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# The relationship between acute exposure to hypoxia and heart rate variability in unacclimatized individuals

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The Effect of Acute Exposure to Hypoxia on Heart Rate Variability in Unacclimatized Individuals

A thesis submitted in partial fulfillment of the requirement
for Departmental Honors in Kinesiology & Health Sciences from
The College of William and Mary

by

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April 30, 2019

# THE EFFECT OF ACUTE EXPOSURE TO HYPOXIA ON HEART RATE VARIABILITY IN UNACCLIMATIZED INDIVIDUALS

A thesis submitted in partial fulfillment of the requirement for Departmental Honors in Kinesiology & Health Sciences at The College of William & Mary in Virginia

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**ABSTRACT**

Acute exposure to hypoxia results in a stress response categorized by sympathetic dominance, including hyperventilation to prevent arterial desaturation. As an estimation of overall stress, heart rate variability (HRV) reflects the balance between sympathetic and parasympathetic autonomic regulation. PURPOSE: The purpose of this study was to determine whether resting sea level (SL) HRV is correlated with arterial desaturation and respiratory responses to acute normobaric hypoxia exposure (equivalent 3500 m). METHODS: Resting HRV, $\%SpO_2$, and respiratory rate (RR) were measured in 19 subjects (male n=9, female n=10) at SL for 15 minutes. HRV was measured (Firstbeat Bodyguard2) including RMSSD, HF, and LF components. $VO_2$ peak was estimated using a standard graded exercise test (GXT) on a bicycle ergometer. Subjects later returned and resting HRV, $\%SpO_2$, and RR were measured in a normobaric hypoxic chamber (Colorado Mountain Systems, Inc.) set at 3500 m (treatment) or sea level (control). Subjects exercised on a bicycle ergometer at 65% capacity where HR, %SpO2 and RPE were recorded. RESULTS: There was a significant correlation observed in treatment subjects between RR (PIII – PII) and LFHF ratio (PIII - PII) ($p<0.01$), RR (PIII – PII) and PII LFHF ratio ($p<0.05$), and $VO_2$ peak and exercise-induced change in %SpO2 (PIII) ($p<0.05$). No significant correlation was observed between HRV in the time domain (RMSSD) and %SpO2 at rest, %SpO2 during exercise, or respiratory rate during rest ($p>0.05$) when exposed to hypoxia. CONCLUSION: HRV as a measure of overall stress and sympathovagal balance may be predictive of the change in RR, but not in the change in %SpO2. Subsequent studies may require a controlling for key variables (caffeine use, time of day, etc.) or paired study design.

*Keywords: Heart rate variability (HRV), Autonomic Nervous System, Firstbeat Bodyguard, Normobaric Hypoxia, Acute Mountain Sickness (AMS)*

## REVIEW OF LITERATURE

### INTRODUCTION TO HEART RATE VARIABILITY (HRV)

Heart rate (HR) has long been understood to be an excellent indicator of cardiovascular health; however, utility is lost when the complex system is reduced down to a single average number. Fluctuations in HR occur as a result of interactions between multiple body systems, and evaluation of these changes over time provides for a more integrative analysis. Predating the electrocardiograph (ECG), beat-to-beat changes were studied as a diagnostic tool for aging, illness, and psychological states for centuries (Billman, 2011; Ernst, 2014). Stephen Hales (1677-1761) and Carl Ludwig (1816-1895) pioneered early research surrounding these concepts by recording oscillations in heart rate and arterial pressure related to the respiratory cycle (Hales, 1733; Ludwig, 1847). Modernly, this phenomenon is known as heart rate variability (HRV) and is defined as the variation in time between consecutive heartbeats measured by interbeat intervals, as shown in Figure 1 (Ernst, 2014; Appelhans & Luecken, 2006).

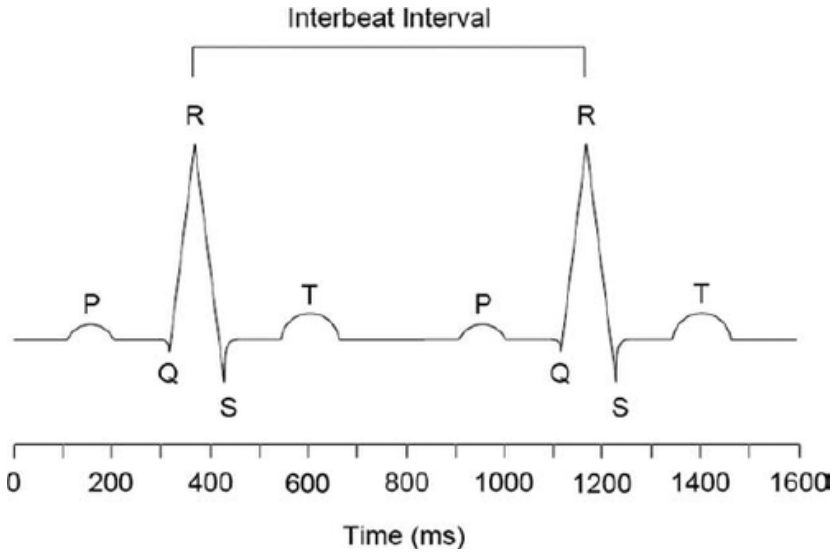


***Figure 1***. *ECG segment representing two heartbeats. The P wave reflects atrial depolarization and contraction. The QRS wave reflects ventricular depolarization followed by contraction. The T wave reflects ventricular repolarization and relaxation. The interbeat interval is defined as the temporal distance between R-spikes, the waveforms corresponding to depolarization of the heart's ventricles (Source: Appelhans and Luecken, 2006).*

The first clinical application of HRV noted that fetal distress could be identified by changes in HRV before changes in HR (Hon & Lee, 1963). Currently, HRV has clinical importance in patient monitoring for acute and chronic care including conditions which pose widespread epidemiologic significance, such as congestive heart failure and diabetes (Adamson et al., 2004; Rothberg, Lees, Clifton-Bligh, & Lal, 2016). Further, with the recent upsurge in high-performance wearable technology, HRV as a factor of physical and psychological stress has been increasingly explored by researchers. This study, among others, aims to quantify this stress response and utilize its predictive value.

As a measure of neurocardiac function, HRV provides a non-invasive way of measuring autonomic nervous system (ANS) activity (Ferreira & Zanesco, 2016; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The ANS controls the function of smooth muscle, heart, endocrine, and exocrine glands and consists of two major components, the excitatory sympathetic and inhibitory parasympathetic, which work antagonistically to influence cardiac activity. In order to maintain homeostasis, the ANS regulates heart rate, blood pressure, respiratory rate, body temperature, and other vital activities. Although the unconscious ANS accomplishes this, it is controlled by centers located in the spinal cord, brain stem, and hypothalamus.

At rest, both sympathetic and parasympathetic systems are active (Levy, 1990). Both branches regulate heart rate by influencing the activity of the sinoatrial (SA) node, the primary pacemaker of the heart, which is located in the right atrium. The SA node generates action potentials, which cause regions of the myocardium to contract and produce a heartbeat. The balance between sympathetic and parasympathetic activity, the sympathovagal balance, provides an estimation of stress in an individual at any given time (Pagani et al., 1984). Sympathetic

dominance, reflected by a low HRV, is indicative of physical, psychological, or other internal or external stressors. This is controlled through the mediation of norepinephrine (NE) which excites cardiac muscle and increases heart rate (Kemp & Quintana, 2013). Parasympathetic dominance, reflected by a high HRV, has the opposite effect and is associated with recovery status or stress toleration. In contrast to the sympathetic nervous system, the parasympathetic nervous system releases acetylcholine (ACh) which inhibits cardiac muscle and slows HR (Kemp & Quintana, 2013). These two different signaling methods vary by their temporal effects on cardiac activity. The activity of the sympathetic branch produces a relatively slow response, resulting in a peak effect about 4 seconds after neurotransmission (Appelhans & Luecken, 2006). In contrast, parasympathetic influence produces a rapid response with a peak effect only 0.5 seconds after neurotransmission and, because of this, it is the dominant system in HRV (Appelhans & Luecken, 2006). Due to these differences, oscillations in heart rate produced by the two branches occur at different speeds, or frequencies, and serve as the basis of frequency-based HRV analyses, which will be discussed in detail later.

Intrinsically, HR is between 100-120 beats per minute (bpm) without extrinsic neural or hormonal influence in healthy adults (Jose & Collison, 1970). In order to adjust to the rapidly changing environment to meet cell demands for oxygen and nutrients, the cardiovascular system must be capable of variation. An individual's ability to adjust on a moment-to-moment basis is indicative of the flexibility of the ANS, or the capacity to respond to environmental stimuli; however, problems arise when the body is unable to adapt. For example, during physically demanding or stressful periods, the body requires an increase in sympathetic activity to perform the task. Similarly, parasympathetic activity should be dominant during periods of recovery and sleep in order to restore energy levels. Consequently, a long-term imbalance of sympathetic and

parasympathetic activity may lead to poor health outcomes (Thayer, Yamamoto, & Brosschot, 2010).

