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Resting Heart Rate Variability at Sea Level Predicts Arterial Desaturation during Acute Exercise in Hypoxia

Kelsey Short

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Resting Heart Rate Variability at Sea Level Predicts Arterial Desaturation During Acute Exercise in Hypoxia

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

by

Kelsey J. Short

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RESTING HEART RATE VARIABILITY AT SEA LEVEL PREDICTS ARTERIAL DESATURATION DURING ACUTE EXERCISE IN HYPOXIA

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

by

Kelsey J. Short

Williamsburg, Virginia

May 2020
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ABSTRACT

Predicting responses to acute hypoxia based on physiologic measures at sea level may be valuable in anticipating adverse responses to acute hypoxia. The purpose of this study was to examine the relationships between heart rate variability (HRV) and arterial oxygen desaturation in response to acute hypoxia exposure at rest and during exercise. METHODS: 13 subjects (6 male, 7 female) aged 20.6±0.3 years rested in a supine position for 10 min after steady state respiration was achieved. Average resting HRV and arterial oxygen saturation (%SpO₂) were calculated from the last 5 min. A VO₂peak test to determine aerobic fitness (44.1±3.0 ml/kg/min) was performed on a stationary cycle at sea level. Subjects also performed a submaximal bike test for 10-15 min at 65% of HR max achieved during the VO₂peak test in normobaric hypoxia equivalent to an altitude of 3500 meters. RESULTS: HRV (RMSSD 54.5±5.7) was significantly correlated (r=0.227, P>0.05) to the percent change (9.7±0.7) in %SpO₂ from rest (97.4±0.2) to exercise (88.0±0.8) in normobaric hypoxia. CONCLUSIONS: HRV at rest appears to be predictive of the degree of arterial desaturation during exercise in hypoxia equivalent to an altitude of 3500 meters. Current wearable technology that can measure HRV could be used to predict potential adverse responses to exercise in hypoxic conditions such as altitude.

Keywords: Heart Rate Variability (HRV), Acute Hypoxia, Arterial Desaturation, Acute Mountain Sickness (AMS)
REVIEW OF LITERATURE

INTRODUCTION TO HEART RATE VARIABILITY (HRV)

Physiology has been based on the principle that cells, tissues, and organs work to maintain a constant “steady-state” condition, however, biological processes vary in multifaceted ways even throughout these “steady-state” conditions. Observations of heart rate (HR), for example, from signal processing technologies acquiring continuous time series data have led to the understanding that optimal utility results from continuous interactions among multiple control systems at local and central levels (McCraty & Schaffer, 2015). The evaluation of beat-to-beat changes within HR advanced furthermore with the invention and standardization of the electrocardiogram (ECG) (Billman, 2011). Steven Hales (1677-1761) was first to state that the arterial pressure interval and the beat-to-beat interval vary during the respiratory cycle through his research in conducting blood pressure measurements in animals via inserting cannulas into arteries and subsequently measuring how high the column of blood rose (Hales, 1733). Later on in 1847, Carl Ludwig (1816-1895) recorded through the use of a kymograph that periodic oscillations in the timing and amplitude of the arterial pressure waves vary during respiration, or respiratory sinus arrhythmia (RSA) (Ludwig, 1847). Early research sought to explain RSA in terms of baroreceptor responses along with respiratory changes in thoracic pressure, leading to the finding of heart rate variability (HRV) (Berntson et al., 1997). In RSA, the R-R interval is shortened during inspiration and is lengthened during expiration (Crespo-Ruiz et al., 2018). The variation within beat-to-beat intervals in HR or the duration of the R-R interval has come to be known as HRV. These beat-to-beat intervals between consecutive heartbeats are also called interbeat intervals (IBIs). Physiology centers around the idea of homeostasis, the tendency towards maintaining a relatively steady-state equilibrium within the body, and these oscillations.
of a healthy heart continuously change, thus allowing for the cardiovascular system to rapidly adjust to sudden functional challenges posed to homeostasis (Shaffer & Ginsberg, 2017).

Though the invention of the ECG brought physicians and scientists a better method of monitoring normal and abnormal electrical conduction through the myocardium, the origins of the study of HRV predate the ECG (Berntson, Bigger, et al., 1997). Norman Holter (1914-1983) further expanded ECG recordings through his invention of a small portable recorder where ambulatory ECGs could be obtained and recorded over more extended time periods (Holter, 1961). An ECG readout depicts the P wave, where atrial depolarization occurs, the QRS complex wave, where ventricular depolarization occurs, and the T wave, where ventricular repolarization occurs. Depolarization occurs when the action potential, caused by an electrical impulse causing different concentrations of ions to cross the cell membrane, is activating muscle cells to contract. With repolarization, the action potential is completed, muscle cells are relaxed, and the ions return to their previous resting state. Figure 1 displays ECG recordings showing the beat-to-beat variations derived from a conscious dog (Billman, 2011).
Figure 1

Heart rate variability: representative electrocardiogram (ECG) recordings from a conscious dog that illustrate beat-to-beat variations in both R–R interval and heart rate. (Source: Billman, 2001).

Early research into HRV focused on RSA, primarily diverging into two directions: one being a trend towards understanding the underlying physiological mechanisms that mediate heart rate rhythms, and another being a trend towards identifying specific relationships between HRV and possible clinical applications (Berntson, Bigger, et al., 1997). One of the first clinical applications of HRV investigated fetal ECGs and indicated fetal distress through diminished variability post-contractions (Hon & Lee, 1965). Current clinical applications of HRV and its measurements have been found useful in evaluating the function of the autonomic nervous system with respect to cardiac function. Previous to this, Eppinger & Hess (1915) suggested that HRV could be used to display some abnormalities in autonomic regulation in disease (Billman,
2011; Eppinger & Hess, 1915). The application of HRV has been recognized in many clinical conditions, including one condition, where it acts as a predictor of risk for sudden cardiac death after an acute myocardial infarction (AMI) or arrhythmic events and in another condition, where it works as a clinical marker of evolving diabetic neuropathy (Stys & Stys, 1998). Kleiger et al. (1987) also found that the relative risk of mortality was 5.3 times higher in a group with HRV of less than 50ms as compared with a group with HRV of more than 100ms in individuals surviving an AMI. HRV has also become an important diagnostic tool in the detection of autonomic impairment and the extrapolation of prognosis in many neurological disorders and thus provides insight overall into the autonomic nervous system tone (Cyganiewicz & Zareba, 2013).

Formerly, Galvani’s and Volta’s work with frogs and observations with the electrophysiology of the heart generated work on the effects of nerve transection on the activity of the heart focusing on the vagus and intercostal nerves and lead to their invention of the galvanometer, allowing for the measurement of slight electrical currents through the use of magnetic induction to rotate a pointer (Ernsberger & Rohrer, 2018; Piccolino & Bresadola, 2013). This research brought about the thought that the heart operates autonomously while also being modified by the vagus and intercostal nerves (Ernsberger & Rohrer, 2018). Eduard Weber and Ernst Heinrich used an electromagnetic rotation apparatus to provide the first substantial report on the antagonistic relationship of heart activity via the sympathetic and vagus nerve in 1845 (Fye, 2000; Ernsberger & Rohrer, 2018). Through this electromagnetic rotation apparatus for their experiments, originally in frogs and later confirmed in mammals, Weber and Heinrich found that galvanic excitation of the vagus nerve weakens the heart and slows or interrupts the heartbeats, whereas the excitation of the sympathetic nerve enhances and restores the movement of the heart (Ernsberger & Rohrer, 2018). HRV studies are sometimes based on the paradigm
that decreased parasympathetic tone is associated with increased sympathetic tone and increased parasympathetic tone is associated with decreased sympathetic tone; thus HRV has been regarded as a parameter for the complex interaction between the cardiovascular system and the brain (Ernst, 2017).

Sympathetic and parasympathetic systems influence the activity of the sinoatrial (SA) node and thus regulate the HR. The SA node comprises of a collection of cells located in the upper portion of the wall of the right atrium, where electrical impulses are generated via action potentials and a heartbeat then occurs. Postganglionic parasympathetic terminals at the SA node release acetylcholine, which then decelerates the rate of SA node depolarization through binding to muscarinic cholinergic receptors and then activating a transmembrane potassium channel (Berntson et al., 1997). Counter to this process, sympathetic terminals on the SA node release norepinephrine, accelerating the SA node rhythm by means of a $B_1$ receptor-mediated second messenger cascade of signals within the cells.

