial pressure of inspired oxygen and FiO_2 is fraction of inspired oxygen

A reduction in atmospheric pressure and PiO_2 result in consequent drop in the partial pressures of oxygen at all major stages of oxygen delivery (i.e., oxygen "cascade"; ***Figure 8***) (Beall, 2007). Lower partial pressure of oxygen reduces diffusion of oxygen as a result of smaller pressure differences, reducing saturation of hemoglobin and arterial oxygen content (Beall, 2007; Peacock, 1998). This downward shift in the oxygen cascade effects oxygen delivery, requiring reflexive adjustments to compensate. The drop in arterial oxygen (PaO_2) is rapidly sensed via the peripheral chemoreceptors, which initiate a corrective increase in ventilation and compensatory autonomic and cardiovascular changes to mitigate the decrease in oxygen availability. Carotid body chemoreflex activation is dependent in part on the duration and severity of hypoxic exposure (Allwood et al., 2018; Powell, 2007), but the accepted 'threshold' for initiation of these responses is defined as PaO_2 <60mmHg (Marshall, 2015).

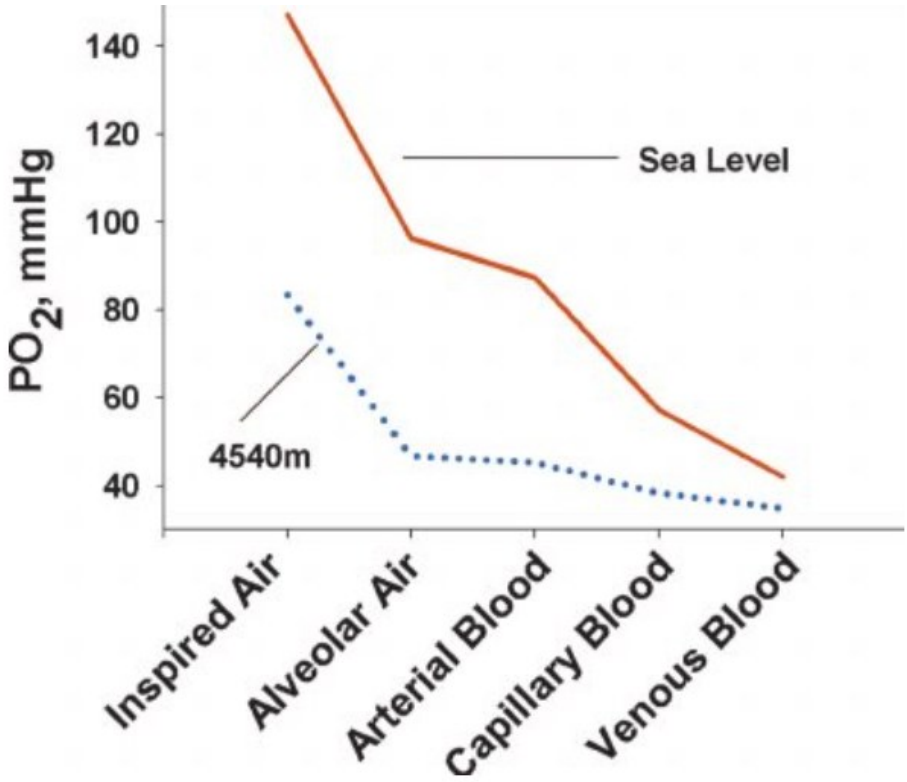

FIGURE 8. Partial pressure of oxygen at high altitude for each step of oxygen cascade. Partial pressure of inspired air is reduced at high altitude, which consequently reduces partial pressure of oxygen at each subsequent step of the oxygen cascade. Figure from Beall 2007.

2.4.1 Integration of peripheral chemoreflex afferent information and efferent outflow

The carotid body chemoreceptors regulate arterial chemical composition primarily through reflex changes in ventilation (Nurse, 2005). The carotid bodies are highly vascularized organs located bilaterally in the carotid bifurcation (López-Barneo et al., 2008). They are composed of Type I glomus cells and Type II substantencular cells, although it is broadly accepted that Type I glomus cells are the primary receptors for circulating chemostimuli (López-Barneo et al., 2008; Nurse, 2005). Briefly, when low PaO_2 is sensed, voltage gated potassium (K^+) channels on the glomus membrane are inhibited, resulting in membrane depolarization and opening of Ca^{2+} channels, leading to increased Ca^{2+} influx. Calcium ion influx promotes release of dopamine from the presynaptic cleft, which binds to receptors on the carotid sinus nerve at the post synaptic cleft (López-Barneo et al., 2008). Dopamine binding increases carotid sinus nerve discharge, and this signal is propagated along the glossopharyngeal nerve to the nucleus tractus solitarius (NTS) within the brainstem where it is integrated (Dampney et al., 2002; Guyenet, 2000). Direct glutaminergic (excitatory) transmission from the NTS to the respiratory pattern generator (RPG) drives an increase in ventilation (Guyenet, 2014). This increase in ventilation and consequent increase in discharge of lung stretch afferents inhibits cardiovagal outflow, in addition to the direct inhibitory influence of RPG activation on cardiovagal preganglionic neurons (Guyenet, 2014), ultimately producing an increase in heart rate. Afferent information integrated at the NTS also targets the rostral ventrolateral medulla (RVLM), raising sympathetic outflow (Guyenet, 2014). Further discussion of autonomic changes in response to hypoxia will be reviewed in Section 2.4.

2.4.2 Necessity of Peripheral Chemoreceptors in Response to Hypoxia

Hypoxic exposure results in an acute robust increase in ventilation (hypoxic ventilatory response; HVR), and time dependent increases in ventilation and PaO_2 throughout prolonged exposure (e.g., days to weeks) (Robbins, 2007). There is extensive evidence to support that the carotid body chemoreceptors are obligatory for the initial hypoxic ventilatory response as well as ventilatory acclimatization to high altitude (VAH). Early evidence of this comes from studies assessing carotid body responses to systemic hypoxia (Barnard et al., 1987; Nielsen et al., 1988; Vizek et al., 1987), direct carotid body stimulation by sodium cyanide and or hypoxia (Barros et al., 2002; de Burgh Daly, 1997), and carotid body denervation (Bisgard et al., 1986; Dempsey et al., 2014; Forster, 2003; Timmers et al., 2003). Importantly, all these studies corroborate a necessary role of intact carotid body chemoreceptors for the acute ventilatory response to hypoxia and VAH. Additionally, they provide evidence of specificity of hypoxic stimulus for these responses; for example, carotid body response to hypercapnia does not result in the same ventilatory response or in change in sensitivity (VAH) over time (Nielsen et al., 1988; Smith et al., 1986). Although it is apparent that intact carotid body and peripheral chemoreflex are necessary for the ventilatory response and VAH (***Figure 7***), it is less well characterized how the integration of peripheral chemoreflex, central chemoreflex, and other respiratory brain centers interact over time at high altitude; this remains an area of interest for future research.

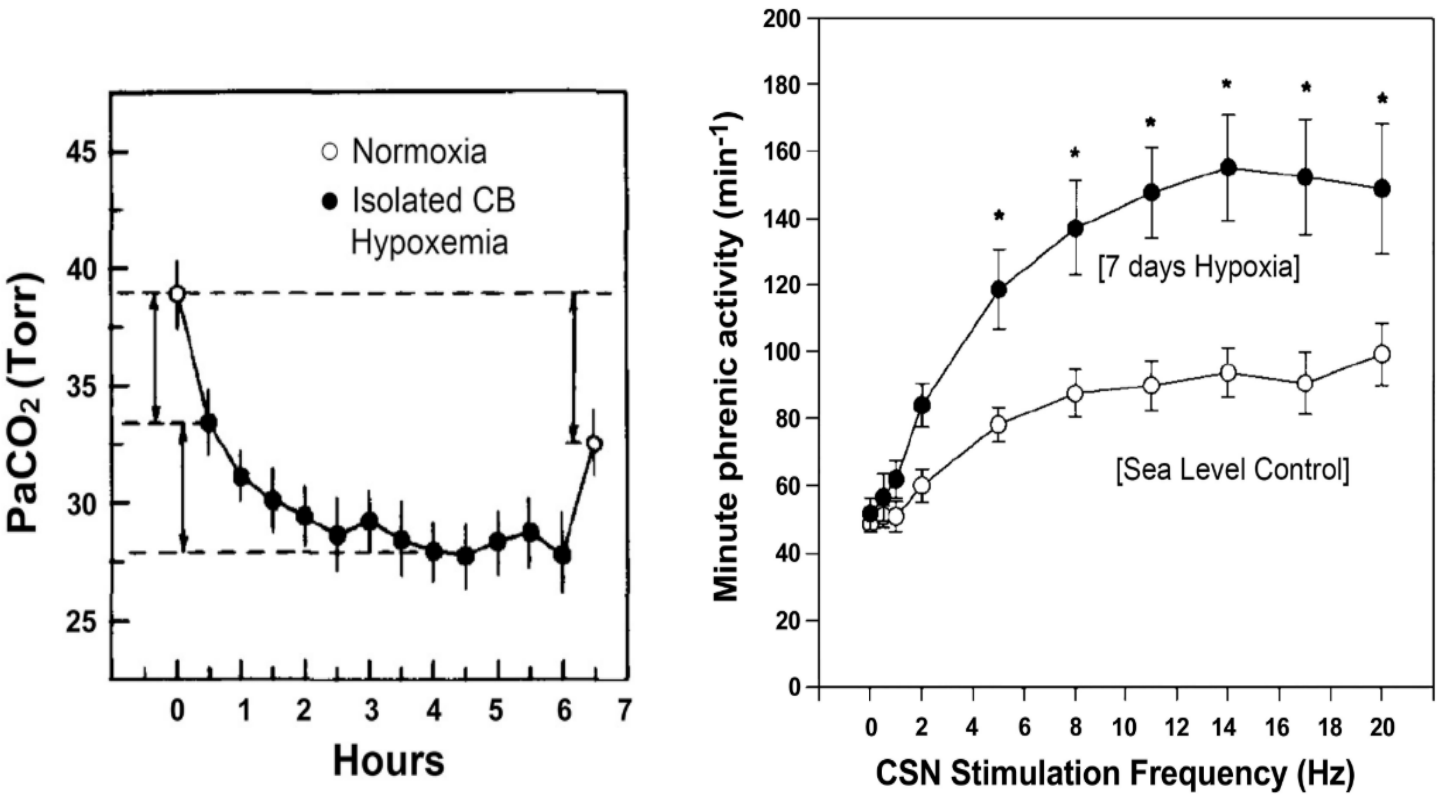

FIGURE 9. Peripheral chemoreflex response to hypoxia and increase in sensitivity over time. Left**:** Increases in ventilation (evidenced by drop in arterial CO_2; $PaCO_2$) upon hypoxic exposure. In this experimental preparation, the carotid body was isolated to demonstrate that hypoxic ventilatory response is specific to the peripheral chemoreflex and not dependent on arterial hypoxemia. Note, this response is not seen with hypercapnic stimulus (Dempsey et al., 2014). Right: Sensitization of peripheral chemoreflex overtime. Several days at high altitude resulted in an elevated ventilatory response (i.e., higher phrenic nerve activity) for any given carotid sinus nerve (CSN) stimulation compared to the sea level control. Figures from Bisgard & Forster 1996 (left) and Dwinell & Powell 1999 (right).

2.5 Cardiovascular Response to Hypoxia

2.5.1 Acute Hypoxia

Hypoxia affects autonomic outflow and ventilation via the peripheral chemoreflex, and also exerts local effects at the level of the vasculature (Bärtsch & Gibbs, 2007a; Marshall, 1999). The immediate increase in ventilation, or the HVR, (as described above), is complete within minutes of hypoxic exposure, whereas VAH is time dependent and occurs over several days to weeks (Robbins, 2007). Immediate effects of peripheral chemoreflex activation also include a reflexive increase in sympathetic outflow.

