THE DUAL EFFECT OF NORMOBARIC HYPOXIA ON HEART RATE VARIABILITY AND SUBSTRATE PARTITIONING FOLLOWING INTERVAL CYCLING

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Abstract

Recent studies have shown the importance of the beat-by-beat changes in heart rate influenced by the autonomic nervous system (ANS), or heart rate variability (HRV). The purpose of this study was to examine the lasting effects of hypoxic exercise on HRV, and its influences on substrate usage. Results from this study could lead an increased understanding on this topic. Eight active healthy males (age: 31±11 years; height: 180±7 cm; weight: 83±8 kg; $\dot{V}O_2max$ (maximal oxygen consumption): 4.4±0.6 $L \bullet min^{-1}$) underwent normoxic and hypoxic (F_iO_2= 0.15) conditions during high-intensity interval (HIIT) cycling (70%-high interval, 35%-rest interval). Cycling intensity was determined by a peak power output cycling test. Each experimental session consisted of a basal metabolic rate determination, up to 45-minutes of HIIT cycling, and three 30-minute post-exercise metabolic rate measurements (spanning 3 hours and 15 minutes after exercise). During exercise, RPE was higher ($p<0.01$) and LAC (lactate) increased ($p=0.001$) at each point of time in hypoxia, with no change in normoxia. After hypoxic exercise, the SNS/PNS ratio (overall ANS activity) was significantly higher ($p<0.01$) and significantly decreased through time in both conditions ($p<0.01$). In addition, a significant interaction between time and conditions ($p<0.02$) showed a decrease in LAC concentration through time post-hypoxic exercise. The findings showed that a single bout of hypoxic exercise alters ANS activity post-exercise along with shifting substrate partitioning from glycolytic to lipolytic energy production. The significant decrease in LAC concentration post-hypoxic exercise supports the notion that hypoxic HIIT induces a greater muscle glycogen depletion leading to increased fat oxidation to sustain glycogenesis and gluconeogenesis to maintain blood glucose level during recovery.

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List of Symbols, Nomenclature or Abbreviations

AMI – Acute Myocardial Infarction

AMS – Acute Mountain Sickness

ANOVA- Analysis of Variance

ANS – Autonomic Nervous System

ATP – Adenosine Triphosphate

AR – Autoregressive model

ARMA – Autoregressive moving average model

BP – Blood Pressure

BMI – Body Mass Index

BMR – Basal Metabolic Rate

CHO - Carboydrate

CO_2 – Carbon Dioxide

CTW – Continuous Wavelet Transform

DC – Direct Calorimetry

ECG – Electrocardiogram

EP – Energy Production

FAT – lipid

FFA – Free Fatty Acid

FFT – Fast Fourier Transform

HIIT – High Intensity Interval Training

HF – High Frequency

HR – Heart Rate

HRV – Heart Rate Variability

IC – Indirect Calorimetry

IE – Interval Exercise

LAC – Lactate

LF – Low Frequency

LF/HF ratio – Low Frequency/High Frequency

LHTL – Live-high, train-low method

LLTL – Live-low, train-low method

N_2 – Nitrogen

NN Intervals – Normal-to-Normal Intervals

MA – Moving Average

MR – Metabolic Rate

O_2 – Oxygen

PAR-Q – Physical Activity Readiness Questionnaire

PEMR – Post-exercise Metabolic Rate

PNS – Parasympathetic Nervous System

PO_2 – Partial Pressure of Oxygen

PPO- Peak Power Output

RER – Respiratory Exchange Ratio

RPE – Rate of Perceived Exertion

SA Node – Sinoatrial Node

SD – Standard Deviation

SNS- Sympathetic Nervous System

SP – Substrate Partitioning

S_PO_2 – Blood Oxygen Saturation

SUMSF – Sum of Skinfolds (chest, abdomen, thigh)

TP – Total Power

WC – Waist Circumference

WP – Welch Periodgram

VLF – Very low frequency

$\dot{V}O_2max$ – Maximal Oxygen Consumption

$\dot{V}O_2peak$ – Peak O_2 Uptake

List of Appendices

Chapter 1: Introduction

Thesis Overview

This thesis report entitled "The Dual Effect of Normobaric Hypoxia on Heart Rate Variability and Substrate Partitioning following Interval Cycling."

Chapter 1 is an introduction into the different components of this manuscript, including: 1. heart rate variability (HRV), 2. substrate partitioning (SP), and 3. hypoxia (HYP). A history on these aspects is also discussed.

Chapter 2 is a comprehensive review of the current literature on the main components to this study. These components include: 1. HRV, 2. SP, and 3. HYP. Also, the relevance of the study is discussed.

Chapter 3 starts the manuscript for this study. This chapter examines in great detail the study methodology, including the participants, materials and equipment utilized, schedule and sessions for the study, and the different variables measured.

Chapter 4 shows the overall results from this study. The findings include lactate (LAC), heart rate (HR) and rate of perceived exertion (RPE) during exercise and LAC, HRV values and SP values following exercise.

Chapter 5 discusses the results from the study. The effects of HYP on ΔHR and ΔLAC during exercise, as well as the post-hypoxic effects are examined in great detail. More specifically, findings for ΔSNS, ΔPNS, ΔSNS/PNS ratio, ΔLAC, ΔFAT (lipid), and ΔCHO (carbohydrate) oxidation as well as hypotheses are argued. Lastly, the effects of eating a light snack 90 minutes after exercise is discussed.

Chapter 6 concludes the manuscript by reviewing the results and discussing the importance of the findings.

Background of Study

The beating heart has been of clinical interest since the dawn of medicine. In fact, in the early 18th century John Floyer was the first physician to measure HR in beats per minute (Lauer, 2011), because HR was an important measurement of human health. In the 19th century, there were many early investigations on the mammalian heart, including cats, dogs, rabbits, hedgehogs, guinea pigs, and rats (McWilliam, 1888). Through his studies, McWilliam learned many aspects of heart physiology including the "refractory period," cardiac rhythm when excited and inhibited, and the origin of a contraction in the heart (Sino-atrial node or SA node). The clinical significance of heart rate variability (HRV) was first elicited in 1965 when researchers Hon and Lee discovered alterations in interbeat intervals during fetal distress (Camm, 1996). Wolf et al. (1978) were the first to show that reduced HRV correlated with mortality rates. Very soon after this publication, Akselrod et al. (1981) first introduced spectral analysis, which is one of the methods used to analyze HRV today.

Altitude physiology studies began in the late part of the 19th century and early 20th century by physically active physiologists, which included Bert, Douglas, Haldane, and Barcroft (Brooks et al., 2005). More research was needed when there were aviation problems during World War II. Astrand and Astrand (1958) published one of the first studies that examined the heart during hypoxia. Their study (1 female, 3 males; extensive data from a 35 year old male) looked at muscular work performed first at sea level, then during four weeks at an altitude of 14,250 feet, then during reacclimatization to sea level, and lastly, during acute exposure using an altitude chamber. Their results showed how HR was affected during these changes in altitude. During acute exposure to HYP, HR

increased by 15 to 30 beats per minute. When hypoxia was prolonged, HR gradually decreased due to acclimatization. At 17 days at altitude, they performed an experiment where the participants exercised with ambient air or with O_2 breathing. Their HR was considerably lower during the O_2 breathing. Lastly, they observed S-T depression on the electrocardiogram (ECG) during work at high altitudes. Altitude research has become an important area to study and further our knowledge for many reasons, which include an increase in high-altitude hiking and an increase in athletic competitions held at moderate altitudes (Brooks et al., 2005).

More current research has shown the significance of measuring substrate partitioning (SP), that is, the contribution of CHO, FAT, and protein, to the production of energy within the body. For instance, high intensity exercise leads to more CHO usage than FAT when compared to low intensity exercise (Malatesta et al., 2009). As a consequence, FAT usage increases post-exercise, that is, during the recovery period (Kuo et al., 2005). These changes in SP are, to some extent, mirrored in the regulation of the ANS. Drager et al. (2010) states that SNS activation (caused by exercise, hypoxia, etc.) causes lipolysis, which in turn reduces insulin-mediated whole body glucose uptake. This evidence gives reason to examine the cumulative effect of exercise and hypoxia on the sympathovagal balance and its potential association with the shift in SP during recovery.

Purpose of the Study

The objective of this study was to assess the interactions between the autonomic nervous system (ANS) and substrate partitioning (SP). Further analysis explored how hypoxia plays a role in this interaction. The activity of the ANS was measured using heart

rate variability (HRV) three hours post-exercise. Post-exercise SP was split amongst glucose and lipids. It was hypothesized during post-exercise, when lipid oxidation increased, ANS activity would increase in tandem. Additionally, hypoxia would strengthen this relationship between these two variables.

Significance of the Study

Currently, there is little known about the relationship between the ANS and SP post-exercise. Results from this current study could lead to further understanding this association between the ANS and metabolism. By looking at the ANS and SP post-hypoxic exercise, recovery patterns emerge. These patterns could be useful information in many areas such as athletics and hiking. For example, recovery patterns in the field of athletics can be very beneficial so no overtraining or injury occurs (Dupuy et al., 2012). The same can be apparent for hikers and includes the physical stressor of hypoxia. In addition, increased knowledge on this topic could assist in further study on this topic.

Chapter 2: Review of Literature

Introduction

In this section, the variables of this study (HRV, SP and hypoxia) will be discussed in detail. HRV measures ANS activity at rest, and this variable will display the lasting effect of exercise, such as a prolonged decrease in PNS activity. This sustained decrease in PNS activity will also lead to a prolonged increase in HR. This is magnified after hypoxic exercise (Haddad et al., 2012). Hypoxia can also affect SP following exercise, by increasing lipid usage (Kelly, 2015). From these findings, one can hypothesize that the ANS and SP are linked following exercise.

The Autonomic Nervous System

The nervous system is comprised of the central and peripheral nervous systems. The peripheral nervous system is further divided into the ANS and somatic nervous systems. According to Freeman et al. (2006), the ANS is mostly an efferent system that transmits impulses from the central nervous system to the peripheral organs. The ANS primarily controls smooth muscles that, in turn, monitor blood flow and blood pressure (BP) while controlling smooth muscle contractions in other organs. Although mostly efferent fibers, there are also some afferent ANS fibers. These fibers innervate the baroreceptors and chemoreceptors located in the carotid sinus and the aortic arch. Primarily, these fibers control HR, BP, and respiratory activity. The ANS is divided into the PNS division, SNS division, and the enteric division. The PNS and SNS divisions are responsible for different physiological responses in the body, but the most important task is controlling the SA node and by extension, regulation of HR (Yamamoto et al., 1996).

The Parasympathetic Nervous System

As a part of the ANS, the primary function of the PNS is to conserve energy (Freeman et al., 2006). The PNS decreases HR and BP and aids in digestion, absorption of nutrients, and discharge of wastes. During PNS activation, the arteries become dilated. The chemical transmitter at the synapses responsible for triggering these responses in the PNS is acetylcholine. One of the most important anatomic locations of the PNS is the innervation at the heart, via the Vagus Nerve (X). This nerve is important because it carries fibers to many areas of the body, including the heart and lungs.

The Sympathetic Nervous System

The primary functions of the other branch of the ANS, that is, the SNS, is to respond to physiological challenges, such as fight or flight, respiratory failure, or exercise (Freeman et al., 2006). Responses to these challenges imply increasing HR, BP, and cardiac output. Also, blood flow from non-important areas of the body, such as the skin, is diverted and supplied to areas in need such as the skeletal muscle. Most of the SNS nerves originate at the thoracolumbar outflow. The flight or fight response is caused by the adrenal medulla, which is innervated by preganglionic fibers. When this gland is stimulated by the nicotinic acetylcholine endings, adrenaline is released. At times of greater stress, more adrenaline is released. Another important stress hormone is norepinephrine; also released during the fight or flight response, this hormone increases and decreases blood flow to skeletal muscles. This hormone is mostly present on the postganglionic SNS endings.

Heart Rate Variability

General

HR increases linearly with workload leading to changes on a beat-by-beat basis (Buchheit et al., 2004). To measure this beat-by-beat change, HRV is used. HRV reflects changes in the activity of the ANS and could indicate an imbalance within the body related to exercise, recovery, and environmental-induced stress such as hypoxia (Montano et al. 2009; Povea, et al., 2005). At the beginning stage of recovery from exercise (after three minutes), there is a decrease in cardiac SNS activity and an increase in cardiac PNS activity. Most commonly, HRV is utilized at this stage to measure this beat-by-beat change in HR caused by the SNS and PNS. In addition, HRV can be used hours after exercise to observe the lasting effects on the ANS. According to Dupuy et al. (2012), HRV is one of the most common forms of measuring cardiac ANS regulation and cardiac PNS reactivation post-exercise. As an added bonus, this test is non-invasive, so it can be conducted on patients easily. HRV can be measured in two different ways, in the time domain or the frequency domain (Camm, 1996). As can be seen in Figure 1, there are steps that need to be taken in order to properly measure HRV.

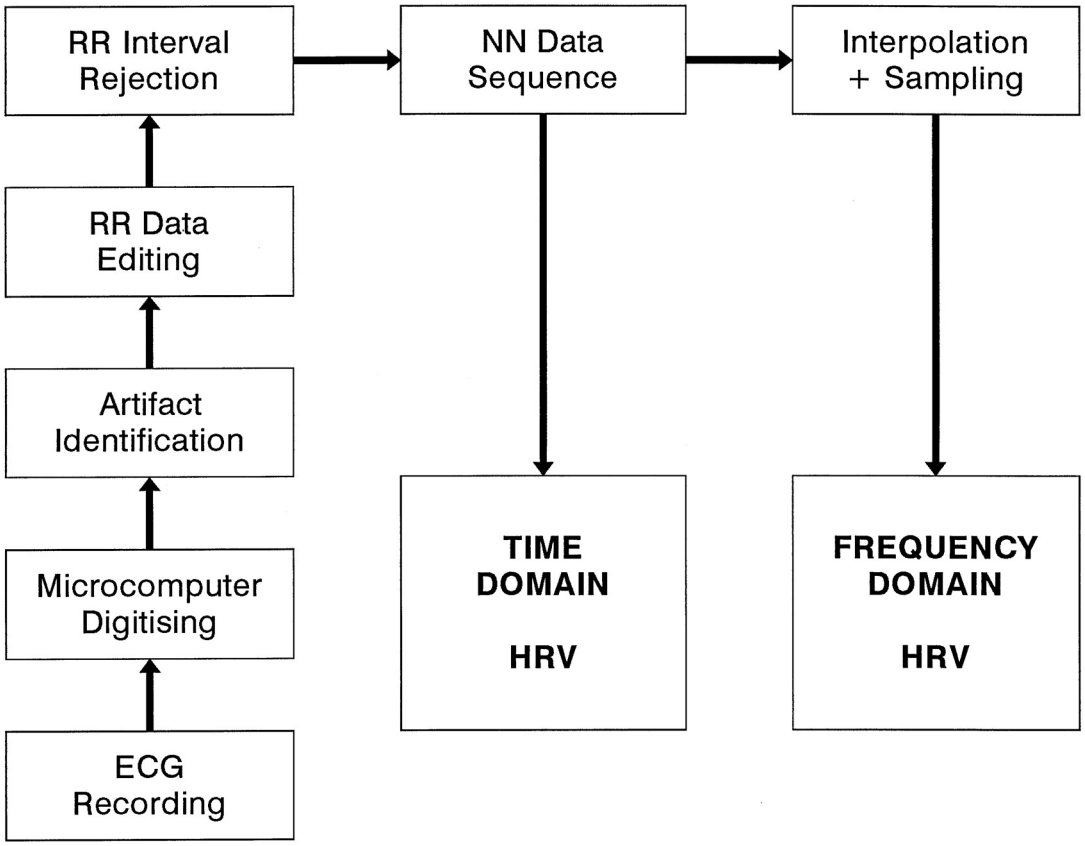


Figure 1: All of the steps, described below, needed to obtain data for HRV analysis (Camm, 1996).

