THE EFFECTS OF AN ACUTE SESSION OF BLOOD FLOW RESTRICTION EXERCISE ON AUTONOMIC MODULATION

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Andrew Robert Garner

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Health, Nutrition, and Exercise Sciences

March 2024

Fargo, North Dakota

North Dakota State University
Graduate School

Title

THE EFFECTS OF ACUTE BLOOD FLOW RESTRICTION EXERCISE ON AUTONOMIC MODULATION

By

Andrew Robert Garner

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Kyle Hackney, PhD
Chair

Ryan McGrath, PhD

Jacob Erickson, PhD

Approved:

March 12th, 2024
Date

Yeong Rhee, PhD
Department Chair

ABSTRACT

PURPOSE: How an acute training session of blood flow restriction (BFR) exercise affects autonomic modulation during a unilateral knee extension exercise. **METHODS:** Fourteen physically active males completed three different sessions while performing a unilateral knee extension exercise. The dependent variables measured: Heart rate variability (HRV), muscle oxygen saturation (SmO_2), and rating of perceived exertion (RPE). Repeated measures ANOVAs were used to analyze HRV and SmO_2 data. A paired t-test was used to analyze RPE data. **RESULTS:** Significant time-effect differences were found in lnRMSSD, lnHF, and lnLF at baseline to 15 minutes post-exercise and 15 to 30 minutes post-exercise ($P < 0.05$). Time and group-effect differences were significant in SmO_2%, oxygenated, and deoxygenated hemoglobin in the vastus lateralis (VL) muscle in BFR compared to control ($P < 0.05$). RPE increased when BFR was applied ($P < 0.05$). **CONCLUSION:** Exercise protocol may need to be altered to show autonomic modulation changes.

ACKNOWLEDGMENTS

I would like to give a special thanks to the Health, Nutrition, and Exercise Sciences Department for the funding of my thesis. Additionally, I would like to thank the entire Health, Nutrition, and Exercise Sciences faculty, my research committee, and all the researchers involved who helped in my data collection and analysis.

DEDICATION

I would like to dedicate this research to my family and fiancé who have always supported me throughout my education. I would like to thank my advisor Dr. Kyle Hackney for always setting me up for success and providing me with great knowledge and insight on research. Additionally, I want to thank my other committee members Dr. McGrath and Dr. Erickson for their continuous support and assistance. Lastly, I would like to thank Jacob Fanno for assisting and helping in data collection and analysis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I. INTRODUCTION

Heart disease remains the leading cause of death in the United States (U.S.) (Xu et al., 2020). An increase in age increases the risk of developing cardiovascular disease (CVD). For males, life expectancy in 2019 was 76.3 years, and for females 81.4 years (Xu et al., 2020). That's an astonishing 5.1-year difference between genders. Individuals who are older than 20 years of age are 49.2% more prevalent to develop some form of CVD both in males and females (Aparicio et al., 2021). CVD has been attributed to being the highest U.S. health expenditure cost from 2016-2017, with heart disease attributing the most cost of all CVD at $219.6 billion (Aparicio et al., 2021).

Heart rate variability (HRV) has become a valuable health predictor in adverse outcomes in various diseases (Tsuji et al., 1996). The autonomic nervous system (ANS) is composed of the sympathetic (fight or flight) and parasympathetic (rest and digest) nervous systems, which, both are responsible for controlling your heart rate (HR). A healthy individual will have a good balance between the two systems in which they counteract each other equally (*How the Heart Works | NHLBI, NIH*, n.d.). Autonomic modulation can be used to treat patients with CVD, depression, neural diseases, heart arrhythmias, and heart failure (Stavrakis et al., 2021). Autonomic modulation utilizes neural tissue to stimulate neural remodeling which can obtain a therapeutic benefit (Stavrakis et al., 2021). Previous research using acute and long training sessions to improve HRV has been heavily studied. Furthermore, previous research that studied how blood flow restriction (BFR) can improve HRV during a long training program and an acute bout of BFR training has not been well established (Santos et al., 2022; Tai et al., 2019; Rossow et al., 2011; Okuno et al., 2014).

The purpose of this study will be to determine how an acute bout of BFR exercise affects autonomic modulation during a unilateral knee extension exercise. This experimental study will examine heart rate variability (HRV) and muscle oxygen uptake while performing a unilateral knee extension exercise at 20% of one-repetition max (1RM) during a control session without BFR and an intervention session containing BFR. Before testing the control and intervention, an exercise session will be held to determine each participant's 1RM on the knee extension exercise. A couple of research questions were proposed when reading the previous literature: 1) What are the acute physiological responses to the cardiovascular system when BFR is applied during a unilateral knee extension exercise? 2) How does BFR influence local muscle oxygen saturation between the intervention and control?

CHAPTER II. LITERATURE REVIEW

Cardiovascular System

The human body is composed of many systems that keep it functioning daily. Among these systems is the cardiovascular system (CVS). CVS is defined by the National Institute of Health (NIH) as the heart and blood vessels of the body, with the heart being the center of the CVS (*How the Heart Works | NHLBI, NIH*, n.d.). From the heart, blood vessels branch off to the rest of the body carrying oxygen and other nutrients to organs for proper functioning. The electrical system of the body controls the rate and rhythm of the heartbeat (*How the Heart Works | NHLBI, NIH*, n.d.). The hindbrain, which contains the spinal cord, brain stem, and cerebellum, controls vital functions such as respiration and heart rate (*Brain Basics: Know Your Brain | National Institute of Neurological Disorders and Stroke*, n.d.). The body needs enough blood with a healthy heart rate (HR) ranging between 60-100 beats per minute (bpm) (Laskowski, 2015) for organ function (*How the Heart Works | NHLBI, NIH*, n.d.). A physically active individual may have a lower resting heart rate of around 40 bpm (Laskowski, 2015).

Cardiovascular Health

The health of CVS is important for living a long and sustainable life. Life expectancy for the United States (U.S.) population in 2019 was 78.8 years, with an increase of about 0.1 years from the following year. In 2019, heart disease remains the leading cause of death in the U.S., remaining unchanged from data collected in 2018 (Xu et al., 2020). An increase in age increases the risk of developing cardiovascular disease (CVD). For males, life expectancy in 2019 was 76.3 years, and for females 81.4 years (Xu et al., 2020). That's an astonishing 5.1-year difference between genders. Individuals who are older than 20 years of age are 49.2% more prevalent to develop some form of CVD both in males and females (Aparicio et al., 2021). Cardiovascular

(CV) health is important not only in preventing CVD and increasing life expectancy but reducing the economic burden. CVD has been attributed to being the costliest U.S. health expenditure from 2016 to 2017, with heart disease attributed to the highest cost of all CVD at $219.6 billion (Aparicio et al., 2021).

Anatomy & Physiology

For the body to function, the CVS is one of the most important driving factors to keep humans alive. The heart is located between the lungs in the middle of the chest and slightly behind the sternum (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). The heart is an organ that weighs between 200-425 grams (7-15 ounces) which is roughly the size of a fist (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). By the end of a long life, the heart would have beat more than 3.5 billion times, with an average of 100,000 times per day pumping 7,571 liters (2,000 gallons) of blood (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). Heart size differs among each other based on many factors such as height, weight, and physical activity (PA) level. The heart is made up of multiple layers such as the endocardium, myocardium, and pericardium *How the Heart Works | NHLBI, NIH*, n.d.; *Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). The endocardium is a thin inner layer that forms the surface of the different heart chambers and valves. The myocardium is a thick middle layer of muscle, which allows the heart chambers to contract and relax to circulate blood throughout the body. Lastly is the pericardium, an important sac that is made up of a thin layer of tissue responsible for holding the heart in place and provides fluid between the layers to help the heart reduce any unwanted friction *How the Heart Works | NHLBI, NIH*, n.d.; *Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). The pericardium also protects major blood vessels around the heart and uses ligaments to

attach itself to the spinal column and diaphragm (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.).

The different heart chambers and valves allow blood to get pumped and circulated through them, in which pulmonary arteries pump de-oxygenated blood to the lungs and the aorta pumps oxygenated blood to the rest of the body (*How the Heart Works | NHLBI, NIH*, n.d.). The heart is separated into two halves, the first half being the right atria and right ventricle which contains de-oxygenated blood. The second half contains the left atria and left ventricle which contains oxygenated blood. The left ventricle is the largest and most powerful chamber around 1.27 cm (1/2 inch) in width (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). This is because it needs to produce enough force to pump blood throughout the body (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). Both halves operate like a dual pump (*How the Heart Works | NHLBI, NIH*, n.d.). A wall of muscle called the septum separates the right and left ventricles (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). The right atrium is the first chamber where deoxygenated blood flows through first. Between this chamber and the right ventricle is the right atrioventricular valve (tricuspid). This valve will open when the atria pressure is greater than the ventricle pressure, allowing blood to flow from the right atria into the right ventricle (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). Within the right ventricle chamber sits the pulmonary semilunar valve. This valve regulates blood flow from the right ventricle to the greater arteries that supply the lungs with deoxygenated blood. The pulmonary semilunar valve is controlled due to pressure changes in the right ventricle (*How the Heart Works | NHLBI, NIH*, n.d.; *Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.).

Oxygenated blood arrives back from the lungs and enters the left atria. Between the left atria and left ventricle is the left atrioventricular valve (bicuspid). Like the right atrioventricular valve, this valve operates in the same manner such as allowing blood to flow from the left atria into the left ventricle (*How the Heart Works | NHLBI, NIH*, n.d.; *Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). The oxygenated blood then flows into the left ventricle and once the pressure changes the aortic valve opens and the blood is pushed out of the aorta to the rest of the body. All heart valves are one-way valves, meaning they prevent the backflow of blood. Heart valve diseases can cause blood flow to be slowed throughout the heart and sometimes even cause backflow of blood through the heart (*How the Heart Works | NHLBI, NIH*, n.d.).

Adding oxygen to the blood is important to keep muscle and organ tissue alive. De-oxygenated blood enters our heart through large veins called the superior and inferior vena cava. This blood enters through the right atrium and is pumped to the right ventricle which is pumped to the lungs. The main artery involved in the deoxygenated blood transfer to the lungs is the pulmonary artery. Once the blood arrives at the lungs it becomes infused with oxygen. Gas exchange occurs between alveolar air and the blood in the pulmonary capillaries (Powers & Dhamoon, 2021). The capillaries saturate the alveoli in the lungs where the process of diffusion takes place. Oxygen diffuses into the bloodstream when the partial pressure of oxygen is greater in the alveoli than in the blood (Powers & Dhamoon, 2021). The gases must pass through the alveolar surfactant, alveolar epithelium, basement membrane, and capillary endothelium for blood to be saturated with oxygen (Powers & Dhamoon, 2021). The diffusion of gas across the alveolar membrane can increase with an overall surface area of the membrane, increase alveolar pressure difference, increase in solubility of the gas, and decrease in membrane thickness

(Powers & Dhamoon, 2021). De-oxygenated blood from the pulmonary arteries has a partial pressure of 40 mmHg and oxygen in the alveoli has a partial pressure of 100 mmHg, in which a movement of oxygen will diffuse across the membrane into the blood capillaries until equilibrium has been reached (Powers & Dhamoon, 2021). Carbon dioxide pressure will decrease from its partial pressure of 46 mmHg to 40 mmHg in the alveolar capillaries to retain its equilibrium (Powers & Dhamoon, 2021). Once the diffusion process has been completed, the oxygen blood then returns to the heart through the pulmonary veins. The blood enters the left atria and ventricle to be pumped throughout the aorta to the rest of the body (*How the Heart Works | NHLBI, NIH*, n.d.).

Blood delivers oxygen and nutrients to every cell in the body (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). The blood travels through a complex pathway of arteries, arterioles, and capillaries (*How the Heart Works | NHLBI, NIH*, n.d.; *Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). Arteries take the oxygen and nutrients to working tissues such as muscles and organs, and in return veins and venules carry the used-up oxygenated blood back to the heart to be recirculated. This is a cycle that continues to occur throughout life (*How the Heart Works | NHLBI, NIH*, n.d.).

The more important arteries and veins are those that surround the heart. The cardiac arteries branch off the aorta and consist of the left coronary artery, circumflex artery, left anterior descending artery, right coronary artery, marginal arteries, and posterior descending artery. The left coronary artery delivers blood to the left side of the heart, this includes the left atrium, left ventricle, and the septum between the ventricles. The circumflex artery branches off the left coronary artery to supply part of the left ventricle with blood. The left anterior descending artery branches off the left coronary artery and supplies blood to the right and left ventricles. The right

coronary artery supplies blood to the right atrium and both ventricles. The marginal arteries branch off the right coronary artery and supply blood to the right atrium. Lastly, the posterior descending artery branches off the right coronary artery and supplies blood to the bottom of both ventricles (*How the Heart Works | NHLBI, NIH,* n.d.). These arteries are responsible for keeping the heart tissues alive and well to ensure the tissues are not damaged.

Once the heart tissues use the oxygenated blood from the arteries. The cardiac veins carry the deoxygenated blood back to the right atrium to be recirculated throughout the heart to be pumped back to the lungs to become oxygenated once again. The cardiac veins include the anterior cardiac veins, the great cardiac vein, the middle cardiac vein, and the small cardiac vein (*How the Heart Works | NHLBI, NIH,* n.d.). The arteries and veins throughout the body that surround the heart are a complex interconnected system that could stretch 96,500 kilometers (60,000 miles) if laid end-to-end (Powers & Dhamoon, 2021). Arteries and veins keep our muscles and organ tissues alive and well. This helps the body function and allows us to perform everyday activities.

The nervous system is an integral part of the functioning of the CVS. The autonomic nervous system (ANS) is often called the involuntary nervous system because it happens without any conscious thought. There are two parts of the autonomic nervous system, the parasympathetic and sympathetic systems. The parasympathetic system is responsible for telling the heart to beat at a slower pace or as result lower the heart rate. The sympathetic system is the opposite of the parasympathetic in which tells the heart to beat faster. This system is often referred to as the "fight or flight response" which our body uses as a protective mechanism. A chemical signal of norepinephrine is released to cause the heart to beat faster. This chemical also causes the heart to beat harder with a larger contraction. A healthy individual will have a good

balance between the two systems in which they counteract each other equally (*How the Heart Works | NHLBI, NIH*, n.d.).

Understanding the electrical pathway of the heart is important for how the heart contracts and produces a heartbeat. The heart has its cardiac conduction pathway. The cardiac conduction system controls the rate and rhythm of the heart. With each heartbeat, an electrical signal travels from the right atrium to both ventricles to allow muscle contraction of the heart. The signal starts in pacemaker cells located in the sinoatrial (SA) node in the top right of the atrium (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). The SA node is often referred to as the "natural pacemaker" (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). This signal passes through the muscle fibers of the atria and causes them to contract and pump blood into the ventricles (*How the Heart Works | NHLBI, NIH*, n.d.; *Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). The SA node sends electrical impulses at a certain rate, although physical demands, stress, and hormonal factors may change the heart rate (*Heart Information Center: Heart Anatomy | Texas Heart Institute*, n.d.). An electrical signal then moves through pacemaker cells called the atrioventricular (AV) node which sits between the atria and ventricles. This signal is significantly slowed to allow enough time for the ventricles to fill up with blood. The AV node fires another signal along the walls of both ventricles causing them to contract and pump blood either to the lungs or out to the rest of the body. Once the ventricles relax, this process will take place again starting with the SA node (*How the Heart Works | NHLBI, NIH*, n.d.). Understanding how the CVS and ANS systems integrate gives us a better understanding of how the human body functions throughout our daily lives. The body cannot function without either one of the systems.

Health Assessments

An increase or decrease in blood pressure (BP) is a common sign that an individual may be diagnosed with either hypertension (high BP) or hypotension (low BP). Understanding how to take a BP measurement accuracy is important in determining BP status. Accurate measurement of blood pressure is critical for making an appropriate clinical decision in primary health care (Frese et al., 2011). The American Heart Association (AHA) used the term BP for estimates obtained through the non-invasive way of measurement, often referred to as the "office BP" (Muntner et al., 2019). This BP is measured traditionally with a BP cuff and an observer listening through a stethoscope and watching a sphygmomanometer (Muntner et al., 2019). Other methods to measure BP using semiautomated and automated oscillometers. This method detects the amplitude of the BP oscillations on the arterial wall (Muntner et al., 2019). Systolic (SBP) and diastolic (DBP) are the most commonly used measures in research and clinical practice since they are well-established in identifying CVD risk factors (Muntner et al., 2019). According to BP requirements for physical therapists, BP screening should occur every two years in individuals with a BP less than 120/80 mmHg, and every year for individuals with an SBP of 120-139 mmHg or DBP of 80-89 mmHg (Frese et al., 2011). Hypertension is the most common medical diagnosis in the U.S. affecting 29% of the adult population. It's a major risk factor for coronary heart disease (CHD), stroke, and renal failure (Frese et al., 2011).

Myers et al., (2011) investigated the differences and quality between manual office BP and automated BP while using awake ambulatory BP as the gold standard (Myers et al., 2011). Patients under the care of a primary health provider from 67 practices participated (N=303). Due to the large sample size, alpha was set at ($P < 0.001$). The mean SBP and DBP were recorded as 135.6/77.7 mmHg for the automated office SBP/DBP measurement, and 149.5/81.4 mmHg for

the manual office BP. This may be explained due to the fact the automated BP does not need a physician to administrate the test, which as result could reduce "white coat syndrome." The overall accuracy of the automated office BP compared to the manual office BP showed a significant difference (Myers et al., 2011). Similarly, Godwin et al., (2011) compared office-based manual BP to automated ambulatory BP. The investigators found automated office BP predicts results more superior to the average of three manual office BP measurements. The investigators found this to be significant, and automated ambulatory BP to be overall more accurate (Godwin et al., 2011).

Frese et al., (2011) found normative blood pressure to have an SBP lower than 120 mmHg, and a DBP lower than 80 mmHg. Pre-hypertension BP ranges from an SBP of 120-139 mmHg, with DBP ranging from 80-89 mmHg. Stage 1 hypertension values range from an SBP of 140-159 mmHg, with DBP ranging from 90-99 mmHg. Stage 2 hypertension values range from SBP greater than 160 mmHg, with DBP ranging greater than 100 mmHg (Frese et al., 2011). Muntner et al., (2019) found the pre-hypertension, and stage 1 and 2 hypertension values to be different. Muntner et al., (2019) report the pre-hypertension value to have an SBP of 120-129 mmHg and a DBP of less than 80. Stage 1 hypertension SBP value ranges from 130-139 mmHg, and DBP of 80-90 mmHg. Stage 2 hypertension SBP value greater than 140 mmHg, and DBP greater than 90 mmHg (Muntner et al., 2019). Aram et al., (2003) also found different values for pre-hypertension, and stage 1 and 2 hypertension. Pre-hypertension value had an SBP range of 120-139 mmHg and DBP ranging from 80-89 mmHg. Stage 1 hypertension had an SBP range of 140-159 mmHg, with a DBP ranging from 90-99 mmHg. Stage 2 hypertension had an SBP greater than 160 mmHg, with a DBP greater than 100 mmHg (Bakris et al., 2003). All three reports found the same normative BP value, but Frese et al., (2011) and Aram et al., (2003) both

found the same pre-hypertension and stage 1 and 2 hypertension values. Aram et al., (2003) is the only report that states it combined stage 3 hypertension into stage 2 hypertension category (Bakris et al., 2003).

Controlling for errors in BP is important for the most accurate BP that can be measured. A source of error referred to as “white coat syndrome”, is an error that affects men and women in which BP may rise when a primary care provider is administrating the test (Frese et al., 2011). Frese et al., (2011) define this syndrome as having a persistently elevated BP greater than 140/90 mmHg with an average ambulatory reading of less than 135/85 mmHg (Frese et al., 2011). That’s an astonishing 5 mmHg difference between SBP and DBP. Likewise, Pickering et al., (2005) reported that 15-20% of individuals will experience “white coat syndrome” when a primary care provider administrates the measurement (Pickering et al., 2005). It occurs in the majority of hypertensive patients but can be reduced significantly using oscillometric automated devices (Pickering et al., 2005). As previously stated in Myers et al., (2011) and Godwin et al., (2011), automated devices are more accurate in reading BP and provide better quality to the patient (Myers et al., 2011; Godwin et al., 2011).

Miscuffing results in the most commonly frequent error in measuring BP (Frese et al., 2011). Pickering et al., (2005) report a proper cuff has a bladder length of 80% and a width of 40% of arm circumference (Pickering et al., 2005). Cuff size should be determined between the halfway point of the olecranon and acromion process (Pickering et al., 2005). When administering a BP measurement patient should be seated with feet flat on the floor, with the upper arm bare without constrictive clothing (Bakris et al., 2003; Pickering et al., 2005). The observer must find and palpate the brachial artery which is located on the antecubital fossa, and place the midline bladder of the cuff over the arterial pulsation. Place the stethoscope over the

brachial artery located near the antecubital fossa. Inflate the cuff approximately 30 mmHg above at which point the pulse cannot be heard. Deflate cuff 2-3 mm/s, a deflation greater than this can underestimate SBP and overestimate DBP. The first Korokoff sound will be the SBP and the last Korokoff sound will be the DBP (Frese et al., 2011; Bakris et al., 2003; Pickering et al., 2005). Individuals who have hypotension in which SBP and DBP can be hard to hear. A doppler probe can be used over the brachial artery to determine the patient's SBP and DBP (Frese et al., 2011).