A decrease in arterial oxygen content and hemoglobin saturation necessitates a compensatory increase in cardiac output (Siebenmann & Lundby, 2015), which is accomplished through increased heart rate (Siebenmann et al., 2015) and increased myocardial contractility (Bärtsch & Gibbs, 2007a), although stroke volume is reduced (Siebenmann et al., 2013; Stembridge et al., 2015). Reflex increases in heart rate at high altitude appear to be a result of hyperventilation and increased pulmonary afferent feedback; however, in the absence of repetitive lung stretch, the direct effect of peripheral chemoreflex stimulation with hypoxia is bradycardia (Kato et al., 1988). Hypoxia may also act directly on the heart and stimulate bradycardia through increased local release of adenosine (Marshall, 1999).

2.5.2 Chronic hypoxia

Throughout acclimatization to high altitude there are continued ventilatory, autonomic, and hemodynamic adjustments which facilitate increased arterial oxygen content and delivery. Ventilatory acclimatization, defined as a time dependent increase in ventilation and PaO_2 and concomitant decrease in $PaCO_2$, occurs during days to weeks of exposure and is one of the most important features of acclimatization to high altitude (Ainslie et al., 2013). The time course of

VAH response is also altitude and species dependent (Ainslie et al., 2013; Robbins, 2007). Notably, VAH does not restore PaO_2 to sea level values, but rather is a marker of integrative processes of acclimatization which begin to mitigate the environmental hypoxic challenge (Ainslie et al., 2013).

Centrally, cardiac output is normalized to sea level values with acclimatization, while stroke volume is decreased and heart rate remains elevated (Bärtsch & Gibbs, 2007a; Naeije, 2010). Although heart rate remains elevated, it begins to decline towards sea level values throughout prolonged exposure (Richalet et al., 1992), indicating either a decrease in cardiac adrenergic sensitivity, increase in muscarinic sensitivity with acclimatization, or both. Changes at the level of cardiac receptors are evident following exogenous adrenergic stimulation (e.g., isoproterenol), where increases in heart rate are attenuated (Kacimi et al., 1993; Richalet et al., 1992), and during maximal exercise, where maximal heart rate is still reduced following prolonged hypoxic exposure (Boushel et al., 2001). Further, it has been demonstrated that there is an increase in muscarinic receptor affinity, density, and sensitivity during chronic hypoxia (Kacimi et al., 1993; Richalet et al., 1992). These changes affect cardiac control at high altitude in addition to affecting the response to changing autonomic drives (i.e., parasympathetic versus sympathetic dominance).

Despite established evidence that the peripheral chemoreflex is responsible for the initial elevation in SNA upon exposure to hypoxia, heightened sympathetic activity over time at high altitude appears to be mediated through an alternative mechanism. Both Fisher et al (2018) and Hansen & Sander (2003) demonstrated only a mild decrease in muscle sympathetic nerve activity when peripheral chemoreflex input was inhibited with low dose dopamine and hyperoxia (respectively) following weeks of hypoxic exposure. Recently, Simpson et al (2020b)

demonstrated that elevated pulmonary pressure and increased afferent input from pulmonary artery baroreceptors may contribute to sustained SNA over time at high altitude, although whether this is the primary mediator requires further investigation. Sustained SNA over time at high altitude may be necessary to preserve arterial pressure in face of hypoxic vasodilation (Simpson et al., 2020). Indeed, arterial pressure is relatively maintained with prolonged hypoxic exposure (Berthelsen et al., 2020), indicating a balance between local vasodilatory mechanisms and sympathetically mediated constriction. While mechanisms have yet to be fully established, heightened peripheral sympathetic activity throughout high altitude exposure has been relatively well characterized, although less work has focused on specific cardiac effects of sustained sympatho-excitation. Even fewer studies have investigated concomitant parasympathetic cardiac effects of chronic hypoxia, limiting our understanding of autonomic adaptation to hypoxia.

2.6 High Altitude: a "proarrhythmic" environment?

There is evidence that a high altitude environment may increase risk of developing cardiac arrhythmias (Alexander, 1995; Karliner et al., 1985; Malconian et al., 1990; Windsor et al., 2011; Windsor et al., 2010; Woods et al., 2008, 2011) and sudden cardiac death (Burtscher & Ponchia, 2010; Lo et al., 2013). Although a large focus on these events has been conducted in older age participants or in those with pre-existing conditions (e.g., coronary artery disease), cardiac arrhythmias at high altitude have also been shown to occur in younger, healthy individuals during exercise (Boos et al., 2017), during sleep (Karliner et al., 1985; Malconian et al., 1990), and more recently, during voluntary apnea (Busch et al., 2018; Busch et al., 2020).

Busch et al. (2018) provided the first evidence of arrhythmia during voluntary apnea at high altitude. In this study, apnea at high altitude elicited a heightened bradycardic response and

cardiac arrhythmias which were not observed at low altitude (Busch et al., 2018). Interestingly, individual HVR was predictive of whether participants would develop irregular rhythm (e.g., ectopic beats, heart block) during apnea and arrhythmias were absolved following supplemental (100%) oxygen administration, implicating a specific role of the peripheral chemoreflex in this phenomenon. The findings of Busch et al (2018) were supported in a follow up study in acclimatizing lowlanders at 4300m (Busch et al., 2020), providing evidence that this is a true physiological phenomenon unmasked at high altitude. In both 2018 and 2020 investigations, Busch et al demonstrated these cardiac events in lowlanders following 4-10 days at high (>4000m) altitude, but not in indigenous high altitude residents (Nepalese Sherpa and Peruvian Andeans), suggesting this response is specific to acclimatization rather than adaptation. Additionally, most recently it has been demonstrated that this cardiac response to apnea does not occur following acute (<5 hours) hypoxic exposure (Busch et al., 2021). Taken together, this series of studies demonstrates necessity of prolonged hypoxic exposure to unmask this heart rate and rhythm response to apnea. However, the exact time course required (e.g., duration of hypoxic exposure necessary) is still unclear.

2.6.1 Clinical Implications of Apnea at High Altitude

Although use of apnea at high altitude has served as a functional model for assessing autonomic control of the heart, it may hold direct relevance to instances of apnea experienced during sleep at high altitude. Above ~2500m, the majority of individuals sojourning to high-altitude experience central sleep apnea (Ainslie et al., 2013). CSA at high altitude is characterized by recurrent periods of apnea with no ventilatory effort, triggered by transient arterial hypocapnia (low $PaCO_2$) during sleep (Burgess & Ainslie, 2016). Previous assessments of continuous ECG overnight at high altitude have shown 1) that periods of apnea are associated

with bradycardia (Masuyama et al., 1990) and 2) that arrhythmias do occur during sleep (Karliner et al., 1985; Malconian et al., 1990). It has yet to be confirmed whether the changes in heart rhythm during sleep are associated with (or occur during) periods of apnea, but if apneic periods of CSA are associated with cardiac arrhythmias it would add direct clinical relevance to the current investigations of the mechanism(s) regulating the response to apnea at high altitude.

2.7 Summary

Heart rate is generally governed by the SA node, although abnormal electrical activity within the heart can disturb sinus rhythm (Perry & Illsley, 1986). Tonic autonomic activity as well integrative reflex responses modify ANS outflow and effect heart rate and contractility via sympathetic and parasympathetic drives. The peripheral chemoreflex is key in responding to hypoxia and initiates an increase in ventilation and autonomic activity to compensate for reduced PaO_2. The peripheral chemoreflex also initiates the reflexive responses to apnea, driving a reduction in heart rate and elevation in peripheral sympathetic activity. Apnea has been linked to cardiac rate (bradycardia) and rhythm (arrhythmia) disturbances in elite divers (Costalat et al., 2020; Hansel et al., 2009), in sleep apnea disorders (Geovanini & Lorenzi-Filho, 2018; May et al., 2017; Mehra et al., 2009), and more recently, at high altitude (Busch et al., 2018; Busch et al., 2020). It is apparent that these arrhythmias during apnea at high altitude are only present following several days to weeks of exposure; however, the exact time course to the onset of these cardiac events is unclear.

CHAPTER 3 STUDY DESIGN AND DATA COLLECTION

3.1 Research Design

This study was conducted as a part of a research expedition to Barcroft Station, White Mountain, California, USA, in August of 2019 (ascent profile ***Figure 8***). The expedition included numerous independent investigations with a primary goal of assessing integrative physiological responses to prolonged hypoxic exposure. Researchers took care to ensure there were no overlap between distinct studies, and each study addressed an independent a priori question. All participants provided informed consent prior to inclusion in any research protocols. Local Ethical approval was obtained for the expedition by the University of Alberta Biomedical Research Ethics Board (Pro00089322, date of approval 7/12/2019), University of Calgary Conjoint Human Research Ethics Board (Protocol REB18-0374) and the Mount Royal University Human Research Ethics Board (Protocol 101879). Due to the nature of field expeditions, participants are selected from a convenience sample and were all members of the expedition team.

Participants were tested once in Calgary, Alberta, Canada (1045m) prior to the expedition to establish a low altitude baseline (Day 0). The same participants were tested over 8 consecutive days (Day 1-8) at high altitude. A total of 22 participants (8 females) were included in this study. Self-assessed Lake Louise score was recorded for each participant on each day at high-altitude; no participant recorded a score of over 4 (out of 10) on any day at high altitude. No participant experienced any other altitude-related illness (e.g., high altitude pulmonary edema, high altitude cerebral edema). All participants were free of any known respiratory, cardiovascular, metabolic and neurological disorders as determined by a self-reported health history questionnaire. However, two participants reported taking prescription anti-anxiety/anti-depression medication (Alprazolam, Sertraline, Lorazepam), 1 participant recorded prescription medication for migraines

(Sumatriptan), 2 participants reported taking attention deficit hyperactivity disorder medication (Ritalin, Vyvanse, Concerta), and one participant recorded taking Prednisone and Dupixent (for eczema and inflammation). Participants abstained from caffeine, alcohol and strenuous exercise for a minimum of 12 hours prior to testing. All females were premenopausal and testing of females was not standardized to a particular point in the menstrual cycle. However, 7 out of 8 female participants were using an intra-uterine device (IUD brands: Mirena, Kyleena, Jaydess, and Liletta); one female was not taking any form of birth control. A full day to day description of participation is outlined in ***Table 3*****.** Participants in this study were tested in the morning and had not consumed any alcohol, caffeine, or exercise strenuously within the previous 12 hours. Participant demographics are reported in ***Table 1***.