Time Domain Analysis Method

The time domain refers to how the HRV signal changes over time (Persson et al., 2001). For this method, HR can be detected at any point in time or in intervals (Camm, 1996). Intervals are between consecutive normal complexes (interval from one QRS complex to the other). Normal-to-normal (NN) intervals or instantaneous HR results from this method. Using the mean NN interval from the data set, many physiological measurements can be calculated including the mean HR and the difference between the longest and shortest NN interval (see table 1). The time domain method is often utilized

in long-term HRV recordings (such as over 24 hours) using a Holter monitor.

Geometric Methods – RR Interval

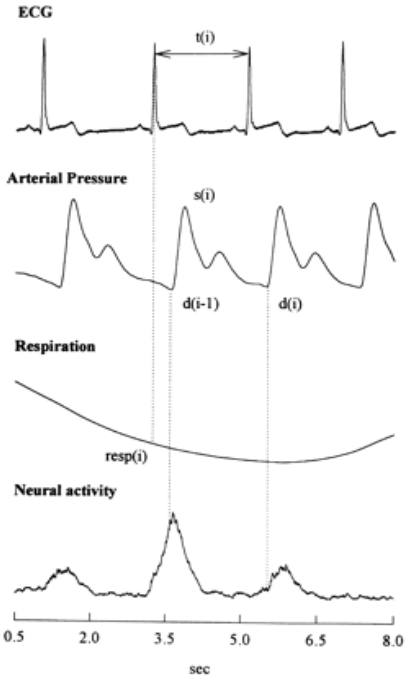


Figure 2: Arterial pressure, respiration and neural activity during an ECG

The series of NN intervals can be converted into geometric patterns such as RR intervals (Camm, 1996). Using an ECG (ex: figure 2), RR intervals (time between one R wave and the next R wave) show fluctuations that occur due to continuous interactions of inhibitory and excitatory reflexes caused by the PNS and the SNS (Pagani et al., 1997). The simplest way to measure HRV is by quantifying variations in RR intervals (Maliani et al., 1994). Amongst other calculations (see table 1), the most important, standard deviation (SD) can then be calculated from these variations. Again, this assessment of HRV relies on the time domain or how HRV signal changes over time (Persson et al., 2001). According to Malliani et al., (1994), in physiological conditions, such as exercise, which leads to tachycardia and an increase in SNS activity, the SD of HRV increases. During recovery, or when PNS activity is at its pinnacle, the SD of HRV decreases. The use of RR interval SD has been proven as a useful tool to measure ANS activity in many populations, such as diabetic and cardiac patients. But this technique is limited because it does not measure changes in the sympatho-vagal balance (PNS and SNS).

Table 1: Selected time domain measures of HRV (Camm, 1996).

Variable	Units	Description
Statistical Measures		
SDNN	ms	Standard deviation of all NN intervals
SDANN	ms	Standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals
SDNN index	ms	Mean of the standard deviations of all NN intervals for all 5-minute segments of the entire recording
SDSD	ms	Standard deviation of differences between adjacent NN intervals
NN50 count	n/a	Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording; three variables are possible counting all such NN intervals pairs or only pairs in which the first or the second interval is longer
pNN50	%	NN50 count divided by the total number of all NN intervals
Geometric Measures		
HRV triangular index	n/a	Total number of all NN intervals divided by the height of the histogram of all NN intervals measured on a discrete scale with bins of 7.8125 ms (1/128 seconds)
TINN	ms	Baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of all NN intervals
Differential index	ms	Difference between the widths of the histogram of differences between adjacent NN intervals measured at selected heights (e.g. at the levels of 1000 and 10 000 samples)

Logarithmic index	n/a	Coefficient ϕ of the negative exponential curve $k \cdot e^{-\phi t}$, which is the best approximation of the histogram of absolute differences between adjacent NN intervals

Frequency Domain Method – Power Spectral Analysis

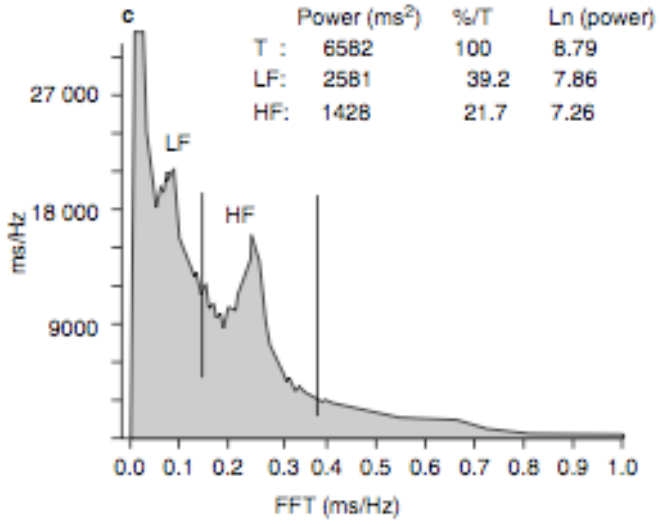


Figure 3: HF and LF Frequency Peaks (Pagani, et al., 1997).

In addition to the time domain, the frequency domain method is commonly used to assess cardiac ANS activity by examining the PNS and SNS activity of the heart non-invasively (Malliani et al., 1994). Spectral analysis is the most common linear method in the frequency domain and calculates how much of the HRV signal lies within each given frequency band within a range of frequencies (Persson et al., 2001). There are three main frequency peaks (as can be seen in figure 3) in the HR spectrum and these peaks include a high- (HF; 0.15-0.40 Hz), low- (LF; 0.04-0.15 Hz), and very low-frequency (VLF; below 0.04 Hz; Malliani et al., 1994).

Regulated by hormones and other influences, these three frequency peaks measure different activities. Primarily, the HF peak measures the activity of the cardiac PNS (vagal; Malliani et al., 1994). The LF peak measures mostly the cardiac SNS and is influenced by the baroreceptor reflex arch. In addition, it measures the vasomotor activity

(dilation or constriction of blood vessels; Malliani et al., 1994). Not much is known about the VLF peak, but it may be affected by the activities of the renin-angiotensin system, peripheral vasomotor function and chemoreceptors. Many studies (Pagani et al., 1997; Dupuy et al., 2012; Bigger et al., 1992; James et al., 2012; Kaikkomen et al., 2007; Torres et al., 2008), have verified the importance of these peaks and how these peaks are affected by the SNS and PNS.

Methods for Calculation of HRV in the Frequency Domain

Methods for calculating HRV in the frequency domain can be classified as parametric or nonparametric (Camm, 1996). Parametric methods are chosen models that result with relatively smoother spectral components than the nonparametric methods. Nonparametric methods are fast methods that utilize simple algorithms. Simple algorithms commonly used are the Fast Fourier transform (FFT) or the Autoregressive (AR) models. In a study by Mendonca et al. (2009), FFT and AR models were compared in 16 participants (9 males, 7 females; physical education students, young adults; active-about 9 hours of physical activity a week). Three protocols were used to measure HRV: resting, submaximal, and maximal. The submaximal protocol consisted of two five-minute walks at 4 km/h at treadmill grades of 0 and 7.5%. The maximal protocol was an incremental test conducted until exhaustion. Results showed significant differences between the FFT and the AR models. Therefore, these models cannot be used interchangeably. The AR model is more sensitive to the effects of dynamic exercise than the FFT model.

Statistics for the Frequency Domain

Variable	Units	Description	Frequency Range
Analysis of short-term recordings (5 min)			
Total power	ms^2	Variance of NN intervals over the temporal segment (NN intervals are subsequent RR intervals)	≤ 0.4 Hz
VLF	ms^2	Power in very low frequency (VLF) range	≤ 0.04 Hz
LF	ms^2	Power in low frequency (LF) range	0.04-0.15 Hz
LFnu	nu	LF power in normalized units [LF / (total power - VLF) x 100]	
HF	ms^2	Power in (HF) range	0.15-0.4 Hz
HFnu	Nu	HF power in normalized units [HF / (total power – VLF) x 100]	
LF/HF		Ratio of LF(ms^2)/HF(ms^2)	
Analysis of 24 hour recordings			
Total power	ms^2	Variance of all NN intervals	≤ 0.4 Hz
ULF	ms^2	Power in ultra low frequency (ULF) range	≤ 0.003 Hz
VLF	ms^2	Power in VLF range	0.003-0.04 Hz
LF	ms^2	Power in LF range	0.04-0.15 Hz
HF	ms^2	Power in HF range	0.15-0.4 Hz

Amongst the many frequency domain measures of HRV (see table 2), a new statistic, the LF/HF ratio, has recently been used more often in the field of kinesiology (Malliani et al., 1994). This ratio is used to quantify the alternating relationship between SNS and PNS activities (sympatho-vagal balance; Billman, 2013). During several physiological or pathophysiological conditions, this balance seems to be affected by total power (TP; Malliani et al., 1994). TP reflects the total variance in the HR pattern (R-R intervals) over time. According to Malliani et al. (1994), in physiological conditions, such as exercise, which leads to tachycardia and an increase in SNS activity, the TP of HRV

increases. While during vagal activity, or PNS activity, the TP of HRV decreases. Use of the LF/HF ratio, especially in cardiac patients, is very important and will become more important in the future as our population ages.

Comparison of Equipment and Frequency Analysis Methods

An important study was conducted by Weippert et al. (2010), which compared three mobile devices (ECG, Polar S810i, and Suuto t6) that measure R-R intervals and HRV and compared different HRV spectral analysis methods. R-R intervals were recorded during three different tests: supine to sitting rest test, moderate dynamic test, and moderate to vigorous static exercise of the upper and lower limb test. HRV was then analyzed in the frequency domain using FFT, AR, a Welch periodgram (WP), and a continuous wavelet transform (CWT). Results showed an excellent intra-class correlation between all devices. But the different frequency analysis methods showed varying results. CWT showed the VLF and LF powers most accurately, while FFT showed the HF power most correctly.

Another important study was conducted by Acharya et al. (2008) to compare frequency analysis methods (FFT, AR, moving average (MA) model, and an autoregressive moving average (ARMA) model). There were 352 cardiac patients tested in this study (participants were classified into one of nine categories: normal sinus rhythm, congestive heart failure, atrial fibrillation, ventricular fibrillation, preventricular contraction, left bundle branch block, complete heart block, ischemic/dilated cardiomyopathy, and sick sinus syndrome). ECG signals were recorded while each patient was lying down for 10 to 15 minutes. Results show that the ARMA model gives

better resolution and would be the best to use in a clinical setting. More specifically, FFT had 80% sensitivity, 100% specificity, and 82% average accuracy; this model had a low reliability for the disease class, but it works well for the normal, healthy class. While AR had 80% sensitivity, 100% specificity, and 82% average accuracy; like FFT, this did not work well for those with high HRV (the cardiac disease class), but it was found suitable for the normal and ischemic/dilated cardiomyopathy classes. Lastly, ARMA had 84% sensitivity, 82% specificity, and 100% average accuracy; it worked well for all patients except for the atrial fibrillation patients.

Limitations

Although HRV can measure the beat-by-beat differences in HR, it can be limited. For instance, Perini and Veicsteinas (2003) state that the HRV peaks do not accurately reflect the changes in SNS and vagal activities during exercise. Also, many physiologists cite the signal selection and analysis as being a major limitation to HRV (Faber, 1996; Dupuy et al., 2012). But Melanson (2000) had a high reliability of HRV in his study because he rigidly controlled factors that influence HRV, such as the testing environment, time of day, and timing of the last meal. Overall, Dupey et al. (2012) found HRV to be moderately reliable in measuring cardiac PNS reactivation.

In addition, there are apparent limitations in the different ways to measure HRV. When measuring in the time domain, errors often result due to the imprecision of the NN interval sequence (Camm, 1996). When measuring HRV, it is better to measure in the frequency domain. Although there have been many studies that successfully used the time domain, the frequency domain is better because it shows the fluctuations in ANS

regulation in different physiological situations (Acharya et al., 2008). In addition, FFT is limited because it does not calculate HRV accurately in participants with cardiac diseases.

Lastly, there are limitations apparent in the HRV statistics. In a HRV review, Billman (2013) states that the LF/HF ratio does not accurately measure cardiac sympatho-vagal balance for healthy people and those with disease. In studies he cited, the LF/HF ratio was unaffected by exercise or acute myocardial ischemia. Also, Billman discusses the roles of the LF and HF components in his review. The LF component, according to Billman, does not provide an index of cardiac SNS drive but instead it reflects a mix of SNS, PNS, and other factors. While the SNS may alter the HF peak by at least 10%, even though most physiologists think the HF peak is only influenced by the PNS. It is important to note, Malliani et al. (1994) stated that many studies have shown the LF and HF components do indeed accurately represent the SNS and vagal activities.

Effects of Body Position on HRV

At rest, HRV can vary due to the body position. According to Perini and Veicsteinas (2003), there are differences in HRV between sitting and a supine position in a young male. Specifically when the participant went from supine to sitting, there was an increase in LF peak power from 20% to 50%, and a decrease in HF peak power from 36% to 12%. These changes in peak power are caused by the ANS. In particular, the decrease in HF power reflects the decrease in vagal activity produced by the orthostatic load. In addition, the increase in LF power reflects the increase in SNS activity caused by the change of body positions. Similarly, the LF/HF ratio also shows this shift.

Specifically, the ratio increased from 0.4 in the supine position to 4.1 in the sitting position.

Effects on Gender on HRV

Along with body position, there appears to be differences in HRV between males and females. According to Mendonca et al. (2010), it is commonly known that women exhibit a more favorable resting ANS profile because of enhanced PNS input. Greater PNS input can be protective during times of stress. For instance, the risk of sudden death with an acute myocardial infarction (AMI) is higher in men than women (Airaksinen et al., 1998).

Mendonca et al. (2010) conducted a study to examine these differences in HRV between genders during early recovery from supramaximal exercise. In this study, the subjects (13 males and 12 females, healthy, nonsmokers, normotensive and sedentary for at least 6 months, ages 20 to 30) first completed a cycle ergometer warm-up, which increased the HR to between 140 and 150 beats•min-^{1}. Then the participants cycled at full speed (unloaded) for 5 to 8 seconds. Following this time period, participants cycled at full speed (loaded; 7.5% of subjects body mass in kilograms) for the next 30 seconds. Next, the participants assumed the supine position while ECG measurements were recorded for 10 minutes.