HR holds important and prognostic information that can be associated with all-cause mortality and CVD. Lower HR has been associated with lower all-cause mortality and less chance of CVD, however, higher HR is associated with higher all-cause mortality and a greater chance of CVD (Avram et al., 2019). AHA defines normal resting HR to range between 60-100 bpm (Mason et al., 2007). This is the accepted range commonly used by health providers to determine resting HR. HR can be measured at the carotid or radial artery using the index and middle fingers. Find either artery and count the number of beats up to 15 seconds. Multiply this number by 4 to calculate the HR (Laskowski, 2015). Likewise, Harvard Medical School uses the same technique to measure HR (Harvard Health Publishing, 2021). Factors that may influence HR are age, PA level, smoking, CVD, stress, medications, and body mass index (BMI) (Laskowski, 2015). BMI is defined as total body mass divided by the body height squared. Measuring resting HR should occur after 2 hours of strenuous activity or a stressful event. Resting HR can stay elevated for 1-2 hours after strenuous activity or a stressful event (Harvard Health Publishing, 2021). Normal range varies among individuals, while at rest under 60 bpm is considered bradycardia, and, above 100 bpm is considered tachycardia (Laskowski, 2015). Tachycardia reflects the imbalance between the autonomic nervous system tone. This high sympathetic activity can explain the link between atherosclerosis and a cardiac event occurring

(Perret-Guillaume et al., 2009). HR can be an early predictor of CV and non-CV mortality (Harvard Health Publishing, 2021).

Perret-Guillaume et al., (2009) studied different subgroups in the French population according to age, gender, and BP levels. The study included 936 subjects between the ages 40-69 years, who had a routine health examination over 3 years. The study found an increase in HR by 10 bpm was associated with an increase in cardiac death by 20% (Harvard Health Publishing, 2021; Perret-Guillaume et al., 2009). Likewise, Hozawa et al., found a home-measured resting HR of 5 bpm was associated with a 17% increase in 10-year CV mortality (Perret-Guillaume et al., 2009). High HR is an important indicator of CV health and leads to greater all-cause mortality (Perret-Guillaume et al., 2009).

Heart rate variability (HRV) has been investigated for many years in clinical and experimental settings across the world. HRV has become a valuable health predictor in adverse outcomes in various diseases (Tsuji et al., 1996). HR and HRV differ from one another, in that, HR measures the average beats per minute, and HRV measures specific changes in time between two successive beats (Tsuji et al., 1996). Tsuji et al., (1996) studied the relationship between HR and HRV in 2,722 subjects with a mean age of 55 +/- 14 years. Tsuji et al., (1996) conducted three multiple linear regression analyses that revealed higher HR, older age, beta-androgenic blocking agent use, history of myocardial infarction (MI) or congestive heart failure (CHF), diuretic use, DBP greater than 90 mm Hg, diabetes mellitus (DM), consumption of three or more cups of coffee per day and smoking were all associated with a lower HRV (e.g, suggesting more sympathetic activity versus parasympathetic activity). Age and HR were the two determinants that were the most significant (partial R^2 values 0.125 to 0.389), with $R^2 \geq 0.005$ [20]. Tsuji et al.,

(1996) confirmed that HR must be taken into account when assessing HRV, and are significantly associated with each other.

HRV Parameters

HRV has many indexes and metrics that can be used throughout the research to explain the autonomic function of the heart when under stress. HRV is often looked at as how well the body can adapt and recover to different stressors placed upon it. Measuring autonomic function can be looked at by measuring different indexes that are collected through electrocardiogram (ECG), HR monitors, and pulse wave velocity (PWV) recordings. The HRV recording times can be measured using time and frequency domains, and also with non-linear measurements (Shaffer & Ginsberg, 2017; Guidelines et al., 1996). A combination of the three measures can be used in a single research study.

Time-Domain Measures

Time-domain indices of HRV count the variability between interbeat intervals (IBI), which is the period between successive heartbeats. The values may be expressed as units of the natural logarithm (Ln) to express a more normal distribution (Table 1) (Shaffer & Ginsberg, 2017; Guidelines et al., 1996). NN intervals are interbeat intervals in which artifacts have been removed. RR intervals are interbeat intervals between all successive heartbeats (Shaffer & Ginsberg, 2017; Guidelines et al., 1996). Malik, (1997) explained there are two categories of time-domain methods. The Spectral method treats the RR intervals as a time-ordered series and the non-spectral method processes the sequence of RR intervals or their pairs without looking at the timing of each interval. The non-spectral method reports data on HRV in milliseconds (ms) and for this reason, it is termed the time-domain method (Malik, 1997).

Methods of gathering HRV have predominantly been done by ECG and are recognized as the gold standard of data collection. Any premature beats or pauses found on the ECG must be accounted for and removed to get an accurate HRV recording. Malik, (1997) termed "NN" normal-to-normal intervals. These intervals have been widely accepted when determining HRV measurements (Malik, 1997).

Table 1.

Time-Domain Indexes

SDNN (ms)	The standard deviation of NN intervals
SDRR (ms)	The standard deviation of RR intervals
SDANN (ms)	The standard deviation of the average NN intervals for each 5 min segment of 24-hour HRV recording
PNN50 (%)	Percentage of successive RR intervals that differ by more than 50 ms
HR max – HR min (bpm)	The average difference between the highest and lowest HRs during each respiratory cycle
RMSSD (ms)	Root mean square of successive RR interval differences
HRV Triangular index	The integral of the density of the RR interval histogram divided by its height
TNN (ms)	Baseline width of the RR interval histogram
SDNN index (ms)	Mean of the standard deviations of all the NN intervals for each 5 min segment of a 24-hour HRV recording

Ultra-Short-Term Recording Norms

Ultra-short-term measurements are defined as HRV recording times less than 5-minutes. This method has often been criticized due to its criterion for acceptable validity (Shaffer & Ginsberg, 2017). A 1-minute brief recording may be sufficient in obtaining HR, SDNN, and RMSSD data only if artifacts are carefully removed (Shaffer & Ginsberg, 2017). Munoz et al., (2015) used three, 10-second ECG recordings on each participant to measure SDNN and

RMSSD data. Investigators calculated HRV by using the Bland-Altman method. The Bland-Altman method uses 95% limits of agreement and Cohen's d statistics to be used as an agreement analysis technique. By using this method Munoz et al., (2015) computed the ultra-short-term ECG recordings to obtain measurements of SDNN and RMSSD to calculate HRV (Munoz et al., 2015). Nussinovitch et al., (2011) hypothesized that ultra-short-term HRV would prove to be more reliable than the short-term recording time. The method of the study was to conduct a 1-minute long series and a 10-second long series with the control being a standard short-term HRV recording. Agreement between measurements was obtained using the intraclass correlation coefficient. A good correlation between average RR intervals was found between 5-minute, 1-minute, and 10-second recordings, along with the RMSSD. Investigators did not use a metronome to control respirations, which could have caused a good correlation in the RMSSD parameter (Nussinovitch et al., 2011). Salahuddin et al., (2007) used ultra-short-term HRV to measure parameters intervals starting with HR and RMSSD (10 sec); pNN50, HF n.u., LF n.u., LF/HF (20 sec); LF ms^2, VLF ms^2 (50 sec); SDNN (60 sec); HTI and TINN (90 sec) (Salahuddin et al., 2007). The parameters were used to estimate a total of 150 seconds of recording time for each HRV parameter being measured (Nussinovitch et al., 2011). Similarly, Nussinovitch et al., (2011) used 10-second intervals as well to gather data using the ultra-short-term recording to measure the total time for each parameter being measured. Shaffer et al., (2016) used 10-second intervals to estimate 5-minute values for each HRV parameter being measured. Each parameter was then estimated for a total of 5-minute recording time (Shaffer et al., 2016).

Ultra-short-term recording times can be accomplished but must be calculated to estimate a 5-minute recording time for all data to be valid. This can be observed in Nussinovitch et al., (2011) and Shaffer et al., (2016) where timing intervals separated each parameter and were

estimated for a total of 5-minute recording to give accurate results. Salahuddin et al., (2007) used the same intervals as Nussinovitch et al., (2011) and Shaffer et al., (2016) but only estimated the recording times to be 150 seconds, which could not be long enough for accurate results.

Short-Term Recording Norms

Short-term recording can be defined as recording times of at least 5-minutes (Shaffer & Ginsberg, 2017). Berkoff et al., (2007) describe using a 5-minute short-term HRV recording in 147 track and field athletes between endurance and non-endurance athletes at rest to stabilize their HR. Once HR was stabilized the subjects were informed to another 2.5 minutes in the supine position. The investigators used the Fast Fourier Transformation (FFT) method to run a spectral analysis of data. The investigators found females to show greater values for pNN50 and HF n.u. than male athletes, while male athletes had a greater value of LF n.u. and LF/HF ratio than females. The type of sport did not have any effect on any HRV parameter (Berkoff et al., 2007). Abhishekh et al., (2013) used a short-term ECG recording of 5-minutes in the supine position in 189 healthy subjects. Subjects were informed to perform 12-15 breaths per minute to help control for any respiration interference within the parameters of SDNN and RMSSD. Investigators found a negative correlation between RMSSD, SDNN, and total power with age. There was also a negative correlation between HF n.u. with age, but a positive correlation with LF/HF ratio with age. Investigators concluded that sympathetic tone increases with age (Abhishekh et al., 2013). Seppala et al., (2014) investigated 465 children 6-8 years old. Recording times at rest were obtained at 5-minutes and 1-minute, with 1-minute being the control. The investigators found that HTI, TINN, LF ms2, SD2, relative LF, and HF power, and SD1/SD2 require at least 5 minutes of recording due to longer rhythms containing larger LF band activity (Seppälä et al., 2014).

Short-term intervals of 5-minutes require less calculation when taking ultra-short-term intervals and estimating them to total 5-minutes. Seppala et al., (2014) compared 1-minute to 5-minute recordings and even found that certain parameters could not be obtained due to the short recording period. While Berkoff et al., (2007) and Abhishekh et al., (2013) both obtained all of the same measures as Seppala et al., (2014) using the 5-minute short-term method but Berkoff et al., (2007) and Abhishekh et al., (2013) also did not measure parameters HTI, TINN, LF ms2, SD2, relative LF and HF power, and SD1/SD2. The previous studies discussed have shown that a short-term 5-minute recording is needed to obtain all the parameters in HRV recording. It also shows that fewer calculations are to be made compared to the ultra-short-term recordings to estimate for a short-term recording to get accurate results.

Long-Term Recording Norms

Long-term recordings can be defined as 24-hour recordings. 24-hour recordings can be used to calculate more complex statistical time-domain measures. The calculation can be separated into two classes. The first class is those values derived from direct measurements of the NN intervals or instant HR, and the second class is derived from differences between NN intervals (Guidelines et al., 1996). SDNN is the simplest variable to calculate and is often calculated over a 24-hour recording. Long-term recordings also are designed for calculating ULF and VLF domains. Long-term recordings are only used when collecting data on ambulatory subjects (Shaffer & Ginsberg, 2017).

Umetani et al., (1998) used 24-hour Holter ECG monitoring to investigate if age and gender affected HRV over a decade. The investigators collected the time-domain measures of SDNN, SDANN, SDNN index, RMSSD, and pNN50. Also, investigators collected the frequency domains of HF, LF, and VLF. Two-hundred and sixty healthy subjects aged between 10-99 years

old. Both males and females were recruited for the study. The investigators analyzed the relationship between each time-domain measure with HR and age in 10 years. Several HRV parameters declined with age, after 65 years old, subjects fell below cutoffs, and mortality increased. Before the age of 30 females had lower HRV measurements than males, but gender differences disappeared after age 50 (Umetani et al., 1998). Beckers et al., (2006) also performed 24-hour ECG recording in 276 healthy subjects consisting of males and females 18-71 years old. Investigators performed a spectral analysis using the FFT method and divided recordings into day and night time. Berkoff et al., (2007) also used this same method while performing short-term power spectral analysis in elite American track and field athletes (Berkoff et al., 2007). Beckers et al., (2006) used the time-domain measures; SDNN, RMSSD, and pNN50, frequency-domain measures; total power, HF, LF, and LF/HF ratio, non-linear measures; DFA a1, DFA a2, CD, percent of CD difference, S value, and Lyapunov exponent. Investigators found both non-linear and linear measures decreased with age. Non-linear values were not different between sexes and decreased with age (Beckers et al., 2006). Likewise, Bonnemeier et al., (2003) investigated 166 healthy subjects between the ages of 20-70 years old, obtaining 24-hour ECG Holter recordings of day and night time patterns. Time-domain meausres used were RMSSD, SDNN, SDNNI, SDANN, NN50, and HTI. The study concluded that SDNNI, NN50, and RMSSD are strongly correlated with age. Mean 24-hour RR intervals were higher in males compared to females and there were minor gender differences with increasing age (Bonnemeier et al., 2003).

Increasing age seems to show minor gender differences. Umetani et al., (1998) found that after age 50, HRV measurements between genders seem to be minor. Beckers et al., (2006) also found that as you increase in age, HRV measurements between genders are also minor.

Likewise, Bonnemeier et al., (2003) found minor differences between genders with age. Umetani et al., (1998) found before the age of 30, HRV measurements showed a significant decrease in females compared to males. Bigger et al., (1993) performed a study outlining the importance of recording time lengths. The investigators found that the SDNN parameter is more accurate in long-term readings compared to ultra and short-term readings. Longer recording times provide more data about cardiac functions and reactions when placed upon an environmental stimulus. Cardiorespiratory regulations and increased measurement periods can obtain the heart's response to changing workloads and circadian processes such as sleep-wake cycles. Long-term recording activates and increases the SNS component in HRV (Leicht & Young, 2012). SDNN Parameter is the "gold standard" for medical cardiac risk stratification when recorded within 24 hours. SDNN values below 50 ms are considered unhealthy, 50-100 are compromised, and greater than 100 are healthy (Bigger et al., 1993). Long-term recording may be more applicable in clinical use for individuals who may be at risk for CV diseases.

Frequency-Domains Measures

Frequency-domain measurements estimate the relative or absolute power into four frequency bands. The four bands consist of: ultra-low-frequency (ULF), very-low-frequency (VLF), low frequency (LF), and high frequency (HF). Power is produced by the signal found within the frequency band. Absolute power is calculated as the millisecond (ms) squared divided by the cycles per second (ms^2/Hz). Relative power is the percentage of total HRV power or normal units (nu), which divides the absolute power of the LF and HF bands. HF bands have been widely accepted as parasympathetic activity and can be influenced by respiration. LF bands are accepted as sympathetic activity. While the LF/HF ratio means sympathy-vagal balance (Pinheiro et al., 2016). Relative power can be used to compare frequency domains between two

subjects (Shaffer & Ginsberg, 2017; Guidelines et al., 1996). Shaffer et al., (2017) and "Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology" have shown the same normative values of the different frequency domains that can be measured (Table 2) (Shaffer & Ginsberg, 2017; Guidelines et al., 1996).

Table 2.

Frequency-Domain Indexes

ULF power (ms^2)	The absolute power of the ULF band (≤ 0.003 Hz)
VLF power (ms^2)	The absolute power of the VLF band (0.0033-0.04 Hz)
LF peak (Hz)	The peak frequency of the LF band (0.04-0.15 Hz)
LF power (ms^2)	The absolute power of the LF band (0.04-0.15 Hz)
LF power (nu)	The relative power of the LF band in normal units (n.u.) (0.04-0.15 Hz)
LF power (%)	The relative power of the LF band (0.04-0.15 Hz)
HF peak (Hz)	The peak frequency of the HF band (0.15-0.4 Hz)
HF power (ms^2)	The absolute power of the HF band (0.15-0.4 Hz)
HF power (nu)	The relative power of the HF band in normal units (n.u.) (0.15-0.4 Hz)
HF power (%)	The relative power of the HF band (0.15-0.4 Hz)
LF/HF ratio (%)	The power ratio between the HF and LF band

The normative values of frequency ranges can be explained by numerous studies. Borchini et al., (2018) investigated the stimulation of the ANS using high and low-frequency domains as well as the LF/HF ratio. Thirty-six healthy nurses were measured on their work stress. 24-hour ECG recordings were used to measure the different frequency domains. The low-frequency bands were measured using the range 0.04-0.15 Hz, while the high-frequency bands were measured using the range 0.15-.40 Hz (Borchini et al., 2018). These are the same ranges used by Shaffer et al., (2017) and by the "Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Shaffer & Ginsberg, 2017; Guidelines et al., 1996). Seon-Ah Cha et al., (2018) measured low and high-frequency bands in patients who had type II diabetes mellitus (T2DM) and who also may be at risk of CVD. The band range for the low-frequency measurement was 0.04-.015 Hz and for high frequency was

0.15-0.40 Hz (Cha et al., 2018). Berkoff et al., (2007) measured HF and LF bands in American track and field athletes. HF was measured at 0.15-0.4 Hz and LF at 0.04-0.15 Hz (Berkoff et al., 2007). The low and high band frequencies are the same compared to the study by Borchini et al., (2018) Normal values of the different frequency ranges can be accepted when performing new research using the proven frequency ranges.

Non-Linear Measures

Non-linear measurements are defined as relationships between variables that cannot be plotted in a straight line (Table 3.) (Shaffer & Ginsberg, 2017). These measure the unpredictability of time series which come from the mechanisms that measure HRV. Non-linear measurements correlate time and frequency domain measurements when generated by the same processes. Different stressors and diseases can depress non-linear measurements (Shaffer & Ginsberg, 2017). Stein et al., (2005) demonstrated this in patients who had a myocardial infarction (MI). The investigators found that non-linear measurements could be used to utilize risk stratifications of patients with different CVDs (Stein & Reddy, 2005).

Table 3.

Non-Linear Measures

S (ms)	Area of the ellipse of total HRV
SD1 (ms)	Poincare plot standard deviation perpendicular to the line of identity
SD2 (ms)	Poincare plot standard deviation along the line of identity
SD1/SD2 (%)	The ratio of SD1 and SD2
ApEn	Approximate entropy; measures the complexity of time series
DFA a1	Detrended fluctuation analysis; describes short-term fluctuations
DFA a2	Detrended fluctuation analysis, which describes long-term fluctuations
D_2	Correlation dimension, which estimates the minimum number of variables required to construct a model of dynamics

HRV Devices

HRV is a variable to estimate cardiac autonomic function. Electrocardiography (ECG) is well known for being the gold standard way of measuring HRV (Shaffer & Ginsberg, 2017). New technological devices have been challenging the ECG in terms of accuracy and feasibility. HR monitors can be used to measure HRV through a wireless transmitter that connects to a mobile device allowing more versatility in different environments. Pulse wave velocity (PWV) is another method used to measure HRV. PWV can be defined as, the velocity at which the pressure waves generated by a systolic contraction of the heart, propagate through the arterial tree of the human body (Pereira et al., 2015). PWV also can be used to evaluate arterial health in the body (Pereira et al., 2015; Elgendi, 2012). There are various methods to measure PWV. The non-invasive use of photoplethysmography (PPG) is a feasible option that includes measurements of arterial stiffness and HRV (Pereira et al., 2015). PWV is considered the gold standard way of measuring arterial stiffness, and a tool for evaluating damage composed to the arterial system, vascular adaptation, and therapeutic efficacy. HRV research is often measured using frequency and time domains to measure the stimulation of the ANS. Devices such as ECG, HR monitors, and PWV can measure these different parameters using short or long-term recordings.

Methodology Using ECG

ECG has been the gold standard way of measuring ECG and has been validated throughout research when performing long-term HRV. Tsuji et al., (1996) Performed a study measuring 8 different time-domain and frequency-domain variables in a large cross-sectional study. The investigators studied the major determinants of HRV. The instrumentation used was a 2-hour ECG recording across 2,722 eligible participants with a mean age of 55 years.

Ambulatory ECG recordings were used to measure HRV recordings using the Cardiodata/Mortara Mk5 Holter analysis system. Frequency power variables were computed with a 2-hour recording time (Tsuji et al., 1996). The parameters of the frequency power follow the same parameters found in the studies of Shaffer et al., (2017), Borchini et al., (2018), Seon-Ah Cha et al., (2018), and by the "Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology". The investigators found that age must be taken into account when measuring HRV. Other covariates such as beta-blockers, diuretics, coffee, heart disease, and CV risk factors all decreased HRV (Tsuji et al., 1996). It's important to screen for such variables before measuring HRV.

HRV can measure CV physiological changes in athletes based on the type of training they acquire for specific sports. Toufan et al., (2012) investigated HRV differences between the dynamic and static types of athletes. HRV time and frequency domain parameters were measured. The sample measured was 50 professional athletes and 50 non-athletes with an age range of 20-35 years. A standard short-term 12-lead ECG was used to measure HRV in a resting supine position before any testing. Respirations were recorded at 25 mm/second while at rest. Along with HRV all subjects went under transthoracic echocardiography and measured Left ventricle ejection fraction (LVEF), left-ventricle end-diastolic diameter (LVEDD), left ventricle end-systolic diameter (LVESD, left ventricle mass, left atrial volume index (LAVI), early mitral velocity and early diastolic mitral annular velocity ratio, and pulmonary arterial pressure (PAP) were recorded. ECG short-term Holter monitoring was performed for 15 minutes after subjects had been resting in a supine position for 30 minutes. Before testing all subjects sustained from exercising for 12 hours. Statistical software SPSS was used to analyze the data. One-way analysis of variance (ANOVA) was used to measure the quantitative variables between the two

groups along with a posthoc test in cases of significance. For changes in qualitative data, the Chi-square test was used with a $P < 0.05$ to be considered significant. Investigators found no differences in HRV parameters between both frequency and time domains between the two groups (Toufan et al., 2012).