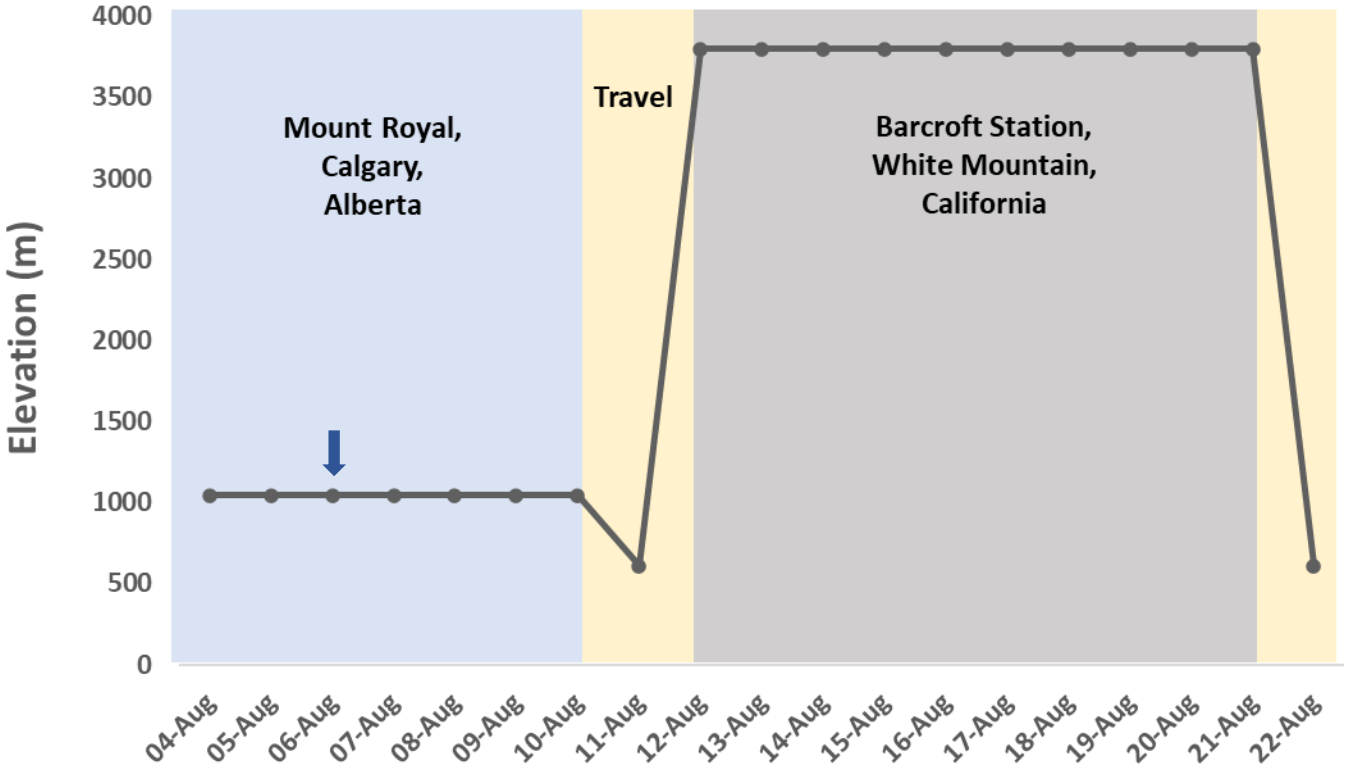

FIGURE 10. Participant testing locations and ascent profile. Participants were tested once (August 6th; indicated by blue arrow) at low altitude (1045m) at Mount Royal University in Calgary, Alberta, Canada. All baseline testing for all expedition studies took place between August 4th to August 10th. On August 11th, participants flew from Calgary to Las Vegas, Nevada, USA (610m). On August 12th, participants travelled via car (~6 hours) from Las Vegas to Barcroft Station, White Mountain, California, USA (3800m). The high altitude portion of this study was conducted between August 13th to August 20th (Day 1 to Day 8). No testing was conducted August 21st. Participants descended from Barcroft Station and travelled back to Las Vegas via car on August 22nd.

3.2 Instrumentation

All participants were tested in the supine position. Heart rate and rhythm (Electrocardiogram leads II and V2) and oxygen saturation (SpO2; pulse oximetry, ADInstruments, ML320 Oximeter Pod, Pod series, Australia). were collected continuously at 1 KHz (ADInstruments, Chart Pro v8.3.1, Australia) each day during baseline and apnea protocol. Lead V2 was incorporated only for use in confirmation of cardiac events during apnea. Heart rate (HR) was calculated from the ECG R-R interval (Lead II). Resting blood pressure was obtained while participant was supine, using an average of three manual sphygmomanometry readings (model BP786n; Omron, San Ramon, CA, USA) taken at least 1 minute apart. Resting ventilation and end-tidal PCO_2 (Torr) were recorded over one minute using a respirometer (nSpire Haoscale, Colorado, USA) portable capnograph (EMMA, Masimo, Danderyd, Sweden), respectively. Participants breathed through a mouthpiece connected to both the respirometer and capnograph (***Figure 11***). Researchers allowed ventilation to stabilize prior to collecting 1 minute of resting ventilation data. The obtained ventilatory data were used during analysis to calculate stimulus index (SI; $P_{ET}CO_2/SpO_2$) and steady state chemoreflex drive (SS-CD; ventilation [L/min] divided by SI).

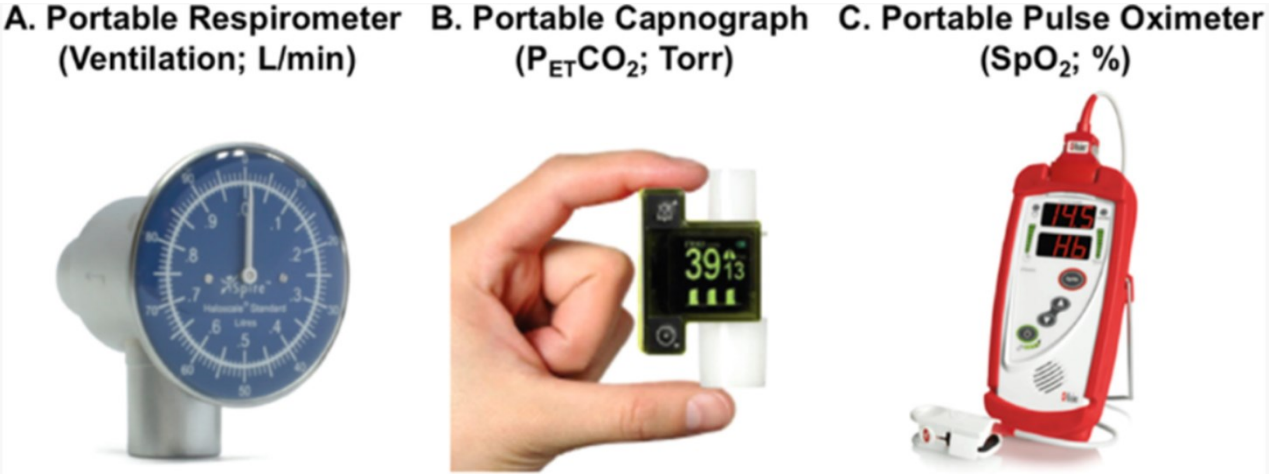

Figure 11. Ventilatory measures. Images of type of portable respirometer (A), portable capnograph (B), and portable pulse oximeter (C) used for the study. Respirometer and capnograph were attached to a mouthpiece. The pulse oximeter was placed on the participants index finger. Figure retrieved from Bruce et al., 2018.

Following instrumentation and resting measurements, participants lay supine for a 2 minute period while breathing room air. Participants then performed one maximal end-expiratory apnea maneuver. End-expiratory apnea was used to minimize the influence of lung stretch receptors and to avoid alterations in venous return and cardiac output (i.e., Valsalva maneuver). Apnea was kept within an individuals' tidal volume (i.e., end of normal expiration). Participants were instructed in advance to hold their breath for "as long as possible" during each protocol until volitional breakpoint. Volitional breakpoint for each individual was confirmed visually when participant ceased apnea. Each apnea was followed by a brief (~1 minute) recovery period to allow cardiovascular (HR, SpO_2) measures to return to baseline (i.e., pre apnea) values. This protocol was performed once at low altitude (1045m; Day 0) and then consecutively for 8 days at high altitude (3800m; Days 1-8). Only a single apnea was performed during each trial (i.e., single apnea per participant per test day).

3.3 Analysis of ECG Morphology and Conduction Abnormalities

ECG cardiac cycles (Lead II) over a two-minute baseline period were over-laid, aligned with the initial R-wave, and analyzed using automated software (ECG analysis module, LabChart Pro 8.3.1) in order to assess electrophysiological characteristics (***Table 4***). To assess the occurrence of arrhythmias, data were extracted for a 30 second period immediately preceding apneas, for the complete apnea duration and for 20 second immediately following the apnea attempt. Data were de-identified and blinded for analysis. Cardiac events were manually screened by a trained researcher (LFB) and interpreted by a cardiologist (SVD) who were blinded to study conditions. Rhythm abnormalities from ECG waveforms were identified for both baseline and apnea conditions. Cardiac arrhythmias were classified based on origin and type (see ***Table 5*** for

categorization). To quantify the HR response to apnea, 10 cardiac cycles preceding the apnea breakpoint were analyzed in order to account for variations in apnea duration. The nadir heart rate from the last 10 cardiac cycles was analyzed in relation to the baseline period to determine the magnitude of bradycardia during apnea. Cardiac events were classified based on origin (e.g., atrial) and type (e.g., ectopy).

3.4 Analysis of Ventilation, Stimulus index and Steady state chemoreflex drive

Ventilation data was recorded for each participant during 1 minute of rest. SpO2 was collected continuously during this time and used for calculations of stimulus index (SI) and steady state chemoreflex drive (SS-CD; described below). SI was calculated as $P_{ET}CO_2$ divided by resting oxygen saturation (%); $P_{ET}CO_2/SpO_2$, as previously described (Leacy et al., 2020; Pfoh et al., 2017). SS-CD was calculated as ventilation (L/min) divided by the stimulus index. Calculations for SI and SS-CD have been previously used to characterize ventilatory acclimatization to high altitude (Bruce et al., 2018; Leacy et al., 2020; Pfoh et al., 2017). To provide an additional assessment of chemoreflex activity, a Dejours test was conducted using data obtained in a subset of participants early (Day 2 and 3; n=12, 6 females) and late (Day 7 and 8; n=7, 4 females) at high altitude. The Dejours test involves a transient hyperoxic stimulus, which provides a more specific assessment of peripheral chemoreflex contribution to ventilation at rest (Prasad et al., 2020). Participants breathed through a mouthpiece connected to a pneumotach (Hans Rudolf, Inc., United States) for 2-3 minutes to collect room air ventilation. They were then switched to breathing a 100% O_2 (hyperoxic) gas. The first 3 minutes of hyperoxia were averaged to determine ventilation during hyperoxia. The change in ventilation from room air to hyperoxia (quantified as ventilation during hyperoxia minus ventilation in room

air) was taken to represent the peripheral chemoreflex contribution to basal ventilation (i.e chemoreflex activity).

3.5 Data and Statistical Analysis

Ventilation, manual blood pressure, oxygen saturation, and heart rate and rhythm data obtained on each day at high altitude (Day 1-8) were compared to low altitude (Day 0) values using a repeated measures ANOVA design, with a Post-Hoc analysis (Holm-Sidak method) where main effect of time was significant. ANCOVA analyses were incorporated to account for desaturation during apnea and duration of apnea. An additional two-way ANOVA (mixed effects model) was performed to determine whether there was an interaction between sex and time (day at altitude) on the bradycardic response to apnea. Receiver Operator Characteristic (ROC) curve analyses were used to determine the specificity and sensitivity of SS-CD and the Dejours test for predicting arrhythmias. All ANOVA analyses were performed using Prism (GraphPad Software, San Diego, California USA); ANCOVA and ROC analyses were performed using SigmaPlot (Systat Software, Chicago, IL). Statistical significance was set *a priori* $p<0.05$. Statistical significance was set a priori $p<0.05$.

CHAPTER 4 RESULTS

4.1 Results

4.1.1 Participant demographics and baseline metrics.

Participant demographics and baseline metrics are reported in ***Table 1***. A total of 22 participants took part in this study. Diastolic blood pressure was elevated and systolic pressure was lower on all days at high altitude compared to low altitude (both main effect of time, $p<0.001$). As a consequence, mean arterial pressure was generally maintained at altitude, with the exception of lower values on days 3 ($p=0.02$) and 6 ($p=0.03$). Resting heart rate was significantly higher for all days at high altitude compared to low altitude (main effect of time, $p=0.002$).

Table 3 (https://doi.org/10.6084/m9.figshare.13099949.v1) contains a full description of day-to-day participation for each participant.

4.1.2 Ventilation, Stimulus index and Steady state chemoreflex drive

$P_{ET}CO_2$, SpO_2, SI, and SS-CD and ventilation are reported in ***Figure 12***. Main effect of time was identified for all variables ($p<0.001$). Oxygen saturation was significantly lower at high altitude compared to low altitude for all days ($p<0.0001$). Ventilation was significantly elevated for Days 1 and 2 versus low altitude ($p=0.04$ and $p=0.009$), returning towards low altitude values over days 3-6, before increasing again on Day 7 ($p=0.04$). $P_{ET}CO_2$ was significantly lower than low altitude for all days at high altitude ($p<0.0001$). SI was also significantly reduced compared to low altitude for all days at high altitude ($p<0.05$). SS-CD was not significantly different than low altitude for Day 1 ($p=0.15$), Day 2 ($p=0.13$), or Day 3 ($p=0.10$). However, on days 4-8, SS-CD was significantly elevated compared to low altitude (Day 4, $p=0.02$; Day 5, $p=0.004$; Day 6, $p=0.003$; Day 7, $p=0.005$; Day 8, $p=0.02$). The change in ventilation in response to the Dejours

test (acute hyperoxia) was similar early and late at high altitude (p=0.195), with hyperoxia decreasing ventilation similarly at both time points (Early: room air 13.1 ± 3.2 L/min, hyperoxia 11.0 ± 2.5 L/min; Late, room air 13.0 ± 3.6 L/min, hyperoxia 12.8 ± 5.1 L/min). Neither SS-CD nor the Dejours test were predictive of whether an individual would develop arrhythmia during apnea (AUC: 0.524 and 0.579, respectively).