Adrian et al. (1932) made the first recordings of the autonomic nervous system (ANS) through their recording of sympathetic nerve impulses that showed a rhythm in phase with the cardiac cycle and respiration (Adrian et al., 1932). The ANS supplies smooth muscle, thus influencing the internal organs of the body, and maintains homeostasis through its regulation of functions including heart rate, blood pressure, respiratory rate, body temperature, and other biological functions unconsciously. The ANS includes the branches of the sympathetic nervous system and the parasympathetic nervous system and is the primary controlling mechanism of the fight-or-flight response encompassed within the sympathetic nervous system. Low HRV reflects dominance of the sympathetic nervous system, indicating some external stressors, whether physical, or psychological. In turn, high HRV reflects dominance of the parasympathetic nervous system.
system, indicating an association with better stress tolerance and recovery and usually reflecting healthy function and intrinsic self-regulatory capacity.

However, sometimes higher HRV values do not always indicate better health. In some instances, pathological conditions such as atrial fibrillation (AF) and other similar electrical conduction abnormalities can produce a higher HRV value, which in turn is linked to a greater risk of mortality (Shaffer & Ginsberg, 2017). Close examination of ECG readings can reveal whether elevated HRV is due to such electrical conduction problems. In a study comparing a group of individuals with atrial fibrillation versus a group of individuals with normal sinus rhythm of the heart, a higher baseline HRV has been observed in the atrial fibrillation group (Berg et al., 1997). This study not only displays a higher baseline HRV in atrial fibrillation, but also the same significant relation in vagal tone, also referred to as vagal cardiac control (VCC), as seen in individuals with normal sinus rhythm (Berg et al., 1997).

The cardiovascular regulatory centers located within the medulla oblongata of the brainstem control the HR by means of the vagus nerve and the vagal tone reduces HR through the inhibition of the SA node (Young & Benton, 2018). HR and CNS functioning involve the combined output of multiple different regulatory mechanisms, including some that are not able to be measured simply through linear approximations like standard deviation (Young & Benton, 2015). Young & Benton (2015) concluded that nonlinear methods of summarizing HR indices captured additional information, which allowed for a greater percentage of variance in behavior to be rationalized, therefore providing a new way of relating brain functioning and behavior as well. Without extrinsic influences such as the neural impacts stated above, HR is between 100-120 beats per minute (bpm). Figure 2 displays an ECG trace where the R-R intervals are measured (Young & Benton, 2018).
As explained above, the variability in the differences between these R-R intervals includes what is of interest in the present study. HRV can be estimated through the use and measurement of the R-R interval or through HR signals, which will be explained in more detail later, however these do not produce the same end results and are inversely correlated to each other (Sacha & Pluta, 2005; Sacha, 2013). The importance of the natural interrelation between HR and HRV is seen, for example, a higher HR provides less time between heartbeats for variability to occur, thus lowering the overall HRV, whereas a lower HR provides more time between beats for more variability (Ernst, 2017). This phenomenon is termed cycle length dependence. However, this relationship between HR and HRV is lost to the point that HRV does not increase in any aspect and would in turn decrease with reductions in HR in the elderly with ischemic heart disease along with other cardiovascular ailments (Ernst, 2017).

Figure 2
A heart-rate trace. A typical electrocardiogram trace is illustrated. R is the peak of the QRS complex (electrocardiogram trace) and heart-rate variability is measured by considering consecutive R–R intervals. The R–R interval is not constant, but varies within a normal range of 0.6–1.2 s. It is the degree of this R–R variability that is of interest as greater variability is associated with better health. (Source: Young & Benton, 2018).
The clinical relevance of HRV is displayed through the simple derivation of HRV, which has provided cardiologists and researchers alike with a noninvasive tool to assess cardiovascular health and fitness. However, the clinical relevance of HRV has only been clearly demonstrated in two clinical conditions: the first being impaired HRV can be used alone or in combination with other factors to predict the risk of arrhythmic events after MI, and the second being a decrease in HRV is a useful clinical marker for evolving diabetic neuropathy (Stys & Stys, 1998; Special Report of the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology, 1998; American College of Cardiology Cardiovascular Technology Assessment Committee, 1993).

HRV has been associated with diseases, including ones affecting the central and peripheral nervous systems, though some affected diseases independently affect HRV through modulating sympathetic and vagal fluctuations of HRV (RenuMadhavi & Ananth, 2012). Examples that reflects this independent effect that RenuMadhavi & Ananth give include depression and severe brain damage, in both of which the normal rhythm of HR is reduced. HRV can give some indication of neurologic state of an individual, and in psychiatric disorders such as depression, HRV analysis proves vital because it detects sympathovagal imbalances existing in such conditions as well (RenuMadhavi & Ananth, 2012).

Sympathetic and parasympathetic nerve endings release neurotransmitters that bind to their corresponding receptors sited on the surface of immune cells and thus initiate immune-modulatory responses (Kenney & Ganta, 2014). The response of immune cells is thus interrelated to both the sympathetic and the parasympathetic nervous systems, which also play a key role in HRV through their influence on the activity of the SA node regulating the HR. The immune system and metabolic regulation have a highly integrated relationship reflecting a
central homeostatic mechanism that can result in chronic metabolic disorders including cardiovascular disease if the mechanism becomes dysfunctional (Hotamisligil, 2006). Through the vagus nerve, the nervous system reduces the immune response and monitors peripheral inflammation (Matteoli & Boeckxstaens, 2013; Young & Benton, 2018). HRV is also associated with higher levels of C-reactive protein (CRP), which the liver produces as a response to inflammation, the levels of which increase after the secretion of interleukin (IL)-6 from macrophages and T cells (Young & Benton, 2018). This protein works through binding to lysophosphatidyl choline on the surface of dead cells along with enhancing the ability of phagocytic cells and antibodies in order to remove pathogens (Jarczok et al., 2014). Dehghan et al. (2007) found that higher levels of CRP were associated with a higher risk of diabetes along with hypertension, cardiovascular disease, as well as other related ailments. Higher HF HRV values have been associated with lower levels of CRP (Jarczok et al., 2014).

As previously stated, the importance of HRV as an index of functional status was seen in one of the first clinical applications which investigated fetal ECGs and indicated that fetal distress follows a reduction in HRV before any changes in HR occur (Hon & Lee, 1965). Since then, HRV has been implicated in relation to many diseases. Ewing et al. (1976) found that HRV was shown to predict autonomic neuropathy in diabetic patients preceding any symptoms through reduction in the time-domain parameters of HRV. Patients with depression have a higher risk of cardiovascular mortality, correlating with a decrease in HRV, however the significance of HRV in relation to depression is still not quite fully understood (Balogh et al., 1993). The findings of Balogh et al. (1993) indicate that the pharmacologic treatment leading to improvement in major depressive disorder (MDD) is associated with an increase in HRV and
may reflect improved autonomic function and thus decrease the risk of cardiovascular mortality found in patients with MDD.

Low HRV reflects sympathetic dominance, which indicates external stressors, and in turn high HRV reflects parasympathetic dominance, indicating an association with better stress tolerance and recovery, as discussed earlier. Kenneth Hambly defines stress as a maladaptive state where the sympathetic nervous system is over-activated, which in turn causes acute, or chronic physical, behavioral, and psychological problems (Campkin, 2000). Many studies have discussed the relation between HRV and stress and the idea that HRV can be used as a measurement and overall reliable index of stress. Vrijkotte et al. (2000) showed that the detrimental effect of work stress is partly mediated through increased HRV to a stressful work day along with a decreased vagal tone through the monitoring of HRV on two work days and one non-work day. Dishman et al. (2000) showed that individuals with more perceived stress, figured through self-ratings of perceived emotional stress and trait anxiety, indicate a lower cardiac vagal component of HRV. In concordance with many studies relating HRV and stress, HRV can be used as an objective assessment of stress and mental health (Kim et al., 2018). With this being said, psychiatric illnesses still have various symptoms and causes, so the psychological and medical histories of an individual should be taken into consideration when interpreting HRV results and HRV therefore can be a tool considered to reflect overall heart activity and autonomic health versus a tool for assessing specific mental illnesses or diseases in general (Kim et al., 2018).