Most conveniently and precisely, heart rate variability is found by measuring distances between QRS complexes in milliseconds. This period may be referred to as the R-R interval, interbeat interval, or heart period (Berntson et al., 1997). This measurement may take place over a time period ranging from minutes to days - short-term HRV is classified as between 5-20 minutes, while long-term HRV is classified between 12-24 hours (Ernst, 2014). Variation due to respiration, a concept known as respiratory sinus arrhythmia (RSA), accounts for a major portion of the temporal changes in heart rate and is necessary to improve efficiency of gas exchange (Yasuma, 2004). During inspiration, the R-R interval is shortened, while during expiration, the R-R interval is prolonged as demonstrated in Figure 2 (Crespo-Ruiz, B., Rivas-Galan, Fernandez-Vega, Crespo-Ruiz, C., & Maicas-Perez, 2018). Because this phenomenon is mediated entirely by the parasympathetic nervous system, RSA is used as an estimate of parasympathetically mediated HRV.

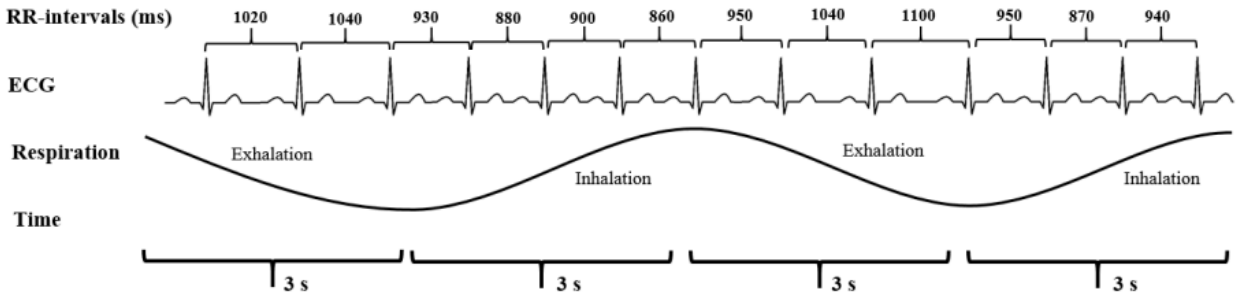


***Figure 2***. *ECG exhibiting RSA. R-R interval shortens (HR increases) during inspiration and R-R interval lengthens (HR decreases) during expiration (Source: Crespo-Ruiz, B., Rivas-Galan, Fernandez-Vega, Crespo-Ruiz, C., & Maicas-Perez, 2018).*

Recent studies concerning HRV have reported higher indexes to be associated with reduced morbidity and mortality (Thayer, Yamamoto, Brosschot, 2010), psychological well-being (Kemp & Quintana, 2013), and physical fitness (Teisala et al., 2014). Similarly, chronic

and acute stress, both physical and psychological, has been associated with lower HRV (Thayer, Åhs, Fredrikson, Sollers, & Wager, 2012). Sympathetic dominance, characteristic of low HRV, is associated with cardiovascular diseases such as hypertension and heart failure (Carter & Chester, 2014). Of the many benefits of physical activity, one is the improvement of the body's adaptive abilities. Physical activity protects against stress by giving the individual more resources for flexible adaptation of the neuroendocrine and cardiovascular systems (Gerber and Pühse, 2009). In a theory known as "cross-stressor adaptation," regular physical activity leads to adaptations in response to both exercise and psychological stressors (Forcier et al., 2006; Sothmann et al., 1996).

**QUANTITATIVE MEASUREMENT OF HRV**

HRV may be quantitatively assessed by both time and frequency domain indexes which reflect ANS activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Within the time domain approach, descriptive variables such as the mean R-R interval, mean heart rate, and the range (longest R-R minus shortest R-R) are calculated. Statistical analysis can then be applied to obtain the root mean square successive R-R interval, or RMSSD, measuring the total variability that arises from both periodic and random sources.

While valuable for ease of calculation, time domain measures provide less detailed information than those of the frequency domain. Power spectral density analysis produces a decomposition of the total variance of a series of heart beats into its frequency components (Berntson et al., 1997; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). This technique provides a quantification of low- and high-frequency oscillations of R-R intervals, reflecting autonomic

modulation of the SA node. The spectral power for a given frequency can then be found by determining the area under the curve within a specific range of frequencies (Berntson et al., 1997). Spectral methods used for analysis are classified as parametric and nonparametric. One such nonparametric method commonly used is the fast Fourier transform (FFT) algorithm. The FFT decomposes a function of time into the frequencies that make it up and assumes that the series only contains deterministic components (Berntson et al., 1997). The autoregressive (AR) method is parametric and treats data as a composite of deterministic and stochastic components (Berntson et al., 1997). This may be advantageous as smoother spectral components can be distinguished, allowing for an accurate estimation of power spectral density (PSD) by calculating low- and high-frequency power components. Both methods are shown in Figure 3 as analyzing the given R-R interval time series in the frequency domain (Pichon et al., 2004).

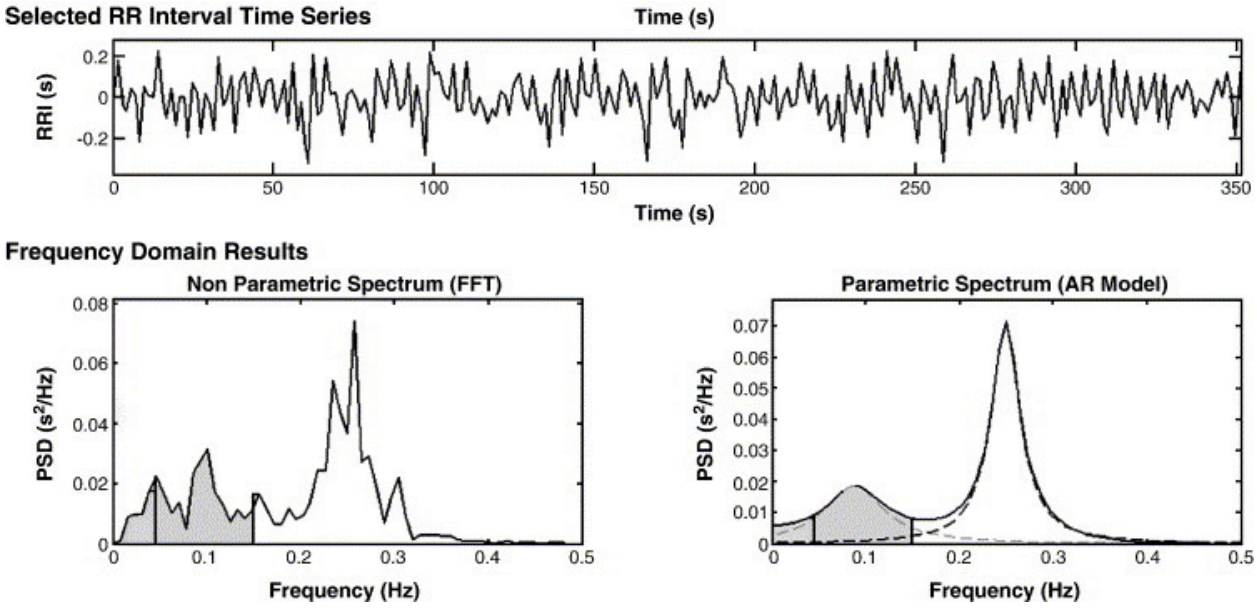


***Figure 3.*** *Tachygram of R-R interval time series (after detrending); HRV representations of nonparametric (FFT) and parametric (AR) for a single subject (Source: Pichon et al., 2004).*

From the FFT and AR method, frequencies are categorized into three main peaks: very low frequency (VLF) < 0.04 Hz, low frequency (LF) 0.04-0.15 Hz, and high frequency (HF) 0.15-0.4 Hz. The HF component estimates cardiac vagal tone, and the LF component estimates

sympathetic activities (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The HF band corresponds to breathing frequency and is associated with parasympathetic modulation of cardiac activity. The LF band corresponds to oscillations in arterial pressure and is associated with mixed sympathetic and parasympathetic modulation. The LF/HF ratio has been used as a marker of autonomic interaction, or sympathovagal balance, although this concept has been challenged by recent literature (Billman, 2013; Pagani et al., 1984). Research indicates that an increase in total spectral power and HF modulation is associated with increased performance (Hamlin et al., 2011).

**FIRSTBEAT TECHNOLOGIES**

Firstbeat software uses HRV to calculate values and assign them to variables that indicate the current state of the body. In this study, the Firstbeat Bodyguard 2 device was used to record heart rate variability. The Firstbeat Bodyguard 2 is a R-R interval and movement data recording device that can be used for short- and long-term measurements. It is designed for 24-hour recordings and can be used during exercise, recovery, and sleep. The device is unobtrusive and is attached directly to the skin with two chest electrodes and and data is recorded automatically. Data from Bodyguard 2 was downloaded directly to Firstbeat SPORTS software. The Firstbeat Bodyguard 2 device has been used in a variety of research studies and by healthcare professionals as a diagnostic tool (Hallman, Ekman, & Lyskov, 2014; Teisala et al., 2014).