Table 1: Participant demographics and baseline cardiovascular measures

	LA Day 0 n= 22 (8 F)	HA Day 1 n= 22 (8 F)	HA Day 2 n= 21 (8 F)	HA Day 3 n= 22 (8 F)	HA Day 4 n= 22 (8 F)	HA Day 5 n= 22 (8 F)	HA Day 6 n= 22 (8 F)	HA Day 7 n= 22 (8 F)	HA Day 8 n= 22 (8 F)
Age (yrs)	30 ± 9								
Height (cm)	174 ± 10								
Weight (kg)	76 ± 15								
BMI (kg/m²)	26 ± 5								
Heart Rate (bpm)	64 ± 9	74 ± 9	71 ± 9	73 ± 10	72 ± 11	72 ± 11	70 ± 9	71 ± 12	71 ± 11
Mean Arterial Pressure (mmHg)	89 ± 10	89 ± 12	87 ± 11	84 ± 10	86 ± 10	85 ± 9	84 ± 8	84 ± 8	86 ± 10
Systolic Arterial Pressure (mmHg)	123 ± 15	104 ± 13	103 ± 12	100 ± 12	101 ± 11	101 ± 9	99 ± 8	100 ± 9	103 ± 13
Diastolic Arterial Pressure (mmHg)	72 ± 9	82 ± 11	79 ± 10	76 ± 10	78 ± 10	77 ± 9	77 ± 8	77 ± 9	77 ± 9
Peripheral Oxygen Saturation (%)	98 ± 2	88 ± 4	89 ± 3	89 ± 4	89 ± 3	90 ± 2	90 ± 3	89 ± 2	91 ± 3

BMI, Body Mass Index; Mean, systolic and diastolic pressure averaged over 3 manual readings; LA, low altitude; HA, high altitude. Data are mean±SD.

Table 2. Event Progression throughout duration at high altitude

	LA Day 0		HA Day 1		HA Day 2		HA Day 3		HA Day 4		HA Day 5		HA Day 6		HA Day 7		HA Day 8	
PARTICIPANT	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**
†1				a				a,b,c		b		b,d,e		a,b,c,d,e,f		a,b,g		
*‡2								h										
*3			b	a,f	b		b,p	a,b,c,e,f	b,p	a,b,e,f		a,b,e,f		a,b,c,e,f		a,b,f		a,e,f
*4									p	a,f				a,c,g				
5										a,f	p	a,f		b		a,b,g		a,f
6				b														
7		a,e,f					p	a,c				a,f,i		a,f		a,f		
*†8																		
9							p	d, *				b,d,f		a,d,e,f		d		
10			b	b	b		b		b									
11											p	a,c,e		a,c,e,f,g		a,c		a,e,f,g
‡12										b				a,b,f		c,l		a,b,f
13												b	p	c				
*14												e		a,f		a,f		a,f
*‡15	c	b,c		c				c,e		a,c,f		c,e	p	b,c		b		
†16																		
†17	b	a,c,d,g		c				a,c,f		a,b,g,m		a,g		a,b,g		a,b,e,g,n		
†*18						c		a,a,f		a,f		a,f		a,g		a,e,g		a,c,g
19																		
20												a,c						
*21																		
22								e,o				a,d,e,f		a,f		a,c,f,a,c		a,f

* Indicates female participant (n=8)

† Indicates partipant >40 years(n=4)

‡ Indicates participants using sympathomimetic drugs (n=3)

	NO DATA
a	sinus pause/arrest
b	PVC
c	ectopic atrial rhythm/beat/escape
d	non conducted beat (PAC, p wave atrial, sinus beat)
e	PAC
f	junctional escape (rhythm/beat)
g	ventricular escape
h	Mobitz I
i	3rd degree heart block
j	ectopic atrial bradycardia
k	PJC
l	T wave inversion
m	multifocal PAC
n	1st degree heart block
o	atrial bigeminy
p	sinus arrhythmia
*	grouped beating, unclear etiology

Table 3. Reasons for exclusion

	DAY 0 (n=22)	DAY 1 (n=22)	DAY 2 (n=18)	DAY 3 (n=21)	DAY 4 (n=21)	DAY 5 (n=20)	DAY 6 (n=21)	DAY 7 (n=20)	DAY 8 (n=14)
PARTICIPANT									
1									c
2					a	a	a	a	a
3									
4						d			d
5									
6									
7									c
8									
9									
10								c	c
11			b						
12									
13									c
14									
15			b						
16				d					
17									c
18									
19									
20			d						c
21									
22			a						

Some data were not collected or were lost due to factors including scheduling constraints/overlap, ECG artifact, or AMS symptoms on the day of testing

a = AMS related illness

b = ECG artifact

c = Scheduling constraints

d= Did not participate

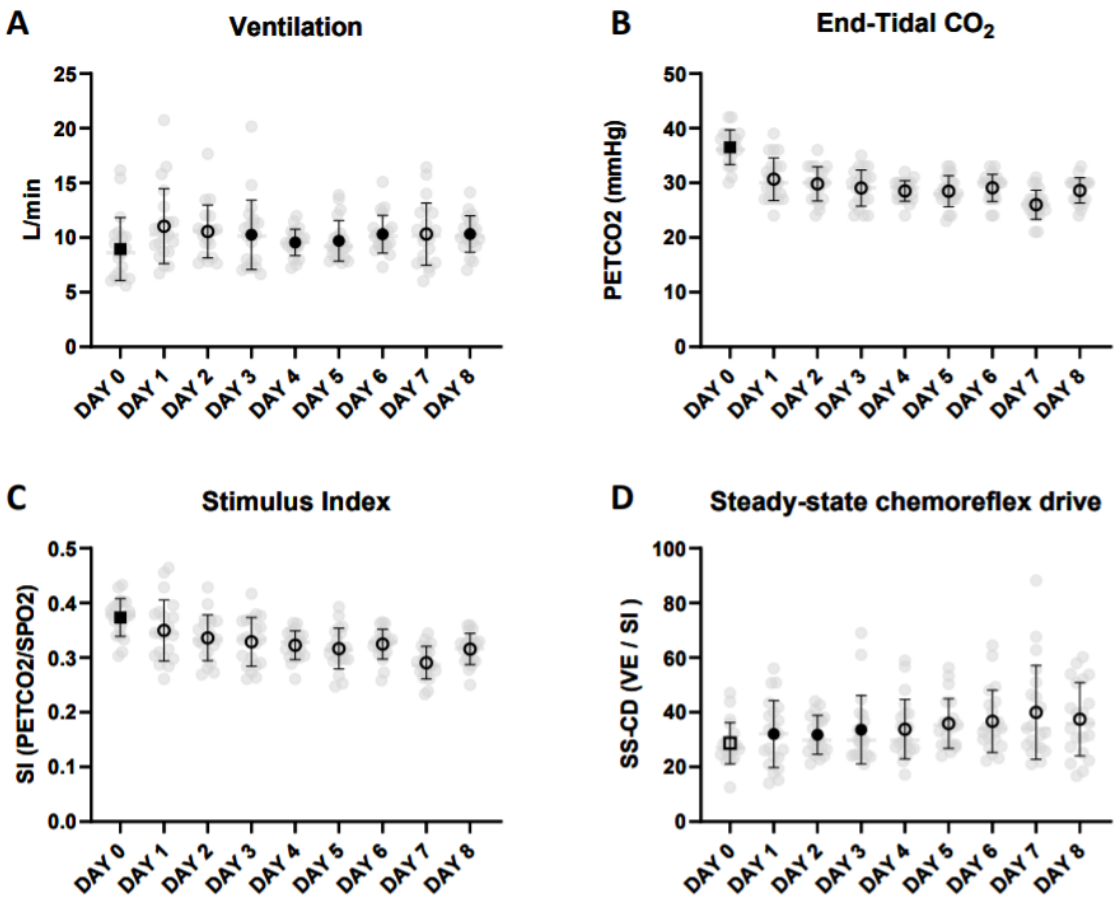

Figure 12. Ventilatory data from low altitude (Day 0; black square) and high altitude (Days 1-8; circles). Significance versus Day 0 ($p<0.05$) is shown by white circles; non-significant versus Day 0 is shown by black circles. Individual data is represented by grey circles. Data was collected during quiet rest (steady state) prior to apnea protocol. Panel A: Ventilation was significantly elevated compared to low altitude on Day 1, 2 and 7 ($p<0.05$). Panel B: End tidal CO_2 was significantly lower on Days 1-8 compared to low altitude ($p<0.001$). Panel C: Stimulus index was significantly lower for all days at high altitude compared to low altitude ($p<0.05$). Panel D: Steady state chemoreflex drive (SS-CD) was not different than low altitude on Days 1-3; on Days 4-8, SS-CD was significantly elevated compared to low altitude ($p<0.05$).

4.1.3 Baseline electrocardiographic characteristics

Basic electrophysiological characteristics for each day (Day 0- Day 8) are reported in ***Table 4***. Changes in ECG morphology at high altitude were compared to ECG morphology at low altitude (see ***Figure 13***). A main effect of time was identified for P wave duration ($p=0.008$), with duration increasing at high altitude. P-R interval was reduced on Day 1 at high altitude ($p<0.001$) but returned towards low altitude value over subsequent days at high altitude. P wave amplitude was similar to low altitude on days 1 ($p=0.863$), 2 ($p=0.863$), 3 ($p=0.129$) and 8 ($p=0.067$), but was elevated on Days 4-7 ($p=0.047$; $p=0.017$; $p=0.048$; $p=0.047$). R wave amplitude was depressed on Day 1 ($p=0.008$) but elevated for all subsequent days at high altitude (main effect of time, $p=0.004$). A main effect of time was identified for QRS duration ($p<0.001$), where QRS was widened on Day 1 at high altitude ($p<0.001$) but returned towards low altitude values over the following days at high altitude. QTc was significantly lengthened for all days at high altitude (main effect of time, $p=0.003$). T wave amplitude (main effect of time $p=0.03$), was slightly elevated compared to low altitude for Days 1-4 and lower than low altitude for Days 5-8, although these comparisons did not reach statistical significance. ST segments were depressed on Day 1 ($p=0.005$; reduced by -0.02) and Days 5-8 (reduced by -0.05 for days 5-8; $p=0.007$; $p=0.004$; $p<0.001$; $p=0.007$, respectively) compared to low altitude.

