EFFECTS DIFFERENT PERCENTAGES OF BLOOD FLOW RESTRICTION WHILE WALKING ON MUSCLE OXYGEN SATURATION

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DEDICATION

This work is dedicated to my wife, Lillianne. She showed tremendous love and support while I committed time towards this project. Thank you for pushing me to advance in my academic and professional endeavors.

ABSTRACT

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PURPOSE: The purpose of this investigation was to determine the effect different relative pressures of blood flow restriction (BFR) had on muscle oxygen saturation (SmO₂) while walking at 3.0 mph. METHODS: Fifteen physically active healthy adults performed seven 5-minute stages of walking at 3.0 mph with a blood flow restriction cuff applied to the proximal portion of the left or right leg while bilateral SmO_2 changes were measured using near infra-red spectroscopy (NIRS) bilaterally on the medial head of the gastrocnemius (GM) and vastus lateralis (VL) muscles. Other measurements including heart rate (HR), blood pressure (BP), rating of perceived exertion (RPE), and ground contact time balance (GCTB) were also collected. SmO₂ measurements were analyzed using two-way repeated measures ANOVA while other measurements were analyzed using one-way repeated measures ANOVA. **RESULTS**: We observed a significant main effect of LOP (limb occlusion pressure)% on the difference in total area of desaturation that occurred during each occlusion stage (ADS), F (1.432, 40.08) = 32.74, p < 0.0001, initial Δ SmO₂, F (1.8, 52) = 28, p < 0.001, and final Δ SmO₂, F (1.359, 38.04) = 9.631, p = 0.0016. Tukey's post hoc analysis of differences in ADS revealed significant differences for all comparisons except at 40% vs 80% LOP (p = 0.0821) for the GM. Post hoc analysis of initial Δ SmO₂ revealed a significant difference for all comparisons except at 40% vs 80% (p = 0.555) for the VL. Multiple comparisons for final Δ SmO₂ only showed a significant difference at 40% vs. 100% (*p* = (0.0029) and 80% vs 100% (p = 0.0079) for the VL. CONCLUSION: The results did not support our hypothesis that there would be no significant difference in SmO₂ between 40%, 80%, and 100% LOP. Multiple comparison results differed between ADS, initial Δ SmO₂, and final Δ SmO₂. ADS data was used to reflect the magnitude of SmO₂ desaturation on the VL and GM during each occlusion stage. The magnitude of SmO₂ desaturation was statistically significant between LOP% except for between 40% and 80% LOP of the GM.

KEY WORDS: Blood flow restriction, Oxygen saturation

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CHAPTER I

Introduction

Statement of the Question

Blood flow restriction (BFR) is a clinical practice of applying pressurized tourniquet cuffs to the proximal portion of the upper or lower limbs during various exercise modes. Pneumatic cuffs are pressurized to partially restrict arterial inflow and fully restrict venous outflow in working musculature (Patterson et al., 2019). The utilization of BFR combined with both resistance (Loenneke et al., 2012; Slysz, Stultz, and Burr, 2016; Lixandrao et al., 2018) and aerobic (Silva et al., 2019; Bennett and Slattery, 2018) training can help stimulate favorable adaptations in most populations, especially when traditional exercise recommendations are not practical or contraindicated (Hughes, Paton, Rosenblatt, Gissane, and Patterson, 2017).

Inducing acute skeletal muscle ischemia from BFR has been suggested to be an important driver for metabolic stress in a targeted limb (Pearson and Hussain, 2015; Biazon et al., 2019; Takarada et al., 2000; Kon, et al., 2012). Studies have utilized near-infrared spectroscopy (NIRS) to provide data on the concentration or oxygenation of light-absorbing molecules such as hemoglobin (Hb) and myoglobin (Mb) in skeletal muscle while performing BFR exercise in order to provide indirect insight on localized tissue perfusion (Biazon et al., 2019; Cayot, Lauver, Silette, and Scheuermann, 2016; Corvino et al., 2017; Lauver, Cayot, Rotarius, and Scheuermann, 2017; Mahoney, Dicks, Lyman, Christensen, and Hackney, 2019). Relative pressures between 60-80% of limb occlusion pressure (LOP) have been used to maintain an anaerobic environment as shown in prior research (Ferguson et al., 2018; Hunt, Stodart, and Ferguson, 2016; Ilett,

Rantalainen, Keske, May, and Warmington, 2019). Findings from Kilgas et al., (2018) and Reis et al., (2019) found that cuff pressures between 60%-80% LOP while performing low-load BFR exercise to be effective at producing a comparable decline in tissue oxygenation. However, the relationship between cuff pressure and skeletal muscle deoxygenation from BFR walking at recommended occlusion pressures for aerobic exercise (Patterson et al., 2019) has not been investigated. The purpose of the study was to compare the effects different percentages of individualized BFR have on muscle oxygen saturation while walking.