The Firstbeat Bodyguard 2 device records R-R interval data, which is then scanned through an artifact detection filter to correct for falsely detected, missed, and premature heartbeats. The artifact-corrected R-R intervals are then re-sampled at a rate of 5 Hz by using linear interpolation. Low frequency trends and variables below and above the frequency band of

interest are removed using a polynomial filter and digital FIR band-pass (0.03-1.2 Hz) filter. The software then calculates values for the following variables: root mean square successive R-R intervals (RMSSD), high-frequency power (HFP, 0.15-0.40 Hz), low-frequency power (LFP, 0.04-0.15 Hz), and amplitude of the respiratory sinus arrhythmia (RSA). Using these variables as well as neural network modeling of the data, the software calculates values representing physiological phenomena, such as respiration rate (RR), oxygen consumption (VO2), and excess post-exercise oxygen consumption (EPOC). These variables are used to determine the individual's current physiological state from one of five categories as shown in Figure 4 (Firstbeat Technologies, 2014). Stress reactions, shown in red, are characterized by dominance of the sympathetic nervous system and are associated with high HR, low HRV, increased respiratory rate, and an oxygen uptake less than 20% of $VO_2$max. Recovery periods (green) are characterized by parasympathetic dominance and are associated with low HR, high HRV, lower respiratory rate, and oxygen uptake less than 20% of $VO_2$ max. Physical activity (dark blue) is marked by an intensity greater than 30% of maximal capacity. Daily physical activity (light blue) is lower-level physical activity (i.e. walking, housework, etc.) where intensity is between 20-30% of maximal capacity. The other state (white) is recovery from exercise, short awakenings during sleep, or otherwise missing data periods.

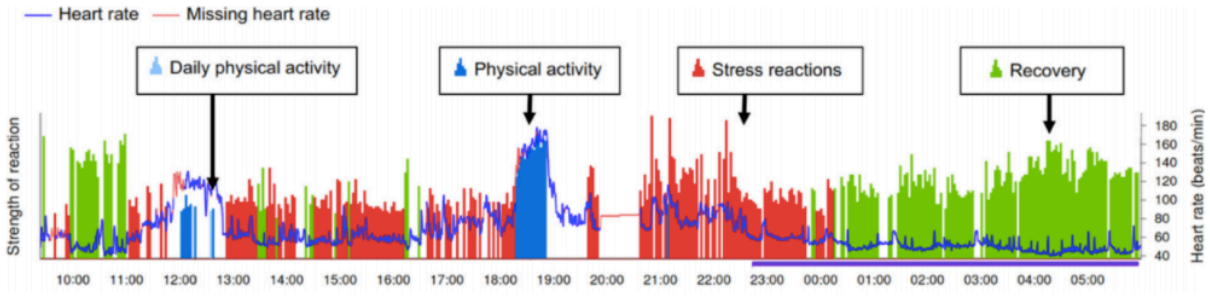


***Figure 4.*** *An example of a Firstbeat report. Five physiological states are characterized by stress (red), recovery (green), physical activity (dark blue), daily physical activity (light blue), and white (missing data periods). (Source: Firstbeat Technologies Ltd., 2014).*

**HYPOXIA**

Hypoxia is a state of decreased oxygen availability in body tissues occurring in environments of higher altitude. In these environments, the partial pressure of oxygen is lower compared to sea level. This causes a reduction in the partial pressure of arterial oxygen, thus reducing arterial oxygen saturation (%$SpO_2$). High-altitude conditions may be simulated in a normobaric laboratory setting with equipment that reduces the fraction of inspired oxygen. The reaction to hypoxia is immediate and is characterized by increased ventilation, heart rate, stroke volume, systemic arterial pressure, and cardiac output (Reeves, Mazzeo, Wolfel, & Young, 1992). This phenomenon is referred to as the hypoxic ventilatory response (HVR) (Powell, Milson, & Mitchell, 1998). Frequency (respiratory rate) and amplitude (volume) increase immediately after exposure; however, frequency tends to decline during the stimulation period (Powell et al., 1992). Hypoxia stimulates peripheral chemoreceptors to activate the sympathoadrenergic axis, inducing physiological adjustment process to direct blood flow to organs with the greatest metabolic need (Maher, Manchada, Cymerman, Wolfe, & Hartley, 1975; Somers, Mark, Zavala, & Abboud, 1989). This increase in sympathetic activation is categorized by increased plasma concentration of catecholamines (Reeves et al., 1992) and β-adrenergic receptor desensitization (Richalet, Kacimi, & Antezana, 1992).

Several studies have investigated the correlation between hypoxia and heart rate variability in both temporal and frequency domains. Several studies have demonstrated a relative increase in LF and LF/HF ratio indicative of sympathetic dominance (Roche et al., 2002) while other studies noted a decrease in RMSSD (Povea et al., 2005). One study by Buchheit et. al (2004) investigated the sympathovagal balance, inferred by measurement of HRV, as a response to acute hypoxia at rest and during exercise in order to predict the ability of an individual to

adapt to high altitude. Subjects were evaluated during a standardized hypoxic tolerance test, developed by Rathat et al. (1992), consisting of four periods, alternating rest and moderate exercise at 50% $VO_2$ max in normoxic and normobaric hypoxic (11.5% O2, simulated 4,800 m) conditions. The ventilatory responses and HRV indexes were measured for the last five minutes of each period. These indexes help to stratify subjects as a function of their response to the hypoxic environment. The results of this study found that hypoxia at rest induced a decreased RMSSD and absolute HF power in well-tolerant subjects. Also, the HF/(LF+HF) ratio was significantly increased during exercise in hypoxia compared to normoxia, along with ventilation and tidal volume. These results are indicative of vagal control withdrawal under hypoxia at rest.

Acute mountain sickness (AMS) is a syndrome encountered at altitude. Symptoms include headache, nausea, malaise, dizziness, and difficulty sleeping. Exercise is known to exacerbate acute mountain sickness (Roach, et al., 2000). This is significant as many athletes, particularly those in endurance events, choose to train at altitude to increase their performance (Lundby, Millet, Calbet, Bärtsch, & Subudhi, 2012).

## METHODS

### SUBJECTS

Nineteen apparently healthy, male (n=9) and female (n=10) 19-to 35-year-old informed volunteer subjects were tested in the Jack Borgenicht Normobaric Hypoxia Chamber, Department of Kinesiology & Health Sciences at the College of William & Mary, Williamsburg, VA 23187. The study was approved by the College of William & Mary Protection of Human Subjects Committee. All of the subjects were nonsmokers, none were born at an altitude greater than 1,500 m or had traveled to altitudes greater than 1,500 m during the preceding 6 months, and all were screened through a medical history questionnaire (Appendix A) for evidence of any

condition that would make participation in the study more hazardous. All health history forms were reviewed by the project medical director. If approved for the study, each subject gave written, informed consent (Appendix B) prior to their first session in which they were familiarized with all testing procedures and the testing environment.

**RESEARCH LOCATION**

The Jack Borgenicht Hypoxia/Altitude Physiology Research Facility (JBARF) is located in Adair Hall, Room 108 on the campus of The College of William & Mary in Williamsburg, Virginia. The laboratory is located at an altitude of approximately 15 meters (49 feet) with a standard barometric pressure of 752 mmHg which is approximately sea level but varies slightly depending on weather conditions. The facility consists of a normobaric hypoxia chamber (Colorado Altitude Training Systems, Boulder, CO) within which the partial pressure of oxygen can be adjusted to simulate atmospheres found at altitudes from sea-level to 7,645 meters (25,000 ft). The normobaric hypoxia chamber operates at a "normal" sea level atmospheric pressure of approximately 760 torr. The air units associated with this chamber extract oxygen from external air that is then pumped into the chamber and maintain a preset simulated altitude while subjects are inside.

The fraction of $O_2$ in the atmosphere remains the same at 20.9% regardless of the altitude. Therefore, the atmospheric pressure at sea-level (760 mmHg) corresponds to a 159 mmHg partial pressure of $O_2$. The balance is made up of nitrogen, inert gases, and a very small amount of carbon dioxide. At 4,300 meters (14,100 feet), the barometric pressure is 462 mmHg, producing a partial pressure of oxygen of 96 mmHg (20.9% $O_2$ fraction). This reduced partial pressure pushes oxygen into the bloodstream across a lower gradient, resulting in less oxygen carried by the blood to the body tissues. This environment of lower oxygen (hypoxia) can result in Acute

Mountain Sickness (AMS) and, in extreme cases, more serious illnesses such as High-Altitude Pulmonary Edema (HAPE) or High-Altitude Cerebral Edema (HACE).