Specifically, Mendoca et al. (2010) found women had a greater change in the LF/HF ratio compared to their male counterparts. Therefore, the cardiac ANS regulation in women is more affected by supramaximal exercise than in men. Their findings conclude that even though women have a more favorable ANS resting profile, they are

equally as vulnerable to myocardial electrical instability as males during supramaximal exercise.

Furthermore, HRV also differs among genders during intermittent hypoxia. Wadhwa et al. (2008) compared the HRV of males and females during and following intermittent hypoxia at rest. In this study, subjects (15 healthy men and 15 healthy women, matched on the basis of age, BMI, and race) were exposed to eight 4-minute episodes of intermittent hypoxia (8% O_2 and 92% N_2) followed by 15 minutes of recovery. After each episode, there was five minutes of normoxia (recovery).

They found ANS differences between males and females. In males they found a greater level of SNS activity and depressed PNS activity (a greater LF/HF ratio in males) when compared to females. Unlike the male participants, HR in the female participants returned to resting levels during each recovery period (enhanced PNS, depressed SNS). Therefore, there are apparent differences among genders in ANS activity after exposure to intermittent hypoxia.

In addition, a study by Perini et al. (2000) aimed to assess if there are differences between HRV in elderly males and their elderly female counterparts. The subjects' (12 males and 11 females, physically active and highly functional; none of them took medication that would interfere with cardiovascular function) HRV was recorded two ways: 1) at rest in both the supine and sitting position for 10 minutes each, and 2) during dynamic exercise in two different workloads. After performing a warm-up, males pedaled at 0.5kp and 1.5kp and females pedaled at 0.25kp and 0.75kp. From the pedal rate, workload was estimated. Through the first and second protocol, ECG was continuously recorded. They concluded that there were no differences between elderly males and their

elderly female counterparts in the different body positions at rest. During exercise, as exercise intensity increased both genders saw a decrease in the TP of HRV. This reflects the withdrawal of vagal activity, which serves to increase HR in increasing exercise intensities. Overall, there were no differences attributed to gender in the LF, HF, or VLF. Therefore, even though there may be differences in HRV between young males and young females, the HRV differences seem to disappear with age. Further studies should be conducted to delve deeper into these gender differences.

Effects of Body Composition and Activity Level on HRV

Along with gender, body composition and activity level play an important role in HRV. It is well known that endurance trained athletes have a lower resting HR (Esco et al., 2011). Because of this occurrence, Esco et al. (2011), Perini and Veicsteinas (2003), and Luque-Casado et al. (2013) state that trained subjects tend to have an enhanced vagal tone and reduced SNS activity. Therefore, the LF/HF ratio is lower in trained subjects compared to sedentary subjects.

A study by Esco et al. (2011) showed that cardiovascular ANS regulation is significantly related to maximal aerobic fitness and body composition. They measured BMI, waist circumference (WC), sum of skinfolds across the chest, abdomen, and thigh regions (SUMSF), and HRV from 50 subjects. To assess HRV, each subject rested in a supine position for five minutes. Although the results showed that SUMSF seem to be the best indicator of HRV and the LF/HF ratio, WC was also an important factor. Therefore, there seems to be a poor ANS regulation in individuals with greater SUMSF and WC, and/or those who have lower activity levels. When used in combination with traditional

cardiovascular evaluations, HRV can be good tools to measure fitness and cardiovascular health.

Effects of Exercise Modes and Intensities on HRV

Besides gender and activity level, the type of exercise can also affect HRV. Leicht et al. (2008) studied 17 healthy, active males (age: 21.6±5.8 years; mass: 78.6±9.4 kg; $\dot{V}$O2max: 57.0±8.1 ml kg^{-1} min^{-1}). Exercise was performed three times using either upper body (arm ergometer), lower body (cycle) or whole body (treadmill) modes. Separated by 1 week and in random order, participants completed these three tests. All three tests were done the same with three 15 minute stages: 15 minutes seated rest, 15 minutes at 50% HR_{max}, and 15 minutes 65% HR_{max}. Only the last 10 minutes of each stage was used for HRV analyses.

The primary finding from this study was that HRV was significantly greater for the upper body exercise (arm ergometer) when compared to the whole or lower body exercise. More specifically, HRV was greater within the VLF and LF bands. Authors concluded this was possibly caused by a greater dual ANS activation (the PNS and SNS were both higher) via the enhanced baroflex. This conclusion is supported by a greater respiratory exchange ratio (RER) during upper body exercise.

In addition, the intensity of exercise can also influence HRV. Kaikkonen et al. (2007) studied thirteen sedentary women. The participants completed two low-intensity exercises (3500m and 7000m; 50% of the velocity of their $\dot{V}O_2$max), two moderate-intensity exercises (3500m and 7000m; 63% of the velocity of their $\dot{V}O_2$max), and one high-intensity exercise (3500m; 74% of the velocity of their $\dot{V}O_2$max) on a treadmill.

Following each exercise, HRV was analyzed with short time FFT during the 30-minute recovery.

They found a significant relationship between intensity, HF power and TP. Specifically, an increased intensity caused slower HF power recovery (decrease in HRV) and therefore, lower HF frequency and TP levels during the first five minutes of recovery. Thus exercises exceeding 50 to 60% $\dot{V}O_2$max will slow recovery of cardiac ANS regulation. Interestingly, doubling of the distance on the treadmill had no impact on HRV. From this study, the authors suggest that as HRV decreases closer to zero during exercise, the recovery pattern may be delayed.

In addition, James et al. (2012) conducted a study with sixteen experienced runners (14 men, 2 women; average of 3.7 sessions of endurance training a week). In this experiment, participants completed a progressive exercise test on a treadmill at two intensities: moderate (about 75% $\dot{V}O_2$max) and severe (about 95% $\dot{V}O_2$max). Results show that male and female runners had lower HF frequency and higher LF frequency (prolonged decrease in PNS activity and increase in SNS activity) for at least an hour following severe intensity exercise (95% $\dot{V}O_2$max). In contrast, moderate intensity exercise resulted in no significant changes in either the SNS or PNS influences on the heart.

Effects of Age and Cardiac Disease on HRV

Among different age groups, there also seems to be differences in HRV. Lakatta (1993) states that with advanced aging, the heart can change in many ways, including increased fat accumulation around the SA node. Also, usually by age 60, there is a

noticeable decline in the number of pacemaker cells in the SA node. A decrease in pacemaker cells as well as an accumulation of fat around the SA node lead to a decrease in HR during rest and exercise. All of these occurrences lead to a modest elongation of the P-R interval (interval that reflects conduction through the AV node). For some, a longer P-R interval may be indicative of a first-degree block. In addition, variation in HRV is reduced. This is thought to occur because of a reduction in both SNS and PNS modulation (mostly caused because of the decreased efficiency of the SA node).

In addition, Perini and Veicsteinas (2003) state that due to these SNS activity changes, peak power begins to shift with age. For instance, it was shown that the VLF power is more than 50% of the TP in subjects greater than 70 years old (Perini et al. 2000). This was found in both supine and sitting positions. Also, it was found that when elderly subjects change from a supine position to a sitting position, the LF and HF powers, and the LF/HR ratio do not change. This is caused by a lack of ANS activity prompted by orthostatic intolerance (upright to a supine position, or a tilt test) in the elderly population.

Lastly, along with aging, there is an increased risk of cardiac disease that is caused by reduction in HRV. According to Melanson (2000), a reduced HRV lowers the threshold for the creation of arrhythmias. Therefore, individuals (likely the elderly) that have a lower HRV often have an electrically unstable myocardium. Consequently, these reduced levels of HRV are related to all-causes of mortality and increases the chance of new cardiac events. These cardiac events include angina pectoris, AMI, coronary heart disease or congestive heart failure.

Hypoxia

General

In addition to aging and cardiac disease, hypoxia can also affect HRV. Hypoxia is defined as an increase in altitude that triggers a decrease in the PO_2 (Brooks et al., 2005). Moreover, hypoxia can be classified as permanent, long-term, short-term, or intermittent. There are different types of hypoxia: hypobaric hypoxia versus normobaric hypoxia and active versus passive. Normobaric hypoxia occurs at a barometric pressure of 760 mmHg, whereas hypobaric hypoxia occurs at a barometric pressure less than 760 mmHg (Millet et al., 2012). While active hypoxia occurs during exercise and passive hypoxia occurs during rest (Buchheit et al., 2004). These types of hypoxia can affect the body differently.

The decrease in PO_2 during hypoxia directly affects the saturation of hemoglobin, which consequently affects O_2 transport in the human body. In response to delayed O_2 transport, as well as the reduction of inspired PO_2 and maximal cardiac output, $\dot{V}O_2max$ is reduced in hypoxia (Favret & Richalet, 2007). In short-term hypoxia, tachycardia can also be induced. But in long-term hypoxia, resting and exercise HR can decrease; this decrease is caused by acclimatization. There are two hypotheses that relate to the SNS and the PNS: hypoxia either causes a decrease in the responsiveness of the SNS to stimulation or an increase in PNS or both. Both of the hypotheses can be validated through experimental evidence.

Exposure to high altitude hypoxia causes increased ventilation, HR, stroke volume, systemic arterial pressure, and cardiac output (Povea et al., 2005). All of these changes are the result of stimulated chemoreceptors during acute systemic hypoxia. Stimulation of chemoreceptors will then activate the sympathoadrenergic axis. Following

this activation, physiological responses lead to redistribution of blood flow (toward vital organs with greater metabolic need).

Classifications

Hypoxia can be classified as permanent, long-term, short-term, or intermittent (Hoppeler et al., 2003). Permanent hypoxia occurs in higher altitude natives that live under hypoxic conditions from birth to death. Long-term hypoxia is when low-level natives (often near sea-level) are brought to higher altitudes for weeks to months; this window of time will allow for acclimatization processes. Short-term hypoxia is when a subject is exposed to continuous bouts of hypoxia ranging from several minutes to hours (i.e. an exercise bout). Intermittent hypoxia (periodic hypoxia or episodic hypoxia) is when a subject is exposed to short periods of hypoxia (minutes) that are combined with similarly short periods of normoxia (Clanton and Klawitter, 2001; Hoppeler et al., 2003).

There are two further types of hypoxic exposures: hypobaric and normobaric (Millet et al., 2012). Normobaric hypoxia has a barometric pressure of 760 mmHg and hypobaric hypoxia has a barometric pressure less than 760 mmHg. Although disputed, many researchers suggest that hypobaric hypoxia is a more severe condition that leads to different physiological effects from normobaric hypoxia. These physiological effects include but are not limited to ventilation, fluid imbalance, acute mountain sickness (AMS), nitric oxide metabolism, and sports performance.

In a study by Faiss et al. (2013), physiological reactions were compared in hypobaric hypoxia and normobaric hypoxia during exercise and rest. Ten subjects (healthy, well-trained males; middle-aged) were randomly exposed for 24 hours to

hypobaric hypoxia or normobaric hypoxia conditions. Prior to exposure and at every 8-hour marker during exposure, O_2 saturation (S_PO_2), HR, and gas exchanges were measured during a 6-minute submaximal cycling test. Results show that hypobaric hypoxia led to a lower ventilatory response in rest and exercise, as well as a lower plasma pH level, exaggerated oxidative stress, and lower exhaled amounts of nitric oxide compared to the normobaric hypoxia condition. These findings can be very useful in adaptation and physical performance at altitude.

Also, there are different physiological responses with active hypoxia and passive hypoxia. Active hypoxia occurs during exercise and passive hypoxia occurs during rest. According to Buchheit et al. (2004), short-term passive hypoxia significantly increases HR when compared to normoxia at rest. More specifically, under resting conditions exposure to mild or moderate hypoxia triggers an increase in SNS activity, which causes peripheral vasodilation (Wilkins et al., 2006). This initiates a redistribution of blood flow to different areas of the body. When comparing active hypoxia to passive hypoxia, active hypoxia causes an additional desaturation of hemoglobin (Povea, et al., 2005). When comparing active hypoxia to active normoxia, active hypoxia leads to a more prominent decrease in HRV.

Acute Active Normobaric Hypoxia

According to Engelen et al. (1996), active normobaric hypoxia can affect the peak O_2 uptake ($\dot{V}O_2$peak) and the lactic acidosis threshold (the threshold for accumulation of blood LAC). In their study, seven healthy men and one healthy woman breathed three different O_2 concentrations (21%- room temperature, 15%- mild hypoxia, and 12%-

moderate hypoxia) during cycling. The participants completed incremental and constant work rate (moderate and heavy) tests. Results showed that the baseline HR (during unloaded cycling) was elevated in proportion to the severity of hypoxia. They suggest this may be due to elevated circulating catecholamines. Furthermore, as intensity increased the amplitude and kinetics of HR were similar at 12%, 15% and 21% O_2. Lactate acid threshold also decreased for the 12% and 15% O_2 when compared with the 21% O_2.

In addition, Haddad et al. (2012) also completed a study comparing normoxia and hypoxia during exercise. In this study they also used healthy participants, but only men. Participants completed five minutes of submaximal running, followed by a fifteen-minute seated passive recovery. Each participant then ran a twenty-second all-out supramaximal sprint, which was then followed by a fifteen-minute seated passive recovery. This protocol was performed in hypoxia and normoxia. HR was monitored and kept the same in both conditions during the submaximal portion. Results indicated that hypoxia only affected the PNS reactivation after submaximal running, not supramaximal running. Because there is elevated SNS activity following supramaximal exercise, the authors suggest that this causes the activation of the metaboreflex and central chemoreflex. Activation of these two systems will lower muscle pH (through increases in pCO_2 and LAC). It will also slow PNS reactivation following exercise. The authors hypothesize that the already maximal SNS activity caused no differences between conditions following supramaximal exercise.

In contrast, Koelwyn et al. (2012) tested eleven trained men who breathed normoxic air and hypoxic air (16.5 ± 0.5% O_2) and cycled a 10 km time trial. HRV and

HR responses were measured. While they did not find a significant difference between normoxia and hypoxia, they did find that those who had greater than 10% decrease in mean S_pO_2 between hypoxia and normoxia trials experienced greater changes in HRV, which points to inter-individual response differences. If all participants were desaturated at a fixed S_pO_2 (such as 85%), then the physiological stress would be equivalent and the response the same. Although the results from these studies were informative, they were conflicting. These three articles could have contrasting results because Engelen et al. (1996) and Haddad et al. (2012) had "healthy" subjects while Koelwyn et al. (2013) had trained athletic subjects. Koelwyn et al. (2013) also had a higher percent O_2 for their study.