PARAMETERS AND ANALYSIS OF HRV

Currently, many techniques have been developed to quantify HRV in an attempt to provide some indices of cardiac autonomic regulation in health and disease (Task Force of the
The two main approaches to the analysis of HRV include time domain and frequency domain methods (Berntson et al., 1997; Billman, 2011). Table 1 and table 2 quantify specific measurements that are calculated through time domain methods and frequency domain methods, respectively (Shaffer & Ginsberg, 2017). Time domain measures generally tend to have easier calculations, however provide less detailed information than frequency domain measures. Different approaches to the time domain method share the same feature that either the intervals between successive normal heartbeats or heart rate at any point in time, also referred to as instantaneous HR, are derived from ECG recordings (Billman 2011). For this calculation, only the normal QRS complexes of the HR are used. Normal QRS complexes to be included would only be those heartbeats which result from the normal electrical activation pattern within the heart, starting with depolarization at the SA node. Abnormal heartbeats in this case, including arrhythmias, would be excluded from this calculation. The interval between adjacent normal QRS complexes is thus termed the normal-to-normal (NN) interval (Billman, 2011). This NN interval or the instantaneous HR is derived along with other descriptive variables, some of which include mean HR, mean NN interval, and the range of the NN interval (longest NN interval minus shortest NN interval). The standard deviation (SD) of the NN interval (SDNN), or the square root of the variance of the NN interval, measures the total variability resulting from periodic and random sources, and is a widely implemented time domain index of HRV on account of the ease of calculation (Shaffer & Ginsberg, 2017).

The most commonly used measures derived from differences in the intervals include the number of interval differences of the successive NN intervals greater than 50ms (NN50), the square root of the mean squared differences of successive NN intervals (RMSSD), and the
proportion calculated from dividing NN50 by the total number of NN intervals (pNN50) (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Of these three, the RMSSD method is preferred due to the fact that it yields better statistical properties. The method of selection of a measurement of HRV should correspond to the aim of each individual study.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN</td>
<td>ms</td>
<td>Standard deviation of NN intervals</td>
</tr>
<tr>
<td>SDRR</td>
<td>ms</td>
<td>Standard deviation of RR intervals</td>
</tr>
<tr>
<td>SDANN</td>
<td>ms</td>
<td>Standard deviation of the average NN intervals for each 5 min segment of a 24 h HRV recording</td>
</tr>
<tr>
<td>SDNN index (SDNNI)</td>
<td>ms</td>
<td>Mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording</td>
</tr>
<tr>
<td>pNN50</td>
<td>%</td>
<td>Percentage of successive RR intervals that differ by more than 50 ms</td>
</tr>
<tr>
<td>HR Max – HR Min</td>
<td>bpm</td>
<td>Average difference between the highest and lowest heart rates during each respiratory cycle</td>
</tr>
<tr>
<td>RMSSD</td>
<td>ms</td>
<td>Root mean square of successive RR interval differences</td>
</tr>
<tr>
<td>HRV triangular index</td>
<td></td>
<td>Integral of the density of the RR interval histogram divided by its height</td>
</tr>
<tr>
<td>TINN</td>
<td>ms</td>
<td>Baseline width of the RR interval histogram</td>
</tr>
</tbody>
</table>

Table 1. HRV time-domain measures. (Source: Shaffer & Ginsberg, 2017).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ULF power</td>
<td>ms²</td>
<td>Absolute power of the ultra-low-frequency band (≤0.003 Hz)</td>
</tr>
<tr>
<td>VLF power</td>
<td>ms²</td>
<td>Absolute power of the very-low-frequency band (0.0033–0.04 Hz)</td>
</tr>
<tr>
<td>LF peak</td>
<td>Hz</td>
<td>Peak frequency of the low-frequency band (0.04–0.15 Hz)</td>
</tr>
<tr>
<td>LF power</td>
<td>ms²</td>
<td>Absolute power of the low-frequency band (0.04–0.15 Hz)</td>
</tr>
<tr>
<td>LF power</td>
<td>nu</td>
<td>Relative power of the low-frequency band (0.04–0.15 Hz) in normal units</td>
</tr>
<tr>
<td>LF power</td>
<td>%</td>
<td>Relative power of the low-frequency band (0.04–0.15 Hz)</td>
</tr>
<tr>
<td>HF peak</td>
<td>Hz</td>
<td>Peak frequency of the high-frequency band (0.15–0.4 Hz)</td>
</tr>
<tr>
<td>HF power</td>
<td>ms²</td>
<td>Absolute power of the high-frequency band (0.15–0.4 Hz)</td>
</tr>
<tr>
<td>HF power</td>
<td>nu</td>
<td>Relative power of the high-frequency band (0.15–0.4 Hz) in normal units</td>
</tr>
<tr>
<td>HF power</td>
<td>%</td>
<td>Relative power of the high-frequency band (0.15–0.4 Hz)</td>
</tr>
<tr>
<td>LF/HF</td>
<td>%</td>
<td>Ratio of LF-to-HF power</td>
</tr>
</tbody>
</table>

Table 2. HRV frequency-domain measures. (Source: Shaffer & Ginsberg, 2017).
Frequency domain methods approximate the distribution of relative or absolute power into four separate frequency bands: ultra-low frequency (ULF), very-low frequency (VLF), low frequency (LF), and high frequency (HF) bands (Shaffer & Ginsberg, 2017). Power is defined as the signal energy found within a frequency band and frequency domain measurements can be expressed in either relative or absolute power (Shaffer & Ginsberg, 2017). Power spectral density analysis (PSD) produces the basic information of the way in which the power of a series of heartbeats distributes as a function of frequency, and only an estimate of the PSD can be found through mathematical algorithms (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). The calculation of PSD is classified into two categories: parametric and nonparametric. The two most common approaches to spectral analysis of HRV include the fast Fourier transform (FFT) technique, which falls under the nonparametric category, and the autoregressive (AR) modeling technique, which falls under the parametric category (Akselrod et al., 1981; Pagani et al., 1986). Figure 3 displays both FFT and AR methods of analysis in the selected R-R interval time series within the frequency domain (Pichon et al., 2004).
The FFT technique assumes that the time series contains only deterministic components, and the spectrum computed with this method is derived from all the data irrespective of how well they fit a model based off of peaks in the spectral distribution (Berntson et al., 1997). The AR technique treats data as a composite of deterministic and stochastic components, and with this the time domain data are used to identify a best fit model based on peaks in the spectral distribution and attempt to exclude “noise” (Berntson et al., 1997). The AR technique may be seen as more advantageous when compared with the FFT technique, however in practice this distinction can be blurred through the application and usage of smoothing algorithms to stabilize the variation from FFT, leading to predominantly equal results from the two methods of analysis (Berntson, 1997). However, there are some instances where one method is preferred over the other and each method is not interchangeable. Chemla et al. (2005) compared spectral components of short-term HRV obtained by both the FFT method and the AR method and found that in diabetic patients, FFT analysis is preferred and the two methods are in fact not
HEART RATE VARIABILITY AND ARTERIAL DESATURATION

interchangeable. In a comprehensive comparative study reviewing data from humans and rats during a pharmacological blockade in rats and a postural test in humans and their hypertensive state, it was found that there was a 43% disagreement between AR and FFT methods (Silva et al., 2009). In this study, AR and FFT analysis did not agree on variables such as LF_{nu} and HF_{nu} after vagal blockade and under basal conditions, along with AR and FFT detecting some variables that the other could not, including AR detecting LF and HF for systolic BP whereas FFT did not, and FFT detecting the reduction in both BP variance and total power (Silva et al., 2009).

Frequency domain methods distribute relative or absolute power into the four frequency bands mentioned briefly above, (ULF, VLF, LF, and HF bands). The sympathovagal balance, the ratio of absolute low-frequency (LF) to absolute high-frequency (HF) power, reflects the balance between sympathetic and parasympathetic activity (Pagani et al., 1984). The LF band of HRV is influenced within the range of 0.04Hz to 0.15HZ, reflecting sympathetic nervous system outflow, and the HF band of HRV within the range of 0.15Hz to 0.4Hz, reflecting the parasympathetic nervous system, where efferent vagal activity appears to be a foremost contributor (Rosenberg, 2017). Increases in vagal activity are seen affecting the HF component of HRV in response to autonomic movements such as electrical vagal stimulation (Malliani et al., 1991). Unlike the HF component of HRV, the LF component has more variety of interpretation. Some authors believe LF can be a marker of sympathetic modulation of vasomotor activity, and others see it as including both vagal and sympathetic influences (Malliani et al., 1991; Kamath & Fallen, 1993). The 0.1HZ component to the LF band is also referred to as the Mayer wave or 10-s wave. Therefore, an increased power in the LF band demonstrates a more prevalent activity of
the sympathetic nervous system, and an increased power in the HF band demonstrates a more prevalent activity of the parasympathetic nervous system (Rosenberg, 2017).