## EXPLANATION OF VARIABLES

In addition to heart rate variability variables described above, several other parameters were measured in this study. $VO_2$ peak was calculated by performing a standard graded exercise test (GXT) on a stationary bicycle ergometer. $VO_2$ peak is defined as the highest value of $VO_2$ attained on a particular test designed to bring the subject to the limit of tolerance (Whipp, 2010). $VO_2$ peak does not necessarily equate to the $VO_2$ max which is the highest value attainable by the subject and is dependent on a particular set of criteria to be met. Typically, standard graded exercise tests on a bicycle are not considered to be $VO_2$ peak tests unless the subject is an experienced cyclist. The $VO_2$ peak will occur ideally right as the subject begins to plateau in their volume of oxygen consumption, as demonstrated by the open circle in Figure 5 (Whipp, 2010). Rating of Perceived Exertion (RPE) was also measured during this time by asking subjects how they felt at specific time intervals. The RPE chart used was on a scale of 6 to 20, with 20 being the absolute most effort they are physically capable of exerting.

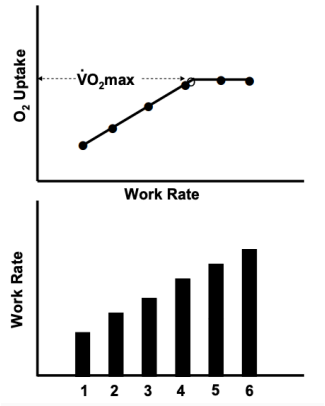


***Figure 5.*** *Schematic and idealized representation of the end-bout oxygen uptake to a series of constant work rate tests performed to the steady state or to the limit of tolerance (Source: Whipp, 2010).*

Percentage of oxygen saturation (%$SpO_2$) was another variable that was assessed in this study. %$SpO_2$ is the percentage of oxygenated hemoglobin compared to the total amount of hemoglobin in the blood. %$SpO_2$ can be measured by pulse oximetry, a non-invasive method that works by emitting and absorbing a light wave passing through blood vessels in the fingertip. The degree of oxygen saturation causes variation's in the bloods color. Normal $SpO_2$ values, at sea level, vary between 95 and 100% (Mayo Clinic, 2018). If the level is below 90%, it is considered hypoxemia and is considered a clinical emergency (World Health Organization, 2011).

End-tidal carbon dioxide ($EtCO_2$) monitoring measures the partial pressure of carbon dioxide during expiration which is expressed as a percentage of $CO_2$ or in mmHg. $EtCO_2$ is reflective of ventilatory status, including cardiac output (CO) and pulmonary blood flow. This non-invasive technique makes use of a capnograph. The normal range of values for $EtCO_2$ is 5 to 6% $CO_2$, equivalent to 35-45 mmHg (American Association of Sleep Technologists, 2018).

**PROTOCOL**

After approval and voluntary informed consent, each subject was familiarized with the testing procedures, equipment, and environment in the Jack Borgenicht Altitude Physiology Research Facility. Subjects were divided into two groups; Control Group (n=6) and Treatment Group (n=13). After the familiarization session, subjects returned for their sea level measurements and VO2peak test. The third session was conducted between 48 hours to one week after the second session. All treatment subjects were exposed to a normobaric simulated altitude of 3,500 m (11,500 feet) for approximately 45 minutes during the third session. Controls followed the same protocol except the chamber air was not filtered of any oxygen as it passed through the air units, thus maintaining sea level partial pressure of oxygen in the chamber

atmosphere. Measurements were made of height (standard fixed stadiometer) and weight (Pelstar Health-O-Meter, McCook, IL), oxygen saturation ($SpO_2$) (Nonin 8500 Pulse Oximeter, Plymouth, MN), respiratory rate (RR) and resting end-tidal $CO_2$ ($EtCO_2$) (Nellcor N-85 Capnograph, Pleasanton, CA), heart rate (HR) (Polar H10, Kempele, Finland), ventilation (VE) (Firstbeat BodyGuard2, Jyväskylä, Finland), and heart rate variability (HRV) (Firstbeat BodyGuard2, Jyväskylä, Finland).

During the second session, each subject sat quietly at SL for approximately 15 minutes while having their oxygen saturation of hemoglobin (% SpO2), heart rate (HR), heart rate variability (HRV), and end-tidal carbon dioxide (EtCO2) measured. Subjects then warmed up in the chamber on a stationary bicycle ergometer (Lode 906900, Groningen, Netherlands) for 3 minutes. Subjects then underwent a standard graded exercise test (GXT) at SL on a bicycle ergometer to voluntary exhaustion. This maximum physical effort test yielded VO2peak data from which hypoxia test parameters were calculated. VO2 and SpO2 (VacuMed Vista MX and Calibringe, Ventura, CA), HR, and, rating of perceived exertion (RPE) were recorded at rest and at every 2-minute stage of the GXT. Once started, the entire GXT test lasted no longer than 12 minutes. Between 48 hours and one week following GXT, the subject returned to the laboratory and entered the normobaric hypoxia chamber in which the atmospheric content of oxygen was reduced to a percentage whose partial pressure is equivalent to that found at an altitude of 3,500 meters (11,500 ft.) or, in the case of control subjects, sea level. After entering the chamber, the subject rested quietly for approximately 15 minutes during which time EtCO2, HR, and SpO2 was again measured. The subject then warmed up in the chamber on a stationary bicycle ergometer for 3 minutes, then pedaled the ergometer at a HR equivalent to that recorded at 65% of their SL VO2peak. By matching target HR, the relative exercise intensity is equal at SL and at 3,500 meters. At rest and every 2 minutes during 10 minutes of bicycle exercise at a HR equal

to that recorded at 65% SL VO2peak, SpO2, HR, and RPE was recorded and compared to measurements taken during SL testing. Resting ventilation SpO2, HR, and EtCO2 at altitude was also compared to SL data.

Heart rate variability analysis was performed in each subject by manual selection of a 10-minute time interval. For the resting state at SL, this interval was chosen by visually identifying when the subject was experiencing a steady state of rest. This typically began about 2 minutes after the subject began resting. For the resting state at altitude, this interval included the first 10 minutes just after sitting down, in order to properly represent the hypoxic ventilatory response (HVR) which occurs immediately after exposure to hypoxia.

## STATISTICAL ANALYSIS

Statistical analysis was performed using the IDE RStudio for R using Version 1.1.456 for Mac from (http://www.rstudio.org). Relationships between variables were analyzed by calculating Pearson correlation coefficients. Differences were considered statistically significant when $p < 0.05$. Paired t-tests were conducted to assess if there was a significant difference between variables in hypoxia versus normoxia.

## RESULTS

Table 1 presents the demographic data of the participants, indicating the distribution of Asian (15.7%), white (63.2%), and Hispanic/Latino (21.1%) subjects within the study population. Table 2 presents further descriptive data of the participants including height (m), weight (kg), BMI ($kg/m^2$), age (years), and $VO_2$ peak (mL/kg/min). The mean BMI of female and male subjects was 22.4 and 23.0 $kg/m^2$, respectively, with all but two subjects within the normal limits of 18.5 to 24.9 $kg/m^2$. Subjects ranged in age from 19 to 35 years old with a mean age of 21 years old. The mean $VO_2$peak of female and male subjects was 45.6 and 55.8

mL/kg/min, respectively. This indicates that the average female subject was in the "above average" range (Table 3) and the average male subject was in the "good" range (Table 4). This is graphically represented in Figure 6 to further highlight the skew in the data towards more "fit" participants.

At rest, exposure to acute normobaric hypoxia increased ventilation (VE) ($p<0.05$) and decreased SpO2 ($p<0.0001$) and EtCO2 ($p<0.05$). There was no significant difference between HR ($p>0.05$) or RR ($p>0.05$) at hypoxia versus normoxia. There was no significant difference in the HRV in the temporal or frequency domain including RMSSD ($p>0.05$), LF component ($p>0.05$), HF component ($p>0.05$), and LFHF ratio ($p>0.05$). These changes are all expressed as percentage change in treatment and control subjects in Figure 7. Additionally, exercise in hypoxia caused a decrease in $SpO_2$ ($p<0.0005$).

Figure 8 demonstrates the change in oxygen saturation (% $SpO_2$) from Phase II to Phase III in control and treatment subjects. Treatment subjects experienced a significantly greater desaturation upon entering the chamber. Figure 9 demonstrates the change in oxygen saturation (% $SpO_2$) from rest to exercise in control and treatment subjects during Phase III (in chamber). Treatment subjects experienced a significantly greater desaturation during the duration of exercise.

There was a significant correlation observed in treatment subjects between the difference in RR (PIII – PII) and difference in LFHF ratio (PIII - PII) ($p<0.01$), difference in RR (PIII – PII) and PII LFHF ratio ($p<0.05$), and $VO_2$ peak and $\%SpO_2$ (PIII exercise – PIII rest) ($p<0.05$). These results are graphically represented in Figures 10, 11, and 12, respectively. No significant correlation was observed between HRV in the time domain (RMSSD) and %SpO2 at rest

(p>0.05), %SpO2 during exercise (p>0.05), or respiratory rate during rest (p>0.05) when exposed to hypoxia.