Table 4. ECG Metrics during baseline

	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
P amplitude	0.11 ± 0.03	0.11 ± 0.04	0.11 ± 0.05	0.13 ± 0.05	**0.13 ± 0.05**	**0.15 ± 0.07**	**0.13 ± 0.04**	**0.12 ± 0.04**	0.13 ± 0.04
R amplitude (mV)	1.16 ± 0.36	**1.00 ± 0.432**	1.33 ± 0.575	1.27 ± 0.492	1.27 ± 0.494	1.23 ± 0.433	1.27 ± 0.390	1.24 ± 0.450	1.33 ± 0.376
T amplitude (mV)	0.28 ± 0.13	0.27 ± 0.15	0.33 ± 0.17	0.29 ± 0.14	0.30 ± 0.14	0.33 ± 0.17	0.33 ± 0.16	0.33 ± 0.14	0.33 ± 0.15
QRS Interval (s)	0.083 ± 0.007	**0.121 ± 0.033**	0.086 ± 0.027	0.083 ± 0.024	0.083 ± 0.024	0.082 ± 0.018	0.083 ± 0.006	0.083 ± 0.006	0.083 ± 0.007
QTc interval (s)	0.38 ± 0.02	0.42 ± 0.02	0.40 ± 0.02	0.40 ± 0.02	0.41 ± 0.03	0.41 ± 0.04	0.41 ± 0.02	0.40 ± 0.03	0.41 ± 0.02
P duration (s)	0.075 ± 0.017	0.075 ± 0.023	0.078 ± 0.028	0.075 ± 0.027	0.081 ± 0.029	0.089 ± 0.018	0.086 ± 0.018	0.086 ± 0.017	0.092 ± 0.022
P-R Interval (s)	0.15 ± 0.07	**0.13 ± 0.04**	0.15 ± 0.05	0.14 ± 0.05	0.15 ± 0.05	0.15 ± 0.02	0.14 ± 0.02	0.15 ± 0.02	0.15 ± 0.02
ST elevation (mV)	0.05 ± 0.03	**0.03 ± 0.02**	-0.06 ± 0.03	-0.05 ± 0.04	-0.05 ± 0.04	**-0.004 ± 0.05**	**-0.006 ± 0.04**	**-0.003 ± 0.04**	**-0.004 ± 0.04**

BOLD indicates significant ($p<0.05$) vs Day 0

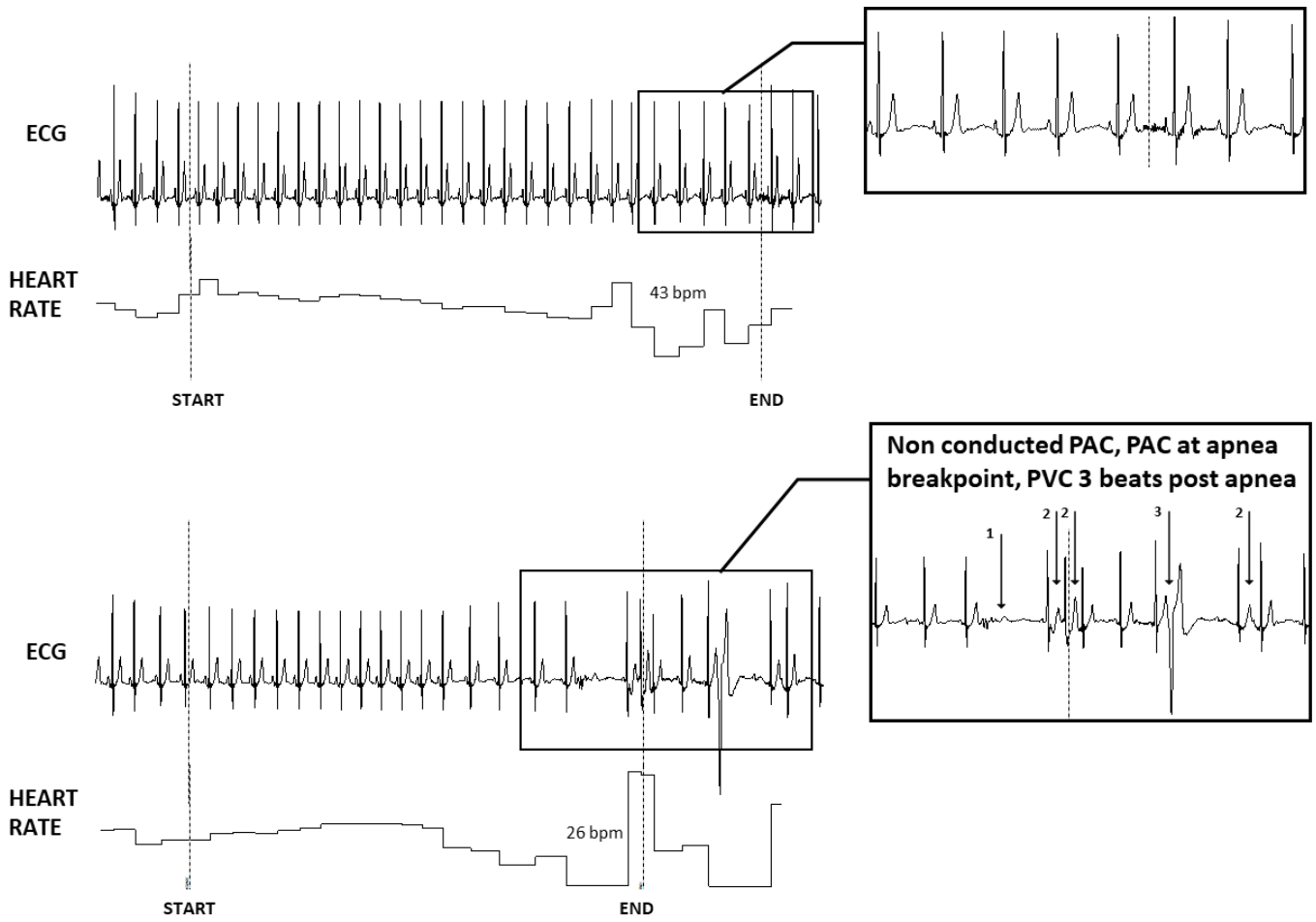

FIGURE 13. Representative figure of apnea in one individual at low altitude (*top*) and high altitude (Day 5; *bottom*). No arrhythmias were identified in this individual at low altitude. At high altitude (Day 5), this same individual developed non conducted premature atrial contraction (PAC; 1) during apnea, PAC at apnea breakpoint (2), and premature ventricular contraction (PVC) immediately post apnea (3).

4.1.4 Heart rate and rhythm during apnea

Breath hold duration was reduced compared to low altitude values on Day 1 (p=0.04), 2 (p=0.008), 3 (p=0.004), 4 (p=0.005), and 7 (p=0.03). Days 5, 6, and 8 were not significantly different than low altitude (p=0.09; p=0.34; p=0.15) (***Figure 14***). The change in SpO_2 (average baseline SpO_2– nadir SpO_2during breath hold) was not significantly different between low altitude and any day at high altitude (main effect p=0.23) (***Figure 14***). However, a main effect of time was identified for the delta change in heart rate (calculated as BL HR – BH nadir) (p<0.0001), as bradycardia became more pronounced with acclimatization. Delta change in heart rate for all days is depicted in ***Figure 14.*** ANCOVA analysis revealed that the magnitude of bradycardia during apnea was not affected by change in saturation (p=0.157) nor breath hold duration (p=0.988). Interestingly, the magnitude of bradycardia was strongly correlated with the percentage of arrhythmias per day (r=0.8; p=0.006). ***Figure 13*** provides a representation of the heart rate and rhythm response to apnea at both low and high altitude in a single participant. Rhythm abnormalities during baseline and apnea are reported for each participant on each day in ***Table 2 (***https://doi.org/10.6084/m9.figshare.13093520.v1). Events during baseline included premature ventricular contractions (PVC) and ectopic atrial beat. At low altitude, 2/22 participants had events (PVC, n=1; ectopic atrial rhythm, n=1) during baseline. At high altitude, two participants had persistent PVCs (>2 per minute) during baseline consistently on Days 1 through 4. On Days 5-8 at high altitude there were no events recorded during baseline. At low altitude 14% (3/22) of participants developed arrhythmias during apnea. By Day 5 at high altitude, >50% of participants developed arrhythmias during apnea. The percentage of arrhythmic events during apnea per day is presented in ***Figure 14***. See ***Table 5*** for details on classification of cardiac events.

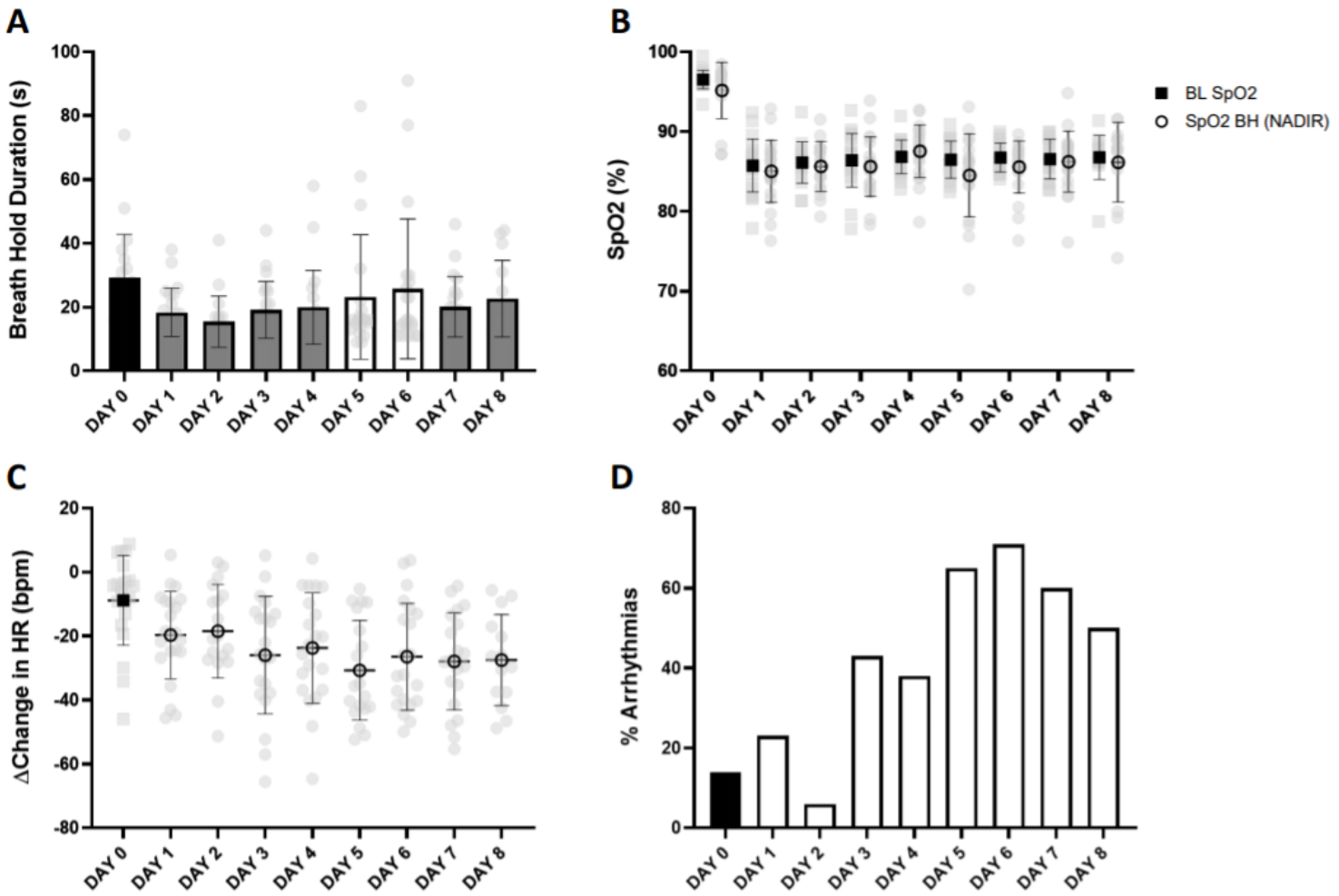

FIGURE 14. Panel A**:** Breath hold durations (s), Panel B: SpO_2 (%) average during baseline and nadir during apnea, Panel C: Delta change in heart rate (bpm) during apnea, Panel D: Percentage of arrhythmias per day (%). Individual data is represented by grey

circles. Day 0 is low altitude (1045m); Days 1-8 are high altitude (3800m). Breath hold duration was significantly different at high altitude versus low altitude on Day 1-4, and Day 7-8 (grey bars). Breath hold duration on Day 5 and 6 were not significantly different than low altitude (white bars). Change in saturation (nadir saturation minus baseline saturation) was not significant between low altitude and any day at high altitude. Change in heart rate during apnea (nadir heart rate minus baseline heart rate) at high altitude is significantly different ($p<0.05$) versus low altitude for all days (white circles). Percentage of arrhythmias per day increased by Day 5 at high altitude and remained at or above 50% over the following days. All percentages represent the onset of newly developed arrhythmias (i.e., those not identified during baseline). At low altitude, 14% of participants developed arrhythmias; on Day 1, 23% of participants developed arrhythmias; on Day 2, 6% of participants developed arrhythmias; on Day 3, 43% of participants developed arrhythmias; on Day 4, 38% of participants developed arrhythmias; on Day 5, 65% of participants developed arrhythmias; on Day 6, 71% of participants developed arrhythmias; on Day 7, 60% of participants developed arrhythmias; on Day 8, 50% of participants developed arrhythmias.