HRV has been characterized at ultra-low frequencies (ULF) of (0.0005 to 0.4) Hz and has shown evidence of neuro-cardiac regulatory mechanisms influenced by emotional valence that operate within the bandwidth of (0.002 to 0.01) Hz (Fisher et al., 2014). There is noteworthy variability in frequencies lower than 0.03Hz, which is where 95% of the total power of the HRV power spectrum resides (Fisher et al., 2014). Though noteworthy variability is found within ULF and VLF components and this is where 95% of the total power spectrum lies, the physiologic correlates of ULF and VLF components are unknown. The methodological constraints attributed to VLF and ULF components under 0.03Hz come into question when deciding whether these frequencies truly represent cardiovascular oscillations (Fisher et al., 2014). However, Bigger et al. (1992) found VLFs and ULFs of HRV to have good predictive power for developing serious arrhythmias and at that better than frequencies above 0.03Hz in relation to these electrical conduction abnormalities.

The measurement of HRV appears to be a well-established tool for research and is also useful in assessing the functionality of central and peripheral modulators that are involved in controlling the beat-to-beat variability of the heart (Stys & Stys, 1998). The Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology (1996) has minimized the number of necessary parameters for time domain HRV analysis to the SDNN, HRV triangular index, standard deviation of the averages of N-N intervals (SDANN), and RMSSD, seeing that there are many different measures of HRV coupled with the fact that many of these measures closely relate with each other.
Firstbeat technologies calculate accurate and reliable HRV values and thereby provide a comprehensive stress and recovery analysis. In this study, the Firstbeat Bodyguard 2 device, which is a R-R interval and movement data recording device, was used to record HRV. The Firstbeat Bodyguard 2 can be used for both short and long term measurements. It is designed for 24-hour recordings and can be used during recovery, exercise, or sleep. This device is unobtrusive and easy to use as it attaches directly to the skin via two chest electrodes and starts recording data automatically. The measurement data is then uploaded to Firstbeat SPORTS software.

After the Firstbeat Bodyguard 2 records R-R interval data, it is then scanned through an artifact detection filter to fix incorrectly detected, premature, and missed heartbeats. The artifact-corrected R-R intervals are then re-sampled at a rate of 5 Hz through the use of linear interpolation. Low frequency trends and variables above and below the frequency band of interest are then removed by means of a polynomial filter and digital FIR band-pass (0.03-1.2 Hz) filter. The software then determines values for variables including RMSSD, HF power (0.15-0.40 Hz), LF power (0.04-0.15 Hz), and amplitude of the RSA. Through the use of these variables, along with neural network modeling of given data, the software calculates values that exemplify physiological data including respiration rate (RR), oxygen consumption (VO₂), and excess post-exercise oxygen consumption (EPOC).

HYPOXIA

Hypoxia is defined as a physiological state of decreased adequate oxygen supply at the tissue level of the body occurring in higher altitude environments where the partial pressure of oxygen is lower than at normal sea level. Four broad categories of hypoxia include hypoxemic
hypoxia, hypemic hypoxia, ischemic or stagnant hypoxia, and histoxic hypoxia. Hypoxemic hypoxia results from low O\textsubscript{2} levels from pulmonary or environmental causes, which included high altitude (Zaoutis et al., 2007). Hypoxemia refers specifically to low oxygen content in the blood, and when there is a reduction in the partial pressure of arterial oxygen, arterial oxygen saturation (%S\textsubscript{PO} \textsubscript{2}) will also decrease (Zaoutis, 20017). With this reduction in the partial pressure of oxygen, anatomical, physiological, and biochemical characteristics are modified so the active tissue cells may receive and appropriately use the oxygen that is transported at low pressure in the circulation of blood (Noel-Jorand, 1996; Hurtado, 1964).

Individuals exposed to an altitude greater than 5500m may lose consciousness, and over 8000m, loss of consciousness can occur within less than three minutes (Brown & Grocott, 2013). However, if the body is trained with gradual increasing altitude exposure, it may be able to adapt and survive in such environments, and this process as a whole is termed acclimatization. It is believed that acclimatization is accomplished through increasing the oxygen delivery through respiratory, cardiac, and general physiologic changes and mechanisms. High altitude generally refers to an altitude greater than 2500m, though some definitions of this vary (Brown & Grocott, 2013). Figure 4 shows the acute effects of hypoxia with respect to increasing altitude and increasing time (Clarke, 2006).
The central chemoreceptor response to hypoxia in turn initially depresses ventilation, ostensibly through depressing oxidative metabolism in neural tissue (Pittman, 2011). The peripheral chemoreceptors are located in the carotid sinus and aortic bodies. The body’s response to this high altitude low oxygen concentration is then seen as an increase in ventilatory response activated by an increase in the firing rate of the carotid body receptors within the carotid sinus from the peripheral chemoreceptors (Khodae et al., 2016). With this being said, all of the stimulatory responses to hypoxia reside in the peripheral chemoreceptors, whereas the central chemoreceptors give a non-specific metabolic response that depresses ventilation (Pittman, 2011). The carotid bodies are related to the respiratory centers in the brainstem and the aortic bodies are connected to the cardiovascular centers in the brainstem (Pittman, 2011). The acute exposure to hypoxia also then causes multiple compensatory changes, including an increase in sympathetic activity accompanied by an increase HR, BP, and cardiac output (Q) (Khodae et al., 2016). These physiologic mechanisms make up the hypoxic ventilatory response (HVR).
This hypoxic ventilatory response then induces a decreased alveolar CO$_2$, respiratory alkalosis, and hypocapnia, thus inhibiting the central respiratory center and reducing any further increases in ventilation (Whayne, 2014; Khodae et al., 2016).

Reduced atmospheric partial pressure of oxygen (PO$_2$) leads to a decrease in alveolar partial pressures of oxygen, therefore leading to an initial reduction in oxygen delivery (Brown & Grocott, 2013). Reduced PO$_2$ at high altitudes results in reduced oxygen loading in the lungs as well, so the blood may not carry a sufficient supply of oxygen to the cells of respiring tissues (Bencowitz et al., 1982). This reduced level of tissue oxygenation may enforce severe restrictions on aerobic metabolism as well as influence food and water requirements and the capacity for internal heat production (Storz, 2007). Two main steps where circulatory adjustments can help minimize the inevitable reduction in tissue PO$_2$ exist and include the gradient between alveolar gas and arterial blood and the gradient between capillary blood and tissues (Storz, 2007). Tissue gas exchange starts at the arterial inlet to the capillary bed and the PO$_2$ then decreases quickly from the arterial side to the venous side while oxygen diffuses from the higher PO$_2$ of the blood to the lower PO$_2$ of the interstitial fluid (Storz, 2007). The arterial-mixed-venous PO$_2$ gradient can estimate mean capillary PO$_2$ and can be minimized by increasing the circulatory conductance of oxygen in the blood, and one of the primary mechanisms for increasing this circulatory conductance involves increasing the oxygen-binding affinity of hemoglobin (Storz, 2007). Evidence exists that modifications of hemoglobin function could repeatedly play a key role in mediating an adaptive response to high altitude hypoxia (Perutz, 1983).

An important mechanism to compensate for reduced arterial PO$_2$ at high altitude includes a shift in the shape and position of the oxygen-hemoglobin dissociation curve (ODC), which
describes how the reversible binding of oxygen by hemoglobin is dependent on the PO$_2$ in the bloodstream (Luft, 1972; Storz, 2007). When the PO$_2$ is low as in hypoxic conditions, the mixed venous and arterial points on the ODC would move towards the left side toward the steeper position of the curve, and thus the slope of the line, also known as the capacitance coefficient, would considerably increase (Storz, 2007). This increase would then bring about an automatic increase in the blood oxygen conductance as discussed above and thus prevent PO$_2$ from falling too low under hypoxic conditions. Moderate hypoxia presents a right-shifted ODC, whereas severe hypoxia presents a more left-shifted ODC position, both of which give the mixed venous and arterial points steep positions on the slope (Shappell & Lenfant, 1975). Figure 5 displays the ODC under physiochemical conditions in arterial blood and mixed venous blood (Storz, 2007).

**Figure 5.** A schematic representation of the oxygen dissociation curve under physiochemical conditions prevailing in arterial blood (open circle) and mixed venous blood (solid circle). The y-axis measures the oxygen concentration in the blood ($C_{O_2}$) and the x-axis measures the partial pressure of oxygen in the blood ($P_{O_2}$) and are the oxygen concentrations in arterial and mixed venous blood, respectively, and are the partial pressures of oxygen in arterial and mixed venous blood, respectively. The slope of the line joining the arterial and mixed venous points on the curve denotes the blood oxygen capacitance coefficient (in equations 2 and 3). (Source: Storz, 2007).
The above physiological adaptations represent the trend that acclimatization restores oxygen delivery to values as seen at sea level through increasing hemoglobin, oxygen saturation, and cardiac output. Hypoxia and resultant hypoxemia thus triggers many physiologic regulatory mechanisms, which mostly favor adaptation, however occasionally may evolve into pathologies including acute mountain sickness (AMS). AMS consists of symptoms including but not limited to headache, vomiting, insomnia, dizziness, and fatigue occurring in unacclimatized individuals at altitudes greater than 2500m (Clarke, 2006). A common feature of AMS includes rapid ascent by otherwise healthy individuals without sufficient time to acclimatize (Imray et al., 2010).