## CONCLUSION

In conclusion, HRV as a measure of overall stress and sympathovagal balance may be predictive of respiratory changes, though not in the change in $\%SpO_2$. Sea level (Phase II) LFHF ratio was predictive of the respiratory response (Phase III – Phase II) during exposure to hypoxia, as was the difference between Phase II and Phase III measures of LFHF ratio. $VO_2$ peak was predictive of oxygen desaturation from rest to exercise during exposure to hypoxia, indicating that more fit individuals may have a maladaptive response during hypoxia exposure. There was no correlation between HRV measures in the time or frequency domain and $VO_2$ peak as a measure of fitness capacity. This result may be due to external factors (caffeine use, medication use, current stress level, psychological disorders) which may affect measures of HRV that this study did not control for. Although there were several observed relationships between heart rate variability components and physiological variables, more subjects may be needed to establish statistical significance of the findings. Similarly, same-day measurements of sea level and hypoxia may be beneficial as HRV is highly variable for any given individual. Future studies should ensure that they recruit individuals more representative of the general population in order to not skew results towards those more physically fit.

## FIGURES

***Table 1.*** *Demographic Data on Participants*

| | Asian | White | Hispanic/Latino | Total |
|---|---|---|---|---|
| Females | 1 | 6 | 3 | 10 |
| Males | 2 | 6 | 1 | 9 |
| Total | 3 (15.7%) | 12 (63.2%) | 4 (21.1%) | 19 |

***Table 2.*** *Descriptive Data on Participants (Mean ± SD)*

| | Height (meters) | Weight (kilograms) | BMI ($kg/m^2$) | Age (years) | $VO_2$ Peak (mL/kg/min) |
|---|---|---|---|---|---|
| Females | 1.66 ± 0.068 | 61.6 ± 9.08 | 22.4 ± 2.84 | 21.4 ± 4.83 | 45.6 ± 7.47 |
| Males | 1.80 ± 0.089 | 75.1 ± 12.1 | 23.0 ± 2.84 | 21.6 ± 2.76 | 55.8 ± 8.51 |

***Table 3.*** *Maximal oxygen uptake norms for men (mL/kg/min)*

| Age | Excellent | Good | Above Avg | Average | Below Avg | Poor |
|---|---|---|---|---|---|---|
| 18-25 | >60 | 52-60 | 47-51 | 42-46 | 37-41 | 30-36 |
| 26-35 | >56 | 49-56 | 43-48 | 40-42 | 35-39 | 30-34 |

***Table 4.*** *Maximal oxygen uptake norms for women (mL/kg/min)*

| Age | Excellent | Good | Above Avg | Average | Below Avg | Poor |
|---|---|---|---|---|---|---|
| 18-25 | >56 | 47-56 | 42-46 | 38-41 | 33-37 | 28-32 |
| 26-35 | >52 | 45-52 | 39-44 | 35-38 | 31-34 | 26-30 |