Rationale

The use of BFR has been a supplemental training tool for clinicians and performance professionals to provide favorable muscle stress on individuals in a rehabilitative setting. The development of an optimal training program with BFR should provide an adequate stimulus while also minimizing both risk and perceived discomfort. Despite a large body of research on BFR, insight on associated mechanisms and their relative contributions in mediating both acute and potentially chronic physiological effects is still needed. One proposed mechanism of BFR includes acute ischemia leading to metabolic stress in skeletal muscles as a result of the external compression of vasculature (Suga et al., 2009; Yanagisawa and Sanomura, 2017; Lauver et al., 2017). For example, by restricting venous outflow Yanagisawa and Sanomura, (2017) observed significant increases in the inorganic phosphate (Pi)-to-phosphocreatine (PCr) ratio during low-load BFR plantar flexion exercise compared to a control group performing the exercise condition without BFR. Thomas, Scott, and Peiffer, (2018) observed increased lactate and carbon dioxide production (VCO₂) in a group performing low-intensity interval cycling at 80% LOP compared to a control group. An investigation by Karabulut and Garcia, (2015) found oxygen uptake (VO₂) to be greater in group cycling with 60% LOP compared to 40% LOP. Ischemia to skeletal muscle and surrounding tissues may be one of several mechanisms mediating the positive vascular and muscle adaptations observed from prior BFR research. Measurements of muscle oxygen desaturation invoked by BFR exercise have been used as an indicator of intramuscular metabolic stress according to Larkin et al., (2012) and Ganesan et al., (2015). NIRS is a noninvasive method to measure relative muscle oxygenation in the capillaries and tissue of targeted skeletal muscle (Barstrow, 2019). Additionally, the use of NIRS to measure muscle deoxygenation has great agreement with measurement of intracellular energetics by P-31 MRS, and provides useful information regarding the energy demands of the muscle (Ryan, Southern, Reynolds, and McCully, 2013).

Cuff pressure used for BFR is a main determinant that is commonly determined by assessing an individual's limb occlusion pressure (LOP) at rest in order to limit risk and increase objectivity (Laurentino et al., 2008). LOP is a measurement of the minimum pressure required for 100% occlusion of arterial blood flow in a limb at rest (Weatherholt, Vanwye, Lohmann, and Owens, 2019) and can be measured through several techniques. A review by Patterson et al., (2019) provided an exercise prescription model for continuous or interval aerobic exercise at 40-80% LOP. Silva et al., (2019) reported AE with BFR at moderate intensities to cause increases in heart rate (HR), blood pressure (BP), and ratings of perceived exertion (RPE) compared to without BFR. Higher cuff pressures have also shown to cause a greater perception of discomfort compared to that of lower pressures (Loenneke et al., 2012). It may be optimal to select external cuff pressures for BFR walking that maximize muscle desaturation in a desired limb without inducing unpleasant cardiovascular or perceptual responses.

Hypotheses

Null Hypothesis: There will be no significant differences between 40%, 80%, and 100% LOP on skeletal muscle oxygen saturation while walking on a treadmill at 3.0 mph.

Alternative Hypothesis: There will be significant differences between 40%, 80%, and 100% LOP on skeletal muscle oxygen saturation while walking on a treadmill at 3.0 mph.

Limitations and Delimitations

Limitations for this study include population demographics and data collection methods. The population demographics were limited to physically active healthy adults to ensure participants were prepared to meet the physical demands of exercise testing and to also minimize risk. This limited how results can be extrapolated to other populations. While NIRS is both non-invasive and cost effective for the purpose of investigating local muscle oxygenation, adipose tissue thickness at the sites of measurement may affect measurements according to a review by Jones, Chiesa, Chaturvedi, and Hughes, (2016).