Berkoff et al., (2007) used an ECG recording to measure HRV in elite American track athletes. Investigators used short-term ECG recording to obtain HRV measurements while athletes were lying supine for 5-minutes during the recording (Berkoff et al., 2007). Toufan et al., (2012) also used the same body position when testing HRV. Both studies utilized using short-term ECG recordings, with Toufan et al., (2012) using a 15-minute duration and Berkoff et al., (2007) using a 5-minute duration. Berkoff et al., (2007) also used a one-way analysis of variance (ANOVA) to measure differences in HRV and sex. A two-ANOVA was used to measure the differences between sex and any track and field event categories. The level of significance was accepted at a P value < 0.05 (Berkoff et al., 2007). Unlike Toufan et al., (2012) and Berkoff et al., (2007), Tsuji et al., (1996) used a 2-hour recording using ECG in a large cross-sectional study measuring different determinants in HRV. Tsuji et al., (1996) used three multiple linear regression analyses between HRV parameters compared to the ANOVA's used by Toufan et al., (2012) and Berkoff et al., (2007), whereas Tsuji et al., (1996) used a large cross-sectional study where multiple regression may have been the more viable option compared to the experimental studies of Toufan et al., (2012) and Berkoff et al., (2007).

Borchini et al., (2018) used 24-hour ECG recording times to measure HRV parameters in nurses who may or may not work in a high-stress environment. Thirty-six healthy nurses participated in the study with each nurse filling out a job strain assessment to measure their work stress. The subjects were split into three groups; stable low stress (SLS), recent high stress

(RHS), and prolonged high stress (PHS). Subjects were placed in specific groups depending on their job strain assessment. Investigators used an ANOVA to measure differences across quantitative variables and a Chi-squared test across qualitative variables. Estimated age and smoking status geometric means for HRV frequency domain parameters across SLS, RHS, and PHS groups were done using ANCOVA linear regression models for subjects working during the day and sleeping at night. SAS software was used when analyzing the data, with a $P < 0.05$ level of significance (Borchini et al., 2018). Similarly, Berkoff et al., (2007) and Toufan et al., (2012) both used an ANOVA when analyzing differences among HRV and different independent measures performed in each study. Although, Borchini et al., (2018) and Tsuji et al., (1996) both used longer ECG recording times by using a Holter monitor to measure HRV.

Goldsmith et al., (1992) studied the differences in parasympathetic activity between endurance-trained and untrained young males. Investigators used Dual-lead ECG recordings that were made on Marquette 8500 recorders. Eight untrained and eight endurance-trained healthy males participated in the study. Each participant underwent a graded exercise VO_2 max test. To be accepted in the study each participant's VO_2 max either had to be > 55 ml/kg for endurance training and > 40 ml/kg for untrained healthy males. After testing 24-hour continuous ECG recording was used to measure HRV. Time and frequency domains were computed both during the day and night time of each participant. Unpaired T-tests were performed to compare the two groups HRV over 24 hours. A two-way ANOVA was used to compare the two groups and day versus night time HRV. Investigators used a 95% confidence interval to determine the significance of the variable (Goldsmith et al., 1992). Casties et al., (2006) also used endurance-healthy individuals who participated in cycling (Casties et al., 2006). Non-linear HRV parameters were performed during heavy exercise and recovery in 7 male cyclists. The VO_2 max

was performed for baseline testing. An electromagnetic brake cycle ergometer was used during VO_2 max testing while being monitored by a 2-lead Holter ECG. During the first session $VO_{2,}$ max was measured. The second session consisted of 10 10-minute rest period in a seated position, followed by a 30-minute exercise on the cycle ergometer and a 50-minute recovery seated position. Investigators used a 10-minute HRV recording at rest and during recovery. During exercise, they used the last 5 minutes of each 8-minute plateau while on the cycle ergometer. Recovery was separated into five-10 minute time windows. Time and frequency domain data were collected and analyzed. A one-way ANOVA with repeated measures was used to test the effect of time on the temporal, spectral, and non-linear dependent measures. Differences between means were tested using Scheffe post hoc for comparisons and significance was set at $P < 0.05$ (Casties et al., 2006). Results of the study concluded that HRV during most intense exercise was significantly lower than at rest due to a decrease in both LF and HF domains. Non-linear analyses showed that HRV remained scattered no matter the intensity level. Non-linear analyses also found that heartbeat dynamics were interrupted by other regulating systems (e.g. heavy breathing and respiratory rate) during recovery compared to at rest. HRV was mainly influenced by other factors than the ANS, and factors such as locomotor and respiratory systems could impact the results especially during HR recovery periods (Casties et al., 2006). This suggests that controlling for neurological factors that affect HRV other than cardiac related should be used to limit disruptions in HRV recording.

ANOVA is a statistical analysis that tests variance between groups or groups of data. HRV involves many different parameters that can be measured over short-term or long-term ECG recording. Researchers can use either a one or two-way ANOVA when comparing mean differences among variables (Berkoff et al., 2007; Borchini et al., 2018; Toufan et al., 2012;

Casties et al., 2006). Positioning when testing HRV varies among studies. Different positional testing includes lying supine or sitting in a chair when collecting data for HRV testing. Conducting 24-hour ECG recordings requires participants to be ambulatory during the day and asleep during the night, in which HRV is monitored during real-time activities (Tsuji et al., 1996; Borchini et al., 2018; Goldsmith et al., 1992). Short ECG HRV recordings are often done in a supine or seated position when the participants are in a controlled environment during testing (Berkoff et al., 2007; Toufan et al., 2012; Casties et al., 2006). ECG can be used during a controlled graded exercise test using a treadmill or cycle ergometer (Berkoff et al., 2007; Goldsmith et al., 1992). HRV can be used before, during, and after a max VO_2 test to determine how well your nervous system is responding to the stimulus. Researchers often look at the recovery phase of a VO_2 max test to see how well your CVS, PNS, and SNS respond after exercises (Goldsmith et al., 1992; Casties et al., 2006). HRV has been shown to decrease with age. The SNS increases with age, which allows the body to not be able to adapt adequately to a certain stimulus placed upon it (Tsuji et al., 1996).

Methodology Using Polar Heart Rate Monitors

HR monitors are commonly used for measuring HRV during short-term time domains. Weippert et al., (2013) investigated HRV and BP responses to dynamic and static exercises (Weippert et al., 2013). There were 23 healthy males with a mean age of 25.5 years who participated in the study. HRV measurements time and frequency domains were measured along with rate pressure product (RPP) and mean arterial pressure (MAP). Assumptions were made that low HR levels lead to greater vasoconstriction in static exercise than in dynamic. Another assumption was made that the vagal efferent activity is increased during static exercise. Investigators hypothesized that BP and HRV measures would be different between static and

dynamic exercise (Weippert et al., 2013). The investigators used a combination of all HRV measures; Time-Domain, Frequency-Domain, and Non-Linear. Participants underwent a dynamic exercise using a cycle ergometer while lying in the supine position, and also a static exercise using a leg press to measure a maximal isometric contraction in the supine position. A polar HR monitor along with Kubios HRV 2.0 was used to process the data. BP was measured minute-by-minute using a manual BP cuff, while the participants were being told to breathe normally when performing the exercises and avoid the Valsalva maneuver. Investigators used T-test analysis and effect sizes for pair-wise comparisons of HR, BP, HRV, SE, and DE. The effect size for significant differences between SE and DE was done using G-Power 3.1 (Weippert et al., 2013). The results of the study concluded that SBP, DBP, MAP, and RPP were significantly increased with SE. HRV parameters all increased with both SE and DE. Autonomic control during SE and DE were qualitatively different despite similar HR levels. There was a difference among BP and HRV indices in SE. HRV indices indicated strong vagal cardiac tone during SE when BP indicated a strong sympathetic efferent activity in the vessels. Investigators suggested that the strong vagal cardiac tone from SE could be a response mechanism from possibly co-activation of sympathetic cardiac afferents. By this HR and LF/HF ratios were similar and LF seemed to increase (Weippert et al., 2013).

Cornelissen et al., (2010) also investigated BP and HRV in healthy sedentary males and females while performing various aerobic training intensities over a 10-week, randomized crossover design (Cornelissen et al., 2010). Unlike Weippert et al., (2013), investigators used participants that were 55 years and older, but both studies measured HRV and BP during aerobic exercise. Cornelissen et al., (2010) used one training modality but different intensities through a 10-week training protocol compared to Weippert et al., (2013) The first and third periods of the

10-week protocol consisted of low-intensity and high-intensity training. During the second period, the participants were asked to be sedentary. The intensity was monitored using a Polar HR monitor. Heart rate recovery (HRR) was measured using the polar HR monitor while HRV measures were obtained using ECG during the maximal graded cycle ergometer test (Cornelissen et al., 2010). Other studies similar to Cornelisson et al., (2010) only use either ECG or Polar HR monitor (Weippert et al., 2013; Bentley et al., 2020; Schneider et al., 2019). Power analysis was measured at LF, HF, and total power in the LF and HF bands. LF and HF units were normalized into absolute power divided by partial power. The LF/HF power ratio was also calculated. An ANOVA was used for the statistical analysis during each stage, intensity, and comparison of HRV and BP. The statistical significance was accepted at $P < 0.05$ for a two-tailed test (Cornelissen et al., 2010). The Results of the study concluded at rest before exercise, during exercise, and after exercise, endurance training at a low or high intensity significantly reduces SBP. The effect of training on HR at rest, during, and after exercise was significant at higher intensities. Endurance training had no significant impact on sympathovagal balance. Participants at an older age during both training programs exerted similar effects of SBP at rest, during, and after exercise. Whereas, HR was more significant after higher intensities (Cornelissen et al., 2010).

Bentley et al., (2020) studied HRV and HRR following maximal exercise in endurance athletes and PA individuals (Bentley et al., 2020). Cornelissen et al., (2010) measured HRV and HRR during maximal exercise, but only used one mode of instrumentation (Cornelissen et al., 2010). Both modes can measure HRV and is interesting that one was used for HRV and the other used for HRR. The purpose of the study was to investigate chronic endurance training effects on HRV and HRR after a maximal exercise test between endurance-trained athletes and PA

individuals (Bentley et al., 2020). The investigators hypothesized that endurance-trained athletes would have greater RMSSD during dynamic and stable HRR periods, and an elevated HF during stable HRR periods. Both males and females participated in the study, with 36 being healthy endurance-trained athletes and 19 being healthy individuals (Bentley et al., 2020). Participants visited the lab on two separate occasions. The first visit entailed anthropometric measurements and completing a 2-week exercise diary in the lab before the first visit. The second visit entailed a maximal treadmill test where HRV and HRR were measured after the test (Bentley et al., 2020). Participants were fitted with a polar wireless HR monitor to measure HRV and HRR. HR was captured before, during, and after exercise. Before exercise and after HRV monitored in a seated position. Ultra short-term duration of 30 seconds was used to measure the time and frequency domains of HRV. HRR was measured 6 minutes after the treadmill test to see the HR plateau. Kubios HRV analysis software was used to measure time and frequency domain data. ANOVA with repeated measures was used to assess HRR in 1-minute intervals and establish HR plateau in 30-second intervals. Independent t-tests were used for frequency domain data. The level of significance for all analyses was set at $P < 0.05$ (Bentley et al., 2020). The results of the study concluded the frequency domains LF and HF were non-significantly elevated after exercise in the EA group, while LF/HF ratio was also non-significant. The data suggest an increase in HRR in EA may come from a lower resting HR. There were no overall differences between the EA and PA LF/HF ratios (Bentley et al., 2020).

Using Polar HR monitors can be used for any type of training, unlike, compared to ECG where movement may be limited due to leads being pulled during exercise. High-intensity interval training (HIIT) requires a short burst of high-intensity exercise followed by long low-intensity recovery periods (*High-Intensity Interval Training: For Fitness, for Health or Both?*,

n.d.). ECG leads may become pulled during a short burst of high-intensity exercise. Polar HR monitors can be chest-worn to prevent disruption.

Schneider et al., (2019) investigated HRV between strength training (ST) and HIIT (Schneider et al., 2019). The study consisted of 37 trained male and female athletes who underwent HR and HRV testing during the four-day baseline and recovery period, with a six-day overload microcycle. The study consisted of two groups; strength and HIIT. Similar to Bentley et al., (2020), and Schneider et al., (2019) both used athletes' incremental treadmill test to measure VO_2 max used to measure aerobic capacity. The maximum isometric contraction was used to measure force output on a parallel squat. One-repetition max parallel squat was used to measure dynamic strength. Repeated sprint ability was measured on a non-motorized treadmill, which recorded peak velocities in 20-second sprints. Intermittent aerobic performance was measured with 40-meter shuttle runs. Based on the results of the different tests, participants were placed in the strength or HIIT group. The two groups underwent 11 total training sessions, with the strength group focusing on multi-joint upper and lower body resistance training exercises, and the HIIT group focusing on straight line runs, sprints, and shuttle runs. A polar HR monitor was used to measure HRV and Kubios software was used to analyze HRV parameter data. Every morning HRV was measured in a 7-minute supine and 5-minute standing position before training and before participants consumed any liquids. It is unusual to measure HRV using two types of positional testing compared to other studies that use one type (Weippert et al., 2013; Cornelissen et al., 2010; Bentley et al., 2020). Schneider et al., (2019) used an ANOVA with repeated measures for the statistical analysis, with a level of significance of $P \leq 0.05$ (Schneider et al., 2019). The study concluded that HIIT training decreased HR and increased Ln RMSSD in the standing position, whereas supine recording was unchanged. During both group and individual

levels, moderate to strong negative correlations were found between HR and Ln RMSSD when analyzing changes between testing days and individual time series. Investigators used 2-4 day averages for a more precise estimation of mean changes with smaller confidence intervals compared to day single-day values. Using average values displays unclear effects for evaluating the association between HR, vagal HRV measures, and performance changes. This is determintal for the classification of individual short-term responses. The autonomic changes between standing and supine recording do not reveal any differences between ST and HIIT performance changes. Thus, could be caused by using rolling averages of groups and individuals rather than a single-day average.

Polar HR monitors compared to ECG are primarily used during short-term HRV monitoring. ECG and Polar HR monitors both can be worn during graded exercise tests (Berkoff et al., 2007; Goldsmith et al., 1992; Cornelissen et al., 2010; Goldsmith et al., 1992; Casties et al., 2006). Although ECG Holter monitoring is more common during 2 or 24-hour monitoring periods (Tsuji et al., 1996; Borchini et al., 2018). This method is a common way of tracking HRV ambulatory and sleep cycles. Polar HR monitors are more commonly used in research to measure HRV in specific training modalities such as dynamic vs static, HIIT vs strength, and strength vs endurance. These methods of training can be done in a controlled lab environment for a short period (Weippert et al., 2013: Cornelissen et al., 2010: Bentley et al., 2020; Schneider et al., 2019). After collecting the data Kubios software 2.0 can be used to analyze the different HRV parameters as shown in previous studies (Weippert et al., 2013: Cornelissen et al., 2010: Bentley et al., 2020; Schneider et al., 2019). Cornelissen et al., (2010) used Labview 6.1 HRV analysis software to find values of different HRV parameters (Cornelissen et al., 2010). HRV analysis software can differ among studies but Kubios software is more common and easy to use.

Positional testing can vary among studies. ECG and Polar HR monitors are used in both standing, sitting, and supine positions when measuring HRV. Respirations need to be controlled when measuring HRV. Weippert et al., (2013) stated using the Valsalva maneuver may cause inaccurate readings. Measuring short-term HRV should be done at rest breathing normally (Weippert et al., 2013). A polar HR monitor and ECG can be used interchangeably when measuring HRV. A polar HR monitor is more generally used for short-term HRV and ECG is more used for long-term.

Methodology Using PPG

PPG uses a probe with an infrared light source to detect cardiovascular pulse waves that propagate through the body (Pereira et al., 2015). PPG is a non-invasive measurement that allows for infrared probes to be gently placed upon the skin. The infrared probes can detect deep arteries within your fingertip to examine arterial pressure waves. The finger probe detects blood movement from the heart and extremities (Tokutaka et al., 2009). Only peripheral body sites can be measured, which is a limitation of using PPG and could provide unreliable results. Measurement of PWV in overweight or obese individuals could vary the results depending placement of the sensor (Pereira et al., 2015).

PPG is an alternative method for detecting HRV, compared to ECG. Pinheiro et al., (2016) investigated HRV indexes from both ECG and PPG measurements. The investigators used the term pulse rate variability (PRV) instead of HRV when assessing participants using PPG. The investigators hypothesized PRV features as surrogates for HRV in healthy subjects at rest healthy subjects after exercise and subjects with CVD (Pinheiro et al., 2016). The study included 51 males and 17 females. The subjects were separated into the three groups they qualified for. A finger probe was used on the index finger to measure PRV in each identified

group. All data were collected at rest in a supine position. Investigators used an ultra-short-term recording of 180 seconds to measure time and frequency domains. All artifacts were removed from analyses. HR and pulse rate (PR) were calculated before statistical analysis of time and frequency domain parameters could be made. HR was extracted from the ECG by measuring R-R intervals, while PR was measured as the period between the characteristic points of two consecutive pulses. Pinheiro et al., (2016) evaluated the time and frequency domains obtained from PRV and compared them to HRV analysis obtained from ECG. One-sample unequal-tailed Kolmogorov-Smirnov test (KST) was performed for each parameter in each data set. The KST was used to determine if the data belonged to a normal distribution. Results show that none of the data fell into this criteria and non-parametric methods had to be used to assess agreement between features. Two-tailed Spearman rank correlation (SRC) was used to assess any agreement between HRV and PRV features. Normalized square root means squared error (NRMSE) formula and Wilcoxon's rank sum test (WRST) were used to normalize error between any of the features of HRV and PRV ($P < 0.05$) (Pinheiro et al., 2016). The study concludes that PRV indexes may be used as replacements for ECG-based HRV in healthy subjects at rest, and after exercise. CVD participants were lower in performance in the estimation of PRV features (Pinheiro et al., 2016).

Previous studies have also compared PPG PRV indexes to ECG HRV, and have used the same methodology and statistical analyses (Giardino et al., 2002; Gil et al., 2010; Lu et al., 2009). Giardino et al., (2002) describe using a finger plethysmograph (FP) to measure PRV indexes compared to ECG HRV indexes in healthy subjects. High correlations between HF and LF domains were recorded at rest. During a Stroop Color-Word test, the correlations were negatively associated with each other. The study concluded that HRV was higher using the FP

device than ECG, but investigators noted ECG is still the recommended device in experimental settings. Test-retest reliability of the FP device needs to be verified (Giardino et al., 2002). Gil et al., (2010) found similar results when comparing PPG and ECG indexes in non-stationary healthy subjects who were resting in a supine or upright position (Gil et al., 2010). Lu et al., (2009) also compared PPG and ECG indexes in healthy subjects. The investigators used a 5-minute recording time to measure HRV indexes. Investigators found PPG to be a valid measurement of HRV indexes compared to ECG. This method of FP can not be used in patients with nervous system disorders such as hyperkinesia. Hyperkinesia causes unwanted electrical signals through the extremities and may cause inaccurate results (Lu et al., 2009).

PRV has been closely studied as an alternative to HRV. Lin et al., (2014) performed a study Comparing PRV and HRV in a healthy population (Lacković et al., 2014). PPG pressure waves are in correspondence to R-R intervals obtained for ECG. Investigators recruited 8 healthy participants. ECG and PPG were collected simultaneously for 5 minutes while participants were sitting in a chair. Participants performed a treadmill test, running 9 Km/h for 3 minutes. ECG and PPG were obtained simultaneously after the test for 5 minutes in a seated position. R-R interval (RRI) was calculated as the time interval of two successive beats from the ECG signal. Peak-to-peak interval (PPI) was call calculated as the time interval of two successive peaks of the first PPG pressure wave. PRV and HRV were measured as the power spectrum (PSD) of the PPI and RRI time series. The strength of LF and HF components in PRV and HRV signals was calculated using mean squared coherence spectra at frequencies of each corresponding band. This was calculated for each subject before and after exercise (Lacković et al., 2014). Results show PSD of PRV corresponds well with HRV when subjects are at rest. However, exercise will decrease the correlation in the HF component of PRV (Lacković et al., 2014). The reason is due to the HF

component being interrupted by a peripheral condition such as arterial blood flow or respirations. This can be observed in populations with extensive premature beats (PVCs), which depending on the population PPG may not apply to certain people (Lacković et al., 2014).

Likewise, Jeyhani et al., (2015) have also investigated the alternative of using PPG compared to ECG (Jeyhani et al., 2015). ECG-HRV and PPG-HRV were compared in 19 healthy male participants. HRV parameters were obtained from ECG signals, interpolation, and R-R wave detection. A similar procedure was used for PPG signals and their second derivatives, which constructs the P-P interval for each parameter. The second derivative method is commonly used to measure PPG pulse-to-pulse wave detection. HRV parameters were all verified using Kubios software. A 5-minute recording was used to measure HRV, PPG signals were filtered through the FIR band-pass filter with cut-off frequencies of 0.5 and 10 Hz (Jeyhani et al., 2015). Interpolation was applied to all signals for better peak detection and resolution. Both ECG and PPG signals were interpolated to 1000 Hz sampled per second. To show the effect of using PPG instead of ECG in HRV the parameters measured were SDNN, RMSSD, pNN50, SD1, and SD2. Poincare plots were constructed by plotting R-R interval signals as a function of itself. All plots were detected for false R-R intervals and pulse waves (Jeyhani et al., 2015). Investigators found PPG to be an alternative method for measuring HRV. Other studies argue this because it ultimately depends on the population. PPG measured in patients with CVD may present different results than that in a healthy population (Jeyhani et al., 2015). Lin et al., (2014) also mentioned this as an ongoing issue that needs to be investigated further.