Recommended health guidelines over the recommended rate of ascension to minimize the risk of AMS along with more serious forms of high altitude pathologies including High Altitude Cerebral Edema (HACE) and High Altitude Pulmonary Edema (HAPE) suggest that above 3000m, there should be no more than a 300m increase in sleeping altitude per day with rest on every third day (British Mountaineering Council, 2006). This means an individual may ascend more than 300m per day, however the individual should descend back to the point of 300m above the previous point of rest the day prior. Nonetheless, some climbers attempt a quicker ascent sometimes based on reasons such as limited amount of time or money, which in turn could lead to AMS, HACE, or HAPE. Table 3 displays a table with a detailed description of AMS, HACE, and HAPE individually, including their symptoms, treatments, and prophylaxis (Khodae et al., 2016; Hoffman et al., 2014).
<table>
<thead>
<tr>
<th>CONDITION</th>
<th>SYMPTOMS AND SIGNS</th>
<th>TREATMENT</th>
<th>PROPHYLAXIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACUTE MOUNTAIN SICKNESS</strong></td>
<td>Headache, anorexia, nausea, vomiting, dizziness, fatigue, weakness, insomnia</td>
<td>Descent, acetazolamide, dexamethasone, supplemental oxygen</td>
<td>Slow ascent, acetazolamide, dexamethasone</td>
</tr>
<tr>
<td><strong>HIGH ALTITUDE PULMONARY EDEMA</strong></td>
<td>Dyspnea at rest, cough, decreased exercise, performance, chest pain/tightness, low pulse oximetry, central cyanosis, tachypnea, tachycardia, rales, wheezing</td>
<td>Descent, supplemental oxygen, nifedipine, phosphodiesterase-5 inhibitors, salmeterol</td>
<td>Slow ascent, nifedipine, phosphodiesterase-5 inhibitors, salmeterol</td>
</tr>
<tr>
<td><strong>HIGH ALTITUDE CEREBRAL EDEMA</strong></td>
<td>Change in mental status or ataxia in a person with AMS or HAPE</td>
<td>Descent, dexamethasone, acetazolamide, supplemental oxygen, portable hyperbaric chambers</td>
<td>Slow ascent, dexamethasone, acetazolamide</td>
</tr>
</tbody>
</table>

Table 3. *Acute high altitude illness summary.* (Source: Khodaee et al., 2016; Hoffman et al., 2014).

Acclimatization overall shows a wide range of inter-individual variability due to the fact that some individuals acclimatize quicker and more effectively than others and are therefore relatively less susceptible to high-altitude pathologies like AMS (Brown & Grocott, 2013). The cardiovascular system responds to hypoxic conditions at high altitude in complex ways ultimately leading to adaptation and acclimatization, though sometimes instead evolving into
acute and chronic pathological conditions related to altitude exposure through hypoxic environments as stated above. Increased sympathetic activity under hypoxic conditions leads to an initial increase in cardiac output (Q) which is achieved through an increase in HR (Brown & Grocott, 2013).

Acknowledging the wide range of inter-individual variability of acclimatization to hypoxia, the awareness for individual susceptibility for AMS would be helpful for preventative strategies. The degree of hypoxia when acutely exposed to high altitude varies markedly depending on the individual HVR (Schoene et al., 1984). Thus, individual HVR has been considered as predictive of the tolerance to acute hypoxia (Moore et al., 1986). Moore at al. (1986) found that eight subjects susceptible to AMS symptoms had lower minute ventilations and higher end-tidal CO$_2$ (EtCO$_2$) values as compared with four non-symptomatic subjects, displaying that an overall lower HVR correlated with the subjects’ symptoms of AMS. Schoene et al. (1984) similarly found that at low levels of moderate exercise, ventilation at sea level and after acclimatization to very high altitude at 6,300 m was higher in the high HVR group, and that climbers with the highest HVR values reached and slept at higher altitudes. They also found that HVR inversely correlated with the decrease in O$_2$ saturation (SpO$_2$) from rest to maximum exercise (Schoene et al., 1984).

In contrast, some studies have shown a close relationship between SpO$_2$ values and AMS susceptibility after prolonged exposure to hypoxia. HVR has often been determined under very acute (five to ten minutes) isocapnic (same CO$_2$ content) hypoxia without consideration of the following hypoxic ventilatory decline (HVD), and the fact that AMS susceptibility is sometimes based on one single altitude exposure (Burtscher et al., 2004). Burtscher et al. (2004) assessed the relationship between individual SpO$_2$ following a 20-to-30-minute exposure to poikilocapnic
hypoxia and the AMS susceptibility based on repeated observations and found that SpO\textsubscript{2} values after the 20 to 30 minute exposures were 4.9% lower in subject susceptible to AMS as opposed to those who were not. Their logistic regression analysis thus revealed altitude-dependent SpO\textsubscript{2} values to be predictive of AMS susceptibility (Burtscher et al., 2004). Roach et al. (1998) also concluded that resting arterial hypoxia is related to the later development of AMS and likely mechanisms include hypoventilation relating to normally acclimatizing individuals or abnormalities of gas exchange, which may be noted through pulse oximetry.

When normal acclimatization is disturbed by factors including infection, high rate of ascent, intense exercise, and insufficient fluid intake, AMS may also be triggered (Schneider et al., 2002). Therefore, the assessment of AMS susceptibility due to repeated altitude exposures could possibly provide a more adequate basis for prediction than occasional observations. Burtscher et al. (2007) showed that 86% of AMS susceptible subjects could be predicted when assessing AMS susceptibility on repeated observations. Rathat et al. (1992) similarly displayed that the measurement of cardiac and respiratory responses to hypoxia at rest and during exercise allows for the detection of those subjects more liable to suffer from AMS. They found through a retrospective study performed on 288 subjects that the most clinically susceptible subjects had at least one abnormal response, especially in exercise tests (Rathat et al., 1992).

Exercise tests measuring cardiorespiratory responses during acute exposure to hypoxia have become increasingly important in the context of athletes electing to train at high altitudes to increase their performance levels. Within the environment of these tests, acclimatization and prevention of high-altitude illnesses also prove as vital information. Paterson et al. (1987) found that maximal scores for oxygen consumption (VO\textsubscript{2}), carbon dioxide production, and HR decreased linearly with increasing hypoxia, and in turn, maximal scores for ventilation and
ventilatory equivalent (VE/VO2) increased linearly with decreasing fraction of inspired oxygen (FiO2). They also found that female subjects had significantly higher VE/VO2 scores as well as a significantly less relative decrease in VO2 max test than male subjects, which led them to conclude that when compared to males of similar age and fit condition, young, highly active females have a stronger adaptive response to acute hypoxia during a maximal treadmill run (Paterson et al., 1987). A better hyperventilation response, as seen here in women, could enhance arterial oxygen saturation and oxygen delivery to muscles, however some studies have found a higher VE/VO2 in women at maximal exercise without a lesser oxygen saturation. Gore et al. (1997) concluded that VO2 peak could be a more appropriate indicator of exercise-induced hypoxia, with results calculating a 67-76% decrease in VO2 peak which could be accounted for by a decrease in oxygen delivery, indicating that reduced oxygen tension at mild altitude leads to impairment of exercise performance in a maximal work session lasting about five minutes.