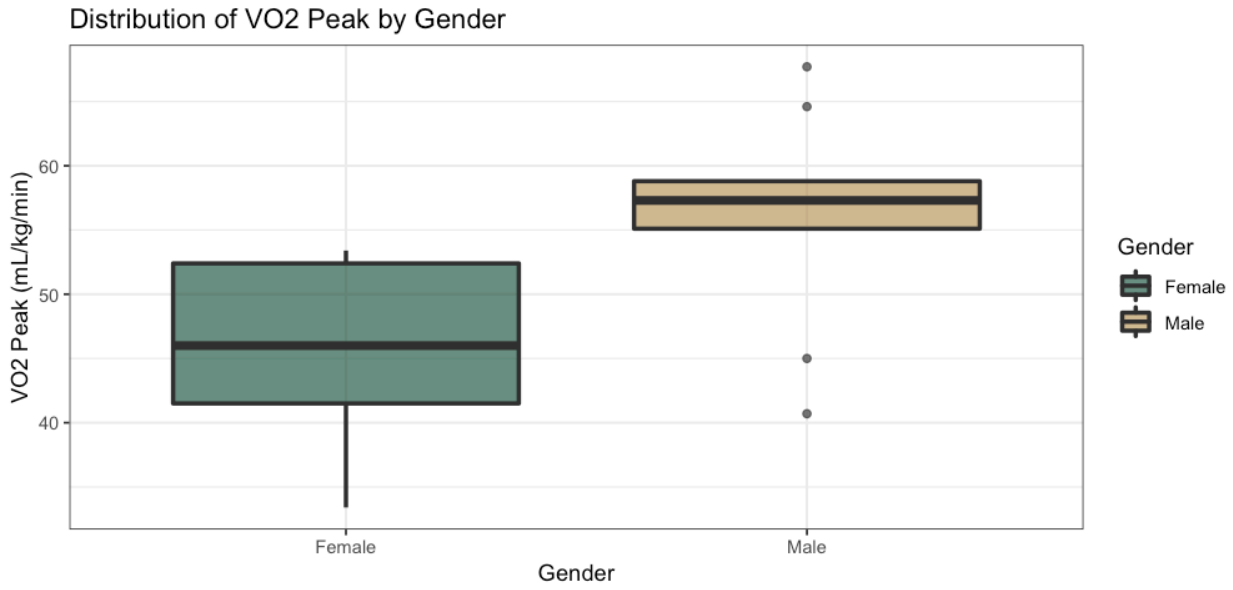


***Figure 6.*** *Distribution of $VO_2$ Peak Values by Gender*

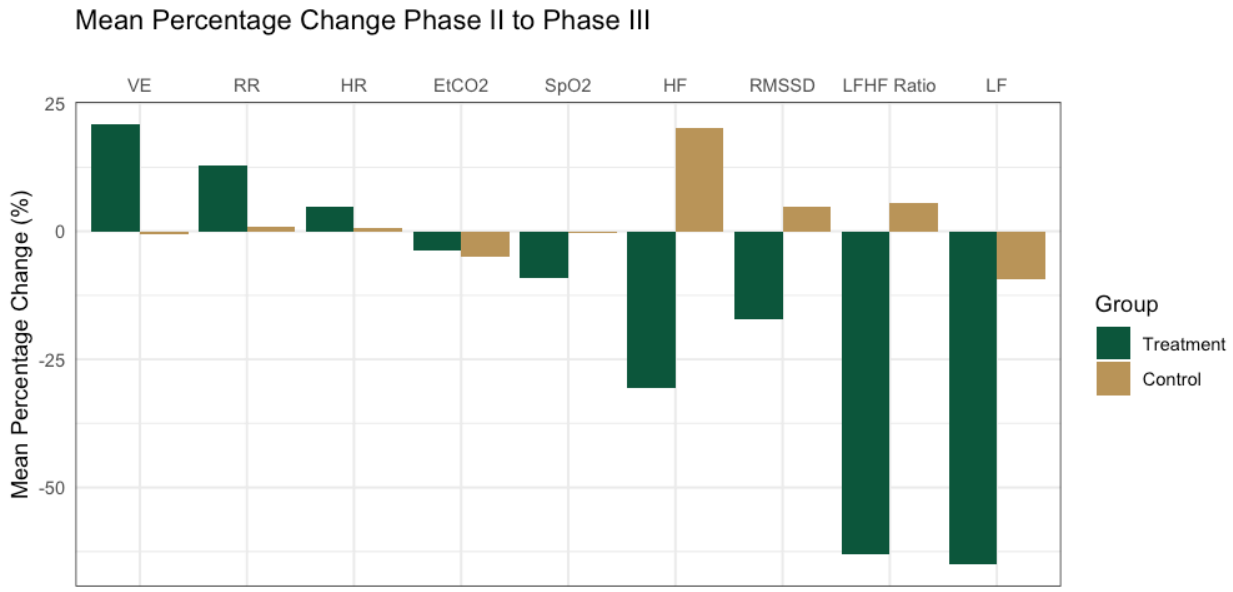


***Figure 7.*** *Mean Percentage Change (%) in Variables in Treatment and Control Subjects*

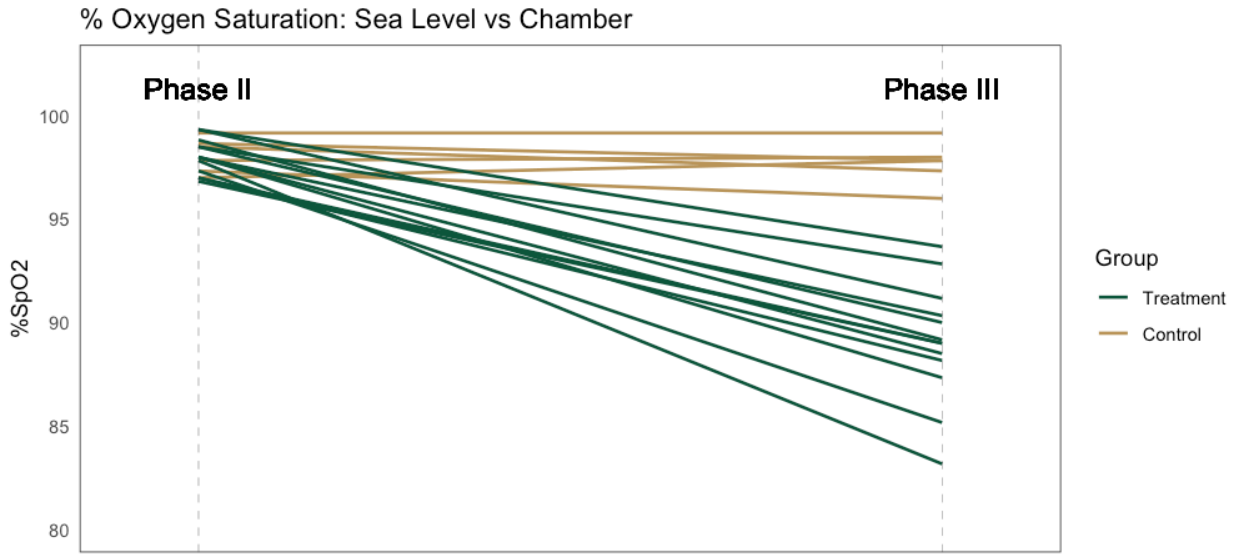


***Figure 8.*** *Oxygen Saturation (%SpO2) During Phase II and Phase III Rest in Treatment and Control Subjects*

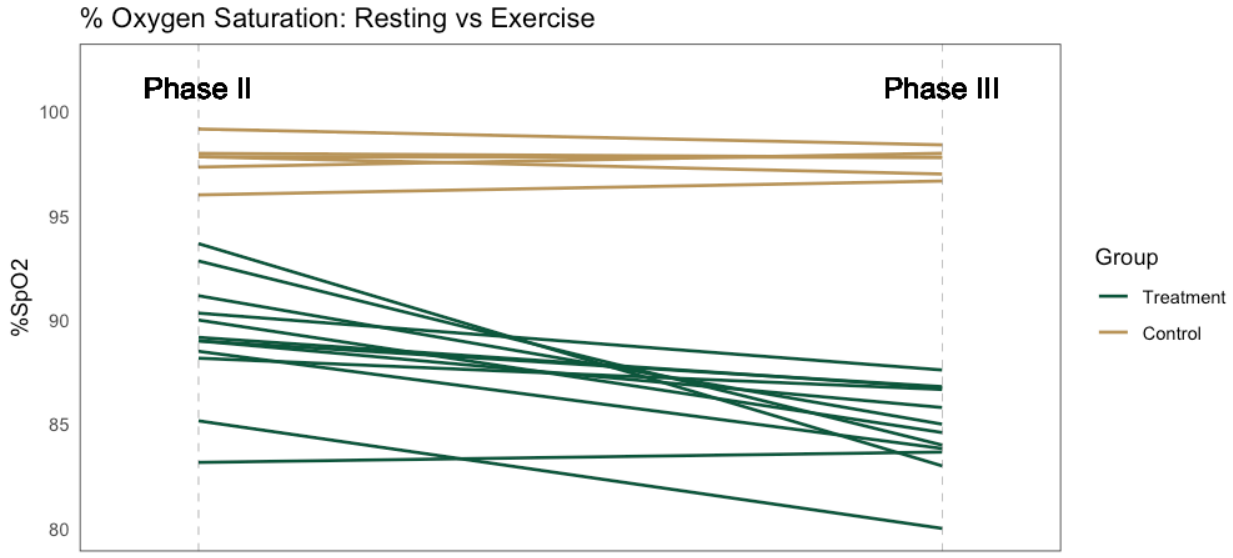


***Figure 9.*** *Oxygen Saturation (%SpO2) During Phase III Rest and Exercise in Treatment and Control Subjects*

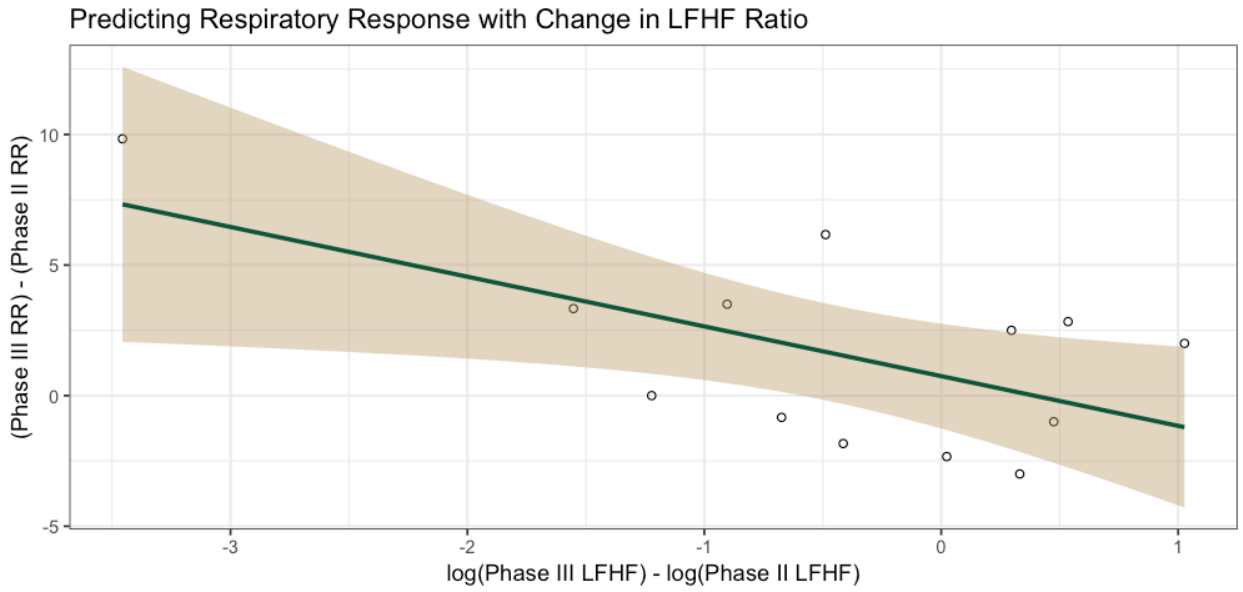


***Figure 10.*** *Change in LFHF Ratio from Phase II to Phase III on a Logarithmic Scale vs Hyperventilatory Response (PIII RR – PII RR)*