Resistance Training and Autonomic Modulation

Resistance training (RT) is performed through physical activities that promote muscle strength and size. RT is often associated with weight lifting and can be done by using concentric,

eccentric, and isometric muscular contractions. Concentric (CON) muscle contractions rely on muscle shortening. Eccentric (ECC) muscle contractions rely on muscle lengthening. Concentric and eccentric movements are both involved during dynamic movements. Isometric (ISOM) muscle contractions rely on no change in muscle length (e.g. Holding your squat parallel to the ground) (Communications, 2009). ECC movements create a greater force per unit muscle than CON and ISOM. Dynamic CON muscular strength is at its greatest when ECC contractions require less motor unit involvement (e.g. Quick descending movements). ISOM contractions can play a major role in nonagonist muscle stabilization during an exercise. (e.g. Squatting while bracing your core to help with stabilization of the spine). ISOM training can help build secondary stability muscles to assist during a CON and ECC movement (Communications, 2009).

Autonomic modulation utilizes neural tissue to stimulate neural remodeling which can obtain a therapeutic benefit (Stavrakis et al., 2021). Different ways to assess autonomic modulation include vagus nerve stimulation, baroreceptor activation, tragus stimulation, renal denervation, and cardiac sympathetic denervation. Autonomic modulation can be used to treat patients with CVD, depression, neural diseases, heart arrhythmias, and heart failure (Stavrakis et al., 2021). HRV is an effective way of measuring autonomic tone, while BP is effective for measuring baroreflex sensitivity (BRS). Autonomic modulation can be affected by exercise, medications, and diet. HRV is emerging as a non-invasive method to assess autonomic modulation. It's important to understand what modality of exercise can impact autonomic modulation the greatest after an acute exercise session. Aerobic exercise is often observed as the modality that promotes CV health the greatest while RT promotes muscular strength. Recent

studies have suggested that RT affects autonomic modulation, particularly the parasympathetic and sympathetic nervous system using HRV (Stavrakis et al., 2021).

HRV After Acute Resistance Training

Chen et al., (2011) investigated parasympathetic power effects on HRV in 7 male weightlifters, with at least 6 years of training at the national or international level of competition. Investigators hypothesized that "parasympathetic nervous activity can reflect recovery status in weightlifting performance after training" (Chen et al., 2011). Participants detrained 10 days before starting the 2-hour acute weightlifting session. Four whole-body exercises (back squat, seated shoulder press, deadlift, and front squat) were performed in the 2-hour training period. Each exercise was completed at the intensities of 60%, 70%, and 80% of 1-RM for 3 repetitions, 90% of 1-RM for 2 repetitions, and 95% of 1-RM for 1 repetition at 90-second rest periods. Baseline HRV resting parameters (VLF, HF, LF n.u., Mean-variance) were measured for 5 minutes in a seated position before starting the training session. HRV was measured at 3, 24, 48, and 72 hours after the training session to measure recovery. Muscular pain assessment (0-10 scale, 0 = no pain, 10 = extreme pain) was used to see if there is a connection between muscle pain and autonomic modulation after an acute training session. One-way analysis of variance with repeated measures was used to compare the difference between all dependent variables 24, 48, and 72 hours after a training session. Mann-Whitney U test was used to compare differences among pre and post-values. Significance level $P < 0.05$ was used on all tests. Results of the study concluded 3 hours after the training session performance and recovery significantly decreased, then were regained during the 72 hours. Muscle pain was significantly elevated during the 24-hour recovery period and remained higher during the 48 and 72-hour periods. HF, VLF, and mean-variance were reduced significantly within 24 hours of recovery and returned to baseline at

the 72-hour mark. LF n.u. was elevated in the 24-hour recovery period but then returned to normal within 48 hours (Chen et al., 2011).

Rezk et al., (2006) investigated the hemodynamics (e.g. BP) and autonomic modulation at different intensities during an acute RT session (Rezk et al., 2006). Seventeen young healthy adults who had all had normal BP were included in the study. Seven days before the RT session, participants underwent a 1RM bench press, 70-degree leg press, lat pull down, leg curl, biceps curl, and 40-degree leg press. In a randomized double-blinded order participants underwent three experimental sessions: Control, exercise at 40%, and 80% of 1 RM. The control group performed no exercise and just rested. The 40% of 1 RM exercising group performed the 6 exercises for 3 sets and 20 repetitions with a rest interval of 45 seconds between each interval and 90 seconds between each exercise. The 80% of 1 RM exercising group performed the 6 exercises for 3 sets of 10 repetitions with a rest interval of 60 seconds between each interval and 90 seconds between each exercise. HRV was taken 10 minutes before the intervention period while BP was taken 20 minutes before the intervention. After testing, BP data were measured at 15, 30, 60, and 90-minute intervals, while HRV data were measured at 10, 20, 40, and 65-minute intervals. Changes in hemodynamic and HRV data were calculated using a two-way ANOVA with repeated measures. A Shapiro-Wilk test was used to verify the distribution of data. Newman-Keuls test was used to show comparisons from a posthoc test. A level of $P < 0.05$ was used to show significance (Rezk et al., 2006). The results of the study concluded there were significant differences between all groups regarding the variables of SBP, DBP, and mean blood Pressure (MBP) from pre to post-intervention. There were significant differences between all groups regarding the variables of cardiac output (CO), systemic vascular resistance (SVR), stroke volume (SV), and HR. R-R interval did not change in the control group but significantly

decreased in the 40% of 1 RM group. R-R interval significantly decreased at the 50 minutes of recovery in the 80% of 1 RM group. There was a significant increase in the HF, while LF and LF/HF significantly decreased in the 40% and 80% of 1 RM groups (Rezk et al., 2006). The main findings were low and high-intensity exercise decreased post-exercise SBP, with only DBP decreasing in the low-intensity group. Independent of the intensity of exercise, post-intervention hypotension was due to a decrease in CO, thus SVR did not completely affect it. CO decreased due to a decrease in SV which was associated with an increase in HR by an increase in sympathetic modulation of the heart (Rezk et al., 2006).

Kingsley et al., (2014) investigated acute RT modalities' effects on autonomic modulation in 17 trained and untrained participants (Kingsley et al., 2014). Participants showed up to 4 sessions. The first session consisted of anthropometric measures and muscular strength. Muscular strength was assessed using the 10 RM method. Exercises that were included were the leg extension and curl, seated row, and chest press. The highest resistance achieved was used for the testing day measurements. The second visit consisted of muscular strength verification, while the third and fourth visits consisted of testing. The acute bouts of training were separated between 72 hours and completed at the same time each day. Participants rested in a supine position for 10 minutes before HRV measurements were taken for 5 minutes. After baseline, HRV testing participants were separated into four groups: whole-body, upper-body, lower-body, and control. RT consisted of 3 sets for 10 repetitions, with the weight lifted being based on 10 RM. Two-minute rest intervals were used between each set for each group. The control group rested in a supine position for 15 minutes. After each group completed their given exercises, participants then returned to a supine resting position for 25 minutes. After 25 minutes HRV was measured for 5 minutes (Kingsley et al., 2014). A one-way ANOVA was used to assess the

characteristics between groups. The Kolmogorov-Smirnov test was used to show total power, LF, and HF values were not normally distributed. Repeated measures ANOVA was used to compare the trained versus untrained groups across time and to the 4 different testing modalities (control, whole, upper, and lower-body) on HRV. If there was a significant between interactions, the Tukey HSD test was used for post hoc comparisons. A $P < 0.05$ was used for the level of significance (Kingsley et al., 2014). The results concluded sample entropy significantly decreased after whole and upper body exercises in the trained group. Acute RT exercise increases parasympathetic modulation regardless of modality in trained or untrained individuals (Kingsley et al., 2014).

Nunes et al., (2021) investigated low-intensity resistance exercise (LI-RE) with short (SSC/LI-RE) and long (LSC/LI-RE) exercise sets of 10 and 20 repetitions (Nunes et al., 2021). Ten healthy males performed one session of both RE protocols. Participants were randomly assigned to either SSC/LI-RE or LSC/LI-RE group. Both protocols started with a warm-up that consisted of 25% of 1 RM. Three sets of 10 (SSC/LI-RE) or 20 repetitions (LSC/LI-RE) at 50% of 1 RM were performed for each exercise: Bench press, bent over barbell row, barbell front raise, barbell biceps curl, lying barbell triceps extension. A 90-second recovery period between each set and exercise was used. A 1 RM test and familiarization session were used before group assignments. Anthropometric measurements were taken along with resting HR and BP for baseline testing. HRV was measured at baseline in a seated position for 10 minutes. After the performance tests, HRV was assessed in a seated position for 60 minutes. Statistical power was set at 80% with a significance level set at $P < 0.05$. ANOVA with repeated measures was used to compare time and the different exercise conditions that were performed. Cohen's d-effect size was calculated with a significance level of $P < 0.05$. Higher HRs were significant during the 10,

20, 30, 40, and 50-minute marks after LSC/LI-RE, and 10 minutes after SSC/LI-RE compared to baseline. There was a significant decrease in values for RRm, rMSSD, pNN50, LnHF, LnTP, SD1, and SampEn at 20-30 minutes after LSC/LI-RE compared to baseline. There was also a significant decrease in values in RRm, pNN50, and SampEn at 50-60 minutes after LSC/LI-RE compared to baseline. A significant decrease in RRm and SampEn at 20-30 minutes was observed after SSC/LI-RE compared to baseline. A significant increase in LnHF/LF at 20-30 minutes after LSC/LI-RE compared to baseline (Nunes et al., 2021). The main findings of the study are LSC/LI-RE caused a significant increase in post-exercise HR and a significant decrease in HRV more than SSC/LI-RE. LSC/LI-RE promoted a sympathovagal imbalance and a decrease in HRV complexity during 60 minutes of recovery (Nunes et al., 2021).

Various methods have been used to investigate acute resistance training effects on HRV. Many studies use an exercise protocol that targets the whole body rather than only targeting the upper or lower body (Chen et al., 2011; Rezk et al., 2006; Kingsley et al., 2014). Nunes et al., (2021) only investigated acute RT using an upper-body exercise protocol (Nunes et al., 2021). Measuring and programming different intensities were mostly done using a percentage of a 1 RM that was tested at baselines (Chen et al., 2011; Rezk et al., 2006; Nunes et al., 2021). Chen et al., (2011) used percentages of 50, 60, 70, 80, 90, and 95, Rezk et al., (2006) used percentages of 40 and 80, and Nunes et al., (2021) used percentages of 25 and 50. Kingsley et al., (2014) did not use any percentages but instead used the 10 RM method at baseline, with the highest resistance achieved being used during testing (Kingsley et al., 2014).

Rest intervals between sets varied for all studies and the appropriate amount of rest was not clearly defined or supported. American College of Sports Medicine (ACSM) position stand recommends 2-3 minute rest intervals between sets when performing multi-joint exercises and 1-

2 minutes when performing muscle isolation exercises According to De Salles et al., (2009), rest intervals should be 3-5 minutes in length when exercising between 50% and 90% of 1 RM to maximize strength and power output (De Salles et al., 2009). When the goal is muscular hypertrophy rest intervals should be between 30 and 60 seconds due to an increase in growth hormone when working at a moderate intensity (De Salles et al., 2009). Chen et al., (2011) and Nunes et al., (2021) used a rest interval of 90 seconds between each set. According to De Salles et al., (2009), the rest interval is too short to maximize strength and power output and should be 3-5 minutes. Rezk et al., (2006) used a rest interval of 45 seconds between each set but used a repetition range of 20 which typically correlates to muscular hypertrophy. Investigators never specified if muscular hypertrophy was being tested along with HRV. This rest interval would be within the range the 30-60 seconds that De Salles et al., (2009) reported. Kingsley et al., (2014) used 2-minute rest intervals between each set which does meet the rest range of 3-5 minutes to maximize strength and power output according to De Salles et al., (2009). Rest periods could affect HRV depending on the length of the interval. The rest interval can affect HRR depending on if it's short or long. Shorter rest periods can mean an increase in intensity than a longer rest period. Rest periods can be used as a form of intensity and should be further investigated in a future study design.

Targeting different areas of the body with exercises could affect HRV. Chen et al., (2011) investigated weight lifters in various whole-body exercises consisting of compound movements. HRV measurements returned to baseline after 24 hours and increased above baseline after 48-72 hours, suggesting pain does not affect HRV since there was more HF activation than LF. Likewise, Rezk et al., (2006) also investigated whole-body exercises consisting of compound movements. Low vs high-intensity exercises promoted post-exercise systolic hypotension in

normative participants. Although, low-intensity exercises decrease diastolic BP. Both intensities increase sympathetic activation more than parasympathetic. Kingsley et al., (2014) investigated the differences between upper, lower, and whole-body movements to determine the effects on HRV. Between upper, lower, and whole-body exercises in trained vs untrained individuals, results show an increase in parasympathetic activation regardless of modality after acute RT. Nunes et al., (2021) investigated how the level of intensity affects HRV in upper-body exercises. A significant decrease in HRV was observed more in the LSC/LI-RE group compared to SSC/LI-RE showing that increased intensity can have an impact on the sympathovagal nervous system.

Blood Flow Restriction

Blood flow restriction (BFR) is the partial occlusion of a limb artery either using a pneumatic cuff or tourniquet (Neal et al., 2023). The cuff is placed at the most proximal part of the limb that is being trained. BFR is used to promote homeostatic disturbances used to increase signaling to enhance muscle adaptations (Santos et al., 2022). An increase in stimulus increases blood capillary supply and mitochondrial function which can improve exercise performance (Santos et al., 2022). Pneumatic cuff placement should have an arterial occlusion pressure (AOP) between 40 and 80% (Neal et al., 2023). Individuals who are unable to perform heavy loads can benefit from using BFR to enhance muscle development safely and feasibly (Neal et al., 2023). BFR is often used in clinical rehab centers for individuals recovering from musculoskeletal injuries. BFR is a way to increase muscular strength using a low-load to mimic a high-load effect. Healthy strength-trained individuals will use BFR in RT programs to promote muscle growth but more research is needed on endurance-trained individuals who strength-trained less than 3 days per week (Ferguson et al., 2021). Neal et al., (2023) investigated the feasibility and

efficacy of BFR in the lower limb. Investigators found lower limb BFR training is safe and effective with the maximum AOP not exceeding 80%. Likewise, Patterson et al., (2019) found a cut-off point of 80% for limb occlusion pressure (LOP) (Patterson et al., 2019). A mean pressure of 241.5 mmHg will fully occlude the artery in the lower limb with an 11.5 cm cuff size, and a pressure of 200 mmHg with a 5 cm cuff size should be the maximum pressure to prevent full occlusion. It's important to have the correct size garment to prevent any harm and obtain accurate results (Neal et al., 2023).

Acute Blood Flow Restriction Exercise and HRV

Tai et al., (2019) investigated autonomic modulation after an acute bench press exercise with and without BFR (Tai et al., 2019). Investigators hypothesized that "autonomic modulation and vagal modulation would significantly decrease with no change in the sympathovagal balance after traditional high-load bench press without BFR compared to low-load bench press with BFR in resistance-trained men" (Tai et al., 2019). The study design consisted of 5 laboratory visits. The first visit consisted of anthropometric measurements, while the second visit consisted of muscular strength testing on the bench press. Each visit was separated 72 hours apart from the other. The next 3 visits consisted of LL-BFR, HL, and a control session for experimental testing. All participants avoided food for at least 3 hours, and caffeine and alcohol for at least 12 hours. Participants were asked to avoid strenuous exercise for 24 hours before any data collection. Participants rested in a supine position for 15 minutes before HRV was assessed for 5 minutes before testing (15-20 min). After each participant completed each training session (LL-BFR, HL, and Control), HRV was assessed in the same way as baseline testing (15-20 min). Except, with an added recording period of 25-30 minutes. A one-repetition max on the bench press was used for baseline testing. The highest weight lifted between the first and second visit was used for LL-

BFR and HL conditions. Four sets on the bench press were used in the LL-BFR session. The sets consisted of 30, 15, 15, and 15 repetitions at 30% of 1 RM with 30 seconds of rest between sets. Knee wraps were used in both arms at the proximal end of each arm. Tightness at 7 out of 10 was used to occlude venous blood flow while maintaining arterial blood flow (Tai et al., 2019). Knee wraps were released after each set to allow complete blood flow to affected muscles. The HL session consisted of 70% of 1 RM for 8 repetitions with 60 seconds of rest in between each set (4 sets total). The control consisted of resting in a supine position for 10 minutes (Tai et al., 2019). ANOVA (3 X 3) with repeated measures was used between each condition. Paired t-tests were used to determine significance using Bonferroni correction for post hoc comparisons. The significance used was $P < 0.05$ (Tai et al., 2019). The results found significance in LnTP, LnHF, and LnRMSSD such as decreased recovery after LL-BFR and HL compared to the Control. No significance between LnLF, LnLF/LnHF ratio, and LnPNN50 (Tai et al., 2019). The main findings of the study found that LL-BFR and HL groups significantly affect autonomic modulation up to 30 minutes after exercise with significant reduction when HL is compared to LL-BFR (Tai et al., 2019).

Santos et al., (2022) investigated the relationship between HRV and eccentric BFR training (Santos et al., 2022). Investigators hypothesized that "there will be a correlation between autonomic responses and performance when using BFR at various loads during eccentric training" (Santos et al., 2022). The study design consisted of 3 weeks of baseline testing. The first week was a familiarization session using an isokinetic dynamometer. Seven days after the first week, anthropometric data were gathered along with eccentric, concentric, and isometric peak torques using the dynamometer. A single-leg hop test was also used during the second week. The third week consisted of peak torques being retested, with the highest eccentric value

used for the 6-week eccentric training program. Participants were randomly assigned to 4 groups: High-load (HL) eccentric exercise with 80% of eccentric peak torque, low-load (LL) eccentric exercise with 40% of eccentric peak torque, high-load eccentric exercise with BFR (HL-BFR) at 80% of peak torque, and low-load eccentric exercise with BFR (LL-BFR) at 40% of peak torque (Santos et al., 2022). Participants underwent a 6-week (18 sessions total) dominant knee-extensor eccentric exercise performed on an isokinetic dynamometer (Biodex). Participants performed a warm-up before completing 4-5 sts of 6-10 eccentric contractions with 1 minute of rest between each set. Total occlusion pressure (TOP) was determined by using a portable vascular doppler. The transducer was placed on the posterior tibial artery, while a nylon cuff was placed on the medial portion of the thigh covering up the femoral artery. The cuff was inflated to 40% of TOP before the eccentric exercise of the quadriceps was performed. HRV was assessed in a seated position for 15 minutes before each training session using kubios HRV software. Data were verified using the Kolmogorove-Simirnov test. Performance parameters were correlated with HRV indices SDNN, rMSSD, LF (nu), HF (nu), LF (ms^2), HF (ms^2), SD1 (ms), and SD2 (ms). A correlation magnitude of 0.00-0.19 is very weak; 0.20-0.39 is weak; 0.40-0.69 is moderate; 0.70-0.89 is strong; and 0.90-1.00 is considered very strong (Santos et al., 2022). The results concluded a significant correlation between performance and HRV in the group's LL and HL-BFR groups, with an increase in parasympathetic indices in the first group along with an increase in performance in the second group (Santos et al., 2022). HRV directly correlates with performance. However, LL had an increase in parasympathetic response without repercussions, and HL-BFR increased performance but had no repercussions in HRV indices (Santos et al., 2022).

BFR can be used with many applications. HRV is less commonly paired with BFR and has not been investigated deeply. Tai et al., (2019), Santos et al., (2022), and Okuno et al., (2014) both investigated how HRV is affected by BFR. Tai et al., (2019) studied how BFR impacted HRV through the bench press exercise where both arms were occluded. Santos et al., (2022) studied only the dominant knee extensor eccentric torque using an isokinetic dynamometer at LL and HL intensities. Likewise, Tai et al., (2019) and Okuno et al., (2014) studied LL and HL intensities. Rossow et al., (2011) the effects of acute BFR training on the effects of post-exercise BP (Rossow et al., 2011). Unlike Tai et al., (2019) and Santos et al., (2022), Rossow et al., (2011) and Okuno et al., (2014) used an acute bout of exercise rather than a long training program. Acute BFR on the effects of autonomic modulation has not been studied heavily, which elicits a gap in research.