Table 5. Classification of cardiac events during baseline and during apnea

	LA Day 0		HA Day 1		HA Day 2		HA Day 3		HA Day 4		HA Day 5		HA Day 6		HA Day 7		HA Day 8	
CLASSIFICATION:	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**	**BL**	**BH**
ECTOPY																		
Premature atrial contraction		1						3		1		6		4		2		2
Premature ventricular contraction	1	1	2	2	2		2	2	2	4		4		6		5		1
Ectopic atrial rhythm/beat/escape	1	2		2		1		5		1		3		6		4		1
Ectopic atrial bradycardia																		
Premature junctional contraction																		
Junctional rhythm or escape		1		1				3		5		6		8		3		6
Atrial bigeminy								1										
Multifocal PAC										1								
Ventricular escape		1								1		1		4		4		2
SINUS NODE DYSFUNCTION																		
Sinus pause/arrest		2		2				5		6		8		11		10		7
CONDUCTION BLOCK																		
Non conducted beat		1						1				3		2		1		
1st degree heart block																1		
Mobitz I								1										
3rd degree heart block												1						
OTHER																		
T wave inversion																1		
Grouped beating unclear etiology								1										

Classification of cardiac events based on origin and type. Events are listed for each day at low and high altitude during baseline (BL) and apnea (BH) conditions. The majority of participants developed multiple arrhythmic events during apnea. All events (including multiple events during a single apnea) are identified here

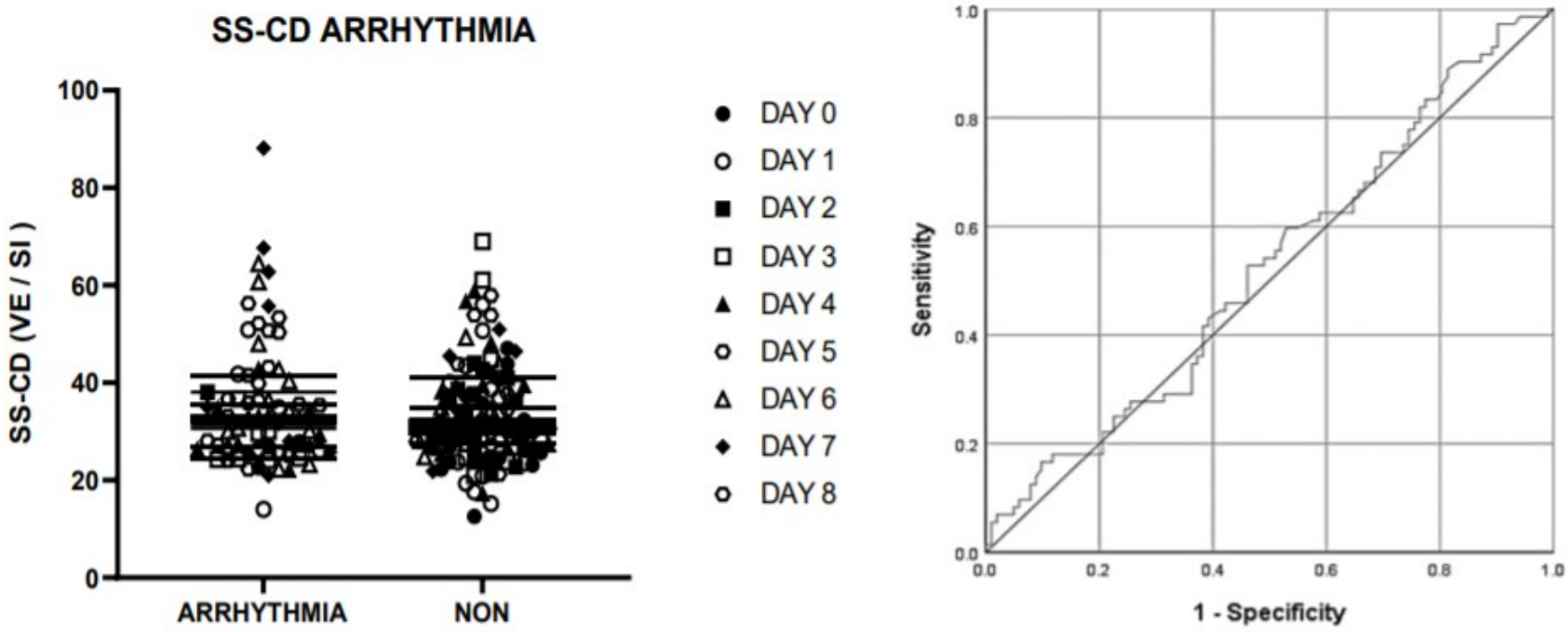

FIGURE 15. Receiver operating characteristics (ROC) curve for steady state chemoreflex drive (SS-CD) and incidence of arrhythmia. ROC was conducted to determine the ability of SS-CD to predict whether an individual would have an arrhythmia during voluntary apnea. Calculated area under the curve was 0.524, suggesting SS-CD has low predictability of whether an individual will have an arrhythmia during apnea.

CHAPTER 5 DISCUSSION

5.1 General Discussion

We aimed to determine how duration of high-altitude acclimatization influences the incidence of arrhythmogenesis during apnea. We demonstrated that bradycardia in response to apnea was more pronounced at high altitude, becoming significantly different as soon as Day 1 of high-altitude exposure. We also observed an increase in the incidence of apnea induced arrhythmias at altitude, but this was only apparent after multiple days of high-altitude exposure, with more than 50% of participants exhibiting arrhythmias during apnea by Day 5. Importantly, the duration of apnea and change in saturation during apnea did not appear to influence the magnitude of bradycardia or incidence of arrhythmia. Collectively, these data indicate there is a time dependent effect of high altitude on the onset and incidence of cardiac arrhythmias during apnea. This study extends our previous findings by suggesting progressive chemoreflex sensitization and concurrent time-dependent alterations in autonomic balance may have a mechanistic role in the genesis of cardiac events during apnea at high altitude. Further, our longitudinal assessment over 8 consecutive days at high altitude highlights a novel time course of autonomic adaptation to hypoxia which has not been previously assessed, despite relatively established ventilatory (Ainslie et al., 2013a; Dempsey et al., 2014; Pamenter & Powell, 2016; Robbins, 2007) and systemic cardiovascular (Bärtsch & Gibbs, 2007b; Hainsworth et al., 2007; Naeije, 2010; Swenson & Bärtsch, 2014) dynamic changes throughout hypoxic exposure. This data also sets a foundation to explore how sensitization to hypoxia may contribute to heart rate and rhythm abnormalities in clinical conditions where elevated chemoreflex or autonomic drive is implicated, such as central and obstructive sleep apnea.

Our current data suggest there is a threshold above which chemoreflex sensitization results in an increased incidence of cardiac arrhythmias during apnea. Here, we demonstrate that arrhythmias become more prevalent during apnea following 5 days at high altitude, suggesting this phenomenon is not hypoxia oriented per se (e.g., chemoreflex activation or cardiac ischemia) but rather is related to progressive chemoreflex sensitization over time. Interestingly, we also observed that the magnitude of bradycardia during apnea was strongly correlated with the incidence of arrhythmia (percentage) per day (r=0.8), suggesting a similar underlying mechanism, or that augmented vagal drive may create a state in which the heart is more susceptible to conduction abnormalities. Although driving heart rate below the intrinsic rate(s) of various steps within the conduction pathway may cause spontaneous depolarization of ectopic pacemakers (i.e., ectopic rhythm), instances of conduction block are not simply explained by reduced heart rate, indicating an external mechanism.

Heightened parasympathetic tone has been previously identified as a primary consequence of peripheral chemoreceptor stimulation (M. de (Michael de) Burgh Daly, 1997; Foster & Sheel, 2005; Jendzjowsky et al., 2018; Kato et al., 1988; Masuyama et al., 1990). Although there is evidence that cardio-acceleration during acute hypoxia is a consequence of vagal withdrawal (Christoph Siebenmann et al., 2019), this has been observed during breathing conditions, rather than during apnea. Ventilation has been clearly shown to suppress sympathetic activity during hypoxia (Busch, Simpson, et al., 2020; Kato et al., 1988), and our previous work has demonstrated a pronounced bradycardia during apnea following prolonged hypoxic exposure, indicating heightened parasympathetic tone that is only unmasked during apnea (5). Additionally, this bradycardic response to apnea is absent when hypoxia is reversed following administration of supplemental oxygen (100% O_2;7) and during apnea at low altitude (Busch et al., 2021). In the

current study, we demonstrated that an augmented bradycardia response is evident during apnea following even 1 day (~24 hours) at high altitude, suggesting that hypoxic exposure results in elevated cardiac parasympathetic tone which becomes apparent in the absence of ventilation. It should also be considered that effects of chronic hypoxia on cardiac tissue are likely related to decreased adrenergic activation of cardiac receptors and increased muscarinic receptor affinity and density following prolonged exposure to hypobaric hypoxia (Kacimi et al., 1993), leading to increased parasympathetic influence on the heart which could independently impact cardiac conduction in response to changing autonomic drive (i.e., during apnea). Future research is needed to experimentally determine influence of hypoxia on vagal tone and cardiac control.

Alternatively, arrhythmogenesis during apnea at high altitude could be attributed to autonomic conflict. In addition to heightened parasympathetic tone during apnea, higher sympathetic outflow in situations of facial immersion (Hansel et al., 2009), cold shock (Shattock & Tipton, 2012), and high altitude (Busch, Simpson, et al., 2020), result in concurrent elevations in both branches of the autonomic nervous system, which deliver opposing signals to the heart and consequently result in conduction abnormalities. It has been well documented that sympathetic nervous activity is increased at high altitude (Hansen & Sander, 2003; Sander, 2016a; Simpson, Steinback, et al., 2020). We observed a higher resting heart rate for all days at high altitude compared to low altitude, consistent with sympatho-excitatory effects of hypoxia. Changes observed in ECG morphology, including enlarged P (Day 4-7) and R (Day 2-8) wave amplitudes and shorter P-R interval (Day 1), are also consistent with increases in sympathetic activity (Busch et al., 2018; Duplain et al., 1999; Hansen & Sander, 2003). These observations are in accordance with what we have previously observed at high altitude and also support the idea that during compounding stress (i.e., high altitude and apnea) autonomic conflict and

delivery of opposing signals to the heart may result in cardiac arrhythmias. However, while adrenergic drive is elevated and remains elevated through hypoxic exposure (Simpson, Steinback, et al., 2020), modulation of adrenergic effects through decrease in adrenergic receptors in parallel with increases in muscarinic receptor density affect the response to changes in autonomic drive. This shift becomes more evident during exercise, where there is a decrease in maximal heart rate during maximal exercise following prolonged hypoxic exposure (Siebenmann & Lundby, 2015). It may also be required to have a background of sympathetic activation (e.g., hypoxia-induced increase in SNA) in addition to elevation in parasympathetic drive (during apnea) that predisposes to arrhythmias as a result of differential stimulation at various steps of the conduction pathway (SA and AV nodes) or as a result of heterogeneity in cardiac stimulation at various regions of the heart (where parasympathetic is largely supraventricular, and sympathetic may have stronger ventricular effects) (Shattock & Tipton, 2012). The alterations in autonomic nervous system function and activity at high altitude are of interest in relation to cardiac control during instances of stress. Future studies should aim to characterize the exact mechanism(s) or autonomic contribution to the observed cardiac events, as the resulting conduction abnormalities have potential clinical implications in pathologies where chemoreflex and or autonomic activity is chronically elevated.