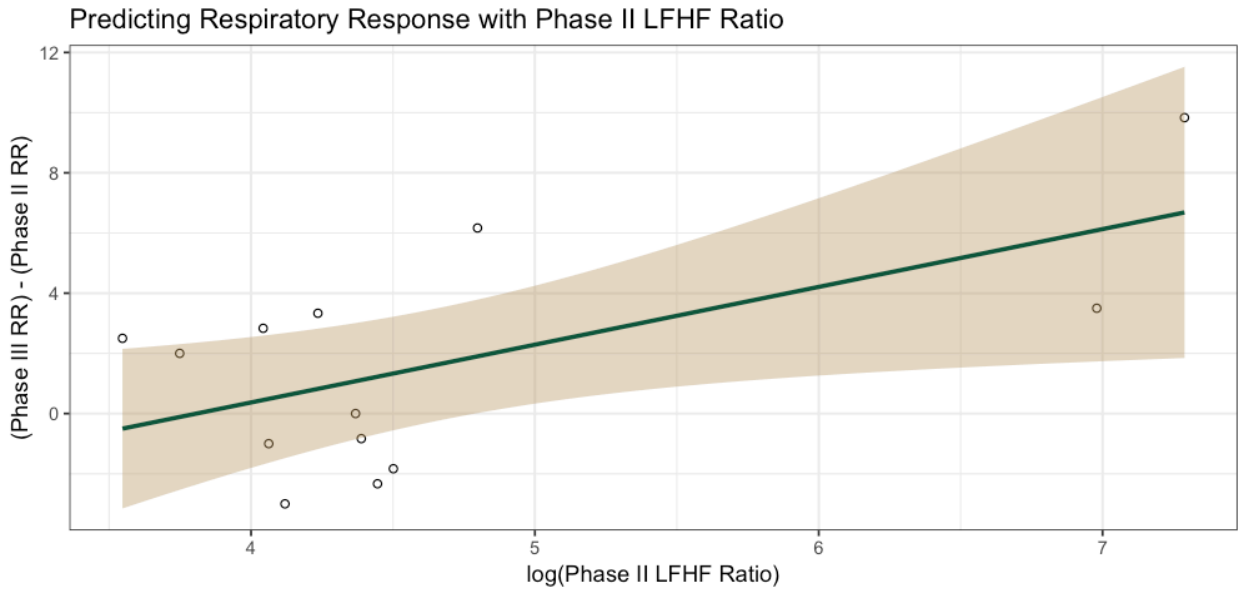


***Figure 11.*** *Phase II LFHF Ratio on a Logarithmic Scale vs Hyperventilatory Response (PIII RR – PII RR)*

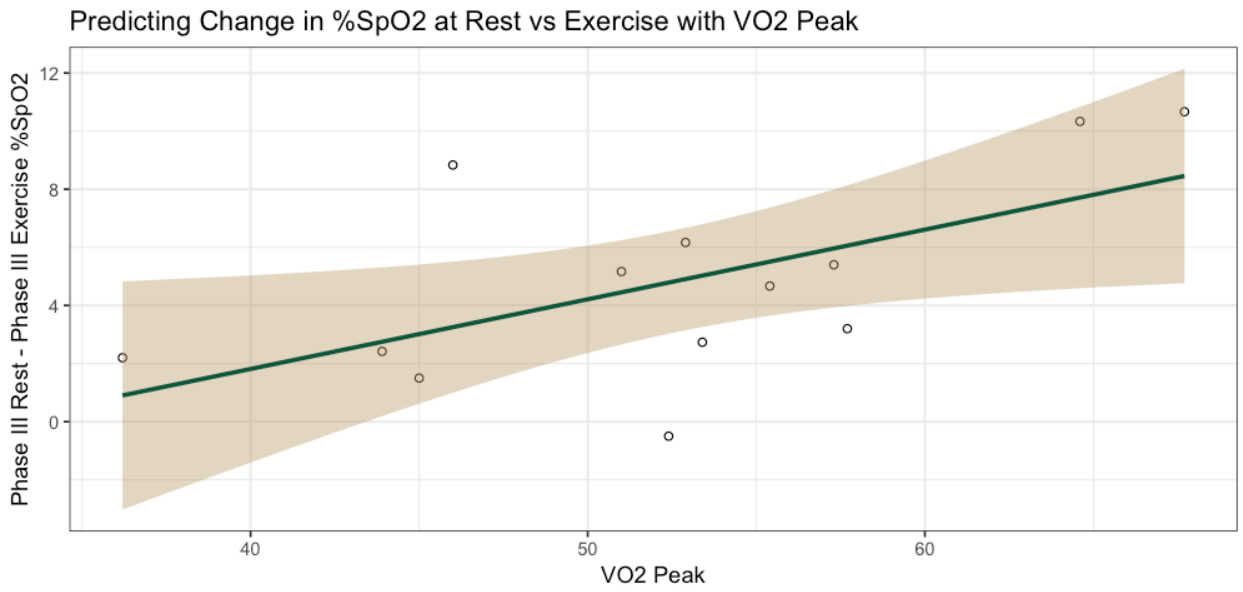


***Figure 12.*** *Change in Oxygen Saturation (%SpO2) at Rest vs Exercise in Hypoxic Conditions Predicted by VO2 Peak*

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I would like to acknowledge several of my peers, Hunter Embry and Kelsey Short, and thank them for their tireless dedication and thoughtful input. Most of all, thank you to all of the volunteers who participated in this study. It takes a certain kind of person to voluntarily expose themselves to adverse conditions for the sake of scientific research. Without an ounce of madness, this research would not have been possible. Thank you.

*Grace Kathryn Becker*

**APPENDIX A**

MEDICAL HISTORY

To act as a volunteer in the research study: PERSISTENCE OF ACCLIMATION TO NORMOBARIC SIMULATED ALTITUDE

Name: _____ Date: _____

Date of Birth: _____ Gender: M F

Contact Phone Number: _____

1. How often do you take part in physical activity or sports?

Not at all: _____. Days per week: _____.

2. What types of physical activity or sports do you usually participate in?

3. How would you compare yourself to others of your own gender and age in terms of physical ability and fitness?

Poor ___ Fair ___ Average ___ Above Average ___ Superior ___

4. Describe yourself in terms of physical activity:

Inactive ___ Moderately Active ____ Active ____ Very Active ____

5. Check which of the following respiratory problems you have or have had:

__Asthma __Emphysema __Bronchitis __Hyperventilation (fast breathing) __Chronic Cough

__Shortness of breath __Other: _____ __None of these

6. Do you presently have any medical problems? Y N

If yes, please indicate the nature of the problem and what therapy and/or medication you are taking:

7. Have you been treated over the past 5 years for anything other than minor illnesses? Y N

If yes, please indicate the nature of the injury or illness, therapy, and length of hospitalization, if appropriate.

8. Have you ever had or have you now?

__Anemia __Sickle cell trait __Sickle cell disease __Hypertension __Diabetes __Tuberculosis __Head injury __Bad headache __Unconsciousness __Sinus problems __Nose/throat trouble __Ear problems __Hearing loss __Ringing in the ears __Eye trouble __Vision problems __Thyroid trouble __Chronic colds __Nervous trouble __Trouble sleeping __Allergies __Dizziness/Fainting __Stomach problems __Stroke __Adverse reaction to __Heart disease __Vascular disease medications __Thalassemia __Family history of __ Nut allergy __ Food allergy heart attack prior to __ Prior history of seizures the age of 50

9. Diet/Medications:

Caffeinated coffee (cups per day): _____. Caffeinated tea (cups per day): _____.

Caffeinated soft drinks or sodas (cans per day): _____. Cigarettes (packs per day): _____.

Cigar (number per day): _____. Pipe (number per day): _____.

Prescription drugs (list if applicable and state reason for use):

**APPENDIX B**

Volunteer Informed Consent

Volunteer Informed Consent
For the research project titled:
INDIVIDUAL VARIABILITY UPON ACUTE EXPOSURE TO A NORMOBARIC HYPOXIC ENVIRONMENT SIMULATING A PHYSIOLOGIC ALTITUDE EQUIVALENT TO 3,500 METERS

I, _____, Date: _____, having full capacity to consent and understanding that I must be at least 18 years old to participate, having attained my ____ birthday, do hereby volunteer to participate in a research study titled: "Individual variability upon acute exposure to a normobaric hypoxic environment simulating a physiologic altitude equivalent to 3,500 meters", Kenneth W. Kambis, Ph.D., Principal Investigator and Professor of Kinesiology & Health Sciences and M. Brennan Harris, Ph.D., Co-Principal Investigator and Associate Professor of Kinesiology & Health Sciences. All research will be conducted in The Jack Borgenicht Altitude Physiology Research Facility, The College of William and Mary. The implications of my voluntary participation; duration and purpose of the research study; the methods and means by which it is to be conducted; and the inconveniences and hazards that may reasonably be expected have been explained to me by Professor Kambis, Contact Phone Number: 757-221-2779, or Professor Harris, Contact Phone Number: 757-221-2757. I have been given an opportunity to ask questions concerning this investigational study. Any such questions were answered to my full and complete satisfaction. Should any further questions arise concerning my rights or study-related injury, I may contact the Chair of the Protection of Human Subjects Committee at The College of William and Mary, Jennifer Stevens, Ph.D. 757-221-3862 jastev@wm.edu. I understand that I may at any time during the course of the study revoke my consent and withdraw from the study without further penalty of loss of benefits. My refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled.

RESEARCH TEAM:
The research team consists of Kenneth W. Kambis, Ph.D., Professor of Kinesiology & Health Sciences (PI) and M. Brennan Harris, Ph.D., Associate Professor of Kinesiology & Health Sciences (Co-PI); and, selected and trained undergraduate students at The College of William and Mary as identified by the PI and Co-PI, who will act as laboratory assistants for this project.

RESEARCH LOCATION:
All aspects of this study will be conducted in The Jack Borgenicht Altitude Physiology Research Facility which is located in Adair Hall, Room 108 on the campus of The College of William and Mary in Williamsburg, Virginia. The facility consists of a normobaric hypoxic room within which the partial pressure of oxygen can be finely controlled to simulate oxygen pressures found in atmospheres at altitudes from sea-level (SL) to 25,000 feet.

OVERVIEW OF STUDY:
Twenty-four apparently healthy, male and female 18-to 35 year-old informed volunteer subjects will be tested in the Jack Borgenicht Normobaric Hypoxia Chamber, Department of Kinesiology & Health Sciences at the College of William & Mary, Williamsburg, VA 23187. The normobaric hypoxia chamber decreases the oxygen content of the chamber atmosphere to a percentage that closely reflects the partial pressure of oxygen in a given hypobaric environment without reducing the atmospheric

pressure. This capability allows for control of oxygen partial pressures similar to those found in hypobaric atmospheres of up to 7,645 meters (25,000 ft.) altitude. Demographics including age, gender, height, weight, and ethnicity will be recorded. A medical history questionnaire will be completed, as will the Environmental Symptoms Questionnaire III. To look for possible gender effects, at least 33% of the volunteer subjects will be women.