Acute Active and Passive Hypobaric Hypoxia

Zupet et al. (2009) state that hypobaric hypoxia can affect HRV. In their study, there were nine healthy, non-acclimatized males who completed step exercises at 400m (normoxia) and 4200m altitude (hypoxia) at 50% and 75% $\dot{V}O_2$max. During passive hypobaric hypoxia, participants at 4200m had a higher HR and a lower HRV when compared to the 400m results. Whereas during active hypobaric hypoxia, they found that HRV in influenced at both 50% (moderate intensity) and 75% (high intensity) of the $\dot{V}O_2$max. They concluded that a reduced PO_2 at 4200m led to a reduction in SNS activity, which in turn led to a compensatory decrease in PNS activity, these changes then decreasing HRV. These low ANS activity levels could be beneficial or harmful to the body. According to Zupet et al. (2009), some researchers state that these low levels at high altitudes could help in protecting organs from excessive SNS activity and stress,

while others think that these levels could signify the inability for the body to respond to a new, challenging environment (such as hypobaric hypoxia).

Intermittent Active and Passive Normobaric Hypoxia

Povea et al. (2005) conducted a study to examine the cumulative effects of exercise and intermittent hypoxia on HRV, and consequently the SNS and the PNS. Twenty national-level male athletes (12 mid-distance runners, 8 swimmers) who lived at sea level prior to the study, trained at moderate altitude for 3 weeks. After determining $\dot{V}O_2max$, six mid-distance runners and two swimmers completed the live-low, train-low method (LLTL) while six mid-distance runners and six swimmers completed the live-high, train-low method (LHTL; slept between 2500 and 3000 meters) for 13 days. Before acclimatization, results show that when comparing exposure to exercise in acute hypoxia and exercise in normoxia, spectral components of HRV decreased with exercise in acute hypoxia. These spectral components include TP, LF, and HF. After acclimatization, the LHTL group had an increase in the LF component and LF/HF ratio. The authors concluded that intermittent hypoxic training increases the response of the ANS mainly by increasing SNS activity.

Substrate Partitioning in Normoxia and HYP

General

Hypoxia can affect the oxidation of substrates within the body. The mechanisms by which substrates are oxidized and utilized to create ATP are referred to as substrate partitioning (SP). According to Taylor et al. (1996), FAT and CHO are the main

substrates used for the production of energy. During exercise, long polymers of CHO (glycogen) and FAT stores are vital in fueling the body. During the recovery period following exercise, FAT usage increases (Kuo et al., 2005). SP is affected by food intake, intensity and duration of the exercise, body composition, and decreased PO_2 levels.

Effects of Food Intake on SP

Stress is placed upon the body following consumption of food, specifically on the hormonal and nervous systems (van Baak, 2008). When these systems are triggered, digestion, absorption, distribution and storage of nutrients are possible. Along with this process, SNS activity will regulate blood flow and BP following ingestion. Overall, the increase in SNS activity will play an important role in the thermogenic effect of food. More specifically, although highly disputed, activation of the SNS after eating a CHO-rich meal will cause BP to stabilize. No such evidence exists for protein or FAT intake. Lastly, the size of the meal affects the level of SNS increase. Therefore, the size of the meal and SNS activity increase in tandem.

Effects of Exercise Intensity and Time on SP

Use of substrates can be affected in multiple ways due to exercise. In a study by Malatesta et al. (2009), they found that alteration of substrate utilization and exercise intensity were related. The subjects (12 physically fit, young men) participated in high intensity submaximal interval exercise and moderate intensity continuous exercise. Both the intensity components were completed on a cycle ergometer. High intensity submaximal interval exercise consisted of 1 minute intervals at 80% of their maximal

aerobic power output, followed by 1 minute active recovery at 40% of their maximal aerobic power output. For the high intensity exercise portion, duration was determined on the basis of each subject, so that it would match the mechanical work output for the moderate intensity. Moderate intensity was comprised of 60 minutes at 45% of their maximal O_2 uptake. Also, a resting control trial was completed for comparison purposes.

Despite similar energy expenditures, subjects in this study used more CHO than FAT in the higher-intensity exercise (Malatesta et al., 2009). CHO and FAT contribution was less in moderate intensity exercise, when compared to the high intensity exercise. During recovery, there were no significant differences between the high and moderate intensities in SP. The authors cite that this is due to matched energy expenditure between the moderate and high intensity components. But it is important to note that post-exercise, FAT oxidation increased while CHO oxidation decreased.

Similar findings were apparent in the experiment by Kuo et al. (2005). In this study, participants (healthy young men and women; 6 participants for each gender) completed two exercise tasks. These two tasks were 1) 86-89 minutes at 45% and 2) 60-minutes at 65% of their $\dot{V}O_2max$. Exercise bouts were matched for energy expenditure. The authors found that during post-exercise recovery, RER decreased which signifies a substantial shift from CHO oxidation during exercise to FAT oxidation during post-exercise recovery. The authors hypothesize that after exercise there is CHO depletion, which is caused by an energy deficit. Because the CHO is depleted, the body turns towards FAT for energy production during the recovery phase.

Effects of Body Composition on SP

In addition, obesity may also play a role in SP. In a study by Goodpaster et al. (2002), 7 obese and 7 lean men (both groups were sedentary and middle-aged; matched for aerobic capacity) cycled for 60 minutes at similar relative (50% $\dot{V}O_2$max) and absolute exercise intensities. Results show the obese men produced most of their energy from free fatty acid (FFA) oxidation and had reduced rates of CHO oxidation (specifically muscle glycogen), when compared with lean men. The authors speculate that this difference is caused by a decreased rate of glycogen storage in muscles in obese participants when compared to lean participants. Furthermore, because FAT and CHO usage are interdependent, the decrease in CHO use during exercise caused an increase in FFA oxidation during exercise.

Effects of Altitude (or hypoxia) on SP

Lastly, altitude (or hypoxia) can affect substrate utilization. It is well known that CHO become the preferred source of energy at altitude because they create a higher yield of ATP per mole of O_2 (Brooks et al., 2005). But, unusually low CHO stores in humans will create a problem at high altitude. In consequence, FAT catabolism increases at higher altitudes if there is an inadequate amount of CHO available. During severe metabolic challenges at altitude, sometimes the body needs to increase the rate of gluconeogenesis as well.

In a study by Workman and Basset (2012), post-metabolic responses were compared between passive acute normobaric hypoxia and passive short-term normobaric hypoxia. Eleven participants (sedentary, overweight, and males) completed the passive

acute normobaric hypoxia session (one single session of three hours of normobaric hypoxic exposure), while the control group completed a simulated normobaric normoxia condition. Six of the eleven participants that completed the passive acute normobaric hypoxia session then underwent an additional six 3-hour sessions on consecutive days. Pre and post-exposure MR were then determined through IC. When comparing post-exposure MR to pre-exposure MR, results show that passive acute normobaric hypoxia increased energy expenditure and affected fuel utilization (increase in FAT oxidation and a decrease in CHO oxidation). The passive short-term normobaric hypoxia group had further magnified results of the passive acute normobaric hypoxia group. The authors concluded that hypoxia has the potential of being a new therapeutic strategy in helping with weight loss in obese and overweight individuals.

Practical Applications

Hikers

With an increase in travel and hiking around the world, high-altitude acclimatory disruption now affect more people (Buchheit et al., 2004). Exposure to altitude and hypoxia can affect the respiratory and cardiovascular systems, and consequently, the metabolic system. Altitude exposure affects the cardiovascular system because it affects the PNS and the SNS neural balance in the heart (sympathovagal balance; Yamamoto et al., 1996). During hypoxic stress, the SNS and the PNS plays a crucial part in the response to acute and chronic hypoxia during exercise (Favret & Richalet, 2007).

Many acclimations occur in the body when a lowlander experiences acute high-altitude exposure, whether simulated or real. These responses depend on the duration of

the hypoxic exposure (Hochachka et al., 1998). Responses to short-term hypoxic exposure occur in the first few hours of exposure. There are five general responses to hypoxia: 1) carotid body O_2 sensors initiate a hypoxic ventilatory response, 2) pulmonary vasculature O_2 sensors initiate the hypoxic pulmonary vasoconstrictor response, 3) O_2 sensors in other tissues initiate angiogenesis in the heart, 4) O_2 sensors in the kidney and liver begin the process of regulating red blood cell mass, and 5) tissue-specific O_2-sensing and signal transduction pathways lead to metabolic reorganization. With continued hypoxic exposure (long-term), acclimatory responses continue. These changes include: 1) increase in the hypoxic sensitivity of the hypoxic ventilatory response, 2) extension of the hypoxic pulmonary vasoconstrictor response, 3) maintain angiogenesis, 4) maintain erythropoiesis and up-regulation of red blood cell mass, and 5) increased CHO preference during exercise. Because of these changes, hikers and athletes may physically benefit from intermittent hypoxia or simulated hypoxic conditions before completing an endurance activity at sea level or at a higher altitude.

Use of simulated hypoxic conditions can be useful to hikers. By using hypoxic preconditioning, there are four important responses in the body: respiratory response, the strength of the antioxidant stress-limiting systems, efficiency of mitochondrial respiration, and production of erythropoiesis-inducing factors (Bobyleva & Glazachev, 2006). In fact, intermittent hypoxia minimizes much of the altitude effects through short- and long-term acclimatory responses. Short-term adaptations are mainly within the SNS and include an increase in the rate of O_2 transfer in blood by increasing blood flow and maintaining adequate blood flow to tissues. Long-term responses are mainly in the PNS and include an increase in PNS regulation of blood flow.

Recently, there has been much discussion about the LHTL method that helps to improve $\dot{V}O_2max$ at sea level (Favret & Richalet, 2007). This method consists of living at higher altitudes to improve arterial O_2 content, and training at low altitude to increase erythropoiesis. Training at low altitude allows the athlete to keep up at high intensities. The LHTL method has been shown to improve $\dot{V}O_2max$ better than the LLTL method. But it is more beneficial to the athlete if they had previous endurance training. As mentioned previously, Povea et al. (2005) found that intermittent hypoxia during LHTL, after acclimation, increased TP, LF, and the LF/HF component. This suggests that intermittent hypoxic training can increase the response of the ANS by increasing SNS activity.

Athletes

Similarly, endurance athletes can also benefit from simulated hypoxic conditions because of the physical changes that occur. Hochachka et al. (1998) conclude the phenotype of endurance athletes is actually very similar to highlanders. This phenotype includes a blunted hypoxic ventilatory response and hypoxic pulmonary vasoconstrictor response, altered expression of metabolic enzymes, CHO as the main fuel source, enhanced endurance, and an enhanced ratio of aerobic/anaerobic contributions to exercise. In addition, hypoxia along with exercise is often utilized in athletes because it induces greater homeostatic disruption that enhances these physiological responses (Koelwyn et al., 2013). But, because of a greater homeostatic disruption during hypoxic exercise, athletes need longer to recover between training bouts. If the recovery is not adequate, then athletes will fatigue to a greater extent and will decrease their

performance.

Also of importance in athletics is discovering an athlete's HRV. Many studies show that an increase in exercise training leads to an increased HRV as part of a chronic response mechanism (Dupuy et al., 2012). But if this training is physically too demanding, then it can impair the athlete's performance. In the athletic training field, it is well known that training an athlete requires a balance between appropriate training and an adequate recovery period (Lamberts & Lambert, 2009). Kaikkonen et al. (2010) state if the training load is too high then it will threaten the homeostasis of cells, tissues and organs within the body. To find if this balance is correct and not detrimental to the athlete, the use of HRV measurements are commonly used (Dupuy et al., 2012). These measures could be important in monitoring fatigue in athletes and to prevent chronic negative adaptations to exercise, such as overreaching or overtraining.

Cardiac Patients

Along with athletes, cardiac patients can also benefit from HRV analysis. According to Torres et al. (2008), a high HRV indicates a healthy individual, while a low HRV indicates an individual with bad health. It can be argued that HRV mimics the metabolic flexibility. A high HRV is better because it shows a good flexibility of the cardiovascular system, while a low HRV or an abnormal HRV indicates a restrictive response. An abnormal HRV is often considered as a risk marker for cardiac death because it is indicative of altered ANS control. In fact, those who experience an AMI have a lower HRV for at least six to twelve months after the incident. A study by Faber et al. (1996) showed high predictability that patients with low HRV index would experience

another AMI in the next two years. Therefore, use of HRV in cardiac patients could be beneficial in assisting with AMI risk stratification.

Conclusion

Presently, with respect to exercise, SP, HRV and physiological responses to hypoxia are very important. Changes in SP occur during exercise and recovery; these fluctuations are very important to measure. In addition, HRV is a critical measurement because it estimates cardiac ANS regulation (Dupey et al., 2012). Following exercise, there are steps the body takes to recover, which involve changes in SP and HRV. Moreover, during more stressful conditions, such as hypoxic exercise, the activity of the adrenergic system somewhat intensifies these fluctuations within SP and HRV. For example, after hypoxic exercise the body's use of lipids furthers, and it takes longer for the body to recover (Cote, 2015; Kelly, 2015). Also, following hypoxic exercise, there is a PNS reactivation delay, while SNS activity prevails (Hadded et al., 2012). But, the question remains, do these two variables affect one another? Discovering the answer to this question could further our knowledge and assist in many different areas, including hikers, athletics, and the cardiology.

Main Questions

Do changes in heart rate variability reflect shift in substrate oxidation following hypoxic interval exercise? Do this dual response related to muscle glycogen depletion induced by hypoxic interval exercise?

Hypotheses

H_0 = post-hypoxic exercise responses of SP and ANS do not mirror each other.

H_1 = post-hypoxic exercise responses of SP and ANS do mirror each other.

Chapter 3: Materials and Methods

Participants

Twelve highly active cyclists were recruited for this study; however only eight completed all the testing requirements. Participant inclusion criteria were comprised of the following: age 19 to 49 years, male, and a cyclist. All participants over the age of 40 were asked to produce a doctor's note stating they were physically fit enough for a maximal test. The age range was chosen based on a review of available literature. Females were excluded from the study because the different stages of the menstrual cycle can affect the metabolic response. The sample size was determined by calculating the statistical power (G*Power software).

Before the start of the study, participants completed a Physical Activity Readiness Questionnaire (PAR-Q). This questionnaire controlled for cardiovascular risks (disease and family history), medication, metabolic disease, smoking habits, and exercise routines. In addition, participants completed a training inventory questionnaire prepared by the researchers (Appendix A). Prior to involvement in this study, participants were verbally informed of all procedures and provided written and informed consent. Research began after the Interdisciplinary Committee on Ethics in Human Research (ICEHR) approved the study.