We have previously observed that hypoxic ventilatory response (HVR) is predictive of arrhythmias during apnea (Busch et al., 2018). Additionally, Masuyama et al (1990) identified that during nocturnal apneas related to CSA at high altitude, a higher HVR was related to a greater drop in heart rate during apnea (Masuyama et al., 1990), emphasizing the connection between chemosensitivity and cardiac responses to hypoxia. Further, the intensity of HVR (increase in chemoreflex gain) and instability in ventilatory control have been related to periodic

breathing at high altitude (Hermand et al., 2015, 2016; Khoo et al., 1996). Collectively these studies support an interactive role of chemosensitivity and ventilatory instability on cardiac control. Thus, in an additional analysis, we attempted to assess whether an elevation in tonic chemoreflex activity over time at high altitude would be predictive of arrhythmic events during apnea. We attempted to quantify this using SS-CD, which provides an indication of resting ventilation against prevailing chemical stimuli (i.e., O_2 , CO_2 and pH) (Bruce et al., 2018; Pfoh et al., 2017), providing insight into the 'set point' of chemoreflex activity. SS-CD has been previously assessed and shown to track ventilatory acclimatization during high altitude ascent (Bruce et al., 2018; Leacy et al., 2020; Pfoh et al., 2017). On Day 4 and beyond, SS-CD became significantly elevated versus low altitude, indicating ventilatory acclimatization. However, ROC analysis revealed relatively low predictability of SS-CD for incidence of arrhythmia (***Figure 4***; AUC, 0.524). The discrepancy between our current findings of SS-CD and previous assessment of HVR could be a result of several factors. First, as SS-CD provides a quantification of ventilation against circulating chemo-stimuli, it provides insight into both central and peripheral chemoreflex activity. However, the Dejours test (change in ventilation with acute hyperoxia), which is more specific to the peripheral chemoreflex, also indicated relatively low predictability for arrhythmia during apnea in a subset of participants (AUC, 0.579). On this point, peripheral chemoreflex sensitivity, or gain, as measured by HVR provides a different indication of chemoreflex function as opposed to "set-point" activity (as measured by SS-CD and the Dejours test). Finally, although chemoreflex activation and sensitivity have been shown to influence ventilatory and cardio-autonomic function, the independent ventilatory and sympathetic responses may not be directly correlated (Prasad et al., 2020). Therefore, it may not be surprising that in the current study ventilatory acclimatization did not have a specific relationship to the

observed responses in heart rhythm during apnea, despite a clear effect of time on increasing incidence of arrhythmias. Based on previous findings of a relationship between HVR and cardiac responses to apnea (Busch et al., 2018; Masuyama et al., 1990), a relationship may exist between ventilation and vagal drive (despite a dissociation between SNA and ventilation), and this may be more so related to individual chemoreflex gain rather than set point of activity. These relationships have yet to be explored but may provide further insight into autonomic control of the heart under conditions of heightened chemoreflex sensitivity. It may be that greater instability between two controller systems (i.e., ventilatory and cardiac) lead to greater variability in both outputs (i.e., arrhythmias during apnea). It should also be considered that alterations in the sensitivity of central autonomic nuclei as well as central chemoreceptors may play a role in the observed time dependent effects of altitude (Day & Wilson, 2009). However, the complex interaction between central and peripheral pathways make it difficult to isolate specific influences of one versus the other, as there are likely redundancies in recruitment of similar or same neural pathways (Guyenet, 2014). Characterization of the relationships between chemosensitivity, ventilation and cardiac control are directly relevant to understanding the causes and consequences of CSA at high altitude. There is evidence that CSA worsens over time at high altitude (Ainslie et al., 2013; Burgess & Ainslie, 2016), and so our findings that the cardiac response to voluntary apnea is time dependent may translate to progressive changes in cardiac electrophysiology overnight with CSA.

5.2 Considerations

We present evidence of a progressive time course leading to a heightened incidence of cardiac arrhythmias during apnea at high altitude. However, it is unclear whether such events would disappear over long-term acclimatization to altitude. In our previous assessments of apnea

in high altitude indigenous populations (Busch et al., 2018; Busch, van Diepen, et al., 2020), we did not observe a significant incidence of arrhythmias during apnea, suggesting a potential cardioprotective phenotype in these high altitude residents. Whether this has developed over generations of incurred hypoxic exposure or whether this may be observed following significant (i.e., >6 months) duration at high altitude has yet to be explored.

Our study included a large age range of participants as well as a relatively high (n=8) proportion of females. While we do not view this as a limitation, as it is more widely representative sample, it should be considered that age and/or sex may have had an influence on our results. However, our previous studies have examined this phenomenon in predominantly young (27 ± 6 and 28 ± 7 years) (5, 7 respectively) male subjects, and our results from the current study are comparable to what we have previously reported. Additionally, only 5 (out of 22) participants were over 40 years old, and there was no apparent increased incidence of arrhythmias during apnea in these participants, nor was there an apparent sex related difference in the incidence of arrhythmia (both identified in ***Table 2***: https://doi.org/10.6084/m9.figshare.13093520.v5). Additional analyses (two-way mixed effects ANOVA) conducted to identify the influence of time (day) and sex on the bradycardic response to apnea revealed a nonsignificant effect of sex (p=0.07) and no interaction between time and sex (p=0.6). Therefore, we do not believe that our sample group significantly impacted our results or interpretation. Finally, inclusion of older age participants may provide a more relevant future model for studying certain pathological conditions involving heightened chemoreflex drive (i.e., obstructive sleep apnea), which become more prevalent with increasing age (Morley et al., 2017).

As we did not continuously measure blood pressure during apnea, baroreflex engagement cannot be excluded as a possible mechanism contributing the reduction in heart rate seen during apneas. During previous investigations of this phenomenon, we have demonstrated that the blood pressure peak does not occur until post apnea, with a lower relative change in blood pressure during apnea at high compared to low altitude (Busch et al., 2018). Additionally, with relatively short (average <1 minute) apnea duration, it seems unlikely that the baroreflex is the primary mediator of the observed bradycardic response (Busch et al., 2021). Finally, our results demonstrate a clear effect of high altitude on both the heart rate (i.e., bradycardia) and rhythm response to apnea, while baroreflex engagement may be similar across conditions, as it has been previously demonstrated that baroreflex gain is similar between low and high altitude (Simpson et al., 2019). Thus, while baroreflex activation may contribute to the heart rate response we do not believe it to be the underlying mechanism driving a greater magnitude of bradycardia or the rhythm abnormalities observed during apnea at high altitude

Ideally, participants would have performed HVR tests at low and high altitude to quantify peripheral chemoreflex sensitivity. Previously (Busch et al., 2018) we have demonstrated a strong predictability of HVR for whether an individual would develop arrhythmia during apnea. Assessments of HVR in the current study would have provided evidence of repeatability of our previous work and determination of a mechanistic role of peripheral chemoreflex sensitivity in occurrence of cardiac arrhythmias during apnea. However, this study was not designed to isolate a mechanism, but rather was intended to be informative in identifying a time course of hypoxic exposure for develop of cardiac events during apnea. Future studies should aim to specifically assess the mechanistic side of this response. However, the current results are still relevant and

important in characterizing that this physiological phenomenon is specific to prolonged hypoxic exposure.

We have focused largely on the elevation in vagal tone that occurs with acclimatization; however, high altitude also results in a large and sustained sympatho-excitation (Hansen & Sander, 2003; Sander, 2016a), and sympathetic activity has also been shown to markedly increases in the absence of ventilation (Busch, Simpson, et al., 2020; Steinback et al., 2010). In the current study, we did not include any direct (i.e., MSNA) or indirect (i.e., arterial pressure) measures of sympathetic activation during baseline or apnea, and so did not capture the peripheral sympathetic activation that is occurring concurrently with elevated cardiac vagal drive. Assessing both sympathetic and parasympathetic aspects of autonomic control is relevant in understanding the integrated response, and may be relevant in the genesis of the observed rhythm disturbances through autonomic conflict (Busch et al., 2018; Shattock & Tipton, 2012). However, the arrhythmias observed seem primarily to be related to vagal drive, based both on origin (i.e., supraventricular) and evidenced by the strong association between bradycardia and percentage of arrhythmias per day, although a background of heightened sympathetic activation may be required for 'autonomic conflict' to manifest. Incorporation of pharmacological intervention and or additional experimental measures are required to confirm the mechanistic basis of this response.

While measures of heart rate variability (HRV) have been previously used to characterize autonomic control of the heart and relative autonomic balance, our baseline periods proceeding apneas were too short in duration (2 minutes) to perform an HRV analysis. However, HRV might not actually correlate with vagal tone (Marmerstein et al., 2021). A notable recent advancement in the field of physiology is the ability to directly record activity from the cervical

vagus nerve in conscious humans (Ottaviani et al., 2020). This impressive advancement in characterizing autonomic physiology will undoubtably help us to further understand autonomic control, and once the technique has become more popularized it would provide a gold standard method to directly assess parasympathetic activity and function at high altitude.

5.3 Perspectives and Conclusion

This study is the first to show a time-dependent effect of chronic hypoxia on the incidence of cardiac arrhythmias during apnea. Although within the current framework we are unable to definitively determine a mechanism for this phenomenon, our results suggest that progressive chemoreflex sensitization throughout acclimatization underlies the development of cardiac arrhythmias during voluntary apnea. The fact that elevated parasympathetic tone is not unmasked with acute hypoxic exposure (Busch et al., 2021), despite a marked increase in sympathetic activity that develops rapidly and is maintained throughout hypoxic exposure (Hansen & Sander, 2003; Sander, 2016b; Simpson, Steinback, et al., 2020), indicates a differential effect of time course on both autonomic limbs which may be related to concurrent changes in chemosensitivity (Busch et al., 2018; Khoo et al., 1996; Masuyama et al., 1990) and or changes in adrenergic/cholinergic receptor density and sensitivity to autonomic drive (Kacimi et al., 1993). Voluntary apnea provides insight into how changing autonomic drive affects cardiac conduction, and thus use of apnea at high altitude may provide a future functional model for exploring the effects of heightened chemoreflex stress on autonomic control of heart rate and rhythm. Future studies should aim to assess whether the same rhythm abnormalities observed during voluntary apnea occur during apneic periods of CSA at high altitude.

CHAPTER 6 FUTURE DIRECTIONS AND CONCLUSION

6.1 Main Findings

In summary, our findings indicate a specific effect of prolonged hypoxic exposure on the occurrence of heart rate and rhythm abnormalities during apnea at high altitude. An augmented bradycardic response to apnea was apparent following even 1 day at high-altitude, while rhythm abnormalities were gradually increased, becoming more pronounced (>50%) at and beyond Day 5 at high altitude. Additionally, the magnitude of bradycardia during apnea was correlated with the incidence of arrhythmia per day, indicating a similar underlying mechanism. Importantly, neither drop in saturation during apnea nor apnea duration were associated with the degree of bradycardia, indicating that this phenomenon is not specific to individual ability to perform maximal apnea. Finally, steady state chemoreflex drive was not predicative of incidence of arrhythmia at high altitude despite previous observations that HVR is predictive of arrhythmia (Busch et al., 2018), suggesting that chemoreflex sensitivity, rather than set point, may be more relevant in the genesis of these cardiac events. From a mechanistic perspective, our findings indicate that prolonged exposure to hypoxia results in elevated vagal tone that is unmasked during instances of apnea, and that this heightened vagal drive may underlie the heart rate and rhythm response to apnea at high altitude. This is supported by our previous studies and by early work demonstrating that hypoxic activation of the peripheral chemoreflex results in bradycardia in the absence of ventilation (Daly & Scott, 1962; Kato et al., 1988).

6.2 Future Directions and Conclusions

The physiology discussed in this thesis provides several avenues for future research in this area. First, while we have repeated evidence of rate and rhythm abnormalities during apnea

at high altitude, we have not specifically elucidated a mechanism. While our results (discussed above) indicate that progressive chemoreflex sensitization and consequently elevated vagal drive may underlie this phenomenon, this hypothesis has yet to be experimentally confirmed. Future studies should aim to assess the cardiac response to apnea under conditions of parasympathetic and or chemoreflex block. Atropine, a muscarinic receptor antagonist, exerts a parasympatholytic effect (i.e., attenuates parasympathetic effect) on heart rate, and has been used previously to assess vagal contribution during exercise (Fontolliet et al., 2018), in relation to baroreflex control of blood pressure (Lin et al., 2002), in response to hypoxia (Koller et al., 1988), and in sleep apnea patients (Januel et al., 1995). However, no study has investigated vagal inhibition during conditions of voluntary apnea. Administration of atropine prior to apnea would confirm whether heightened vagal tone contributes to the observed cardiac rate and rhythm response.