Rossow et al., (2011) compared low (LI) and high (HI) intensities between multiple lower-body exercises. Exercises included supine leg press, seated knee flexion, seated knee extension, and planter flexion. Ten participants (males) were randomly placed into 3 groups: HI, LI, and LI-BFR. BP (brachial artery) and vascular resistance (calf) via ultrasound were measured pre and post-testing. All groups performed a 1-RM on each exercise before testing. HI, group participants completed 3 sets of 10 repetitions using 70% of 1-RM with 1 minute of rest between each set. LI group performed 1 set of 30 repetitions, and 3 sets of 15 repetitions using 20% of 1-RM with 30 seconds of rest between each set. LI-BFR group performed the same protocol as the LI group, except wearing 50 mm width elastic cuffs (Kaatsu-Master System). BFR was used bilaterally on the proximal portion of both legs (Rossow et al., 2011). Cuff pressure was set at 200 mmHg, which Patterson et al., (2019) and Neal et al., (2023) found 200 mmHg to be the upper limit of AOP. A full occlusion of the artery could cause harm to the participant and is

advised to have an AOP of 80% or less (Patterson et al., 2019). Abe et al., (2006) also used an occlusion pressure of 200 mmHg to investigate how muscle size and strength are influenced by Kaatsu-walk training. BFR can also be defined as Kaatsu-walk training, due to being developed in Japan (Patterson et al., 2019; Abe et al., 2006). Tai et al., (2019) did not specify cuff pressure but instead used a tightness scale (1-10). Investigators used two 77 mm-wide knee wraps for occlusion of brachial arteries. When pressure would be rated 7/10, investigators then stopped the tightening of the knee wraps (Tai et al., 2019). This method provides no quantitative data on how many arteries were occluded and how safe. Okuno et al., (2014) used a standard BP cuff inflated to 100 mmHg to occlude femoral arterial blood flow during a unilateral leg press exercise (Okuno et al., 2014) . Santos et al., (2022) used a portable vascular doppler (DV-2001; Medpej) device to measure TOP (Santos et al., 2022). Once TOP was identified, cuff pressure was inflated to 40% of TOP which is the lower TOP that Patterson et al., (2019) recommended using.

Various cuff pressures should be taken into consideration during resistance training. AOP should be between 40-80%, or not exceeding 200 mmHg for cuff pressure. BFR has been used with many different modalities of exercise and is safe and effective in aerobic and anaerobic training. A research question such as, “How does BFR effect autonomic modulation in an acute resistance training session?” needs to be further investigated to show any connection between acute BFR training and an increase in autonomic modulation.

CHAPTER III. METHODS

Research Design

The purpose of this study will be to determine how an acute bout of blood flow restriction (BFR) exercise effects autonomic modulation during a unilateral knee extension exercise. This experimental study will examine heart rate variability (HRV) and muscle oxygen saturation (SmO_2) while performing a unilateral knee extension exercise at 20% of one-repetition maximum (1RM) during a control session without BFR and an intervention session containing BFR. Before testing the control and intervention, an exercise session will be held to determine each participant's 1RM on the knee extension exercise. **Research Questions:**

1. What are the acute physiological responses to the cardiovascular system when BFR is applied during a unilateral knee extension exercise?
    - HRV pre and posttest indices
2. How is local SmO_2 affected when BFR is applied?
    - SmO_2 between control and intervention

Participants

The participants studied will be 15 physically active healthy males, who are regularly active (≥ 150 minutes of moderate activity per week) with at least 6 months of strength training experience, between 18-35 years of age (Bull et al., 2020; Okuno et al., 2014). The sample size is based on previous studies conducted (Santos et al., 2022; Tai et al., 2019; Rossow et al., 2011; Okuno et al., 2014).

Inclusion

Participants will complete a Physical Activity Readiness Questionnaire (PARQ +) to ensure they have no underlying health conditions that prohibit them from performing the

exercise (Warburton et al., 2011). To be considered, participants must be physically active and have at least 6 months of strength training experience (e.g., Bodyweight, free-weight, or strength machines) (Okuno et al., 2014). To be considered physically active, participants must participate in at least 150 minutes of moderate-intensity of activity per week (Bull et al., 2020). Additionally, participants must fill out a Qualtrics survey containing pre-exercising health questions about deep venous thrombosis (DVT) (Patients, 2002). Participants will be included if their DVT risk score of ≤ 2 (Patients, 2002). Before acceptance into the study, researchers will review the Qualtrics survey to determine if the participant is eligible to participate. After acceptance into the study, participants will be informed (verbally and visually) about the study's exercise protocol and what responsibilities they have during exercise testing. Participants will have the opportunity to ask questions or address any concerns throughout the research study. Participants who agree to participate in the study will voluntarily sign the informed consent and will proceed with the research study.

Exclusion

The participant's PARQ + will be reviewed by the principal investigator to determine if they are healthy and ready to perform the exercise protocol. Participants who answer 'yes' to any of the questions on the PARQ + are excluded from the study (Warburton et al., 2011). Any participants with, or at risk, for cardiovascular (e.g., hyper/hypotension, coronary artery disease, and peripheral artery disease), pulmonary (e.g., COPD and asthma), and metabolic (e.g., diabetes, metabolic syndrome, and hyperlipidemia) disease are excluded from the study. Additionally, any participants who currently have or have a family history of cardiovascular disease, exertional rhabdomyolysis, sickle cell anemia, deep venous thrombosis (DVT), recent surgeries, BMI > 35, and orthopedic pain are excluded from the study. Furthermore, participants

who self-report as active smokers, take heart medications (e.g., beta-blockers, hypertensives, and anticoagulants), have implanted medical devices, or inability to consent are excluded from the study. A survey will be used via Qualtrics for participants to complete before testing to screen for DVT, physical activity readiness, strength training experience, physical activity minutes per week, or present injuries that would not enable them to perform a knee extension exercise. Participants are also excluded if they have a DVT risk score of ≥ 3 (Patients, 2002). In addition, participants are excluded if they do not have at least 6 months of strength training experience or obtain 150 minutes of moderate-intensity of activity per week (Okuno et al., 2014; Bull et al., 2020). Participants who are accepted in the study will be scheduled for three separate exercise sessions: 1RM testing, control (without BFR), and intervention (with BFR). An email announcement will be sent to North Dakota State University students and staff to aid in recruiting participants across the university. All protocols and instrumentation devices will be approved by the North Dakota State University Institutional Review Board (Appendix A).

Methods

A stadiometer (Seca 213, Chino, CA) and floor-level scale (Detecto, Webb City, MO) will be used to measure participant height (cm) and weight (kg). A knee extension exercise machine (Cybex International, Owatonna, MN) will be used during all sessions to assess 1RM weight and 20% of 1RM weight for each control and intervention session. A polar heart rate (Polar Electro H10, Kempele, FIN) chest monitor will be used to measure HRV indices at rest and post-exercise. HRV indices are measured in 5-minute increments during pre-and post-test procedures. The Elite HRV app (via Bluetooth) will be used to collect HR data. Kubios HRV software (Kubios HRV Standard version 3.5.0, Kuopio, FIN) will be used to display HRV indices data (via frequency and time domains). BFR (Delfi Medical Innovations Inc., Vancouver BC,

CA) will be used on each leg to restrict femoral venous blood flow to the quadriceps muscle group and lower leg during the knee extension exercise. During the control and intervention sessions, muscle oxygen saturation will be assessed using a near-infrared spectroscopy sensor (Moxy Monitor, Fortiori Design LLC, Hutchinson, MN) placed on the vastus lateralis (VL) muscle.

Anthropometrics

Researchers will use a stadiometer and floor level scale to measure body composition during the first initial session. Body mass index (BMI) will be calculated as mass (kg)/height(m)^2. Additionally, researchers will measure limb circumference of the upper thigh to determine BFR cuff size.

Rated Perceived of Exertion (RPE) Measurement

Researchers will use a 0-10 RPE scale to measure intensity during the intervention and control sessions (Zourdos et al., 2015). RPE will be assessed on the last set completed while performing the knee extension on the right and left leg.

HRV Measurement

A polar HR chest monitor will be placed under the pectoral muscles and wrapped around the sternum. The HR monitor then will be connected via Bluetooth to a cellular device through the app Elite HRV. Before testing during the control and intervention session, Participants will rest in a supine position for 15 minutes. The first initial 10 minutes is for the participants to rest. After 10 minutes, HR data will be recorded for 5 minutes through the Elite HRV app. After completing the control or intervention trial, participants will follow a modified HR recording time outlined by Tai et al., (2019). Participants will lay supine for 10 minutes with a 5-minute HR recording and at 25 minutes with a 5-minute HR recording, consisting of two recovery

intervals at 15 and 30 minutes. Data will then be transferred from the Elite HRV app and sent via email as a file with each participant's corresponding HR data. Once received, data will be saved and uploaded to the Kubios HRV software on a computer. HR data will be processed by Kubios software to generate HRV time and frequency domains. The HRV indices documented are the same indices documented in Okuno et al., (2014): lnLF (ms^2), lnHF (ms^2), LF (nu), HF (nu), LF/HF ratio, and lnRMSSD (ms^2) (Okuno et al., 2014). To avoid any inconsistency in data, participants have only 1 minute after they have completed their last set on the leg extension machine to be laying in a supine position to get ready for HRV recording.

BFR Session (Intervention)

BFR will be performed on each leg of each participant. Two BFR cuffs will be placed at the most proximal position on each leg in the supine position while the participant is resting. After the HRV recording is taken, both cuffs will be inflated to a limb occlusion pressure (LOP) of 80% to identify each participant's personalized tourniquet pressure (PTP) (Patterson et al., 2019). The lowest LOP between each leg will be used to identify PTP for that participant. Patterson et al., (2019) identified a safe LOP between 40-80% (Patterson et al., 2019). Additionally, Patterson et al., (2019) suggested a higher LOP (e.g., 80%) when using weight 20% of the 1RM, and a lower LOP (e.g., 40%) when using weight 40% of 1RM (Patterson et al., 2019). The cuffs will be deflated during the 1-minute rest interval between sets to allow blood flow to the affected quadriceps muscle group. The cuffs will remain on the participant's leg for the entire intervention.

SmO_2

A Moxy monitor will be placed on the participant's vastus lateralis (VL) muscle during each control and intervention session. Researchers adopted the same Moxy monitor placement

protocol that was determined by Crum et al., (2017), where researchers used a single Moxy monitor placed on the VL muscle (Crum et al., 2017). The protocol will consist of the sensor being placed on the right leg halfway between the greater trochanter and lateral epicondyle of the femur. The researchers used the VL muscle because it is a primary knee extension muscle in the quadricep muscle group. Once the monitor is placed, researchers will record SmO_2 throughout the entire control and intervention sessions and time stamp the start and end time of each set. Data will be recorded as percentages (0-100%) via Feldmann et al., (2019) protocol in an Excel spreadsheet for each participant's average SmO_2 levels throughout the entire session (Feldmann et al., 2019).

Procedures

All participants will complete three exercise sessions in order: 1RM testing, intervention, and control. Each session will be separated by at least 72 hours. The sessions will take place Monday through Friday at the Benston Bunker Field House in the Human Performance Lab. Attempts will be made to try and schedule each participant's completed exercise at the same time for each session. Before each session, participants will be told to keep to the same diet pattern and avoid caffeine and alcohol for up to 12 hours before exercise testing. Additionally, participants will also be told to avoid high-intensity exercise 48 hours before each session.

Determining 1RM

Participants will report to Benston Bunker Fieldhouse for their scheduled session. Upon arrival, screening forms (PARQ +) and informed consent are presented to each participant. After height and weight are measured and informed consent is signed, participants will complete a 5-minute warmup on a cycle ergometer before 1RM testing. Researchers will follow a modified version of Okuno et al., (2014) to determine 1RM on the knee extension exercise (Okuno et al.,

2014). Before testing, participants will be asked for their bilateral predicted 5RM knee extension via Qualtrics survey. Researchers will use the equation [1RM = -0.46 + {0.79 X 5(reps)} + {1.08 X load (kg)}] to predict each participant's bilateral 1RM (Julio et al., 2012). Researchers will then divide each participant's bilateral 1RM in two, to estimate a 1RM on each leg. The protocol will consist of 5 sets with 3-minute rest intervals between each set. Before testing, knee extension machine adjustments are made differently for each participant based on limb anatomy and are recorded to ensure consistency for each session. Each participant underwent 1RM testing on each of their lower limbs separately. The order of testing was randomized (e.g., left and right). The protocol to determine 1RM is in order as follows: 50% 1RM for 5 repetitions, 70% 1RM for 3 repetitions, 90% 1RM for 1 repetition, 100% of participants predicted 1RM for 1 repetition, chance at another repetition if 100% of participants predicted 1RM will be completed. Testing will be exclusively done without a BFR cuff applied and a full range of motion (FOM) must be met with each repetition for that set to be completed. If participants develop any orthopedic pain during testing, the test will be immediately terminated. Once 1RM is determined, each participant's data will be documented in an Excel spreadsheet.

Intervention Session

When each participant completes their 1RM, 20% of each participant's 1RM will be calculated (.20 X 1RM). After the HRV recording is taken, a BFR cuff will be inflated on both limbs to measure a limb occlusion pressure (LOP) of 80% to identify each participant's personalized tourniquet pressure (PTP). The lowest LOP between each leg will be used to identify PTP for that participant. Once PTP has been identified, Participants will begin with a 5-minute warm-up on the cycle ergometer without the BFR cuff before testing. Once the warmup has been completed the participants will be fitted with a BFR cuff at their own PTP. A total of 75

repetitions over 5 sets have been shown to increase muscle adaptation significantly in previous studies when BFR is applied. Researchers will use a modified version of Patterson et al., (2019) and Okuno et al., (2014) to obtain a goal of 75 repetitions on the knee extension exercise for each participant (Patterson et al., 2019; Okuno et al., 2014). The protocol will consist of 5 sets of 15 repetitions on each leg with a 1-minute rest interval between each set. Participants will complete all five sets on one leg before switching to the other. During the rest interval, the BFR cuff will be deflated to ensure blood flow to the affected muscles. If a participant could not complete all sets or repetitions due to fatigue or not obtaining FOM, the last set or repetitions completed are documented to ensure consistency during the control session. If participants feel any orthopedic pain during testing, the test will be immediately terminated. Furthermore, if the participant reaches their max HR (220 – age) during any point of testing the test will be terminated.

Control Session

After the intervention session is completed, the control session will consist of the identical knee extension exercise protocol as used in the intervention session. Participants will begin with a 5-minute warm-up on the cycle ergometer and then begin the exercise protocol without BFR. The intervention session will be done before the control portion for the reason that a participant could not complete all the sets or repetitions when BFR is applied. This will allow researchers to control consistency for each participant set and repetitions for each session. All data will be recorded on an Excel spreadsheet. If participants feel any orthopedic pain during testing, the test will be immediately terminated. Furthermore, if the participant reaches their max HR (220 – age) during any point of testing the test will be terminated.

Data Analysis

This study used an experimental design. All participants completed the intervention and control session, with each participant acting as their own control. Anthropometric data is presented using descriptive statistics. A 2 X 3 ANOVA with repeated measures was used to analyze HRV pre and post-test recording times between the intervention and control ($P < 0.05$). Additionally, a 2 X 2 ANOVA with repeated measures was used to analyze SmO_2 exercise and rest time stamps between the intervention and control ($P < 0.05$). RPE data was analyzed using a paired t-test between the intervention and control ($P < 0.05$). All analyses were performed using SPSS statistical software.

CHAPTER IV. RESEARCH ARTICLE

Abstract

BACKGROUND: Autonomic modulation has been used in post-training recovery and can also indicate fitness level. **PURPOSE:** This study determined how an acute training session of BFR exercise affects autonomic modulation during a unilateral knee extension exercise**.** **METHODS:** Fourteen physically active males completed three sessions: 1RM testing, BFR, and control while performing a unilateral knee extension exercise. Heart rate variability (HRV), muscle oxygen saturation (SmO_2), and rating of perceived exertion (RPE) were all measured. Repeated measures ANOVAs were used to analyze all HRV and SmO_2 data, while a paired *t*-test was used to analyze RPE data. A significance level of $P < 0.05$ was used for all data analyses. **RESULTS:** Significant time-effect differences were found in lnRMSSD, lnHF, and lnLF from 15 to 30 minutes post-exercise in both groups ($P < 0.05$). Time effects were also significant in lnRMSSD from baseline to 15 minutes post-exercise ($P < 0.05$) in both groups. SmO_2 data presented time and group-effect differences that were significant in SmO_2% in the vastus lateralis (VL) muscle ($P < 0.05$) in BFR compared to control. Additionally, time and group-effects differences were significant in the oxygenated hemoglobin and the deoxygenated hemoglobin in the VL muscle in BFR compared to the control ($P < 0.05$). RPE was significantly higher when BFR was applied compared to the control session ($P < 0.05$). **CONCLUSION:** Despite the training method, sympathetic (SNS) and parasympathetic (PNS) nervous system activity increased the most from 15 to 30 minutes post-exercise, however, BFR exercise increased in RPE. To show differences in HRV measures among groups, cuff pressure or exercise intensity may need to be altered.

Introduction

Heart disease remains the leading cause of death in the United States (U.S.) (*How the Heart Works | NHLBI, NIH*, n.d.). An increase in age increases the risk of developing cardiovascular disease (CVD). For males, life expectancy in 2019 was 76.3 years, and for females 81.4 years (Xu et al., 2020). Individuals who are older than 20 years of age are 49.2% more prevalent to develop some form of CVD both in males and females (Aparicio et al., 2021). CVD has been attributed to being the highest U.S health expenditure cost from 2016 to 2017, with heart disease attributing the most cost of all CVD's at $219.6 billion (Aparicio et al., 2021).

Heart rate variability (HRV) is a valuable health predictor in adverse outcomes in various diseases (Tsuji et al., 1996). The autonomic nervous system (ANS) is composed of the sympathetic (SNS) (fight or flight) and parasympathetic (PNS) (rest and digest) nervous systems, which, both are responsible for controlling your heart rate (HR). A healthy individual will have a superior balance between the two systems in which they counteract each other equally (*How the Heart Works | NHLBI, NIH*, n.d.). Autonomic modulation can be used to treat patients with CVD, depression, neural diseases, heart arrhythmias, and heart failure (Stavrakis et al., 2021). Autonomic modulation utilizes neural tissue to stimulate neural remodeling which can obtain a therapeutic benefit. Previous research using similar methods and HRV has found that these HRV indices return to baseline within 48 hours after exercise (Caruso et al., 2015; Cornelissen et al., 2010; Bentley et al., 2020; De Oliveira et al., 2019). Furthermore, previous research studied how blood flow restriction (BFR) can improve HRV during a long-term training program, however, an acute session of BFR training has not been well-established (Santos et al., 2022; Tai et al., 2019; Rossow et al., 2011; Okuno et al., 2014). BFR is the partial occlusion of a limb artery either using a pneumatic cuff or tourniquet (Neal et al., 2023). The cuff is placed at the most

proximal part of the limb that is being trained. BFR is used to promote homeostatic disturbances used to increase signaling to enhance muscle adaptations (Santos et al., 2022). An increase in stimulus increases blood capillary supply and mitochondrial function which can improve exercise performance (Santos et al., 2022).

The purpose of this study was to determine how an acute bout of BFR exercise affects autonomic modulation during a unilateral knee extension exercise. This experimental study examined heart rate variability (HRV) and muscle oxygen uptake while performing a unilateral knee extension exercise at 20% of one-repetition max (1RM) during a control session without BFR and an intervention session containing BFR. Before testing the control and intervention, an exercise session was held to determine each participant's 1RM on the knee extension exercise.

Methods

Participants

Participants underwent both intervention and control sessions in a within subjects, cross-over design. The participants included were 14 physically active healthy males, who were regularly active (≥ 150 minutes of moderate activity per week) with at least 6 months of strength training experience, between 18-35 years of age (Bull et al., 2020; Okuno et al., 2014). The sample size is based on previous studies conducted (Santos et al., 2022; Tai et al., 2019; Rossow et al., 2011; Okuno et al., 2014). Inclusion criteria consisted of participants completing a Physical Activity Readiness Questionnaire (PARQ +) to ensure they have no underlying health conditions that prohibit them from performing the exercise (Warburton et al., 2011). Additionally, participants filled out a Qualtrics survey containing pre-exercising health questions about deep venous thrombosis (DVT) (Patients, 2002). Participants were included if their DVT risk score of ≤ 2 (Patients, 2002). After acceptance into the study, participants were informed

(verbally and visually) about the study's exercise protocol and what responsibilities they had during exercise testing. Participants had the opportunity to ask questions or address any concerns throughout the research study. Participants who agreed to participate in the study voluntarily signed the informed consent and proceeded with the research study. Exclusion Criteria consisted of participants who answered 'yes' to any of the questions on the PARQ + were excluded from the study (Warburton et al., 2011). Any participants who were at risk, for cardiovascular (e.g., hyper/hypotension, coronary artery disease, peripheral artery disease, and cardiac arrhythmias), pulmonary (e.g., COPD and asthma), and metabolic (e.g., diabetes, metabolic syndrome, and hyperlipidemia) disease were excluded. Additionally, participants who currently have or had a family history of cardiovascular disease, exertional rhabdomyolysis, sickle cell anemia, deep venous thrombosis (DVT), recent surgeries, BMI > 35, and orthopedic pain were excluded. Furthermore, participants who self-report as active smokers, take heart medications (e.g., beta-blockers, hypertensives, and anticoagulants), have implanted medical devices, or inability to consent were excluded. Participants completed a survey before testing to screen for DVT, physical activity readiness, strength training experience, physical activity minutes per week, or present injuries that would not enable them to perform a knee extension exercise. Participants were also excluded if they had a DVT risk score of ≥ 3 (Patients, 2002). In addition, participants were excluded if they did not have at least 6 months of strength training experience or obtained < 150 minutes of moderate-intensity of activity per week (Okuno et al., 2014; Bull et al., 2020). Participants who were accepted in the study were scheduled for three separate exercise sessions: 1RM testing, control (without BFR), and intervention (with BFR).