Table 3: Anthropometrics and Fitness Characteristics of the Participants

Parameters	Measurements
Age (years)	31 ± 11
Body Mass (kg)	82.6 ± 7.8
Height (cm)	180.1 ± 7.4
VO2max ($ml \cdot min^{-1} \cdot kg^{-1}$)	53.4 ± 6.5
HR Max (bpm)	182 ± 6
PPO (watts)	377 ± 52
Training Experience (years)	13 ± 11
Weekly Cycling ($hours \cdot week^{-1}$)	6.5 ± 1.5
Average Cycling Distance ($km \cdot week^{-1}$)	170 ± 124.3
Cycling Sessions (>70% VO_2max) ($N \cdot week^{-1}$)	1.9 ± 0.6
Weight Training Sessions ($N \cdot week^{-1}$)	1.8 ± 1.6

Experimental Design

A quasi-experimental design with two conditions (treatment: hypoxia, control: normoxia) was used for this study. Participants attended the lab for three different sessions. The first session was the familiarization session where the participants were informed in detail about the study. In addition, participants completed an incremental cycling ramp test for determination of the $\dot{V}O_2$max and were exposed to 20 minutes of resting hypoxia to detect for acute mountain sickness (AMS). The second and third sessions were completed the same, except for the exercise portion was either completed in hypoxia (second session) or normoxia (third session). There was no session randomization because time-matching the exercise portion between sessions was needed in order to accurately compare the data across conditions. These sessions started with a BMR, followed by high intensity interval (HIIT) cycling for up to 45 minutes (if completed) and three post-exercise metabolic rates (PEMRs). The PEMR measurements

lasted 3 hours and 15 minutes after exercise. More specifically, PEMR measurements were 30 minutes each, with a 30 or 45 minute period in between. Measurements during these sessions include HR, HRV, LAC, CHO/FAT usage, and RPE (Borg Scale, Appendix B). The second session took place at least 48 hours after the familiarization session and the third session at least 7 days following the second session (washout period).

Prior to Testing

Before each session, participants were asked to abstain from exercise for 24 hours and to drive or take public transportation into the lab. Before sessions 2 and 3, the participants received standardized meals (780 Kcal; 26g fat, 98g carbohydrate, and 28g protein) to consume the evening prior to testing (before 7pm) in order to control for the thermic effect of food on substrate partitioning. After 7pm, participants were instructed to fast and were only allowed to drink water.

Familiarization Session

The first session was a familiarization session, where the participants were informed in detail about what they would be subjected to during the study. Before the start of session one, participants completed and/or signed the consent form, PAR-Q, and a training inventory questionnaire. Because participants had to eat the provided meal and snack, food allergies or dietary restrictions were disclosed. Anthropometrics (height and weight) and vital signs (BP and resting HR) were taken and seat adjustments (saddle and handlebar height) were made and recorded. Next, participants performed an incremental

cycling ramp test on a magnetic break cycle ergometer (Velotron, Racer Mate, Seattle, Washington, USA) for determination of their $\dot{V}O_2$max and peak power output (PPO). The participants cycled (starting at 50W) at their freely chosen cadence above 60rpm and power was increased by 1W every three seconds. Participants cycled until volitional exhaustion or when the cadence fell below 60rpm. After the PPO test, physiological responses to hypoxia were assessed during rest for 20 minutes. During the hypoxia test, if there were any physiological abnormalities (such as feeling faint, sick, or an S_pO_2 under 85%) the participant was excluded from the rest of the testing. In addition, the Lake Louise Mountain Sickness Score (Appendix C) was utilized. After exposure to resting hypoxia, two participants experienced AMS and were excluded from the rest of the testing.

Treatment Sessions (Hypoxia and Normoxia)

Basal Metabolic Rate

At the beginning of sessions two and three, weight and BP were recorded prior to testing and bladder was voided. Following these pre-measurements, the participant laid under a canopy connected to a metabolic cart and an ECG for a 45-minute period. The atmosphere was calm and quiet so the participant could relax. This protocol (prior to the BMR) was followed consistently for reliable results and to minimize any deviations. A LAC measurement was taken at the end of the BMR to use as a baseline.

Cycle Protocol

Following the BMR, participants completed a cycling interval exercise protocol (IE) for up to 45 minutes while their ECG and S_pO_2 were recorded. For session two, participants cycled in moderate hypoxia (about 15% O_2, equivalent to an altitude of 2750m) and for session three they cycled in normoxia. The IE cycling protocol consisted of 3-minute exercise intervals at 70% of PPO and 3-minute recovery intervals at 35% of PPO (starting at 35% and ending at 35%; 8 low-intensity and 7 high-intensity intervals if completed). This protocol was designed to compare to earlier studies from Kelly (2015) and Cote (2015). HIIT was chosen because it is well known this form of exercise that elevates energy expenditure, as well as a predominant use of CHO during exercise and FAT post-exercise (Gore and Withers, 1990; Brooks, 1997). The high intensity interval at 70% was designed to tax the endogenous glucose (this utilization will increase in hypoxia; Peronnet et al., 2006). Cycling times (to exhaustion) were matched from session 2 to session 3. For instance, if a participant could only cycle 36 minutes in hypoxia, they would only cycle 36 minutes in normoxia. RPE was recorded during the last 30 seconds of each 3-minute interval. Before and after the exercise test, participants sat still for 10-15 minutes on the cycle to record their ECG and heart rate (pre and post). After the exercise test, participants were allowed to rest for 15 minutes.

Post-exercise Metabolic Rate

Following this period, participants completed multiple PEMRs in a supine position (in the same conditions as the BMR) while recording the metabolic rate and an ECG. During this period, SP and HRV were recorded. The PEMRs consisted of three 30-

minute recording periods (pre-snack, post-snack 1, post-snack 2), with a 30-minute and 45-minute rest period in-between. During these rest periods, the participants stayed in the testing room and were allowed to work or relax. The first PEMR (pre-snack) and second PEMR (post-snack 1) were interspaced by a 30-minute rest period. During this rest period participants were given a standardized snack (Clif Crunch Granola Bar- Peanut Butter: 200 Kcal; 9g fat, 26g carbohydrate, and 4g protein; and orange juice: 160kcal; 0g fat, 38g carbohydrate, and 3g protein) to consume in its entirety. The snack was included in the study protocol to look at the affect of a small snack following hypoxic exercise on HRV and SP. The second PEMR (post-snack 1) and the third PEMR (post-snack 2) were interspaced by a 45-minute rest period. In addition, lactate was measured during the end of every PEMR recording (pre-snack, post-snack 1, post-snack 2). Measurements post-exercise totaled about 3 hours and 15 minutes. The total protocol lasted 6 hours to 6 hours and 30 minutes and can be seen in its entirety in figure 4 below.

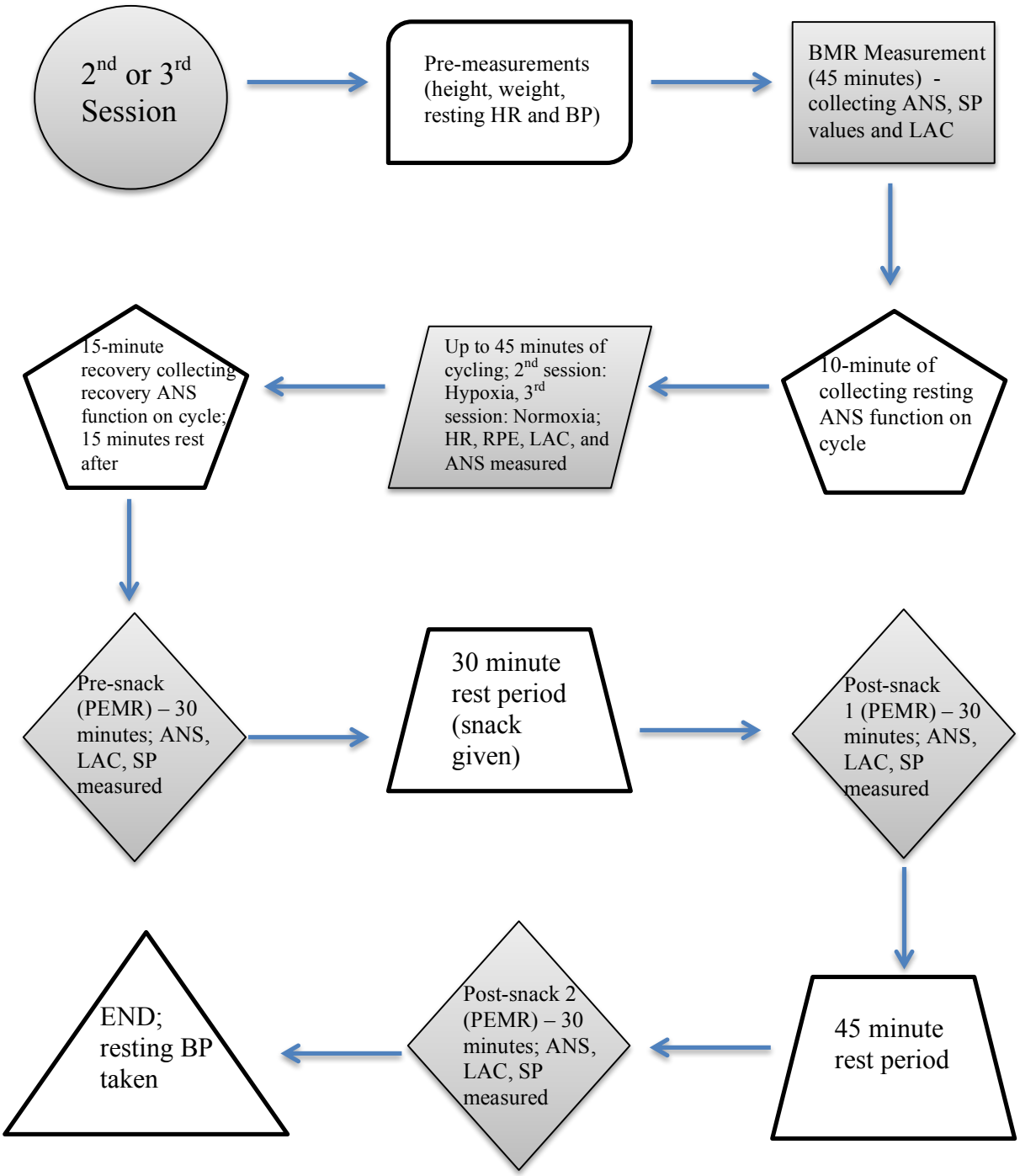


Figure 4: Experimental protocol

Measurements

MR Measurements

For the BMR, MR, and PEMR measurements, a flow through IC system (Sable, Sable Systems International, Las Vegas, NV) was used to measure O_2 uptake ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$). Prior to each session, the O_2 and CO_2 analyzers were calibrated with room air and a reference gas (100% N_2 and 1% CO_2 gases). Furthermore, propane gas calibration was completed to ensure the accuracy of readings at low MRs. Sample gases were dried by passing through a column of magnesium percolate and the sub-sampler pump was calibrated using a flow meter (Gilmont Rotatmer).

During measurements, the system was set to record the fractional amount of O_2 and CO_2, mixing chamber temperature, water vapor pressure, barometric pressure, subsample flow rate, and the mass flow rate in a negative pressure design. For the BMR and PEMR measurements, a canopy was used and the flow rate was set at 75 $L \bullet min^{-1}$. For the exercise measurements, a Hans Rudolph two-way non-rebreathing valve and an oro-nasal mask was used and the flow rate was set at 200 $L \bullet min^{-1}$. A subsample of that flow was pulled at 150 $mL \bullet min^{-1}$ through a water vapor analyzer (RH-300), a dual infrared CO_2 analyzer (CA-10 Carbon Dioxide Analyzer) and a paramagnetic O_2 analyzer (PA-10 Oxygen Analyzer). Fractions of gases in the room were recorded before and after each measurement for baseline references.

HR and HRV Measurements

For the BMR, resting, exercise, recovery and the three PEMRs, HR was recorded at 1 kHz in a 3-lead ECG configuration using a 100C ECG Amplifier (Biopac System

inc, Holliston, MA). Amplifier settings included: gain: 1000, mode: NORM, LPN filter: 35Hz LPN ON, and HP Filter: 0.5Hz HP ON. HRV was measured in a 3-lead ECG (Einthoven's triangle) configuration. The skin was shaved and wiped with an alcohol swap, and electrodes were placed as follows: 1) white electrode just below the right clavicle, 2) the black electrode just below the left clavicle, and 3) the red electrode just below the left pectoral muscles near the apex of the heart (Dubin, 2000). To prevent movement artifact, tape was used on all four sides of the electrodes. In addition, the leads were secured with tape and clips to reduce movement during exercise.

Hypoxia and S_PO_2

For the second session, participants completed the exercise protocol in hypoxic conditions (classified as short-term NH). The GO_2 Altitude System (Biomedtech, Melbourne, Australia) was used to induce hypoxia. This device used a generator that is equipped with a semi-permeable filtration membrane to continuously pump hypoxic air into two 120 L Douglas bags. This system maintained F_IO_2 at 15±0.2% throughout the exercise protocol. The participant was interfaced to the system through a two-way non-rebreathing valve and tubing. Following the familiarization session and the hypoxia session, participants completed the AMS inventory. This inventory determined if the participant felt any hypoxia-induced symptoms and determined the intensities of these symptoms if any. BP was also measured before and after exercise was also utilized for any discrepancies.

S_PO_2 was monitored with a forehead sensor, using a pulse oximeter (Model Rad-8, Massimo Corporation, Irvine, CA), to control for exercise-induced hypoxemia. This

sensor was only used during hypoxic exercise as a safety tool to prevent fainting or sickness. For instance, if S_PO_2 fell below 80%, the exercise test was terminated due to safety reasons.

Lactate

To measure LAC, a blood sample was taken via finger pricks. The LAC device used was an Arkay Lactate Pro blood lactate test meter (Arkay Inc.; Kyoto, Japan). Blood samples were taken after the BMR (initial), during exercise (up to 4) and after each PEMR. During exercise, starting at the 2nd rest interval, blood pricks were taken every other rest interval (2 minutes into the interval). Therefore, there would be up to eight blood samples per session.

Data Reduction

Metabolic Data

Pre and post BMR and PEMR measurements were limited to 20 minutes (out of 45 and 30 minutes total recordings) to decrease any metabolic fluctuations. Using the metabolic data taken during the BMR, pre-snack, post-snack 1, and post-snack 2, CHO and FAT usage was calculated, as well as energy production (EP; see calculations below). For all statistics, all MR values were adjusted by subtracting from the BMR (baseline) values.

HRV Data

Because sampling was short, HRV was analyzed in the frequency domain. A

band-pass FIR filter (0.5hz to 35hz) was applied to the original data (as suggested by BIOPAC System Inc.) to remove any high frequency noise from the signal. After applying the filter, HRV tachograms were also checked for any artifacts. HRV was analyzed in 5-minute segments, which is a standard in the frequency domain (Camm, 1996). SNS/PNS ratio values for all 5-minute samples for each section (BMR, pre-snack, post-snack 1, and post-snack 2) were then averaged. Then the Δ value was found by subtracting the BMR (baseline) average values from the pre-snack, post-snack 1, and post-snack 2 average values. These ratio values were then compared between condition and epoch. HRV was not analyzed during exercise because the signal was too noisy to get accurate values.

Other Measurements

HR was averaged during the last minute of each interval. Because participants ended at different times during exercise, the exercise portion was time aligned to express the beginning, middle and end; this was done for LAC, RPE and HR. Beginning is defined as the first interval, the middle as the end exercise time divided by two, and the end as the last interval. This was done for both the high and low intensity intervals. LAC (exercise and post-exercise) and HR values (exercise) were also calculated by subtracting the baseline to get a Δ value.