Second, it should be determined whether elevated vagal tone at high altitude is a result of progressive chemoreflex sensitization. We have demonstrated that use of hyperoxia (100% O_2) at high altitude to silence the peripheral chemoreflex attenuates the heart rate and rhythm response to apnea (Busch et al., 2018). However, administration of hyperoxia prior to apnea may also affect arterial saturation and or ventilation. To mechanistically determine the role of the chemoreflex in changes in autonomic tone at high altitude, future studies could use low dose dopamine to acutely and directly block the peripheral chemoreflex (Bellville;, 1982; Mozer et al., 2016).

Third, central sleep apnea (CSA) commonly occurs in the majority of individuals during sojourn to high-altitude. Whether the same cardiac events seen during voluntary apnea are relevant to instances of apnea during CSA remains to be determined, although previous studies have identified bradycardia (Masuyama et al., 1990) and arrhythmia (Karliner et al., 1985;

Malconian et al., 1990) during overnight ECG monitoring at high altitude. Although there are several differences in activity of neural networks between wakefulness and sleep, the results of the current study may be of direct relevance to apneas during CSA events at high altitude. Continuous overnight assessment of heart rate and rhythm, in addition to assessment of CSA, will help to determine whether the same types of cardiac events seen during voluntary apnea at high altitude are associated with apneas during sleep.

In conclusion, in this study we have characterized the effect of duration at high altitude on the manifestation of cardiac rate and rhythm disturbances during voluntary apnea. This study has identified that this physiological response becomes more pronounced with acclimatization and may be a result of the progressive chemoreflex sensitization and consequent elevation in vagal tone. The considerations and future directions outlined above will help to translate this work to more mechanistic and applied perspectives.

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

Approval Form

Date:	July 10, 2019
Principal Investigator:	Craig Steinback
Study ID:	Pro00089322
Study Title:	The effects of acclimatization to hypoxia on wakeful and sleeping ECG and heart rhythm
Approval Expiry Date:	Thursday, July 9, 2020
Date of Informed Consent:	Approval Date: 7/10/2019 — Approved Document: Consent Form CLEAN
Funding/Sponsor:	NSERC - Natural Sciences And Engineering Research Council — NSERC

Thank you for submitting the above study to the Health Research Ethics Board - Biomedical Panel. Your application, including the following, has been reviewed and approved on behalf of the committee:

- Consent Form – 09Jul2019
- Health Information Sheet – 01May2019
- ECG Proposal/Protocol - undated
- White Mountain Project Proposals – 11Apr2019
- Study Budget - undated

It is noted that this application has received previous approval by the University of Calgary CHREB, a member of the Alberta Research Ethics Reciprocity Agreement.

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

A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date (Thursday, July 9, 2020), you will have to re-submit an ethics application.

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

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

APPENDIX II: Ethics Approval for Barcroft Field Expedition (University of Calgary)

UNIVERSITY OF CALGARY

Conjoint Health Research Ethics Board
Research Services Office
2500 University Drive, NW
Calgary AB T2N 1N4
Telephone: (403) 220-2297
chreb@ucalgary.ca

CERTIFICATION OF INSTITUTIONAL ETHICS APPROVAL

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

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

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

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

The following documents have been approved for use:

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

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

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

Restrictions:

This Certification is subject to the following conditions:

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

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

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

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

Appendix III: Consent Form for participants

PARTICIPANT CONSENT FORM

Faculty of Kinesiology, Sport, and Recreation

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

Tel: 780.493.5553

Title of Research Study: **The effects of acclimatization to hypoxia on wakeful and sleeping ECG and heart rhythm**

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

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

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

Why am I being asked to take part in this research study?

You are being asked to participate in this research study because you are healthy and participating on a trip to high altitude. The aim of our study is to assess 1) how your heart rate and rhythm change while you hold your breath while breathing a lower than normal amount of oxygen, and 2) whether short pauses in breathing that happen naturally while you sleep at high altitude oxygen influences how heart rate and rhythm change, and 3) the time course of these changes. Our findings could help contribute to a pre-screening protocol which could identify individuals at risk for developing abnormal heart rate and rhythm at altitude.

What is the reason for doing the study?

Right now, we know that there are many mechanisms that are activated when someone holds their breath. These include receptors that detect the amount of oxygen and carbon dioxide in their blood and also the amount of air in their lungs. We also know that when a person holds their breath, the part of the nervous system that controls the heart becomes more active. This part of the nervous system is known as the autonomic nervous system, and it is composed of the sympathetic nervous system (SNS), responsible for the “fight or flight” response, and the parasympathetic nervous system (PNS), responsible for the

“rest and digest” response. The sympathetic nervous system typically acts to increase heart rate and the parasympathetic nervous system acts to decrease heart rate.

During exposure to low oxygen both parts of the nervous system are activated, and even more so when a person hold their breath. This results in an ‘autonomic conflict’ with signals trying to increase and decrease heart rate at the same time. Recently, we showed that when people hold their breath at altitude autonomic conflict caused abnormal decreases in heart rate and irregular heart rhythm. This is very short lasting (resolves after starting breathing again) and in otherwise young healthy individuals is relatively benign. However, in individuals with underlying cardiovascular disease this could pose a risk to the heart, particularly if this occurs chronically and repetitively as may occur at altitude.

Additionally, central sleep apnea (CSA) commonly occurs in healthy people at high altitude. CSA involves short pauses in breathing during sleep. The incidence of irregular heart rhythms as seen during voluntary breath holds may also occur during CSA.

We are attempting to determine the time course of autonomic conflict and abnormal heart rate and rhythm that occur during exposure to high altitude hypoxia.

Am I eligible to take part in this study?

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

What will happen in the study?

If you meet the criteria for this study, you will be asked to be tested a total of fifteen times while awake. You will be tested three times during baseline testing at the Integrative Physiology Lab (B126) in Calgary, AB (1,045m), and every morning during a stay at the Barcroft Station on White Mountain, CA (3,800m). The location of the lab in Calgary is in room B126 at Mount Royal University 4826 Mt Royal Gate SW. It is accessible by city transit. We will need you to stay here for 10-30 minutes total per daytime test. We will also test you twice overnight during your stay at high altitude. You will be tested once early (week 1) and once late (week 2). The test will begin when you are ready to go to sleep for the night, and finish when you wake up in the morning.

Equipment:

Daytime tests:

- One set of small electrocardiogram (ECG) stickers will be used to monitor your heart rate constantly throughout the experiment. The set has five stickers. One sticker goes on your left shoulder, one on your right shoulder, one on your left side, one on your right side, and one on your center-left chest.
- A small clip will be placed on the index finger of one hand to measure the amount of oxygen in your blood.

Overnight tests:

- One set of small electrocardiogram (ECG) stickers will be used to monitor your heart rate constantly throughout the experiment. The set has five stickers. One sticker goes on your left shoulder, one on your right shoulder, one on your left side, one on your right side, and one on your center-left chest.
- A small clip will be placed on the index finger of one hand to measure the amount of oxygen in your blood.
- A nasal cannula, which is a small tube worn across your upper lip with small inserts into your nostrils. This is to measure your breathing.

Once equipment is set up, we will turn on the equipment. The finger clip and ECG leads (and nasal cannula for the overnight test) 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:

Daytime tests:

- Following instrumentation, we will get you to lay still for 5 minutes and relax. This will allow us to obtain measurements of normal values (baseline) for each measure we are recording. Then, we have you perform a breath hold at the end of a normal expiration. One of the researchers will coach you before the breath hold. You will be asked to indicate when you have begun holding your breath. You will also be encouraged to hold it as long as possible.

Overnight tests:

- When you are ready to go to sleep for the night, you will be instrumented and the equipment will be turned on. You will then go to sleep as normal. You will also be instructed on how to turn off and take off the equipment, which you will do when you wake up in the morning.

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

We will go over all procedures in advance and we can address any questions or concerns you might have. Once in the lab, it is important that you are comfortable and relaxed and tell us if anything is wrong or uncomfortable. You will then lay comfortably on a 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?

Reduced Oxygen: Breathing air with a reduced amount of oxygen may cause you to breathe deeper and/or faster. This is a normal response and we encourage you to breathe however you feel most comfortable. In some individuals, breathing deeper or faster may

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

Acute Mountain Sickness (AMS): While breathing a lower than normal level of oxygen you may develop symptoms of acute mountain sickness (AMS). Acute mountain sickness may occur in some people (about 1 in 4) who ascend rapidly to an altitude greater than 2,500m. Because of the drop in oxygen in the air you're breathing in, you may experience AMS. Symptoms of AMS include headache, dizziness, peripheral paresthesia (i.e. tingling or numbness in the arms or legs) and breathlessness. There are no lasting effects of AMS, and symptoms resolve upon descent from altitude.

Pulse oximeter, ECG, and Nasal Cannula: There are no known risks associated with either the pulse oximeter, ECG or ECG stickers, or nasal cannula.

Abnormal findings: Within this study, we take many different measurements that tell us about your heart and blood vessels. It is possible, but rare, that we may identify abnormalities that require further consultation from a medical professional. If any abnormal findings are identified during your participation in the study, we will provide you with full details, contact your chosen medical professional (e.g. your family doctor or obstetrician) and, with your permission, refer you to a responsible medical doctor.

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

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

Do I have to take part in this study?

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

Can my participation in the study end early?

You are free to withdraw from this study at any time for any reason. You can do this by contacting the investigators. If after participating in the study you wish to remove your

information from the study, you have until December 31, 2022 to do so. After this time all information will be used. We may request that you withdraw from the study during the protocol if we are at all worried about your general health (i.e. high blood pressure, irregular heart rhythm etc.). We will notify you of our reason should this occur.

Will I be paid to be in the research?

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

Will my information be kept private?

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

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

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

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

After the study is done, we will still need to securely store your health data that was collected as part of the study. At the University of Alberta, we keep data stored for 5 years after the end of the study.

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

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

What happens if I am injured because of this research?

If you become ill or injured as a result of being in this study, you will receive necessary medical treatment, at no additional cost to you. By signing this consent form you are not releasing the investigator(s), institution(s) and/or sponsor(s) from their legal and

professional responsibilities. If you suffer a research-related injury, please call Dr. Craig Steinback at 780-492-5553. Should you need urgent medical care, please go to the hospital.

What if I have questions?

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

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

Title of Study: The effects of acclimatization to hypoxia on wakeful and sleeping ECG and heart rhythm

Principal Investigator: Dr. Craig Steinback. PhD **Phone Number: 780-492-5553**

Research Coordinator: Lindsey Berthelsen, B.H.K.; Emily Vanden Berg, BSc; Andrew Steele, BSc (KIN)

	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 IV: Lake Louise AMS Scoring System

LAKE LOUISE AMS SCORING SYSTEM (2018 Version)		
Name and Participant ID:		
Date and Time (morning or night):		
Location and Altitude:		
Instructions: Please record the number of each item to correspond to HOW YOU FEEL AT THIS PRESENT MOMENT. PLEASE ANSWER EVERY ITEM. If you do not have the specific symptom, please circle [0]. Whole numbers only. If you feel inclined to use a 0.5 score, then round up.		
Self-Assessment Score		
1. Headache		[0] None at all
		[1] Mild Headache
		[2] Moderate Headache
		[3] Severe Headache. Incapacitating
		Headache Score =
2. Gastrointestinal Symptoms		[0] Good Appetite
		[1] Poor Appetite or Nausea
		[2] Moderate Nausea or Vomiting
		[3] Severe Nausea and Vomiting, Incapacitating
		GI Score =
3. Fatigue and/or weakness		[0] Not Tired or Weak
		[1] Mild Fatigue/Weakness
		[2] Moderate Fatigue/Weakness
		[3] Severe Fatigue/Weakness, Incapacitating
		Fatigue Score =
4. Dizziness/light-headedness		[0] No dizziness/light-headedness
		[1] Mild dizziness/light-headedness
		[2] Moderate dizziness/light-headedness
		[3] Severe dizziness/light-headedness, incapacitating
		Dizziness Score =
Sum 1-4 Total AMS Score =		

Note: A score of at least 1 on the headache scoring and a total of score of a 3 (minimum) constitutes the definition of AMS.

Reference: Roach et al. (2018) High Altitude Medicine and Biology