Instrumentation

A stadiometer (Seca 213, Chino, CA) and floor-level scale (Detecto, Webb City, MO) were used to measure participant height (cm) and weight (kg). A knee extension exercise machine (Cybex International, Owatonna, MN) was used during all sessions to assess 1RM and 20% of 1RM was calculated for the control and intervention sessions. A polar heart rate (Polar Electro H10, Kempele, FIN) chest monitor was used to measure HRV indices at rest and post-exercise. HRV indices are measured in 5-minute increments during pre-and post-test procedures. The Elite HRV app (via Bluetooth) was used to collect HR data. Kubios HRV software (Kubios HRV Standard version 3.5.0, Kuopio, FIN) was used to display HRV indices data (via frequency and time domains). BFR (Delfi Medical Innovations Inc., Vancouver BC, CA) was used on each leg to restrict femoral venous blood flow to the quadriceps muscle group and lower leg during the knee extension exercise. During the control and intervention sessions, muscle oxygen saturation was assessed using a near-infrared spectroscopy sensor (Moxy Monitor, Fortiori Design LLC, Hutchinson, MN) placed on the vastus lateralis (VL) muscle.

Procedures

All participants completed three exercise sessions in the following order: 1RM testing, intervention, and control. Each session was separated by at least 72 hours. The sessions took place Monday through Friday at the Benston Bunker Field House in the Human Performance Lab. Attempts were made to try and schedule each participant's completed exercise at the same time for each session. Before each session, participants were told to keep the same diet pattern and avoid caffeine and alcohol for up to 12 hours before exercise testing. Additionally, participants were also told to avoid high-intensity exercise 48 hours before each session. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and deep venous thrombosis

(DVT) forms were presented to each participant via Qualtrics survey before attending any session. If the participant was eligible to participate, informed consent was signed at their first scheduled session (Appendix B).

Determining 1RM

After each informed consent was signed, height (cm), weight (kg), and right thigh limb circumference (cm) were taken. Participants completed a 5-minute warmup on a cycle ergometer before 1RM testing. A modified version of Okuno et al., (2014) was used to determine 1RM on the knee extension exercise (Okuno et al., 2014). Before testing, participants were asked for their bilateral predicted 5RM knee extension via Qualtrics survey. The equation [1RM = -0.46 + (0.79 X 5 reps) + (1.08 X load kg)] was used to predict each participant's bilateral 1RM (Julio et al., 2012). Each participant's bilateral 1RM was then divided in two, to estimate a unilateral 1RM for each leg. This protocol consisted of 5 sets with 3-minute rest intervals between each set. Before testing, knee extension machine adjustments were made for each participant based on limb anatomy and were recorded to ensure consistency for each session. Each participant underwent 1RM testing on each of their lower limbs separately. The right leg was performed first then the left. The protocol to determine unilateral 1RM is in order as follows: 50% 1RM for 5 repetitions, 70% 1RM for 3 repetitions, 90% 1RM for 1 repetition, 100% of participants predicted 1RM for 1 repetition, chance at another repetition if 100% of participants predicted 1RM was completed. Testing was exclusively done without a BFR cuff applied and a full range of motion must have been met with each repetition for that set to be completed. If participants developed any orthopedic pain during testing, the test was immediately terminated. Furthermore, if the participant reached their maximum HR (220 – age) during any point of the test, the test was terminated.

Intervention Session

Before BFR testing, 20% of each participant's 1RM was calculated (.20 X 1RM). After an HRV recording was taken, a BFR cuff was inflated on both limbs to measure 80% of limb occlusion pressure (LOP) to identify the participant's personalized tourniquet pressure (PTP). The lowest LOP between each leg was used to identify PTP for each participant. Once PTP had been identified, participants began with a 5-minute warm-up on the cycle ergometer without the BFR cuff before testing. After the warmup, participants were fitted with a BFR cuff at their own PTP. A total of 75 repetitions over 5 sets has been shown to increase muscle adaptation significantly in previous studies when BFR is applied (Patterson et al., 2019). A modified version of Patterson et al., (2019) and Okuno et al., (2014) was used to obtain a goal of 75 repetitions on the knee extension exercise for each participant (Patterson et al., 2019; Okuno et al., 2014). The protocol consisted of 5 sets of 15 repetitions on each leg with a 1-minute rest interval between each set. Participants completed all five sets on the right leg before switching to the left. The BFR cuff was deflated during the rest interval to ensure blood flow to the affected muscles. If a participant could not complete all sets or repetitions due to fatigue or not obtaining FOM, the last set or repetitions completed were documented to ensure consistency during the control session. If participants developed any orthopedic pain during testing, the test was immediately terminated. Furthermore, if the participant reached their maximum HR (220 – age) during any point of the test, the test was terminated.

Control Session

After the intervention session was completed, the control session consisted of the identical knee extension exercise protocol as used in the intervention session. Participants began with a 5-minute warm-up on the cycle ergometer and then began the exercise protocol without

BFR. The intervention session was done before the control portion for the reason that if a participant could not complete all the sets or repetitions when BFR was applied. This will allow researchers to control consistency for the participant's set and repetitions for each session. All data was recorded on an Excel spreadsheet. If participants developed any orthopedic pain during testing, the test was immediately terminated. Furthermore, if the participant reached their maximum HR (220 – age) during any point of the test, the test was terminated.

Data Analysis

This study used a within-subject, cross-over, experimental design. All participants completed the intervention and control session, with each participant acting as their own control. Anthropometric data are presented using descriptive statistics (Table 4). A 2 X 3 ANOVA with repeated measures was used to analyze HRV pre and post-test recording times between the intervention and control. Additionally, a 2 X 2 ANOVA with repeated measures was used to analyze SmO_2 exercise and rest intervals between the intervention and control sessions. When significance was detected (time effect, group effect, or group x time interaction), additional post-hoc analysis was completed using Sidak to control for multiple comparisons. Partial eta squared ($p\eta^2$) effect size estimations are also included for interpretation as $p\eta^2$ 0.2 to 0.12 is considered a small effect, 0.13 to 0.25 is a medium effect, and >0.26 is a large effect (Bakeman et al., 2005). RPE data was analyzed using a paired t-test between the intervention and control sessions. All analyses were performed using SPSS statistical software and an alpha level of less than 0.05 was used to determine statistical significance

Table 4.

Anthropometric Data

Variable	Mean ± SD
Age (yr)	21.50 ± 2.69
Height (cm)	177.81 ± 9.08
Body mass (kg)	81.98 ± 11.30
BMI (kg/m^2)	25.85 ± 2.69
Thigh Circumference (right) (cm)	59.71 ± 5.88
KE 1RM left leg (kg)	46.22 ± 14.02
KE 1RM right leg (kg)	46.22 ± 14.02
KE exercise weight left leg (kg)	9.70 ± 3.13
KE exercise weight right leg (kg)	9.70 ± 3.13
Total limb occlusion (mmHg)	179.11± 16.65
80% limb occlusion (mmHg)	143.29 ± 13.32

BMI: Body Mass Index, KE: Knee Extension

Results

HRV using lnRMSSD was the only time-domain variable measured during the study. There was a significant time effect for lnRMSSD (F (1.315, 17.095) = 7.215, P = 0.011, pη^2 =0.357). Post-hoc analyses determined there was a significant decline in lnRMSSD from baseline to 15 minutes post-exercise (P=0.027) and a significant increase from 15 minutes post-exercise to 30 minutes post-exercise with both BFR and control (P=0.005) (Table 5).

The frequency-domain HRV variables analyzed in the study were lnHF, lnLF, HFnu, LFnu, and LF/HF ratio. There was a significant time effect for lnHF (F (2, 26) = 4.165, P = 0.027, pη^2 =0.243). Post-hoc analysis determined a significant increase from 15 minutes post-exercise to 30 minutes post-exercise with both BFR and control (P=0.016). There was also a significant time effect for lnLF (F (2, 24) = 4.157, P = 0.028, pη^2 =0.243). Post-hoc analysis determined a significant increase from 15 minutes post-exercise to 30 minutes post-exercise with both BFR and control (P=0.028). No significant time effects were found for HFnu (F (2, 26) =

0.254, $P = 0.777$, $p\eta^2 = 0.019$), LFnu (F (2, 26) = 0.243, $P = 0.786$, $p\eta^2 = 0.018$), and LF/HF ratio (F (2, 26) = 0.137, $P = 0.873$, $p\eta^2 = 0.069$) (Table 5).

Table 5.

Means and standard deviations of frequency and time domains of HRV at baseline, 15 minutes post-exercise, and 30 minutes post-exercise.

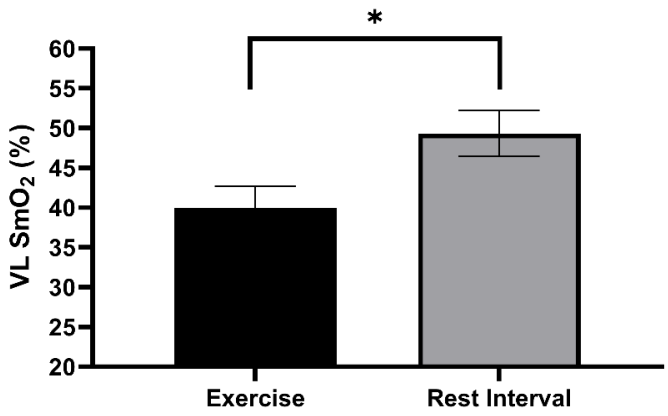
Variable	Baseline			15 min post-exercise			30 min post-exercise		
	BFR Exercise	**Control Exercise**	**Average**	**BFR Exercise**	**Control Exercise**	**Average**	**BFR Exercise**	**Control Exercise**	**Average**
lnRMSSD	4.31 ± 0.39	4.16 ± 0.46	4.34 ± 0.43	3.72 ± 0.87	3.78 ± 0.76	3.75 ± 0.82*	4.00 ± 0.86	4.05 ± 0.60	4.03 ± 0.73#
lnHF	7.43 ± 0.80	6.95 ± 1.34	7.19 ± 1.07	6.65 ± 0.95	6.59 ± 1.35	6.62 ± 1.15	7.23 ± 0.92	7.07 ± 1.01	7.15 ± 0.97#
lnLF	7.42 ± 0.81	7.08 ± 1.05	7.25 ± 0.93	6.80 ± 0.92	6.82 ± 0.81	6.81 ± 0.87	7.41 ± 0.77	7.15 ± 0.91	7.28 ± 0.84#
HF nu.	49.12 ± 17.91	46.32 ± 13.99	47.72 ± 15.95	45.31 ± 17.39	45.66 ± 17.04	45.49 ± 17.22	47.19 ± 17.19	48.22 ± 22.13	47.71 ± 19.66
LF nu.	50.83 ± 17.93	53.65 ± 13.39	52.24 ± 15.66	54.58 ± 17.40	54.27 ± 16.99	54.53 ± 17.20	52.76 ± 17.21	51.72 ± 22.14	52.26 ± 19.68
LF/HF	1.33 ± 0.96	1.43 ± 1.15	1.38 ± 1.06	1.49 ± 0.84	1.48 ± 0.95	1.49 ± 0.90	1.43 ± 0.93	1.59 ± 1.18	1.51 ± 1.06

N=14. *Significant time effect (average of BFR and control) vs. baseline, $P<0.05$. #Significant time effect (average of BFR and control) vs. 15 min-post, $P<0.05$.

There was a significant time effect for VL SmO_2% (F (1, 13) = 17.109, P = 0.001, $p\eta^2$ =0.568). Post-hoc analysis determined that SmO_2% was significantly lower with exercise during both BFR and control compared to the rest intervals (P=0.001, Figure 1).

Figure 1.

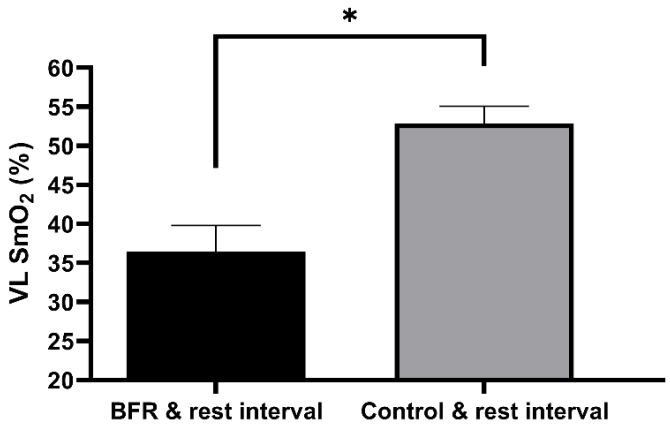
Average of VL SmO2% between exercise and the rest interval in both groups (P<0.05).*



Additionally, there was a significant group effect for VL muscle oxygen saturation SmO_2% (F (1, 13) = 45.537, P < 0.001, $p\eta^2$ =0.770). Post-hoc analysis determined VL SmO_2% was significantly lower during BFR exercise with the associated rest intervals compared to control exercise with the associated rest intervals (P<0.001, Figure 2).

Figure 2.

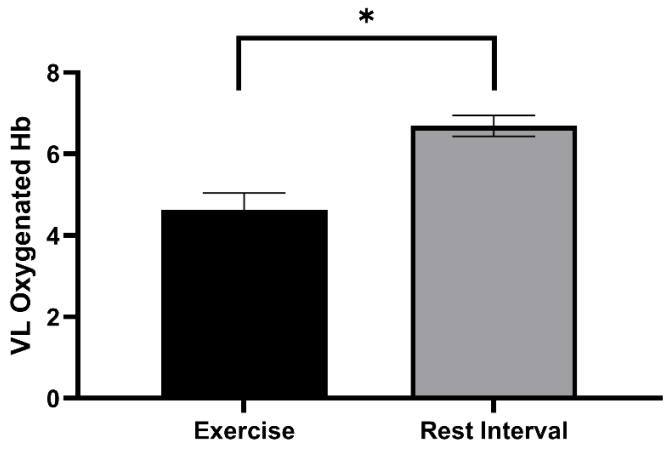
Average of VL SmO2% between both groups when exercise and rest intervals are averaged together (P<0.05).*



There was a significant time effect for oxygenated hemoglobin (F (1, 13) = 35.83, P < 0.001, $p\eta^2$ =0.734). Post-hoc analysis determined oxygenated hemoglobin was significantly lower in the VL in both groups with exercise compared to the rest intervals (P<0.001, Figure 3). There was also a significant group effect of oxygenated hemoglobin (F (1, 13) = 16.131, P = 0.001, $p\eta^2$

Figure 3.

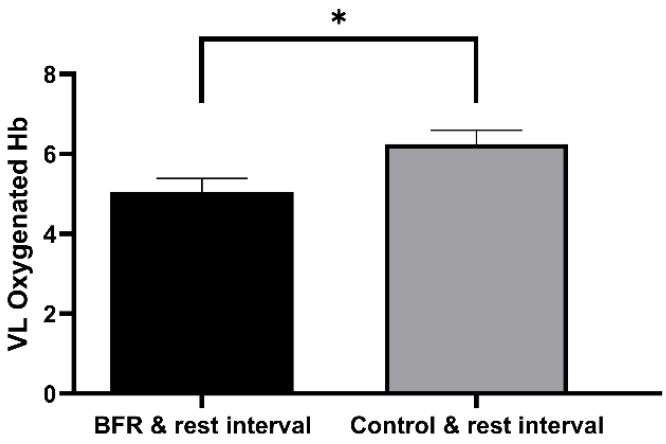
Average of VL oxygenated hemoglobin (Hb) between exercise and rest interval in both groups (P<0.05*).



=0.554). Post hoc analysis determined oxygenated hemoglobin was significantly lower in the VL muscle during BFR exercise with the rest interval compared to the control exercise with the rest interval (P < 0.001, Figure 4).

Figure 4.

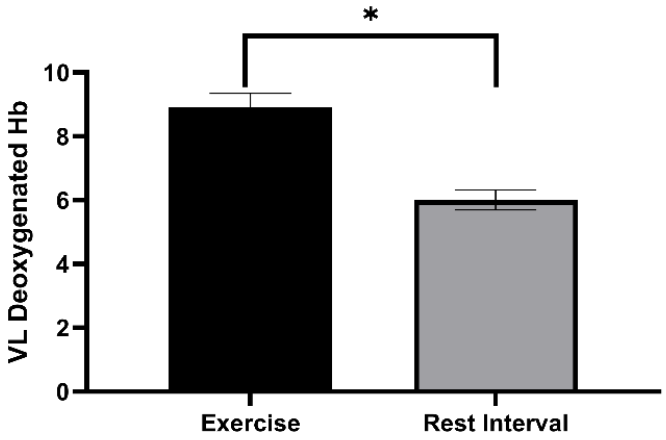
Average of VL oxygenated hemoglobin (Hb) between both groups when exercise and rest intervals are averaged together (P<0.05).*



There was a significant time effect for VL deoxygenated hemoglobin (F (1, 13) = 84.683, P < 0.001, $p\eta^2$ =0.867). Post-hoc analysis determines VL deoxygenate hemoglobin was significantly higher with exercise compared to the rest intervals in both groups (P<0.001, Figure 5).

Figure 5.

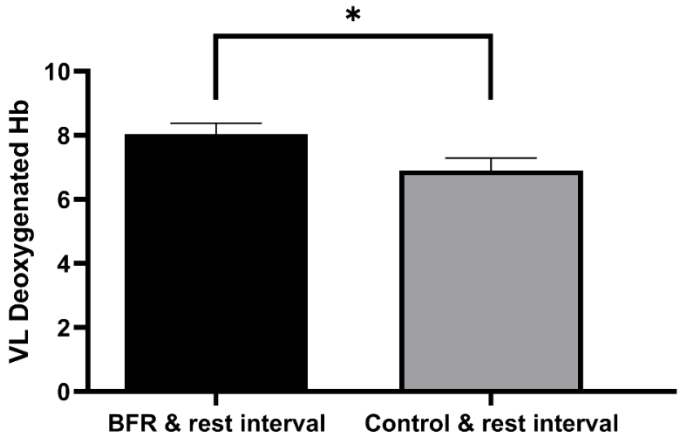
Average of VL deoxygenated hemoglobin (Hb) between exercise and rest interval in both groups (P<0.05).*



There was also a significant group effect for VL deoxygenated hemoglobin (F (1, 13) = 13.817, P = 0.003). Post-hoc analysis determined VL deoxygenated hemoglobin was significantly higher in the VL during BFR exercise combined with the rest interval compared to the control exercise combined with the rest interval P = 0.003, Figure 6).

Figure 6.

Average of VL deoxygenated hemoglobin (Hb) between both groups when exercise and rest intervals are averaged together (P<0.05).*

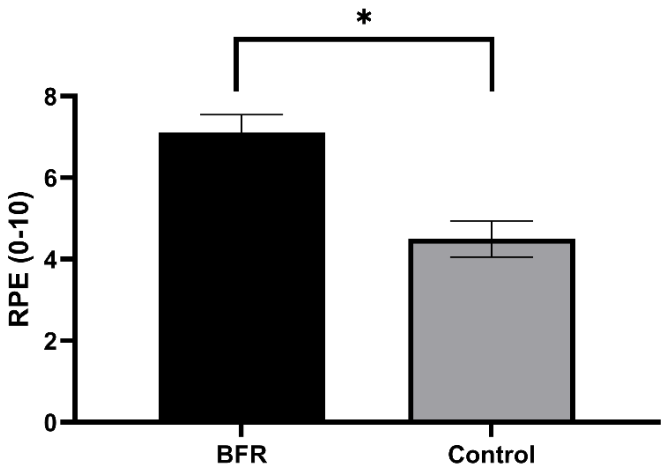


There was no significant time (F (1, 13) =0.609, P = 0.449, $p\eta^2$ =0.045), group (F (1, 13) =0.058, P = 0.814, $p\eta^2$ =0.004), or group by time (F (1, 13) =1.192, P =0.295, $p\eta^2$ =0.84) effects for total hemoglobin.

The RPE for the right and left leg were averaged together for both the BFR and control sessions. The paired *t*-test revealed that RPE was significantly higher when BFR was applied compared to the control session (t (13) = 5.885, P < 0.001, Figure 7).

Figure 7.

Average RPE between both groups (P<0.05).*



Discussion

This is the first study to evaluate the effects of an acute session of BFR exercise on the autonomic nervous system (ANS) and SmO_2 in the VL muscle during a unilateral knee extension exercise. The goal of the study was to establish acute physiological responses in HRV and SmO_2 in the VL muscle in a single session of BFR training. The major findings of the study were that SNS and PNS activity decreased from baseline to 15 minutes post-exercise and increased from 15 to 30 minutes post-exercise in both groups. However, this exercise protocol did not elicit any

HRV differences between groups. Furthermore, major findings were observed in SmO_2%, oxygenated and deoxygenated hemoglobin and RPE.

BFR and HRV

lnRMSSD significantly declined from baseline to 15 minutes post-exercise. This would suggest a disturbance in sympathovagal balance. However, during our study significant increases in lnRMSSD, lnHF, and lnLF were observed in both groups (BFR & Control) 15 to 30 minutes post-exercise. Although, both PNS and SNS activity significantly increased 15-30 minutes post-exercise, this suggests co-activation in both SNS and PNS. Co-activation can occur when the SNS is slow to return to baseline following a stressor, while PNS activity is increased to promote recovery. This event is observed in other studies regarding "vagal rebound" in different stressful situations (Mezzacappa et al., 2001 Page-Gould et al., 2010). These findings suggest that different environmental stressors can elicit a nonreciprocal ANS response from the SNS and PNS (Weissman & Mendes, 2022). Although, during our study subjects were asked to breathe normally while lying in a supine position. Controlled breathing techniques can help elicit respiratory sinus arrhythmia (RSA) which activates the PNS to help facilitate recovery faster (Weissman & Mendes, 2022). By participants breathing not being controlled and a recovery period not long enough for sympathovagal balance to take place, which could have possibly caused the co-activation between the SNS and PNS. Additionally, controlled breathing has been shown to affect thoracic stretch receptor afferents, which can cause a reflex inhibition of the ANS and influence HRV spectral components (e.g. lnHF & lnLF activity).