SP Calculations

Using the $\dot{V}O_2$ and $\dot{V}CO_2$ values from the metabolic data, substrate oxidation rates (for CHO and FAT) were calculated using the following equations (Simonson &

DeFronzo, 1990).

G_{ox} ($g \bullet min^{-1}$) = $4.57\dot{V}O_2 - 3.23\dot{V}CO_2$

L_{ox} ($g \bullet min^{-1}$) = $1.69\dot{V}O_2 - 1.69\dot{V}CO_2$

EP ($kcal \bullet min^{-1}$) = $((3.74*G_{ox}) + (9.46*L_{ox}))/1000$

Statistics

Descriptive statistics are expressed as means ± one SD. All the data (except RPE) were expressed as delta (Δ). A two-way analysis of variance [2 conditions (hypoxia and normoxia) x 3 epochs (pre-snack, post-snack 1, post-snack 2) with repeated measures on metabolic, cardiac parameters, LAC, and RPE was completed. *Post-hoc* tests (with the Bonferroni correction) were performed to detect any difference between conditions and epoch, and to decompose any significant interactions. IBM SPSS Statistics 20 (IBM Corporation, Armonk, New York, USA) was used and statistical significance was set at $p < 0.05$.

Chapter 4: Results

Subject characteristics

Eight participants completed all three sessions (age: 31 ± 11years, weight: 82.6 ±7.8kg, and height: 180.1 ± 7.4cm). On average, they had 13 ± 11 years of cycling experience. During an average week, these participants cycled 6.5 ± 1.5hours and 170 ± 124.3km. From their cycling maximal testing, these participants had a $\dot{V}O_2$max of 53.4 ± 6.5 $ml \bullet min^{-1} \bullet kg^{-1}$. According to the ACSM, for this age group, the $\dot{V}O_2$max is classified as superior and in the 95th percentile ("ASCM's Guidelines for Exercise Testing and Prescription," pg. 84). Also from the maximal ramp cycling test, participants had a HR max of 182 ± 6 bpm and PPO of 377 ± 52 watts; the testing lasted 16.4 ± 2.75 minutes.

Exercise Results

Three out of eight participants completed all 45 minutes of cycling (8 low intensity, 7 high intensity intervals). See Table 4 for the average intervals and minutes completed. On average, the exercise time was 35.6 ± 10.1 minutes. Because the hypoxia session was time matched with the normoxic session, the average exercise time was the same for both sessions. Since the average PPO was 377 ± 52 watts during the maximal tests, the 70% interval was at 264 ± 36.1 watts and 35% interval was at 132 ± 18 watts.

Table 4: Average Intervals and Minutes Completed during Exercise

Interval	Total Intervals Completed	Total Minutes Completed
Rest (132 ± 18W)	6.1 ± 1.89	18.4 ± 5.66
Exercise (264 ± 36.1W)	5.8 ± 1.48	17.3 ± 4.46

Rate of Perceived Exertion

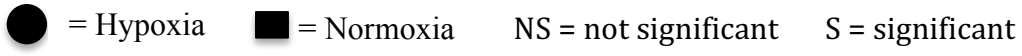
Statistical analysis revealed that condition [$F_{(1)} = 9.179$ (p=0.019)] and epoch [or each point in time; $F_{(2)} = 31.614$ (p=0.0001)] had significant effects on RPE during the rest intervals (35% PPO). More specifically, RPE during the rest intervals increased in hypoxia [hypoxia: (M = 12.7; SD = 2.64); normoxia: (M = 10.7; SD = 3.7)] and increased with time [beginning: (M = 10.3; SD = 2.73); middle: (M = 12.1; SD = 2.36); end: (M = 12.7; SD = 1.68)]. See figure 5 for the RPE results for the rest intervals.

For the high intensity intervals (70% PPO), there was a significant interaction between condition and epoch on RPE [$F_{(2,1)} = 5.444$ (p=0.018)]. The *post-hoc* test showed that RPE significantly differed during the high intensity interval periods for all time points between normoxia and hypoxia [beginning (hypoxia): (M = 14.9; SD = 4.1) and beginning (normoxia): (M = 12.9; SD = 1.8), middle (hypoxia): (M = 16.5; SD = 2.6) and middle (normoxia): (M = 14.4; SD = 4.3) and end (hypoxia): (M = 18.8; SD = 2.5) and end (normoxia): (M = 15.3; SD = 5.2)], but there was no difference between middle normoxia and end normoxia (p=0.598), showing no further increase in RPE during normoxia. See figure 6 for the RPE results for the high intensity intervals.

Heart Rate

Statistical analysis revealed a significant effect of epoch [$F_{(2)} = 49.872$; p= 0.0001] and condition [$F_{(1)} = 10.679$; p= 0.017] on ΔHR during exercise in the rest intervals. More precisely, ΔHR in the rest intervals was greater in hypoxia than normoxia (hypoxia: (M = 89.2; SD = 33.9) and normoxia: (M = 78.4; SD = 38.2)] and increased with time [beginning: (M = 69; SD = 32.2), middle (M = 89.4; SD = 34.4) and end (M = 93.1; SD = 39.9)]. See figure 7 for the ΔHR results during exercise in the rest intervals.

In slight contrast, ΔHR during the high-intensity bouts was only significantly affected by epoch [$F_{(2)} = 37.975$; p= 0.0001] not by condition [$F_{(1)} = 4.293$; p= 0.084]. More specifically, ΔHR during the high intensity bouts increased with time [beginning: (M = 100.4; SD = 32.4), middle (M = 111; SD = 33.2) and end (M = 115.5; SD = 32.2)]. See figures 8 for the ΔHR results during exercise in the high intensity intervals



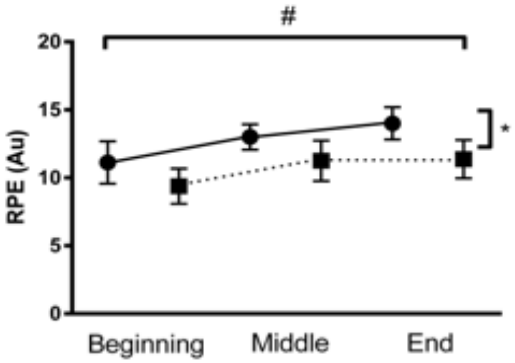


Figure 5: RPE for the rest intervals at the beginning, middle and end of exercise. # = significant epoch effect, * = significant condition effect ($p < 0.05$)

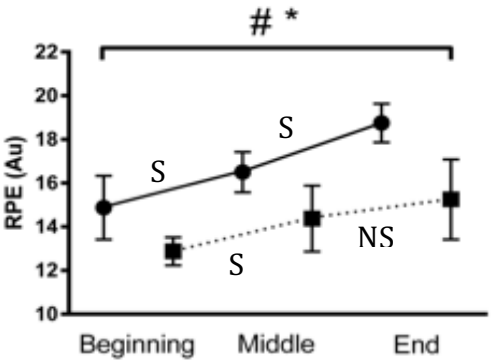


Figure 6: RPE for the high intensity intervals at the beginning, middle and end of exercise. #* = significant interaction between epoch and condition ($p < 0.05$)

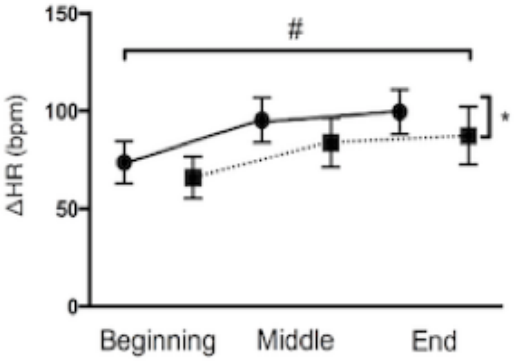


Figure 7: ΔHR for the rest intervals at the beginning, middle and end of exercise. # = significant epoch effect, * = significant condition effect (p< 0.05)

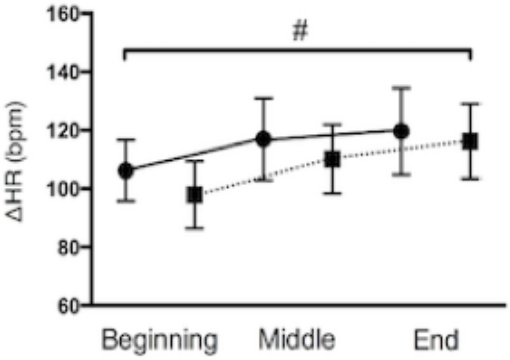


Figure 8: ΔHR for high intensity intervals at the beginning, middle and end of exercise. # = significant epoch effect (p< 0.05)

Lactate

LAC was taken at the end of the rest interval up to five times during exercise (depending on the length). During exercise, there was a significant interaction between condition and epoch with respect to ΔLAC [$F_{(2,1)}$ = 15.074 (p=0.0001)]. *Post-hoc* tests showed that there was a constant increase in the ΔLAC during hypoxia while no change was observed during normoxia [beginning (hypoxia): (M = 5.5; SD = 5.4); end (hypoxia): (M = 12.2; SD = 6.8); end (normoxia): (M = 6.5; SD = 10.2)]. See figure 9 for the ΔLAC results during exercise.

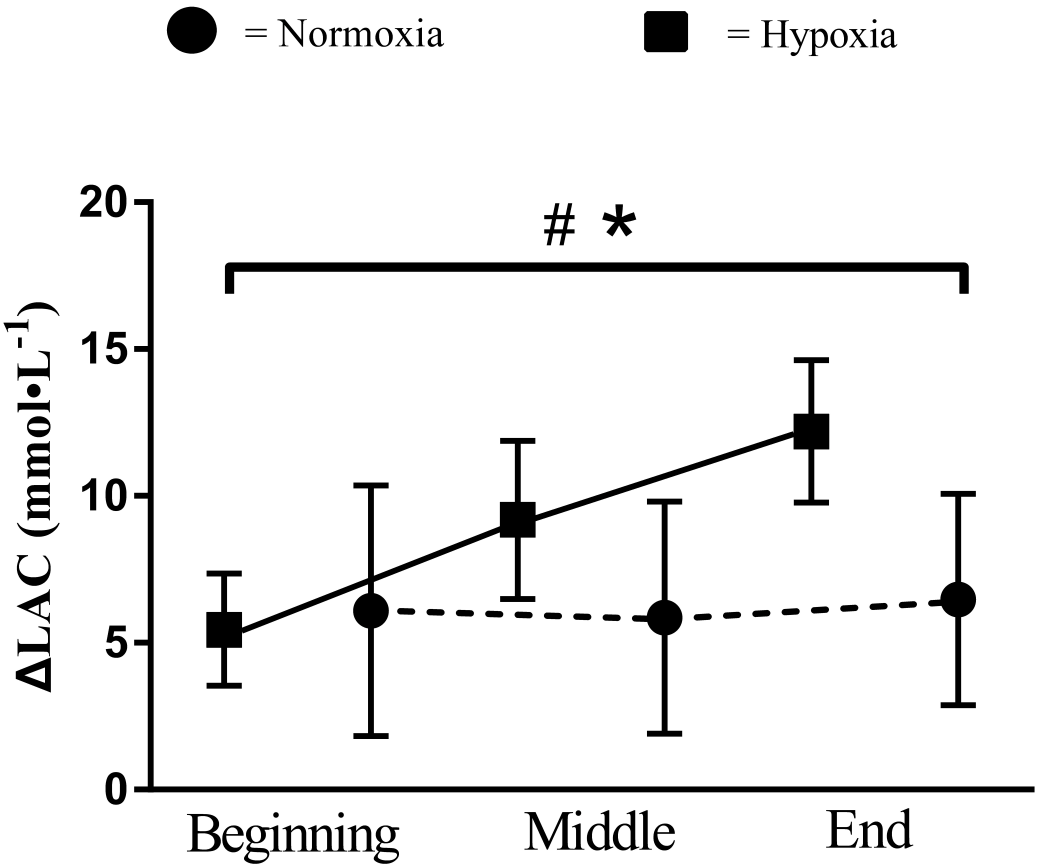


Figure 9: ΔLAC for the beginning, middle and end of exercise (taken during the rest intervals). #* = significant interaction between epoch and condition (p = 0.0001).

Post-Exercise Results

Autonomic Nervous System

Because ΔSNS (and subsequently the ΔPNS) is significantly correlated with the ΔSNS/PNS only the ratio will be reported (see figure 10). There was a significant effect of condition [$F_{(1)}$ = 13.156 (p=0.007)] and epoch [$F_{(2)}$ = 6.061 (p=0.011)] with respect to the ΔSNS/PNS (see figure 10 and 11). The *post-hoc* tests showed significant ΔSNS/PNS differences between conditions only during the pre-snack [$\Delta SNS/PNS_{Hypoxia}$ (M = 1.54; SD = 1.05), $\Delta SNS/PNS_{Noroxia}$ (M = 0.941; SD = 1.35)]**.** By post-snack 1 and 2 there were no significant ΔSNS/PNS differences when comparing conditions (p= 0.220 and p= 0.283). Over time, there is a decrease in ΔSNS/PNS after hypoxic exercise [beginning (M = 1.5, SD = 1) and middle (M = 0.71, SD = 0.9)] but a slight increase at the end (M = 0.86, SD = 0.5)]. While after normoxic exercise, the same trend is followed but to a lesser extent [beginning (M =0.94, SD = 1.35), middle (M = 0.49, SD = 1.1), and end (M = 0.63, SD = 0.66)].