In endurance exercise and sport, most training takes place at altitudes below 3,000 m and as altitude increases, aerobic performance and VO2 max are reduced on account of the reduced partial pressure of O2 in inspired air (Fulco et al., 1998). When comparing the responses of highly trained to untrained subjects’ VO2 max while under acute hypoxic conditions, results have indicated that highly trained subjects experience greater percent decrements in SpO2 and VO2 max as compared with untrained subjects (Martin & O’Kroy, 1993; Lawler et al., 1988). Some results may attribute results similar to SpO2 level, however Cardus et al. (1998) showed that subjects could be limited at sea level by the oxidative capacity of muscle mitochondria. Nevertheless, it has been suggested this larger decline in VO2 max with increasing altitude of higher trained athletes is due to the fact that they already have developed exercise-induced desaturation at sea-level (Chapman et al., 1999; Gore et al., 1996). Fulco et al. (1998) also states
that pre-exposure to altitude effects the degree of VO$_2$ max decline. Another reason for the
decrease in VO$_2$ max under hypoxic conditions includes a reduced maximal exercise intensity
(Peltonen et al., 2001). Wehrlin & Hallen (2005) evaluated VO$_2$ max and performance using the
same absolute exercise intensity from sea level to low and moderate altitudes (ranging from 800 m to 2,800 m) in unacclimatized endurance trained athletes with a VO$_2$ max and performance
test and found that both VO$_2$ max and performance decreased significantly and linearly with
altitude increase, and VO$_2$ max decreased at the same rate between low and high altitudes. The
results of their study also indicated that a reduction in VO$_2$ max is present at altitudes lower then
1,000 m in endurance trained athletes, whereas before based on the sigmoidal shape of the
oxyhemoglobin curve, it has been assumed that the O$_2$ content of the arterial blood and VO$_2$ max
will only be slightly affected by very mild hypoxia (altitude of less than 1,500 m) (Wehrlin &
Hallen, 2005). Figure 6 displays the decline in VO$_2$ max at each altitude, from low to moderate,
in each endurance trained athlete (Wehrlin & Hallen, 2005).
Figure 6. Effect of acute simulated altitude exposure on VO$_2$ max in eight male, sea level resident, ETA. The upper part of the figure shows means ± standard error (SE), the lower part of the figure shows the individual values of the subjects A–H. (Source: Wehrlin & Hallen, 2005).

METHODS

SUBJECTS

Thirteen apparently-healthy male (n = 6) and female (n = 7) 19-to-35-year-old informed volunteer subjects were tested in the Jack Borgenicht Altitude
Physiology Research Facility, Department of Kinesiology and Health Sciences at William & Mary, Williamsburg, VA 23187. The study was approved by William & Mary Protection of Human Subjects Committee (PHSC-2019-05-17-13623- mbharr). All of the subjects were nonsmokers, none were born at an altitude greater than 1,500 meters or had traveled to an altitude greater than 1,500 meters 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) following their first session in which they were familiarized with all testing procedures and the testing environment before the two testing sessions.

**RESEARCH LOCATION**

All aspects of this study were conducted in The Jack Borgenicht Hypoxia/Altitude Physiology Research Facility (JBARF), located in Adair Hall in Room 108 on the campus of The College of William and Mary in Williamsburg, Virginia. The laboratory is located at an altitude of approximately 15 meters with a standard barometric pressure of 752 mmHg which is approximately sea level but varies slightly contingent on weather conditions. The facility consists of a normobaric hypoxic chamber (Colorado Altitude Training Systems, Boulder, CO) within which the oxygen content of the chamber atmosphere in a given hypobaric environment
decreases and thus the partial pressure of oxygen is able to be finely controlled to simulate oxygen pressures found in atmospheres at altitudes from sea-level (SL) to 7,645 meters (25,000 feet). The normobaric hypoxic chamber operates at a “normal” SL atmospheric pressure of approximately 760 torr. The air units that are associated with this chamber extract oxygen from external air that is then pumped into the chamber and thus maintains a preset simulated altitude inside.

The fraction of O$_2$ in the atmosphere remains at the same at 20.9% regardless of the altitude. With this being said, the atmospheric pressure at SL (760 mmHg) corresponds to a 159 mmHg partial pressure of O$_2$. The reduced partial pressure of oxygen in the chamber pushes oxygen into the bloodstream across an overall lower gradient, which results in less oxygen carried by the blood to the body tissues. As previously discussed, this environment containing a lower content of oxygen can result in high-altitude illnesses including AMS, or in more serious cases, HACE or HAPE as well.

**EXPLANATION OF VARIABLES**

In addition to heart rate variability measurements, several other variables were measured in this study. VO$_2$ peak, defined as the highest value of VO$_2$ attained upon an incremental or other high-intensity exercise test, is designed to bring the subject to the limit of tolerance (Whipp, 1990). In contrast, VO$_2$ max refers to the maximum oxygen uptake and is defined as the highest rate of oxygen uptake and utilization by the body during intense, maximal exercise where no more increases in rate of work bring on additional rises in VO$_2$ (Hill et al., 1923). VO$_2$ peak combines skeletal and cardiovascular muscle oxidative function along with pulmonary ventilation and diffusion capacity (Ross et al., 2016). This measurement reflects the integrated
ability to transport oxygen from atmospheric air to the mitochondria to go on to perform work. The VO$_2$ peak will ideally occur right as the subject begins to plateau in their volume of oxygen consumption (Whipp, 1990). Figure 6 displays the oxygen uptake and the work rate of exercise tests performed up to the limit of tolerance (Whipp, 2010).

![Figure 6](image)

**Figure 6.** 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.

Work rate was also assessed through incorporation and use of a Rating of Perceived Exertion (RPE) scale, where subjects were asked how they felt at specific time intervals throughout the exercise tests. The RPE chart was on a scale from 6 to 20, with 6 being very light and 20 being the absolute most effort the subjects are physically capable of exerting.

Percentage of oxygen saturation in the blood, ($\%$SpO$_2$), includes another variable evaluated in this study. Arterial oxygen saturation measured by a pulse oximeter is based off of the guiding principle that oxyhemoglobin and deoxyhemoglobin differentially absorb red and near-infrared light, respectively, and thus the pulse oximeter is able to noninvasively take a measurement of SpO$_2$ at a given time and provide information on respiratory function.
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(Nitzan et al., 2014). The extent and degree of oxygen saturation causes variation in the color of the blood. The normal range of SpO\textsubscript{2} values falls between approximately 95 to 100% (Mayo Clinic, 2018).

End-tidal carbon dioxide (EtCO\textsubscript{2}) is the level of carbon dioxide (CO\textsubscript{2}) released at the end of an exhaled breath, and these levels reflect the capability with which CO\textsubscript{2} is carried in the blood back to the lungs and exhaled (Richardson et al., 2016). Evidence has shown that the measurement of EtCO\textsubscript{2} can provide an indication of cardiac output and pulmonary blood flow (Pokorna et al., 2010). EtCO\textsubscript{2} measurements may be taken non-invasively through two ways: capnometry and capnography. Capnometry provides a numerical value for EtCO\textsubscript{2}, whereas capnography provides a more comprehensive measurement displayed in both numerical and graphical forms, and thus for this reason capnography is most widely recommended for the monitoring of EtCO\textsubscript{2} (Neumar et al., 2010; Richardson et al., 2016). Capnography devices are classified as either sidestream or mainstream devices. In the sidestream configuration of monitoring EtCO\textsubscript{2}, the sensor for CO\textsubscript{2} is located within the monitoring device at a distance from the individual being monitored. The exhaled CO\textsubscript{2} is then veered from the airway into the device through a sampling tube of usually around six to eight feet in length, attached to the breathing device attached to the individual (Richardson et al., 2016). In comparison, a mainstream configuration has the CO\textsubscript{2} sensor as well as the sampling center integrated into a small device that connects directly to the airway between the breathing circuit and the endotracheal tube (ETT) (Richardson et al., 2016). In this study, a sidestream device was employed due to the fact that mainstream devices are mostly limited to intubated individuals. Through the use of this method, an individual’s real-time ventilation status may be monitored. The normal range of
EtCO₂ values is 5 to 6% CO₂, which is equivalent to 35 to 45 mmHg (American Association of Sleep Technologists, 2018).

**PROTOCOL**

After approval and voluntary informed consent, each subject was familiarized with all test procedures and equipment in the Jack Borgenicht Altitude Physiology Research Facility. Subjects were divided into two groups: Control Group and Treatment Group. After the first familiarization session reviewing the forms and procedures, subjects returned for their measurements at SL and VO₂ peak test. The third session was conducted at least 48 hours apart from the second session. All treatment subjects were exposed to a normobaric simulated environment up to 3500m (11,500 ft) for approximately 30 minutes during the third session for a submaximal stationary bicycle test. Control followed the same protocol, except the chamber air was not filtered of any oxygen as it passed through the air units, thus maintaining SL partial pressure of oxygen in the chamber atmosphere.

During the second session, subjects came to the Jack Borgenicht Altitude Physiology Research Facility and first had their height (standard fixed stadiometer) and weight (Pelstar Health-O-Meter, McCook, IL) measured. Subjects then rested quietly at SL for approximately 20 minutes while having their oxygen saturation (SpO₂) (Nonin 8500 Pulse Oximeter, Plymouth, MN), heart rate (HR) (Polar H10, Kempele, Finland), end-tidal CO₂ (EtCO₂) (Nellcor N-85 Capnograph, Pleasanton, CA), ventilation (VE) (Firstbeat BodyGuard2, Jyvaskyla, Finland), and heart rate variability (HRV) (Firstbeat BodyGuard2, Jyvaskyla, Finland) measured. During this procedure, subjects breathed in both air from SL and at the halfway point (10 minutes in) during the resting phase, the inhaled air was switched to draw from the normobaric hypoxia chamber’s
air for treatment subjects. The air from the chamber will have a reduced atmospheric content of oxygen at 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, SL. The control subjects will receive SL air for the entirety of the 20-minute rest period.