Our data suggests an increase in lnRMSSD, lnHF, and lnLF 15-30 minutes post-exercise. Tai et al., (2019) investigated how lnRMSSD, lnHF, and lnLF were affected during a bilateral bench press using two 70 mm-wide knee wraps that were wrapped around both upper arms while

using a rating scale of 7 out of 10 occlusion pressure (Tai et al., 2019). However, Tai et al., (2019) found significant increases in lnRMSSD during both the low-load BFR (25-30 minutes post-exercise) and high-load (15-20 min & 25-30 min post-exercise) exercise sessions. Furthermore, Tai et al., (2019) found a greater increase in lnRMSSD and lnHF during the high-load session compared to the low-load BFR session (Tai et al., 2019). These findings indicate that a greater exercise intensity without BFR could have a greater effect on autonomic modulation than a low-load BFR session to increase lnRMSSD and lnHF activity.

Okuno et al., (2014) investigated autonomic modulation during an acute high and low-intensity session, along with a single session of low-intensity with 40% LOP during a unilateral leg press exercise. RMSSD decreased below resting levels for low-intensity BFR 10 minutes post-exercise and 30 minutes post-exercise for high-intensity before returning to baseline measures (Okuno et al., 2014). However, post-exercise RMSSD during low-intensity exercise remained the same compared to baseline, until 24 hours post-exercise it significantly increased. A decrease in RMSSD measures was observed during the high-intensity exercise compared to the low-intensity exercise session up to 1 hour post-exercise, while the low-intensity BFR exercise session was significantly higher compared to the high-intensity at 10 and 30-minutes post-exercise (Okuno et al., 2014). lnLF decreased from baseline measures to 10 minutes post-exercise in the high-intensity session and during the 1 and 5-hour time period after low-intensity BFR only (Okuno et al., 2014). lnLF significantly increased for low-intensity and low-intensity BFR compared to the high-intensity session at 10 minutes post-exercise. This was different for the low-intensity BFR session where it was significantly lower compared to the low-intensity at 1 and 5 hours post-exercise. High-intensity and low-intensity BFR lnHF significantly decreased up to the 1-hour post-exercise period. High-intensity measures were significantly lower than

low-intensity BFR and low-intensity sessions for up to 30 minutes post-exercise (Okuno et al., 2014).

Our study only utilized 15 and 30-minute HRV recording times compared to Okuno et al., (2014) who used multiple recording times up to 24 hours. Given our study protocol, our study indicated that vagal balance between the SNS and PNS could take longer than 30 minutes to return to baseline measures, unlike compared to Okuno et al., (2014) and Tai et al., (2019). Although lnRMSSD, lnHF, and lnLF did increase from 10 to 30 minutes post-exercise and this was similar to Okuno et al., (2014) and Tai et al., (2019). These differences in exercise protocol/intensity such as repetitions, sets, rest periods, amount of weight, and LOP being used across different studies could affect autonomic modulation differently (Patterson et al., 2019). Differences in total exercise volume and BFR technique used could have played a role in the differences in autonomic modulation between studies.

SmO_2

Findings from our study found that SmO_2% was significantly lower with exercise compared to rest in both groups. However, when BFR was applied SmO_2% significantly decreased compared to the control session. Oxygenated hemoglobin was significantly lower when BFR was applied compared to the control session, and inversely deoxygenated hemoglobin was significantly higher during BFR compared to the control session.

Previous studies have reported similar results in SmO_2%, oxygenated hemoglobin, and deoxygenated hemoglobin. Shriver et al., (2023) reported similar findings during a walking treadmill protocol in SmO_2% where LOP (0%, 40%, 80%, & 100%) were randomized during seven stages of the protocol (Shriver et al., 2023). BFR was used on both legs at the most proximal location of the leg. The VL muscle being monitored during the walking treadmill

protocol showed a significant decrease in SmO_2% at all levels of LOP's. The VL muscle compared to the gastrocnemius muscle showed a significant decrease in SmO_2% at all levels of LOPs while walking (Shriver et al., (2023). These findings suggest a greater LOP indicates an increase in deoxygenated hemoglobin, thus decreasing SmO_2% in the VL.

Reis et al., (2019) investigated how VL tissue SmO_2 and deoxygenated were all affected during a unilateral knee extension exercise (Reis et al., 2019). Investigators used multiple different LOPs (non-BFR, 40%, 60%, and 80%) while occluding the femoral artery blood flow. The change in deoxygenated hemoglobin remained similar between 60 and 80% LOP. However, compared to 60 and 80% LOP, non-BFR and 40% LOP resulted in significantly lower deoxygenated hemoglobin during exercise (Reis et al., 2019). Oxygenated hemoglobin significantly increased during each rest interval in the non-BFR and 40% LOP groups, unlike compared to the 60 and 80% LOP. This data suggests when a higher LOP is used (60 and 80%), deoxygenated hemoglobin increases (Reis et al., 2019).

Our study findings are in agreement with the previous studies that were investigated. Regardless of the study protocol, SmO_2% decreased during exercise. There is enough evidence that BFR is an effective exercise method to increase deoxygenated hemoglobin and reduce muscle tissue oxygenated hemoglobin during low-intensity exercise.

RPE

RPE was recorded during the last rep of each set on both legs. RPE from each leg was averaged together in both groups and the means from both groups were compared. Our study found there to be a significant difference in RPE between the BFR session compared to the control session. RPE was significantly higher following BFR than compared to the control. These results can be replicated in previous studies that were conducted. Shriver et al., (2023)

found when occlusion pressure was higher it resulted in an increase in RPE with no increase in intensity (Shriver et al., 2023). Bartolomei et al., (2022) also investigated the difference in RPE when comparing high and low LOPs. Bartolomei et al., (2022) found that an LOP of 80% had a significantly greater increase in RPE compared to a LOP of 40% during a barbell preacher curl (Bartolomei et al., 2022). Given the differences in exercise protocols and intensities between previous studies, it can be said that a greater LOP will increase RPE due to the muscle being in more of a hypoxic state.

Conclusion

An acute BFR exercise session has been previously shown to promote SNS and PNS deactivation and activation, along with an increase in HRV (Okuno et al., 2014 & Tai et al., 2019). The findings of this study were that lnRMSSD, lnHF, and lnLF significantly increased from 15-30 minutes post-exercise. Although lnLF and lnHF both significantly increased 15-30 minutes post-exercise, this result is different from previous studies conducted (Okuno et al., 2014 & Tai et al., 2019). Despite differences among previous studies' exercise protocols, controlled breathing could be implemented to help avoid inhibiting the ANS and HRV spectral components (Mezzacappa et al., 2001 Page-Gould et al., 2010, Weissman & Mendes., 2022).

Additionally, BFR exercise has also been shown to increase deoxygenated hemoglobin during exercise and at different LOP's (Shriver et al., 2023 & Reis et al., 2019). The findings of this study were similar to previous studies in such that when BFR is applied during exercise it significantly increases deoxygenated and decreases oxygenated hemoglobin in the VL muscle.

Previous studies have shown RPE to increase when BFR has been applied during exercise (Shriver et al., 2023 & Bartolomei et al., 2022). The findings of this study suggest when

BFR is applied it significantly increases RPE compared to a session without BFR. A greater increase in LOP makes the muscle more hypoxic in result producing an increase in RPE.

Performing a unilateral knee extension when BFR is applied could prove to be a useful training method to increase HRV along with musculoskeletal adaptations. Lower-intensity BFR training could potentially lower the risk of developing complicated cardiac diseases or be beneficial for individuals who are advised not to perform high-intensity exercise due to impinging injuries or musculoskeletal diseases.

REFERENCES

Abe, T., Kearns, C. F., & Sato, Y. (2006). Muscle size and strength are increased following walk training with restricted venous blood flow from the leg muscle, Kaatsu-walk training. *Journal of Applied Physiology*, *100*(5), 1460–1466. https://doi.org/10.1152/japplphysiol.01267.2005

Abhishekh, H. A., Nisarga, P., Kisan, R., Meghana, A., Chandran, S., Trichur Raju, & Sathyaprabha, T. N. (2013). Influence of age and gender on autonomic regulation of heart. *Journal of Clinical Monitoring and Computing*, *27*(3), 259–264. https://doi.org/10.1007/s10877-012-9424-3

Aparicio, H. J., Benjamin, E. J., Callaway, C. W., Carson, A. P., Cheng, S., Elkind, M. S. V, Evenson, K. R., Ferguson, J. F., Knutson, K. L., Lee, C. D., Lewis, T. T., Loop, M. S., Lutsey, P. L., Mackey, J., & Matchar, D. B. (2021). Heart Disease and Stroke Statistics-2021 Update A Report from the American Heart Association. In *Circulation*. https://doi.org/10.1161/CIR.0000000000000950

Avram, R., Tison, G. H., Aschbacher, K., Kuhar, P., Vittinghoff, E., Butzner, M., Runge, R., Wu, N., Pletcher, M. J., Marcus, G. M., & Olgin, J. (2019). Real-world heart rate norms in the Health eHeart study. *Npj Digital Medicine*, *2*(1). https://doi.org/10.1038/s41746-019-0134-9

Bakeman, R. Recommended effect size statistic. *Behav Res Methods.* 2005;37(3):379–84. DOI: 10.3758/bf03192707

Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., Jones, D. W., Materson, B. J., Oparil, S., & Roccella, E. J. (2003). *CLINICIAN' S CORNER The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blodd Pressure*. *289*(19). DOI: 10.1001/jama.289.19.2560

Bartolomei, S., Montesanto, P., Lanzoni, I.M., Gatta, G., Cortesi, M., Fantozzi, S. (2022). A Comparison between High and Low Cuff Pressures on Muscle Oxygen Saturation and Recovery Responses following Blood-Flow Restriction Resistance Exercise. *Sensors, 22,* 9138. https://doi.org/10.3390/ s22239138

Beckers, F., Verheyden, B., & Aubert, A. E. (2006). Aging and nonlinear heart rate control in a healthy population. *American Journal of Physiology - Heart and Circulatory Physiology, 290*(6), 2560–2570. https://doi.org/10.1152/ajpheart.00903.2005

Bentley, R. F., Vecchiarelli, E., Banks, L., Gonçalves, P. E. O., Thomas, S. G., & Goodman, J. M. (2020). Heart rate variability and recovery following maximal exercise in endurance athletes and physically active individuals. *Applied Physiology, Nutrition and Metabolism, 45*(10), 1138–1144. https://doi.org/10.1139/apnm-2020-0154

Berkoff, D. J., Cairns, C. B., Sanchez, L. D., & Moorman, C. T. (2007). Heart rate variability in elite american track-and-field athletes. *Journal of Strength and Conditioning Research, 21*(1), 227–231. https://doi.org/10.1519/00124278-200702000-00041

Bigger, J. T., Fleiss, J. L., Rolnitzky, L. M., & Steinman, R. C. (1993). Frequency domain measures of heart period variability to assess risk late after myocardial infarction. *Journal of the American College of Cardiology, 21*(3), 729–736. https://doi.org/10.1016/0735-1097(93)90106-B

Bonnemeier, H., Wiegand, U. K. H., Brandes, A., Kluge, N., Katus, H. A., Richardt, G., & Potratz, J. (2003). Circadian profile of cardiac autonomic nervous modulation in healthy subjects: Differing effects of aging and gender on heart rate variability. *Journal of Cardiovascular Electrophysiology*, *14*(8), 791–799. https://doi.org/10.1046/j.1540-8167.2003.03078.x

Borchini, R., Veronesi, G., Bonzini, M., Gianfagna, F., Dashi, O., & Ferrario, M. M. (2018). Heart rate variability frequency domain alterations among healthy nurses exposed to prolonged work stress. *International Journal of Environmental Research and Public Health*, *15*(1). https://doi.org/10.3390/IJERPH15010113

Brain Basics: Know Your Brain | National Institute of Neurological Disorders and Stroke. (n.d.). Retrieved December 13, 2021, from https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Know-Your-Brain

Bull, F. C., Al-Ansari, S. S., Biddle, S., Borodulin, K., Buman, M. P., Cardon, G., Carty, C., Chaput, J. P., Chastin, S., Chou, R., Dempsey, P. C., Dipietro, L., Ekelund, U., Firth, J., Friedenreich, C. M., Garcia, L., Gichu, M., Jago, R., Katzmarzyk, P. T., … Willumsen, J. F. (2020). World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *British Journal of Sports Medicine*, *54*(24), 1451–1462. https://doi.org/10.1136/bjsports-2020-102955

Casties, J. F., Mottet, D., & Le Gallais, D. (2006). Non-linear analyses of heart rate variability during heavy exercise and recovery in cyclists. *International Journal of Sports Medicine*, *27*(10), 780–785. https://doi.org/10.1055/s-2005-872968

Caruso F. R., Arena R., Phillips S. A., Bonjorno J. C., Mendes R. G., Arakelian V. M., Bassi D., Nogi C., Borghi-Silva A. (2015). Resistance exercise training improves heart rate variability and muscle performance: a randomized controlled trial in coronary artery disease patients. *Euro J Rehab Med.* 51:289-9.

Cha, S.-A., Park, Y.-M., Yun, J.-S., Lee, S.-H., Ahn, Y.-B., Kim, S.-R., & Author Seung-Hyun Ko, C. (2018). Time-and Frequency-domain Measures of Heart Rate Variability Predict Cardiovascular Outcome in Patients with Type 2 Diabetes HHS Public Access. *Diabetes Res Clin Pract*, *143*, 159–169. https://doi.org/10.1016/j.diabres.2018.07.001

Chen, J.L., Yeh, D.P., Lee, J.P., Chen, Huang, C.Y., Lee, S.D., Chen, C.C., Kuo, T.B.J., Kao, C.L., Kuo, C.H. (2011). Parasympathetic nervous system activity mirrors recovery status in weightlifting performance after training. *Strength Cond. 0(*0):1-7.

Communications, S. (2009). Progression models in resistance training for healthy adults. *Medicine and Science in Sports and Exercise*, *41*(3), 687–708. https://doi.org/10.1249/MSS.0b013e3181915670

Cornelissen, V. A., Verheyden, B., Aubert, A. E., & Fagard, R. H. (2010). Effects of aerobic training intensity on resting, exercise and post-exercise blood pressure, heart rate and heart-rate variability. *Journal of Human Hypertension*, *24*(3), 175–182. https://doi.org/10.1038/jhh.2009.51

Crum, E. M., O'Connor, W. J., Van Loo, L., Valckx, M., & Stannard, S. R. (2017). Validity and reliability of the Moxy oxygen monitor during incremental cycling exercise. *European Journal of Sport Science*, *17*(8), 1037–1043. https://doi.org/10.1080/17461391.2017.1330899

De Oliveira, R. M., Ugrinowitsch, C., Kingsley, J. D., Da Silva, D. G., Bittencourt, D., Caruso, F. R., Borghi-Silva, A. & Libardi, C. A. (2019) Effect of individualized resistance training prescription with heart rate variability on individual muscle hypertrophy and strength responses. *European Journal of Sport Science,* 19:8, 1092-1100, DOI: 10.1080/17461391.2019.1572227

De Salles B.F., Simao R., Miranda F., Da Silva Novaes J., Lemos A., Willardson J.M. Rest intervals between sets in strength training. *Sports Med.* 2009;39(9). DOI: 10.2165/11315230-000000000-00000

Elgendi, M. (2012). On the Analysis of Fingertip Photoplethysmogram Signals. In *Current Cardiology Reviews* (Vol. 8). DOI: 10.2174/157340312801215782

Feldmann, A., Schmitz, R., & Erlacher, D. (2019). Near-infrared spectroscopy-derived muscle oxygen saturation on a 0% to 100% scale: reliability and validity of the Moxy Monitor. *Journal of Biomedical Optics*, *24*(11), 1. https://doi.org/10.1117/1.jbo.24.11.115001

Ferguson, R. A., Mitchell, E. A., Taylor, C. W., Bishop, D. J., & Christiansen, D. (2021). Blood-flow-restricted exercise: Strategies for enhancing muscle adaptation and performance in the endurance-trained athlete. *Experimental Physiology*, *106*(4), 837–860. https://doi.org/10.1113/EP089280

Frese, E. M., Fick, A., & Sadowsky, S. H. (2011). Blood Pressure Measurement Guidelines for Physical Therapists. *Cardiopulmonary Physical Therapy Journal*, *22*(2), 5–12. https://doi.org/10.1097/01823246-201122020-00002

Giardino, N. D., Lehrer, P. M., & Edelberg, R. (2002). Comparison of finger plethysmograph to ECG in the measurement of heart rate variability. *Psychophysiology*, *39*(2), 246–253. https://doi.org/10.1017/S0048577202990049

Gil, E., Orini, M., Bailón, R., Vergara, J. M., Mainardi, L., & Laguna, P. (2010). Photoplethysmography pulse rate variability as a surrogate measurement of heart rate variability during non-stationary conditions. *Physiological Measurement*, *31*(9), 1271–1290. https://doi.org/10.1088/0967-3334/31/9/015

Godwin, M., Birtwhistle, R., Delva, D., Lam, M., Casson, I., MacDonald, S., & Seguin, R. (2011). Manual and automated office measurements in relation to awake ambulatory blood pressure monitoring. *Family Practice*, *28*(1), 110–117. https://doi.org/10.1093/fampra/cmq067

Goldsmith, R. L., Bigger, J. T., Steinman, R. C., & Fleiss, J. L. (1992). Comparison of 24-hour parasympathetic activity in endurance-trained and untrained young men. *Journal of the American College of Cardiology*, *20*(3), 552–558. https://doi.org/10.1016/0735-1097(92)90007-A

Guidelines, American, T. N., & Guidelines. (1996). Guidelines Heart rate variability. *European Heart Journal*, *17*, 354–381. https://doi.org/10.1161/01.CIR.93.5.1043

Harvard Health Publishing, H. M. S. (2021). *Want to check your heart rate? Here’s how - Harvard Health*. https://www.health.harvard.edu/heart-health/want-to-check-your-heart-rate-heres-how

Heart Information Center: Heart Anatomy | Texas Heart Institute. (n.d.). Retrieved December 14, 2021, from https://www.texasheart.org/heart-health/heart-information-center/topics/heart-anatomy/

High-Intensity Interval Training: For Fitness, for Health or Both? (n.d.). Retrieved September 6, 2022, from https://www.acsm.org/blog-detail/acsm-blog/2019/08/16/high-intensity-interval-training-for-fitness-for-health-or-both

How the Heart Works | NHLBI, NIH. (n.d.). Retrieved December 13, 2021, from https://www.nhlbi.nih.gov/health-topics/how-heart-works

Jeyhani, V., Mahdiani, S., Peltokangas, M., & Vehkaoja, A. (2015). Comparison of HRV parameters derived from photoplethysmography and electrocardiography signals. *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS*, *2015-Novem*, 5952–5955. https://doi.org/10.1109/EMBC.2015.7319747

Julio U.F., Panissa V.L.G., Franchini E. (2012). Prediction of one repetition maximum from maximum number of repetitions with submaximal loads in recreationally strength-trained men. *Science and Sports. 27,* e69-e76. http://dx.doi.org/10.1016/j.scispo.2012.07.003

Kingsley, J. D., Hochgesang, S., Brewer, A., Buxton, E., Martinson, M., & Heidner, G. (2014). Autonomic modulation in resistance-trained individuals after acute resistance exercise. *International Journal of Sports Medicine*, *35*(10), 851–856. https://doi.org/10.1055/s-0034-1371836

Okuno N.M., Pedro R.E., Leicht A.S., de Paula Ramos S., Nakamura F.Y. (2014). Cardiac autonmoic recovery after a single session of resistance exercise with and without vascualr occlusion. *J Strength Cond Res, 28*(4):1143-1150. DOI: 10.1519/JSC.0000000000000245

Lacković, I., de Carvalho, P., Zhang, Y. T., & Magjarević, R. (2014). The International Conference on Health Informatics: ICHI 2013, Vilamoura, Portugal on 7-9 November, 2013. *IFMBE Proceedings*, *42*(January 2016). https://doi.org/10.1007/978-3-319-03005-0

Laskowski, E. R. (2015). Heart rate: What's normal? - Mayo Clinic. In *Mayo Clinic*. https://www.mayoclinic.org/healthy-lifestyle/fitness/expert-answers/heart-rate/faq-20057979

Leicht, A. S., & Young, F. L. S. (2012). Importance of tachogram length and period of recording during noninvasive investigation of the autonomic nervous system. *Annals of Noninvasive Electrocardiology*, *17*(2), 156. https://doi.org/10.1111/j.1542-474X.2012.00498.x

Lu, G., Yang, F., Taylor, J. A., & Stein, J. F. (2009). A comparison of photoplethysmography and ECG recording to analyse heart rate variability in healthy subjects. *Journal of Medical Engineering and Technology*, *33*(8), 634–641. https://doi.org/10.3109/03091900903150998

Malik, M. (1997). Malik Time-Domain Measurement of HRV Time-Domain Measurement of Heart Rate Variability. *In Cardiac Electrophysiology Review* (Vol. 3). https://www.academia.edu/57757550/Time_Domain_Measurement_of_Heart_Rate_Variability