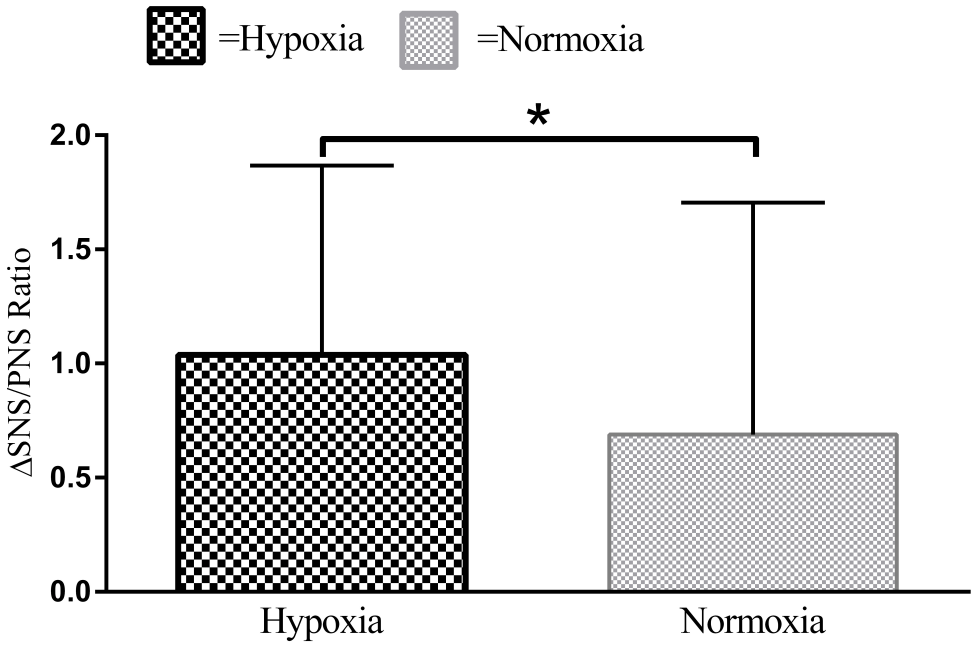


Figure 10: ΔSNS/PNS for each condition. * = significant condition effect (p = 0.011)

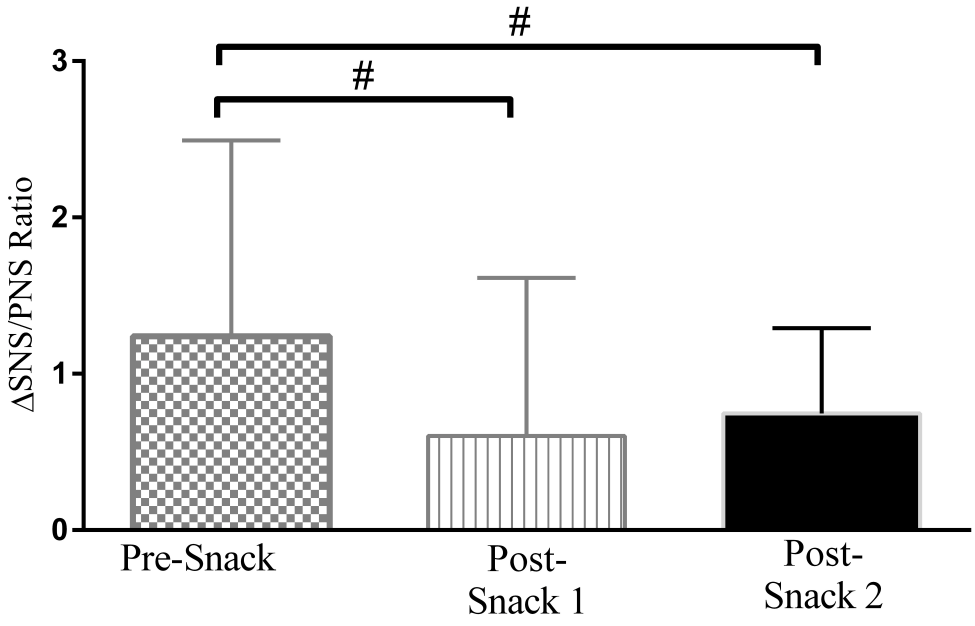


Figure 11: ΔSNS/PNS for the three post-exercise metabolic rates (PEMR). # = significant epoch effect (p = 0.011)

Substrate Partitioning

As shown in Figure 12, statistical analysis revealed a significant effect of epoch on ΔCHO_{oxi} [$F_{(2)}$ = 35.642 (p=0.001)] following exercise. The *post-hoc* tests showed that post-snack 1 ΔCHO_{oxi} (M = 139.7; SD = 268) significantly differed from pre-snack ΔCHO_{oxi} (M = -100.6; SD = 146) and from post-snack 2 ΔCHO_{oxi} (M = -26.8; SD = 86). There is a significant effect of epoch on FAT_{oxi} [$F_{(2)}$= 18.481, p=0.0001] that mirrors ΔCHO_{oxi} as shown by the inverse relationship between the two substrates [pre-snack CHO_{oxi} vs FAT_{oxi} (R=-0.976, p=0.0001); post-snack 1 CHO_{oxi} vs FAT_{oxi} (R=-0.976, p=0.0001); post-snack 2 CHO_{oxi} vs FAT_{oxi} (R=-0.905, p=0.002)] (see figure 13) The *post-hoc* tests showed that the Post-snack 1 ΔFAT_{oxi} (M = -22.9; SD = 129.3) significantly differed from pre-snack ΔFAT_{oxi} (M = 47.7; SD = 82.4) and from post-snack 2 ΔFAT_{oxi} (M = 19.8; SD = 58.7). No effect of condition was revealed for ΔCHO_{oxi} [$F_{(2)}$ = 1.539 (p=0.255); $F_{(2)}$ = 1.820 (p=0.198)] or ΔFAT_{oxi} [$F_{(2)}$ = 1.771 (p=0.225); $F_{(2)}$ = 2.078 (p=0.162)].

In addition, statistical analysis revealed a significant effect of epoch on ΔEP [$F_{(2)}$ = 38.739; p=0.0001]. The *post-hoc* tests showed that ΔEP significantly differed after the snack [(post-snack 1 (M = 0.306; SD = 0.264)] compared to pre-snack ΔEP (M = 0.075; SD = 0.352) and post-snack 2 ΔEP (M = 0.088; SD = 0.336). The increase in energy production during post-snack 1 reflects the thermic of food. No interaction or effect of condition was detected for ΔEP [$F_{(2)}$ = 0.420 (p=0.538); $F_{(1)}$ = 1.314 (p=0.300)] (see figure 14).

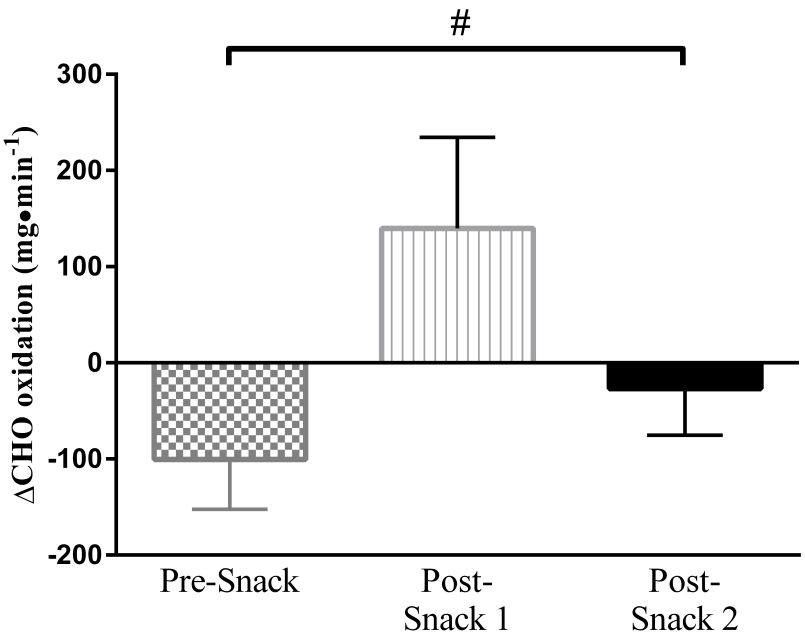


Figure 12: ΔCHO for the three PEMRs. # = significant epoch effect (p = 0.0001), no significant condition effect.

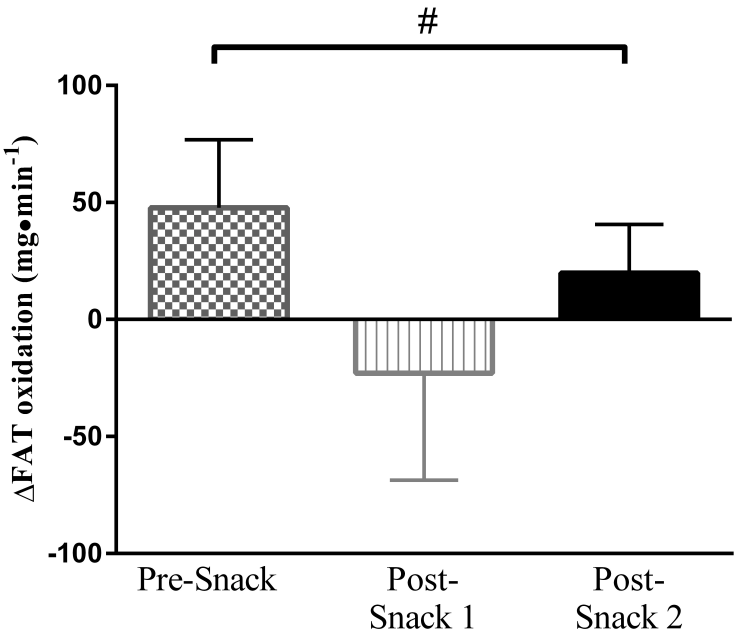


Figure 13: ΔFAT for the three PEMRs. # = significant epoch effect (p = 0.0001), no significant condition effect.

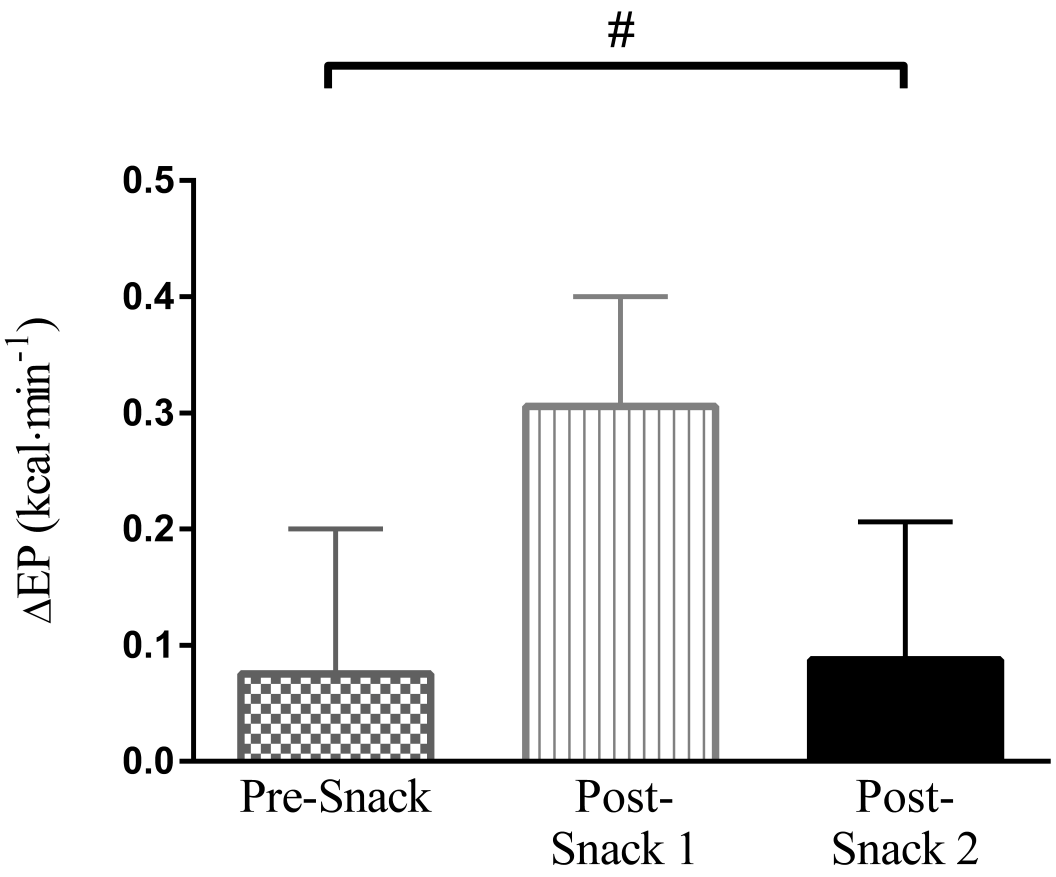


Figure 14: ΔEP for the three PEMRs. # = significant epoch effect (p = 0.0001), no significant condition effect.

Lactate

Statistical analysis shows there was a significant interaction between condition and epoch with respect to ΔLAC after exercise [$F_{(2,1)}$ = 10.068 (p=0.002)]. The *post-hoc* tests showed that the ΔLAC significantly differed between conditions only at pre-snack [hypoxia (M = 2.1; SD = 1.54) and normoxia (M = 0.6; SD = 1.19)]. But, after post-snack 1, ΔLAC decreases after hypoxic exercise (M = 1.8; SD = 1.2) compared to an increase after normoxic exercise (M = 1.9; SD = 1.02). At post-snack 1, there was only a significant time difference for normoxia. At the end of the protocol, about three hours and 15 minutes after exercise, LAC is nearly back to normal for both conditions [hypoxia: (M = 0.35; SD = 0.965) and normoxia: (M = 0.34; SD = 0.965)]. At post-snack 2, there was a significant time difference for both conditions (see figure 15).

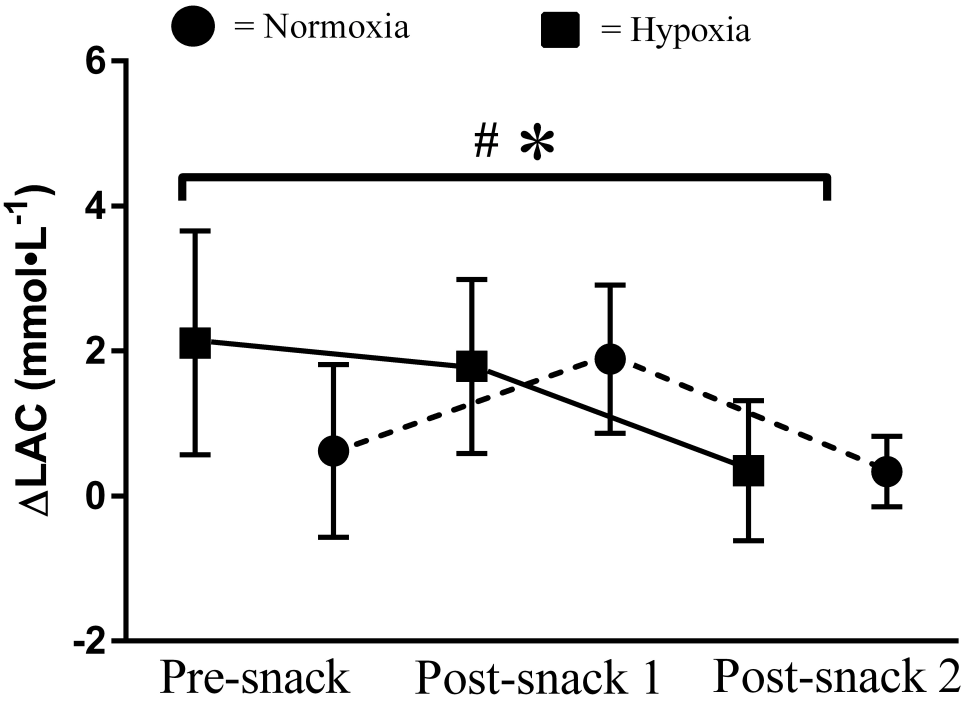


Figure 15: ΔLAC for the three PEMRs. #* = significant interaction between epoch and condition (p = 0.002)

Chapter 5: Discussion

Introduction

The novelty of this study is that it compares two variables, seldom associated with one another, after two different exercise conditions, hypoxia and normoxia. One of these variables is ΔSNS/PNS, which measures the activity of the ANS. This variable is highly reliable in measuring fatigue levels post-exercise and can be used effectively to decrease injury rates (Dupuy et al., 2012; Borresen and Lambert, 2007; Kaikkonen et al., 2007). The other variable is SP, measured here as the oxidation rates of CHO and FAT. Deviations in SP can be affected by exercise duration, intensity, fitness level, body composition and hypoxia. In general, hypoxia affects the whole body, including the cardiovascular system and the metabolic responses (Buchheit et al., 2004).