These tests are non-invasive and require only that subjects sit quietly while breathing through a mouthpiece with a finger clip in place and two electrodes attached to the chest (one under the right clavicle and the other on the lower left ribcage). After the resting phase, subjects rested on the stationary bicycle ergometer for 3 minutes, warmed up for 2 minutes at 25 Watts, and then underwent a standard graded exercise test (GXT) at SL on the stationary bicycle ergometer (Lode 906900, Groningen, Netherlands) to voluntary exhaustion (VO₂ peak test). This maximum physical effort test will yield data from which hypoxia test parameters will be calculated. At rest and every 2-minute stage of the GXT, SpO₂, HR, and, rating of perceived exertion (RPE) will be recorded. Once started, the entire GXT test should last no more than 12 minutes. 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.

No sooner than two days after the GXT, subjects return to the laboratory and enter the normobaric hypoxia chamber in which the atmospheric oxygen content was reduced to a percentage whose partial pressure is equal to those found at an altitude reaching 3,500 m (11,500 ft), or in the case of the control subjects, at SL. Subjects then warm up on a stationary bicycle ergometer for 3 minutes, similar to the VO₂ peak warm up, however then pedal the ergometer at a HR equivalent to that recorded at 65% of their SL VO₂ peak. 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 V02peak, Sp02, HR, CO₂ and O₂ exhaled as well as RPE will be recorded and compared to measurements taken during SL testing. Resting ventilation and metabolic values at altitude will be compared to SL data.

Heart rate variability analysis was performed in each subject through the manual selection of a 5-minute time interval. For the resting state at SL, this interval was carefully chosen through visual identification of where each subject was in a steady state of rest. This typically began approximately 2 minutes after the subject began resting through the 7-minute mark. For the resting portion at altitude for treatment subjects, this interval included the first 5 minutes after switching over to the air of the normobaric hypoxic chamber, in order to most accurately represent the hypoxic ventilatory response (HVR), which occurs immediately after exposure to hypoxia.

STATISTICAL ANALYSIS

Statistical analysis was performed using the data analysis pack in Excel as well as IDE RStudio for R. Relationships between variables were analyzed through calculating linear regression analysis. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Table 1 presents the descriptive characteristics of the subjects, including number of subjects, number of male and female subjects, age (years), height (cm), weight (kg), BMI (kg/m²), and VO₂ peak (ml O₂/min). The subjects ranged from 19 to 35 years of age, with a mean age of approximately 21 years old. The mean VO₂ peak of all subjects overall was 44.1. The average VO₂ peak of male subjects was $51.8\pm3.8$ and the VO₂ peak for female subjects was
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37.7±2.0 kg/m². This indicates that the males fall under the “above average” range (Table 3) and the females fall under the “below average” range (Table 4) for their average VO₂ peak scores.

Table 2 presents the relationships between HRV and oxygen saturation levels under different conditions. In the first column we see the average value for each variable (HRV, oxygen saturation level at SL, oxygen saturation level during hypoxia, oxygen saturation level during hypoxia with exercise, the change in oxygen saturation levels at SL compared with hypoxia, and the change in oxygen saturation level at SL compared with hypoxia with exercise). There is significance, with the p value equivalent to 0.05, when looking at the change in oxygen saturation levels at SL compared with hypoxic conditions with exercise.

Figure 1 displays the relationship between resting HRV (RMSSD) at SL and the change in oxygen saturation (%SpO₂) from SL to exercise in hypoxia equivalent to an altitude of 3500 m. This data displays that with a higher HRV, there is a greater change in oxygen saturation levels, and thus HRV (RMSSD 54.5±5.7) was significantly correlated (r=0.227, P>0.05) to the percent change (9.7±0.7) in %SpO₂ from rest (97.4±0.2) to exercise (88.0±0.8) in normobaric hypoxia. Figure 2 shows the relationship between HRV (RMSSD) and VO₂ peak (ml/kg/min), which yielded a significant linear regression of p<0.01. This displays that overall with a higher VO₂ peak, subjects had a higher HRV. Figure 3 displays the relationship between HRV (RMSSD) and VO₂ peak (ml/kg/min) in females and figure 4 displays this same relationship but in males, both of which yielded a significant linear regression, with p<0.01 and p<0.04 respectively. With a higher VO₂ peak for males and females separately, there is a higher HRV found as well. Figure 5 displays the VO₂ peak (ml/kg/min) at SL compared with the change in oxygen saturation (%SpO₂) from SL to exercise in hypoxia equivalent to an altitude of 3500 m.
The linear regression was not significant, which shows that there was no change with oxygen saturation in relation to fitness overall.

This study has been limited by the number of participants, age group of participants, relatively narrow fitness range of participants, altitude we implemented, and software for data analysis that we used. Our results may not apply to older or younger age groups due to the 18-35-year-old age range. We did not have enough subjects to differentiate between different ethnic groups. The Firstbeat SPORTS software filters the raw data of the RMSSD values, so we were not able to do as much raw data analysis as we would have liked to for analysis as well.

**DISCUSSION**

The application of HRV has been recognized in many clinical conditions. Predicting responses to acute hypoxia based on physiologic measures at sea level may be valuable in anticipating adverse responses to acute hypoxia. The purpose of this study was to examine the relationships between heart rate variability (HRV) and arterial oxygen desaturation in response to acute hypoxia exposure at rest and during exercise. Recognizing the wide range of inter-individual variability of acclimatization to hypoxia and the awareness for individual susceptibility for AMS would be helpful for preventative strategies. We found that HRV at rest appears to be predictive of the degree of arterial desaturation during exercise in hypoxia equivalent to an altitude of 3500 meters. Current wearable technology that can measure HRV could be used to predict potential adverse responses to exercise in hypoxic conditions such as altitude.

Clinical applications including the prediction of the risk of arrhythmic events after MI along with prediction of evolving diabetic neuropathy have been mainly associated with HRV, however observations have shown the applicability of HRV also in relation to physical exercise.
training. Our study agrees with this in that there was a significant direct relationship between HRV and fitness level, where individuals with higher fitness have a higher HRV. In higher trained individuals as compared with sedentary individuals, the sympathovagal balance is altered in response to the duration and different intensities of aerobic training. Pichon et al. (2004) displayed this with their results that identified a sympathetic predominance during comparatively less intense exercise, which was indicated by an increased LF/HF ratio. They also found parasympathetic dominance during comparatively more intense exercise, which was indicated by a decreased LF/HF ratio. Lower HRV reflects sympathetic dominance, indicating external stressors, whereas higher HRV reflects parasympathetic dominance, indicating an association with better stress tolerance and recovery. Acharya et al. (2006) compares the ANS between active and sedentary subjects, showing different HRV values, which could suggest the possibility of monitoring HRV for improving physiological and physical states of being as well. In addition, Berkoff et al. (2007) displayed that elite endurance athletes have a higher parasympathetic tone than recreational athletes or non-athletes, and consequently fitness level and the related conditioning is an important variable influencing the autonomic control of the heart.

Though trained individuals will have a higher fitness level and therefore HRV than untrained or sedentary individuals, a gender difference is still seen in some cases of aerobic fitness levels. Berkoff et al. (2007) also found that there was a difference in both time domain and frequency domain variables, however they concluded it was unclear whether this difference was on account of resistance training, an overall elongated training effect, or some genetic difference. Other studies have looked into significant gender differences in aerobic capacity in relation to physical and physiological differences between males and females. Sharma & Kailashiya (2016) researched this gender difference in aerobic capacity in 30 (17 males, 13
females) young, healthy hockey players competing up to the national level. They measured and analyzed body weight (BW), body fat (BF), body mass index (BMI), resting HR (rHR), hemoglobin concentration (Hb), absolute/relative VO\(_2\max\), and more variables. Even after controlling for BF, BMI, and Hb, a significant difference in absolute/relative VO\(_2\) max was found. The male players had 18.16% higher VO\(_2\) max than the females. Physiological factors that may have impacted the higher aerobic capacity among males who are equally trained with the same competition and nutritional levels as females include a post-pubertal hormonal induced higher lean body mass and Hb content. Females also have a lesser maximum stroke volume and therefore cardiac output on account of their reduced heart size and lesser blood volume overall typically as a result of their smaller body size, which could contribute to a lower aerobic capacity than males (Sharma & Kailashiya, 2016). The finding that males had a higher overall aerobic capacity than females also supports our finding, while also supporting the data that more trained individuals, both male and female, will have a higher fitness level and therefore HRV.