Mason, J. W., Ramseth, D. J., Chanter, D. O., Moon, T. E., Goodman, D. B., & Mendzelevski, B. (2007). Electrocardiographic reference ranges derived from 79,743 ambulatory subjects. *Journal of Electrocardiology*, *40*(3), 228–234. https://doi.org/10.1016/j.jelectrocard.2006.09.003

Mezzacappa, E. S., Kelsey, R. M., Katkin, E. S., & Sloan, R. P. (2001). Vagal rebound and recovery from psychological stress. *Psychosomatic Medicine*, *63*(4), 650–657. https://doi.org/10.1097/00006842-200107000-00018

Munoz, M. L., Van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., De Geus, E. J. C., Gansevoort, R., Lefrandt, J., Nolte, I. M., & Snieder, H. (2015). Validity of (Ultra-)Short recordings for heart rate variability measurements. *PLoS ONE*, *10*(9), 1–16. https://doi.org/10.1371/journal.pone.0138921

Muntner, P., Shimbo, D., Carey, R. M., Charleston, J. B., Gaillard, T., Misra, S., Myers, M. G., Ogedegbe, G., Schwartz, J. E., Townsend, R. R., Urbina, E. M., Viera, A. J., White, W. B., & Wright, J. T. (2019). Measurement of blood pressure in humans: A scientific statement from the american heart association. In *Hypertension* (Vol. 73, Issue 5). https://doi.org/10.1161/HYP.0000000000000087

Myers, M. G., Godwin, M., Dawes, M., Kiss, A., Tobe, S. W., Grant, F. C., & Kaczorowski, J. (2011). Conventional versus automated measurement of blood pressure in primary care patients with systolic hypertension: Randomised parallel design controlled trial. *Bmj*, *342*(7793), 372. https://doi.org/10.1136/bmj.d286

Neal BS, Mcmanus CJ, Bradley WJ, Leaney SF, Murray K, Clark NC. Physical Therapy in Sport The feasibility, safety, and efficacy of lower limb garment-integrated blood flow restriction training in healthy adults. *Phys Ther Sport*. 2023;60:9-16. doi:10.1016/j.ptsp.2023.01.006

Nunes, J. H. C., Locatelli, J. C., Reck, H. B., Porto, F. E., Francisquini Neto, A., & Lopes, W. A. (2021). Cardiac autonomic control following resistance exercise with different set configurations in apparently healthy young men: A crossover study. *Physiology and Behavior*, *230*(November 2020). https://doi.org/10.1016/j.physbeh.2020.113292

Nussinovitch, U., Elishkevitz, K. P., Katz, K., Nussinovitch, M., Segev, S., Volovitz, B., & Nussinovitch, N. (2011). Reliability of ultra-short ECG indices for heart rate variability. *Annals of Noninvasive Electrocardiology*, *16*(2), 117–122. https://doi.org/10.1111/j.1542-474X.2011.00417.x

Page-Gould, E., Mendes, W. B., & Major, B. (2010). Intergroup contact facilitates physiological recovery following stressful intergroup interactions. *Journal of Experimental Social Psychology*, *46*(5), 854–858. https://doi.org/10.1016/j.jesp.2010.04.006

Patients, F. O. R. (2002). Are You at Risk for Osteoporosis? *Clinical Diabetes*, *20*(3), 158–158. https://doi.org/10.2337/diaclin.20.3.158

Patterson, S. D., Hughes, L., Warmington, S., Burr, J., Scott, B. R., Owens, J., Abe, T., Nielsen, J. L., Libardi, C. A., Laurentino, G., Neto, G. R., Brandner, C., Martin-Hernandez, J., & Loenneke, J. (2019). Blood flow restriction exercise position stand: Considerations of methodology, application, and safety. *Frontiers in Physiology*, *10*(MAY), 1–15. https://doi.org/10.3389/fphys.2019.00533

Pereira, T., Correia, C., & Cardoso, J. (2015). Novel methods for pulse wave velocity measurement. *Journal of Medical and Biological Engineering*, *35*(5), 555–565. https://doi.org/10.1007/s40846-015-0086-8

Perret-Guillaume, C., Joly, L., & Benetos, A. (2009). Heart Rate as a Risk Factor for Cardiovascular Disease. *Progress in Cardiovascular Diseases*, *52*(1), 6–10. https://doi.org/10.1016/j.pcad.2009.05.003

Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., Jones, D. W., Kurtz, T., Sheps, S. G., & Roccella, E. J. (2005). Recommendations for blood pressure measurement in humans and experimental animals: Part 1: Blood pressure measurement in humans - A statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Co. *Circulation*, *111*(5), 697–716. https://doi.org/10.1161/01.CIR.0000154900.76284.F6

Pinheiro, N., Couceiro, R., Henriques, J., Muehlsteff, J., Quintal, I., Goncalves, L., & Carvalho, P. (2016). Can PPG be used for HRV analysis? *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS*, *2016-Octob*, 2945–2949. https://doi.org/10.1109/EMBC.2016.7591347

Powers, K. A., & Dhamoon, A. S. (2021). Physiology, Pulmonary Ventilation and Perfusion. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK539907/

Reis J.F., Fatela P., Mendonca G.V., Vaz J.R., Valamatos M.J., Infante J., Mil-Homens P., Alves F.B. (2019). Tissue oxygenation in response to different relative levels of blood-flow restricted exercise. *Frontiers in Physiology. 10:*407. doi: 10.3389/fphys.2019.00407

Rezk, C. C., Marrache, R. C. B., Tinucci, T., Mion, D., & Forjaz, C. L. M. (2006). Post-resistance exercise hypotension, hemodynamics, and heart rate variability: Influence of exercise intensity. *European Journal of Applied Physiology*, *98*(1), 105–112. https://doi.org/10.1007/s00421-006-0257-y

Rossow, L. M., Fahs, C. A., Sherk, V. D., Seo, D. Il, Bemben, D. A., & Bemben, M. G. (2011). The effect of acute blood-flow-restricted resistance exercise on postexercise blood pressure. *Clinical Physiology and Functional Imaging*, *31*(6), 429–434. https://doi.org/10.1111/j.1475-097X.2011.01038.x

Sacha, J. (2014). Interaction between Heart Rate and Heart Rate Variability. *Ann Noninvasive Electrocardiol*, *19*(3), 207–216. https://doi.org/10.1111/anec.12148

Salahuddin, L., Cho, J., Jeong, M. G., & Kim, D. (2007). Ultra short term analysis of heart rate variability for monitoring mental stress in mobile settings. *Annual International Conference of the IEEE Engineering in Medicine and Biology - Proceedings*, 4656–4659. https://doi.org/10.1109/IEMBS.2007.4353378

Santos, I. F., Lemos, L. K., Biral, T. M., de Cavina, A. P. S., Pizzo Junior, E., Teixeira Filho, C. A. T., Vendrame, J. W., & Vanderlei, F. M. (2022). Relationship between heart rate variability and performance in eccentric training with blood flow restriction. *Clinical Physiology and Functional Imaging*, *42*(5), 333–347. https://doi.org/10.1111/cpf.12774

Schneider, C., Wiewelhove, T., Raeder, C., Flatt, A. A., Hoos, O., Hottenrott, L., Schumbera, O., Kellmann, M., Meyer, T., Pfeiffer, M., & Ferrauti, A. (2019). Heart rate variability monitoring during strength and high-intensity interval training overload microcycles. *Frontiers in Physiology*, *10*(MAY). https://doi.org/10.3389/fphys.2019.00582

Seppälä, S., Laitinen, T., Tarvainen, M. P., Tompuri, T., Veijalainen, A., Savonen, K., & Lakka, T. (2014). Normal values for heart rate variability parameters in children 6-8 years of age: The PANIC Study. *Clinical Physiology and Functional Imaging*, *34*(4), 290–296. https://doi.org/10.1111/cpf.12096

Shaffer, F., & Ginsberg, J. P. (2017). An Overview of Heart Rate Variability Metrics and Norms. *Frontiers in Public Health*, *5*(September), 1–17. https://doi.org/10.3389/fpubh.2017.00258

Shaffer, F., Shearman, S., & Meehan, Z. M. (2016). The Promise of Ultra-Short-Term (UST) Heart Rate Variability Measurements. *Biofeedback*, *44*(4), 229–233. https://doi.org/10.5298/1081-5937-44.3.09

Stavrakis, S., Kulkarni, K., Singh, J. P., Katritsis, D. G., Armoundas, A. A., City, O., Hospital, M. G., Service, C. A., & Hospital, M. G. (2021). *HHS Public Access*. *6*(5), 467–483. https://doi.org/10.1016/j.jacep.2020.02.014.Autonomic

Stein, P. K., & Reddy, A. (2005). Non-linear heart rate variability and risk stratification in cardiovascular disease. *Indian Pacing and Electrophysiology Journal*, *5*(3), 210–220. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1431594/

Shriver C.T., Figueroa Y.L., Gifford J., Davis P.R. (2023). Effects of different percentages of blood flow restriction on muscle oxygen saturation while walking. *Int J Exerc Sci. 16*(2): 411-428. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10128124/

Tai, Y. L., Marshall, E. M., Glasgow, A., Parks, J. C., Sensibello, L., & Kingsley, J. D. (2019). Autonomic modulation following an acute bout of bench press with and without blood flow restriction. *European Journal of Applied Physiology*, *119*(10), 2177–2183. https://doi.org/10.1007/s00421-019-04201-x

Tokutaka, H., Maniwa, Y., Gonda, E., Yamamoto, M., Kakihara, T., Kurata, M., Fujimura, K., Shigang, L., & Ohkita, M. (2009). Construction of a general physical condition judgment system using acceleration plethysmogram pulse-wave analysis. *Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*, *5629 LNCS*, 307–315. https://doi.org/10.1007/978-3-642-02397-2_35

Toufan, M., Kazemi, B., Akbarzadeh, F., Ataei, A., & Khalili, M. (2012). Assessment of electrocardiography, echocardiography, and heart rate variability in dynamic and static type athletes. *International Journal of General Medicine*, *5*, 655–660. https://doi.org/10.2147/IJGM.S33247

Tsuji, H., Venditti, F. J., Manders, E. S., Evans, J. C., Larson, M. G., Feldman, C. L., & Levy, D. (1996). Determinants of heart rate variability. *Journal of the American College of Cardiology*, *28*(6), 1539–1546. https://doi.org/10.1016/S0735-1097(96)00342-7

Umetani, K., Singer, D. H., McCraty, R., & Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: Relations to age and gender over nine decades. *Journal of the American College of Cardiology*, *31*(3), 593–601. https://doi.org/10.1016/S0735-1097(97)00554-8

Warburton DER, Jamnik VK, Bredin SSD, and Gledhill of the PAR-Q+ Collaboration. The Physcial Activity Readiness QUestionaire for Everyone (PAR-Q+) and Electronic Activity Readiness Medical Examination (ePARmed-X+). *Health and Fitness Journal of Canada.* 2011; 4(2):3-23. DOI: https://doi.org/10.14288/hfjc.v4i2.103

Weippert, M., Behrens, K., Rieger, A., Stoll, R., & Kreuzfeld, S. (2013). Heart Rate Variability and Blood Pressure during Dynamic and Static Exercise at Similar Heart Rate Levels. *PLoS ONE, 8*(12), e83690. https://doi.org/10.1371/journal.pone.0083690

Weissman, D. G., Mendes W. B. (2022). *Activity During Rest and Acute Stress Tasks*. *617*, 60–68. https://doi.org/10.1016/j.ijpsycho.2021.01.015.Correlation

Xu, J., Murphy, S. L., Kockanek, K. D., & Arias, E. (2020). Mortality in the United States, 2018. *NCHS Data Brief, 355*, 1–8. https://www.cdc.gov/nchs/products/index.htm.

Zourdos MC, Klemp A, Dolan C, Quiles JM, Schau KA, Jo E, Helms E, Esgro B, Duncan S, Merino SG, Blanco R. (2015). Novel resistance training-specific rating of percieved exertion scale measuring repetitions in reserve. *Journal of Strength and Conditioning Research. 30*(1), 267-275. DOI: 10.1519/JSC.0000000000001049.

APPENDIX A. IRB APPROVAL

NDSU NORTH DAKOTA STATE UNIVERSITY

05/24/2023

Dr. Kyle Johnson-Vincent Hackney
Health, Nutrition & Exercise

IRB Approval of Protocol #IRB0004722 , "The Effect of an Acute Session of Blood Flow Restriction Exercise on Autonomic Modulation"

Co-investigator(s) and research team:

- Kyle Johnson-Vincent Hackney
- Andrew Garner

Approval Date: 05/24/2023
Expiration Date: 05/11/2024
Research site(s): Research will be conducted on the North Dakota State University campus at the Benston Bunker Fieldhouse in the Human Performance Lab (Room 14 and 15).
Funding Agency:
Review Type: Full Board, meeting date – 05/12/2023 12:00 PM - Memorial Union, Peace Garden Room

Risk Level: More than minimal

The protocol application and all included documentation for the above-referenced project have been reviewed and approved via the procedures of the North Dakota State University Institutional Review Board.

Additional approval is required:

- Prior to implementation of any proposed changes to the protocol.
- For continuation of the project beyond the approval period.

A report is required for:

- Any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence.
- Any planned or unplanned deviations from the approved protocol.
- Any significant new findings that may affect risks to participants.
- Closure of the project.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

NDSU has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

RESEARCH INTEGRITY AND COMPLIANCE
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | ndsu.research@ndsu.edu
Shipping Address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102

APPENDIX B. INFORMED CONSENT

NDSU NORTH DAKOTA STATE UNIVERSITY

Health, Nutrition, and Exercise Science
1301 Centennial Blvd
Fargo, ND 58108-6050
701-231-6706

The Effect of an Acute Session of Blood Flow Restriction on Autonomic Modulation

This study is being conducted by:
Andrew Garner, Phone: [redacted] Email: andrew.garner@ndsu.edu
Kyle Hackney PhD, CSCS, Phone: 701-231-6706, Email: kyle.hackney@ndsu.edu

Key information about this study

This consent form is designed to inform you about the study you are being asked to participate in. Here you will find a brief summary about the study; however, you can find more detailed information later on in the form.

- We are inviting men who are healthy and physically active between the ages of 18-35 years old, with no recent injuries to participate.
- You will learn more about your autonomic nervous system and how your body responds to the stimulus of exercise be placed upon it.
- This study involves resistance exercise along with blood flow restriction. Blood flow restriction is a term for exercising with very light weights with an inflation cuff around the exercising limb.
- Blood flow restriction is common in training of athletes, rehabilitation from injury, and in treatment of disease.
- You will need to visit our lab 3 times, which will take approximately 60-90 minutes per session.
- Any information we gather from you will be kept private by the researchers.

Why am I being asked to take part in this study?
You are being asked to participate in this study, because we are seeking 15 physically active healthy males, between the ages of 18-35, to complete a knee extension exercise during 3 sessions. Our participants should have at least 6 months of experience with traditional resistance exercise training and have passed the pre-screening questionnaire for the study.

What will I be asked to do?
You will be asked to attend three separate exercise sessions. The first session will consist of the research team presenting information to you regarding the study and informed consent. Basic descriptive such as height, body mass, leg circumference will also be collected. Finally, a one-

repetition max (1RM) test during the knee extension exercise will be completed. In this test, we will determine the maximum amount of weight you can lift with each leg, while kicking outward from a seated position. This will be used to determine the weight lifted for sessions two and three. Session two (experiment) will consist of blood flood restriction (BFR) during the knee extension exercise. While session three (control) will consist of knee extension exercise without BFR. More details on the sessions are below.

- **Information/1RM Session:** You will first attend the information/1RM session where the study details and risks are explained. If you decide to participate you will also sign this form (informed consent form). Then, we will measure your height and weight to calculate your body mass index (BMI). We will also measure the size of both of your upper leg (thighs) with a tape measure to determine which cuff size to use in the experimental session. Next, the 1RM will be tested on both legs during the knee extension exercise. The legs are randomized to determine which one is tested first. Your highest weight lifted on each of your legs will be recorded and used to calculate 20% of your 1RM weight. If a full range of motion (FOM) is not reached, the repetition will not count. If there is any unwanted orthopedic pain, the test is stopped. After this test, we will also familiarize you with the BFR exercise equipment and inflate the cuff around your leg for no more than one minute will you perform a up to 8 repetitions will the weight that will be used in sessions two and three. This will allow you to get a better feel for BFR exercise.

- **Session Two (experiment):** During the second session, you will be asked to lie down on your back in a resting position while the investigators place two BFR cuffs on your thighs, near your hips. You will be given a heart rate monitor to place around your sternum to gather heart rate data. Next, a small oxygen sensor will be placed on one of your thighs below the BFR cuffs. You will then rest for a total of 15 minutes before you start the knee extension exercise. During these 15 minutes heart rate data will be recorded during the last 5 minutes. It is crucial you breathe normally and do not talk or make sudden movements during the data collection period. Next, you will perform 5 sets of 15 repetitions on the knee extension exercise machine on both legs. Exercise will be complete on one leg at a time. Once again, the legs will be randomized. The BFR cuffs will be inflated before starting each set and will be deflated between each set during the rest periods. When the cuffs are inflated it will feel like a blood pressure cuff squeezing your arm except on your thigh. Muscle oxygen will be recorded from start to finish when you start the knee extension exercise. After testing you will rest lying down on your back for 15 minutes and 30 minutes. During the last 5 minutes of each rest interval, a heart rate will be recorded.

- **Session Three (control):** During the third session, you will be asked to follow the exact protocol as used during session two except without the BFR cuffs on each leg. Heart rate and muscle oxygen will be recorded at the same times used in session two.

Where is the study going to take place, and how long will it take?
This study will take place in the North Dakota State University Human Performance Lab, rooms 15 and 14. The first session will take approximately 60 minutes to complete, while sessions two and three will take 90 minutes to complete. All sessions will be separated by at least 72 hours.

What are the risks and discomforts?

It is not possible to identify all potential risks in research; however, reasonable safeguards have been taken to minimize known risks. Some of the most common risks and discomforts include: mild muscle soreness/cramping following exercise, lightheadedness, increased respiration, and an increase in heart rate and blood pressure during the exercise session. However, these generally subside immediately after the exercise session is completed. If you report any orthopedic discomfort/pain the test will end immediately. If new findings develop during the course of the research, which may change your willingness to participate, we will tell you about these findings. Due to a small risk of blood clotting during exercise and the use of BFR, you will leave the final session with a handout that explains possible symptoms, and researchers will contact you within 24 hours of your last exercise session to monitor for any potential negative signs and symptoms that may have developed after the testing sessions.

What are the expected benefits of this research?

Individual Benefits: Possible acute benefits could be an improvement in autonomic modulation along with an increase in muscle strength and size.

Societal Benefits: Possible benefits to others will include a new exercise modality to improve autonomic modulation during an acute session of BFR training in both clinical practices (e.g., athletic trainers and physical therapists) and experienced gym enthusiasts. BFR could be used as another exercise modality not only to increase muscle strength and size but to improve autonomic modulation as well.

Do I have to take part in this study?
Your participation in this research is your choice. If you decide to participate in the study, you may change your mind and stop participating at any time without penalty or loss of benefits to which you are already entitled.

What are the alternatives to being in this study?
Instead of being in this research, you may choose not to participate.

Who will have access to my information?

Identifiable information collected during the course of the research will only be accessed access by the research team. Research data collected will only be used in this study and will not be distributed to other investigators in the future. We will keep all research records with your information private. Your information will be combined with other people partaking in the study and stored in a password-protected data file. During the first session, you will be assigned a participant number and that number will be associated with all your information throughout the study. When we write about the study, we will only write about all the combined data from all participants that we have gathered. We may publish the results in a research journal; however, we will keep your name and other identifying information private.

We will make every effort to prevent anyone who is not on our research team from knowing that you gave us private information, or what that information may be. For example, your name and our research records will be stored separately in different places under lock and key. If you choose to withdraw from the study at any time, your information will be retained in the research records OR removed upon your request, and we will not collect any more information from you.

Can my participation in the study end early?
You may stop participating in the study whenever you wish.

Will I receive any compensation for participating in the study?
You will receive $5.00 for completing session 1, $10.00 for completing session 2, and $10.00 for completing session 3; for a total of $25 dollars.

What happens if I am injured because of the study? [include if applicable]
If you are injured during the course of this study, you should contact Kyle Hackney at 701-231-6706 or Andrew Garner at [redacted]. Treatment for the injury will be available including first aid, emergency treatment, and follow-up care as needed. Payment for this treatment must be provided by you and your third-party payer (such as health insurance). This does not mean that you are releasing or waiving any legal right you might have against the researcher or NDSU as a result of your participation in this research.

What if I have questions? Before you decide whether you would like to participate in this study, please ask any questions that come to mind now. Later, if you have questions about the study, you can contact Dr. Kyle Hackney at 701-231-6706 or kyle.hackney@ndsu.edu or Andrew Garner at [redacted] or andrew.garner@ndsu.edu.

What are my rights as a research participant?
You have rights as a research participant. All research with human participants is reviewed by a committee called the *Institutional Review Board (IRB)* which works to protect your rights and welfare. If you have questions about your rights, an unresolved question, a concern or complaint about this research you may contact the IRB office at 701.231.8995, toll-free at 855-800-6717 or via email (ndsu.irb@ndsu.edu).

Documentation of Informed Consent:
You are freely deciding whether to be in this research study. Signing this form means that

1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

You will be given a copy of this consent form to keep.

Your signature	Date
Your printed name	Date
Signature of researcher explaining study	Date
Printed name of researcher explaining the study	