After familiarization with all test procedures and equipment, you will rest quietly at SL for approximately 15 minutes while having your oxygen saturation of hemoglobin (SpO2), heart rate (HR), and end-tidal carbon dioxide (EtCO2) measured. These tests are non-invasive and require only that you sit quietly while breathing through a mouthpiece with a finger clip in place and two electrodes attached to your chest (one under the right clavicle and the other on the lower left ribcage). You will then undergo a standard graded exercise test (GXT) at SL on a bicycle ergometer to voluntary exhaustion. This maximum physical effort test will yield VO2peak data from which hypoxia test parameters will be calculated. This test has been approved by the PHSC at William & Mary on previous occasions and has been administered to a similar population many times at William & Mary and thousands of times nationwide annually with no untoward outcomes. SpO2, HR, and, rating of perceived exertion (RPE) will be recorded at rest and at every 2-minute stage of the GXT. Once started, the entire GXT test should last no more than 12 minutes. No sooner than two days after the GXT, you will return to the laboratory and enter the normobaric hypoxia chamber in which the atmospheric content of oxygen will be reduced to a percentage whose partial pressure is equivalent to that found at an altitude of 3,500 meters (11,500 ft.) or, in the case of control subjects, sea level. After entering the chamber, you will rest quietly for 30 minutes. During the final 15 minutes of steady state rest, EtCO2, HR, and SpO2 will again be measured. You will then warm up in the chamber on a stationary bicycle ergometer for 5 minutes, then pedal the ergometer at a HR equivalent to that recorded at 65% of your SL VO2peak. By matching target HR, the relative exercise intensity is equal at SL and at 3,500 meters. At rest and every 2 minutes during 10 minutes of bicycle exercise at a HR equal to that recorded at 65% SL VO2peak, SpO2, HR, and RPE will be recorded and compared to measurements taken during SL testing. Resting ventilation SpO2, HR, and EtCO2 at altitude will also be compared to SL data.

If you have ANY questions, before the study starts of after the study starts, the research staff EXPECTS you to ask us. Specifically, call or E-mail the Principal Investigator (Dr. Kenneth Kambis, Williamsburg, VA 757-221-2779; kwkamb@wm.edu) or the Co-Principal Investigator (Dr. M. Brennan Harris, Williamsburg, VA 757-221-2757). If he cannot answer your questions, he likely will be able to provide you with the name of someone or an organization that will.

STUDY PURPOSES:
The proposed research is an important step in quantifying individual responses to acute hypoxia. In addition, the proposed research can possibly develop a single sea level (SL) test or battery of SL tests that can predict an individual's response to acute hypoxic exposure. These data could help prepare people for and protect people from Acute Mountain Sickness (AMS) by reducing the incidence and/or severity of this debilitating disorder. AMS is a debilitating disorder caused by rapid ascent to high altitude resulting in headache, nausea, lassitude and, in some cases, inability to perform even the most basic tasks.

ELIGIBILITY TO PARTICIPATE:
We ask that you read the entire document, ask questions, and take the time to discuss with us anything that you do not understand or that concerns you with the study.

To participate you must:

Be an apparently healthy non-smoking man or non-smoking, non-pregnant otherwise apparently healthy woman over the age of 18 years.

Not have been born at an altitude of greater than 1,500 meters (4,500 feet).

Not have traveled to altitudes greater than 5,000 feet for more than 2 days within the past 6 months.

If you meet the eligibility requirements above, you will be medically screened. The screening will consist of a medical history and review of your medical history by medical personnel or their designate. Volunteers with evidence of anemia of hemoglobin S ("sickle cell") will be excluded. Subjects at risk for Sickle Cell Trait or Disease (African, African-American, Asian-Indian, or Asian Indian-American) will be required to show documentation of having been tested and found negative for Sickle Cell trait or disease. Volunteers with evidence of any physical, mental, and/or medical conditions that would make the proposed study more hazardous will be excluded.

There will be a total of 24 volunteers that will participate in this phase of the study.

SPECIFIC STUDY PROCEDURES:
The first time you participate in a test, the main goal will be to familiarize you with the test and the staff who are performing the test.

1. Graded Bicycle Exercise Test to Exhaustion:
At your initial visit to the Altitude Physiology Research Facility, you will perform a brief (10-12 minutes) exercise test of gradually increasing intensity, culminating in maximal effort, to determine peak oxygen uptake (VO2peak). At rest and at each 2-minute exercise stage, heart rate (HR), SpO2 and Rating of Perceived Exertion (RPE) will be assessed.

2. SpO2, Heart Rate and, EtCO2
In addition to measuring your SpO2 (% of your hemoglobin saturated with oxygen), the padded sensor as well as the two chest electrodes will measure your heart rate (HR). While sitting quietly for 15 minutes, you will breathe through a mouthpiece connected to a gas analyzer. This device (capnograph) will calculate your end-tidal carbon dioxide content EtCO2).

POTENTIAL RISKS AND HAZARDS
Potential risks to you from participation in this study include the risks associated with acute exposure to hypoxia and the risks that are part of the test procedures, measurements, and equipment used in the study.

Risks Associated with Altitude Exposure:
The risks associated with the reduced level of oxygen imposed by this study include Acute Mountain Sickness (AMS). However, because the length of exposure is short (no more than 30-45 minutes) and the simulated altitude is relatively low (11,500 ft.), the risk of you developing AMS is not great. Nevertheless, an investigator will be present to take you to a lower altitude, if necessary.

Risks Associated with Test Equipment:
All instruments to be used in testing will be operated by trained personnel. There will always be at least one assistant or PI present with current CPR/AED certification.

Environmental Symptoms Questionnaire III:
There are no risks associated with the various questionnaires.

Blood Pressure, O2Saturation (SpO2), and EtCO2:
There are no risks associated with blood pressure, O2Saturation tests, and EtCO2 tests.

Graded Bicycle Exercise Test to Exhaustion:
There are no risks associated with blood pressure or RPE measurements. A graded exercise test to exhaustion is a physically stressful test. In those who have heart disease, there is a statistical probability of 1 in 10,000 tests resulting in a lethal heart attack and a 4 in 10,000 tests resulting in a non-lethal cardiac event. This is a very rare occurrence and, since you are apparently healthy and do not have cardiovascular disease, it is reasonable to assume that the risks to you are even lower. You will be medically screened before the test and, if you are deemed not physically fit enough for a maximum effort test, you will be excluded from the experiment.

STUDY COMMITMENT:
It is important that you understand this study and the commitment it will require of you. You are encouraged to ask any questions necessary before or after volunteering. Your participation in this study should require a total of about three (3) hours. Because of the time and expense involved in this study, if you volunteer, we would like you to be reasonably committed to completing the study. However, you have the right to withdraw from the study at any time without adverse consequences or prejudice.

Other Reasons for Your Leaving the Study:
The Principal Investigator may stop your participation without your permission. Your participation may be stopped if you are unwilling or unable to complete the study testing tasks. The Principal Investigator may also stop your participation if you become ill, injured or believes that continuing may not be in your best interest.

BENEFITS TO YOU:
There are no direct benefits to you for participating in this study as a volunteer, except the knowledge of how well you performed on the tests that you participate in. You will be paid $50.00 for your participation in the study. Your payment for participation will not be affected by your responses or by your exercising any of your rights.

INJURY OR SICKNESS NOTIFICATION:
If you become sick or injured as a result of this study, you should immediately notify the Principal Investigator associated with the study.

EMERGENCY MEDICAL CARE:
In the event of a medical emergency, the emergency medical services (EMS) system will be activated by telephone (911), and while awaiting the arrival of EMS, trained personnel (CPR trained) will provide basic life support and first aid. Neither the researchers, the Department of Kinesiology & Health Sciences, or The College of William and Mary can assume responsibility for any medically

untoward outcome. While emergency first aid may be provided by the staff and/or the Student Health Service, any subsequent medical care will be the participant's responsibility.

INVITATION FOR QUESTIONS:
If you have any questions, we expect you to ask us. If you have any additional questions later, further information about the study as well as the aggregate results of the study can be obtained from Dr. Kenneth W. Kambis (757-221-2779) or Dr. M. Brennan Harris (757-221-2757). You may report dissatisfactions with any aspect of this experiment to the Chair of the Protection of Human Subjects Committee, Jennifer Stevens, Ph.D. 757-221-3862 jastev@wm.edu.

Your anonymity will be preserved in that your name will not be connected to your responses nor will your name be associated with any results of this study. You may refuse to answer any question and you may discontinue participation at any time.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PERSON OBTAINING CONSENT, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

_____ _____
Signature Date

_____
Printed Name

THIS PROJECT (PHSC-2018-02-28-12757-mbhar titled Individual Variability Upon Acute Exposure to a Normobaric Hypoxic Environment Simulating a Physiologic Altitude Equivalent to 3,500 Meters) WAS APPROVED BY THE COLLEGE OF WILLIAM AND MARY PROTECTION OF HUMAN SUBJECTS COMMITTEE (Phone: 757-221-3966) ON 2018-04-12 AND EXPIRES ON 2019-04-12.