The relationship between HRV and hypoxia has been explored by some previous studies. Macoun et al. (2017) aimed to assess HRV and the arterial oxygen saturation response to short-term exposure to normobaric hypoxia and examine the association with a normoxic VO\(_2\) max. Supine HRV and SpO\(_2\) were both monitored during normobaric hypoxia for 10 minutes in 28 subjects, with HRV being evaluating using both time domain and frequency domain methods. They divided the subjects then into either the Resistant group (SpO\(_2\) ≥ 70.9%, with n=14) or the Sensitive group (SpO\(_2\) < 70.9%, with n=14). Results showed that VO\(_2\) max was higher overall in the SG (VO\(_2\) max=62.4±7.2 ml/kg/min) compared with the RG (VO\(_2\) max=55.5±7.1 ml/kg/min). In addition, vagal activity was found to be significantly decreased while sympathetic activity was comparatively increased. This displays that subjects with a higher aerobic capacity presented a
greater decline in SpO\(_2\), along with greater autonomic cardiac disturbances in hypoxia (Macoun et al., 2017). These results however are not in accordance with the data displayed in Figure 5 from our study, even though we would have expected to see that the higher the aerobic capacity was, the greater the decline in oxygen saturation would be. Instead, we found that VO\(_2\) peak was not significantly correlated with a change in oxygen saturation level from SL to hypoxia. Since higher HRV has been correlated with a higher fitness level, and our data (Figure 1) displays that higher HRV is significantly correlated with a greater decline in oxygen saturation, we would have expected to see the VO\(_2\) peak significantly correlated with a greater decline in oxygen saturation as well. Reasons that might explain this finding could include a higher altitude. The altitude of the laboratory in the study from Macoun et al. (2017) was 260 m above SL and the normobaric hypoxic environment was then taken up to 6200 m (20,341 ft), whereas the Jack Borgenicht Altitude Physiology Research Facility is located at SL and the normobaric hypoxic environment is taken up to 3500 m (11,500 ft). Additionally, the number of subjects as well as more inter-individual variability could have impacted our results.

**CONCLUSION**

HRV at rest appears to be predictive of the degree of arterial desaturation during exercise in hypoxia equivalent to an altitude of 3500 meters. HRV has been found predictive of clinical illnesses, such as the risk of arrhythmic events after MI along with evolving diabetic neuropathy, however observations have also shown the its importance and applicability in relation to physical exercise training. Although VO\(_2\) peak was not shown to have a significant correlation with the change in oxygen saturation, a higher VO\(_2\) peak is correlated with a higher HRV. Predicting responses to acute hypoxia based on physiologic measures at sea level like HRV may be
valuable in anticipating adverse responses to acute hypoxia. Current wearable technology that can measure HRV could be used to predict potential adverse responses to exercise in hypoxic conditions such as altitude.

**TABLES**

**Table 1.** Descriptive Data on Participants (mean ± SE)

<table>
<thead>
<tr>
<th>Table 1 – Subject Characteristics (mean±SE)</th>
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</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>male</td>
</tr>
<tr>
<td>female</td>
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<tr>
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<tr>
<td>height (cm)</td>
</tr>
<tr>
<td>weight (kg)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>VO₂peak (ml O₂/kg/min)</td>
</tr>
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</table>

**Table 2.** HRV and oxygen saturation levels (mean ± SE)

<table>
<thead>
<tr>
<th>Table 2 – Heart rate variability (HRV) and oxygen saturation levels (%SpO₂) (SL – sea level, H – hypoxia, EX – exercise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean±SE</td>
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<tr>
<td>-----------------</td>
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<tr>
<td>HRV RMSSD (SL)</td>
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<tr>
<td>%SpO₂ (SL)</td>
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<tr>
<td>%SpO₂ (H)</td>
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<tr>
<td>%SpO₂ (H+EX)</td>
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<tr>
<td>Δ%SpO₂ (SL-H)</td>
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<tr>
<td>Δ%SpO₂ (SL-(H+EX))</td>
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**Table 3.** Maximal oxygen uptakes for men (ml/kg/min).

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<thead>
<tr>
<th>Rating</th>
<th>Age</th>
<th>Excellent</th>
<th>Good</th>
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<th>Average</th>
<th>Below Average</th>
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<th>Very Poor</th>
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<td>47-51</td>
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<td>49-56</td>
<td>43-48</td>
<td>40-42</td>
<td>35-39</td>
<td>30-34</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

**Table 4.** Maximal oxygen uptakes for women (ml/kg/min).

<table>
<thead>
<tr>
<th>Rating</th>
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<th>Excellent</th>
<th>Good</th>
<th>Above Average</th>
<th>Average</th>
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<th>Very Poor</th>
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<td>35-38</td>
<td>31-34</td>
<td>26-30</td>
<td>&lt;26</td>
</tr>
</tbody>
</table>
**Figures**

**Fig. 1** Resting Heart Rate Variability at Sea Level
vs
Change in oxygen saturation from sea level to exercise in hypoxia equivalent to an altitude of 3500 m

\[ y = 0.0698x + 5.9057 \]
\[ R^2 = 0.2773 \]

**Fig. 2** Relationship between HRV and VO2peak

\[ y = 1.5988x - 15.228 \]
\[ R^2 = 0.6494 \]
Fig. 5 - VO2peak at sea level vs Change in Oxygen Saturation (%SpO2) from sea level to exercise in hypoxia equivalent to an altitude of 3500m

\[
y = 0.1148x + 4.4995 \\
R^2 = 0.199
\]
CITATIONS


Cardus, J., Marrades, R. M., Roca, J., Barbera, J. A., Diaz, O., Masclans, J. R., … Wagner, P. D.
HEART RATE VARIABILITY AND ARTERIAL DESATURATION


Dishman, R. K., Nakamura, Y., Garcia, M. E., Thompson, R. W., Dunn, A. L., & Blair, S. N.
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HEART RATE VARIABILITY AND ARTERIAL DESATURATION


HEART RATE VARIABILITY AND ARTERIAL DESATURATION

Cardiology, 21(10), 719–724. doi: 10.1002/clc.4960211005


Young, H. A., & Benton, D. (2015). We should be using nonlinear indices when relating heart-rate dynamics to cognition and mood. Scientific Reports, 5(1). doi: 10.1038/srep16619

AKNOWLEDGEMENTS

This research was made possible through the support of the Foundation for Aging Studies and Exercise Research, The Doug Morton/Marilyn Brown Endowment for Biomedical Research, and the Borgenicht Program. Jack Borgenicht was a philanthropist, mountain climber, entrepreneur, and businessman. He made history as the oldest person to summit Mount Rainier at the age of 81. Through his generosity, he aimed to forward research in exercise physiology. Inspired by his goals, I have aimed to conduct and carry on this research.

I would further like to express my sincerest appreciation for my thesis advisor, Dr. Michael Brennan Harris, for his meaningful guidance, assistance, and encouragement in devoting many hours to this study. He has worked tirelessly with me so that I could realize and utilize my potential. I hope that I may one day impact a student’s life the way he did mine. I have been very fortunate to work with someone who I regard so highly.

I would also like to thank Dr. Kenneth Kambis for the operation and oversight of the Jack Borgenicht Altitude Physiology Research Facility. Dr. Kambis was a cherished friend and climbing partner of Jack Borgenicht, and has served as the Director of the Borgenicht Program for Aging Studies and Exercise Science since 1996. I wish to also show my appreciation to Dr. Michael Deschenes and Dr. Margaret Saha, both of whom have provided support, guidance, and input as committee members as well.

I would also like to acknowledge and thank all of the subjects who volunteered to participate in my study. Every single subject has assisted me in bringing this study to life. It takes a dedicated person to voluntarily expose themselves to adverse conditions for the sake of scientific research.

Additionally, I would like to thank my parents, who have supported me with love and understanding. Without you, I could have never reached this current level of success.

Kelsey Jean Short
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
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

For the research project titled:

RESTING HEART RATE VARIABILITY AT SEA LEVEL PREDICTS ARTERIAL DESATURATION DURING ACUTE EXERCISE

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: "Resting Heart Rate Variability at Sea Level Predicts Arterial Desaturation during Acute Exercise in Hypoxia", 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 or 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 13 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-2019-05-17-13623-mbharr) titled RESTING HEART RATE VARIABILITY AT SEA LEVEL PREDICTS ARTERIAL DESATURATION DURING ACUTE EXERCISE IN HYPOXIA WAS APPROVED BY WILLIAM & MARY PROTECTION OF HUMAN SUBJECTS COMMITTEE (Phone: 757-221-3966)