Aforementioned research has shown the significance of measuring SP and HRV pre and post-exercise, but limited studies have compared these two variables. Therefore, the aim of this study was to evaluate the effect of hypoxia and HIIT cycling on SP and HRV during the post-exercise recovery period, and to conclude if they were concurrent. The primary finding of the current study was there was no parallel in SP and ANS responses post-exercise in hypoxia or normoxia. When comparing pre-exercise and post-exercise changes, ΔSNS/PNS was significantly higher after hypoxic exercise, and took longer to recover when compared with post-normoxic exercise. Post-exercise SP results were only affected by epoch and did not follow the same decline as the ΔSNS/PNS.

Effect of Hypoxia on ΔANS and LAC Recovery

Following a 30-minute recovery period after hypoxic exercise, ΔSNS/PNS was significantly elevated which slowed recovery. Over the recovery period, ΔSNS/PNS

decreased. Following normoxic exercise, ΔSNS/PNS followed the same pattern but was less elevated. Condition differences only existed 30 minutes post-exercise ($p = 0.015$). Ba et al. (2009) found that following exercise, a delay in HRV recovery correlated with elevated post-exercise blood LAC levels as well as lower blood O_2 levels. Ba et al. (2009) hypothesized that higher post-exercise blood LAC levels may heighten the chemoreflex control of HR. This stress placed on the chemoreflex may cause a delay in the deactivation of the SNS control of the heart. Furthermore, James et al. (2012) and Kaikkonen et al. (2007) found that moderate to severe intensity exercise (exceeding 50 to 60 percent $\dot{V}O_2max$) significantly increased SNS and reduced PNS influences on the heart up to 1 hour after exercise, thus prolonging ANS recovery. But, it is important to point out that these studies were only examined at the post-normoxic exercise level.

When taking into account the hypoxia factor, Taralov et al. (2015) found that 10 minutes after NH exposure, there was a slight increase in SNS tone and a greater increase in PNS tone (thus decreasing ΔSNS/PNS). This variance from our results could be caused by differences in the type of participant utilized; while we used trained male cyclists, they used healthy males. In addition, no exercise protocol was followed for their study. Haddad et al. (2012) examined the effects of hypoxia following sub- and supra-maximal exercise and also found a delay in PNS activation following exercise. Although, these condition differences were only apparent following submaximal exercise. The authors suggest that under heightened SNS responses (supra-maximal exercise), the effect of blood O_2 (and subsequently a lower blood pH) may limit the PNS to reactivate in a timely manner. This may explain the higher LAC levels and prolonged ANS activity (see figure 10 and 11) following hypoxic exercise during the present study.

Effect of Hypoxia on ΔSP and Lactate Recovery

Another important finding from this study was a significant effect of epoch on FAT oxidation following both hypoxic and normoxic exercise (an averaged value). Following exercise, FAT oxidation increased for both conditions. Although, there was no significant condition effect (as expected from previous studies such as Cote (2015) and Kelly (2015)), there was a higher mean FAT oxidation rate after hypoxic exercise when compared to post-normoxic exercise. But these results were not significantly different. Similarly, Malatesta et al. (2009), Kuo et al. (2005), and Henderson et al. (2007) found no significant differences in post-exercise FAT oxidation between high and moderate intensity bouts. These authors attributed this to matching energy expenditure between intensities. Although not significant, these authors found a slight increase in FAT oxidation and a slight decrease in CHO oxidation after high intensity exercise when compared to moderate intensity exercise. In the current study, hypoxic exercise could have induced a higher metabolic stress, which caused glycogen depletion. It is well known that hypoxia causes increased dependence on blood glucose (Mackenzie et al., 2011). This could place an extra emphasis of FAT oxidation during the recovery phase, as more FAT (and less CHO) is available to oxidize.

Furthermore, Cote (2015) found that hypoxic exercise significantly elevated FAT oxidation rates and reduced CHO oxidation 1 hour after HIIT cycling when compared to normoxia. Also, Kelly (2015) concluded that after hypoxic exercise, there were significantly elevated FAT oxidation rates up to 22 hours post-exercise. Kelly (2015) states that submaximal hypoxic exercise is linked to an increase in LAC production along with elevated FFA mobilization and a subsequent depletion of muscle glycogen. Because

there is little glycogen post-exercise, the body turns to the FFA for energy production. Therefore, increased lipolysis would make FFA more readily available for re-esterification or oxidation. Also it had been demonstrated that following hypoxic exposure, plasma insulin concentrations would be reduced (Mackenzie et al., 2011). It is general knowledge that exercise and insulin are powerful stimuli for glucose transport. When insulin concentration is suppressed, this will contribute to an increased rate of lipolysis post-exercise. In our study, elevation in LAC levels during hypoxic exercise possibly caused an increase in FFA availability post-exercise. Exhausted muscle glycogen levels could have played a role in the body's use of FFA. Because FFA is available, lipolysis rates increase post-exercise.

Effect of Hypoxia on ΔHR and ΔLAC during Exercise

RPE and ΔHR were both significantly affected by hypoxic exercise during the rest-intervals for the current study. During the high-intensity intervals of exercise, ΔHR was not affected by hypoxia despite significant RPE differences. This is in contrast to Koelwyn and others (2013) who found significant differences in HR between conditions during all-out running (at 5km and 10km). Our results could be different from their results because they didn't control for intensity, they only asked their participants to run as fast as possible. In agreement with our results, Cote (2015) found significantly different HRs during hypoxic exercise in the work intervals (70% PPO) but not in the rest intervals (35% PPO). In addition, Engelen et al. (1996) also found an increase in HR during exercise at 12% O_2 when compared to normal air during unloaded cycling. It is important to note that this difference only existed during unloaded cycling (moderate

intensity) and not during heavy workload (high intensity), which is similar to our results. Engelen et al. (1996) hypothesize that there is a shift in baseline HR during hypoxic conditions primarily caused by an increase in catecholamine circulation, or a hormone that is released primarily during times of stress (such an norepinephrine). This is a result of greater SNS activity during hypoxic conditions.

In this current study, ΔLAC steadily increased during hypoxic exercise, while ΔLAC remained constant during normoxic exercise, but differences between conditions existed only at the end of exercise ($p = 0.017$). In contrast, a study by Springer and others (1991) saw a significant decrease in LAC induced by hypoxia during a progressive exercise test. The authors hypothesize that the lowered LAC could be caused by a decreased O_2 availability in the exercising muscle. This decreased O_2 availability could be caused by a smaller diffusion gradient between the mitochondria and blood. The current results could be different because Springer used both children and adults (volunteers) for the study, while we used trained male cyclists. Also, this was a constant work rate, where the current study was HIIT. In comparison to the current study, Katayama, Goto, and Ogita (2010) found that LAC significantly increased during exercise at moderate altitude (2000m) when compared to sea level. The authors state as LAC increases during exercise, lipolysis is inhibited, therefore increasing FFA mobilization. Although the current study did not measure SP during exercise, we can hypothesize that the greater LAC levels during hypoxia may correspond to an increase in CHO usage during exercise. Also in agreement, Murphy, Cuervo, and Hughson (1989) found that hypoxia did not change LAC during exercise at 25W but it did significantly

increase during 105W exercise. Therefore, LAC may only be affected by the higher intensities in hypoxia, as shown in the study.

Effects of the Snack on Post-Exercise Recovery

In the current study, a snack was given an hour after exercise to examine the effects of eating post-exercise on substrate oxidation and partitioning. Results show that after the snack (in both conditions) there was a significant increase in CHO oxidation and a decrease in FAT oxidation (consequently increasing energy production), and a significant decrease in ANS activity. This is in disagreement with van Baak (2008), who states that SNS activity will increase after ingestion of food to assist with regulating blood flow and BP. In the current study, it is possible that SNS activity was still elevated causing no further increase in ANS activity 90-120 minutes post-exercise. In addition, the size of the meal also determines the increase. Therefore, a 360kcal snack may not have an impact on SNS activity. Also during post-snack 1, there was a slight decrease in LAC concentrations after hypoxic exercise. Conflicting results were found after normoxic exercise, as LAC significantly increased post-snack.

The results during post-snack 1 following normoxic exercise are in agreement with Coppack et al. (1996), who gave a 740kcal mixed meal to their obese and non-obese participants. He found increased arterial insulin and lactate levels following this meal which increased glucose oxidation. This increase in LAC is somewhat attributed to adipocytes, which converted 45-80% of their CHO uptake into LAC. In this current study, after normoxic exercise the snack may have caused insulin to increase which stimulated glucose transport and oxidation (Mackenzie et al., 2011). Furthermore, lactate

levels may have increased because adipocytes converted some of the CHO to LAC following the snack. Snack results following hypoxic exercise were somewhat different, as lactate decreased. It is possible plasma insulin concentrations were still reduced, which caused less CHO to be available (Mackenzie et al., 2011). In addition, diminished CHO availability post-exercise may have caused LAC to decrease because of reduced adipocyte activity. It is important to point out that LAC, CHO oxidation, and EP decreased and FAT oxidation increased during post-snack 2, or 1 hour and 15 minutes after the snack. Therefore, these variables were returning to pre-exercise levels. This is in agreement with Kuo et al. (2005) who found that eating a post-exercise snack to match the expended energy (after a bout of exercise at low or high intensity) does not significantly change nutrient oxidation 24-hours after.

Methodological Considerations

There are some inherent methodological considerations for the current study. First, the snack after the pre-snack measurement did not allow us to accurately examine the post-effects of hypoxic exercise on HRV and SP. Although, by including the snack into the study design, we were able to discover the effects of hypoxia after eating on SP post-exercise (a dampening increase in CHO and a decrease in FAT usage post-hypoxic exercise when compared to post-normoxic exercise). A second limitation is that we did not investigate what the participants regularly ate during the week through the use of diet logs. Although participants ate a standardized meal 12 hours prior to the study and then fasted afterwards, we cannot rule out that energy intake was not the same prior to session 2 and 3. It is important to point out that resting substrate values during the BMR were

very similar between sessions. Kelly (2012) and Cote (2012) used diet logs throughout their study but it did not seem to affect their results. In addition, Louis and Punjabi (2009) also had their participants complete a 12-hour fast prior to sessions with no diet logs. Another limitation is the small sample size, which could affect the statistical power. Since this study was so physically demanding, four of the participants could not finish all three sessions. Although, our findings are still significant because the determined sample size of eight was still met (sample size was calculated by the G*Power software). Lastly, another limitation is the variability of HRV. Sources of HRV variability are the testing environment, time of day, timing of last meal, temperature and noise (Melanson, 2000; Dupuy et al., 2012). Although every precaution was taken to decrease HRV irregularity, such as testing in the same location and time, there still may be some HRV inconsistencies, but it is unlikely that this affected our results.

Further Study

More research needs to be done to investigate how intensity can affect the correlation between SP and HRV variables. Future studies could examine further the effects of hypoxia on SP and the ANS in other populations, such as the obese population or sprinters. Discovering differences between populations could further our knowledge in these fields. In addition, it would be interesting to look at these variables during different exercise protocols, such as a ramp protocol or a constant workload.

Chapter 6: Conclusions

Response to the Research Hypotheses

The purpose of this study was to examine if there were any parallels between SP and ANS activity following exercise, and whether hypoxia increased this impact. Our hypotheses stated 1) H_0 = post-hypoxic exercise responses of SP and ANS do not mirror each other; 2) H_1 = post-hypoxic exercise responses of SP and ANS do mirror each other. We found H_0 to be true. Overall, the ΔSNS/PNS ratio was significantly affected by epoch and condition, while SP was only significantly affected by epoch. ANS activity was only significantly elevated up to 90 minutes post-exercise and was not affected by the snack. Following exercise in both conditions, FAT oxidation rates were increased and CHO oxidation rates were decreased; after the snack, this SP was reversed.

Study Summary

Overall, the current study was successful in examining the effects of hypoxia, HIIT cycling and post-exercise snack on ANS activity and SP post-exercise. This experiment allowed the researchers to look at the effects of hypoxia during and after exercise on many parameters including LAC, RPE, CHO/FAT oxidation, energy expenditure and HRV. Recording these variables allowed the researchers to compare ANS activity and CHO/FAT oxidation post-exercise, to see if there were any parallels. This study had many interesting findings, but it also disproved the research hypothesis. The final conclusions drawn from this study are that ANS activity and SP do not correspond after exercise, including following a post-exercise snack.

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Appendices

Appendix A: Training Inventory Questionnaire

1. Age
2. Middle-distance or long-distance cyclist?
3. Number of years of cycling
4. Hours per week cycling
5. Average cycling distance per week (km)
6. The longest distance cycled in one week (km)
7. The longest distance cycled in a single session (km)
8. Participation in other sports, specify which and how many hours per week
9. Number of training sessions per week (include all sessions, whether easy or difficult, but exclude weight training)
10. Number of training sessions per week at or greater than 70% maximal aerobic speed
11. Number of weight-training sessions per week
12. Have you ever cycled at altitude? If so, specify how often

Appendix B: RPE Scale

RPE	Description of Exercise
7-8	Easy
9-10	Very Light
11-12	Fairly Light
13-15	Somewhat Hard
16-17	Hard
18-19	Very Hard
20	Do Not Work This Hard

Appendix C: Lake Louise Score for the Diagnosis of Acute Mountain Sickness (AMS)

Lake Louise Score (LLS) for the diagnosis of Acute Mountain Sickness (AMS)

A diagnosis of AMS is based on:
1. A rise in altitude within the last 4 days
2. Presence of a headache

PLUS

3. Presence of at least one other symptom
4. A total score of 3 or more from the questions below

SELF-REPORT QUESTIONNAIRE

Add together the individual scores for each symptom to get the **total score.**

Headache	No headache	**0**	1
	Mild headache	**1**	
	Moderate headache	**2**	
	Severe headache, incapacitating	**3**	
Gastrointestinal symptoms	None	**0**	
	Poor appetite or nausea	**1**	
	Moderate nausea &/or vomiting	**2**	
	Severe nausea &/or vomiting	**3**	
Fatigue &/or weakness	Not tired or weak	**0**	
	Mild fatigue/ weakness	**1**	
	Moderate fatigue/ weakness	**2**	
	Severe fatigue/ weakness	**3**	
Dizziness/lightheadedness	Not dizzy	**0**	
	Mild dizziness	**1**	
	Moderate dizziness	**2**	
	Severe dizziness, incapacitating	**3**	
Difficulty sleeping	Slept as well as usual	**0**	
	Did not sleep as well as usual	**1**	
	Woke many times, poor sleep	**2**	
	Could not sleep at all	**3**	
	TOTAL SCORE:		

Total score of:

- 3 to 5 = mild AMS
- 6 or more = severe AMS

Note:

- Do not ascend with symptoms of AMS
- Descend if symptoms are not improving or getting worse
- Descend if symptoms of HACE or HAPE develop