Delimitations include population of interest, exercise intensity, and exercise mode. The purpose of delimiting the population to physically active healthy adults was to widen the sample size and to avoid recruiting participants that would be contraindicated for performing BFR. Inclusion criteria were delimited to: being physically active \geq 3 days/week of at least 30 minutes of moderate-intensity physical activity, being between the ages of 18-64 years old, and having a body fat % < 35%. Maximal exercise testing on participants was not used to determine relative exercise intensity. The purpose of not conducting maximal exercise testing on participants was to reduce testing to a single visit, not expose participants to the risks of maximal exercise testing, and minimize risk of possible COVID-19 spread or exposure. Intensities of aerobic exercise with BFR are generally low in nature (Patterson et al., 2019) but are not standardized. A walking speed of 3.0 mph is realistic to a physically active healthy adult and is within the light-moderate intensity for walking. The purpose of delimiting the exercise mode to walking will be due to it being a common mode of aerobic exercise with BFR and because the relationship between recommended occlusion pressures and muscle oxygenation while walking with BFR has not been investigated.

Abbreviations and Definitions

AE – Aerobic Exercise

BFR – Blood Flow Restriction

BP-Blood Pressure

GCTB – Ground Contact Time Balance

Hb – Hemoglobin

HIF – Hypoxia Induced Factor

HR - Heart Rate

LOP – Limb Occlusion Pressure - the minimum pressure required for 100%

occlusion of arterial flow in a limb

Mb - Myoglobin

NIRS - Near-Infrared Spectroscopy

PTP – Personalized Tourniquet Pressure

RPE – Rated Perceived Exertion

 SmO_2 – Muscle Oxygen Saturation – percentage of hemoglobin and myoglobin that is carrying oxygen in the capillaries and tissue of the muscle

tHb – Total Heme – Non-absolute measure of the density of hemoglobin and myoglobin in tissue

VEGF - Vascular Endothelial Growth Factor

Walking – 3.0 mph

CHAPTER II

Review of the Literature

Hypoxia and Ischemia Mechanism of BFR

According to a review by Lundby, Calbet, and Robach, (2009), the term hypoxia refers to an inadequate oxygen supply that may threaten tissue oxygen homeostasis. Hypoxia may occur in situations where an individual is exposed to a high-altitude environment, during physical exercise, ischemia, and diseases (Chaillou, 2018). A review by Sinex and Chapman, (2015) found that exercise in high altitude conditions had positive muscular and systemic adaptations that were either absent or to a smaller degree from training in normoxic conditions. Pathological conditions can have a range of physiological effects due to hypoxia which may be hazardous to organ structure and function (Michiels, 2004). When ATP production through oxidative phosphorylation cannot meet the ATP demands of certain exercise; anaerobic glycolysis is used to meet those ATP demands. According to Michiels, (2004), several systemic, local, and molecular adaptive responses to decreased oxygen concentration may be activated and depend on the duration while under those conditions. Systemic responses of increased ventilation and cardiac output occur in seconds to minutes. Within several minutes, muscle cells will shift to anaerobic metabolism and activate AMPK. Over longer durations, hypoxia-induced factor (HIF)-mediated gene expression promotes improved vascularization and enhanced oxygen carrying capacity of the blood. Ischemia may lead to hypoxia due to low oxygen availability.

Ischemia according to a review by Kalogeris, Baines, Krenz, Korthuis (2014) is the restriction of blood leading to reduced arterial blood flow. The magnitude and

duration of an obstruction may lead to cell dysfunction, injury, and/or death. A reduction in arterial blood flow leads to a decrease in the supply or availability of oxygen and nutrient to tissues. Compromised venous drainage from tissues can decrease metabolite clearance and venous return. BFR exercise predominantly utilizes partially occlusive pressures in order to restrict arterial inflow and minimize venous outflow in a limb (Patterson et al., 2019). A reduction in blood supply to muscle will likely cause oxygen desaturation in restricted tissues. The extent to which BFR impacts skeletal muscle microvascular oxygenation may depend on variables such as cuff pressure, exercise mode, and application duration (Karabulut, Leal, Garcia, Cavazos, and Bemben, 2014; Neto et al, 2016). Localized ischemia from BFR may promote metabolic and endocrine response, increase cellular swelling, and other signaling pathways following resistance exercise (Jessee, et al., 2018). Pearson and Hussain, (2015) found acute muscle ischemia from BFR to be a stimulus for metabolite accumulation in-part from decreased anaerobic metabolism, increased anaerobic glycolysis, and decreased metabolite clearance. Ferguson et al., (2018) and Gaven, Drew, Kubik, Pofahl, and Hickner, (2007) found vascular endothelial growth factor (VEGF) to be elevated in low-load BFR exercise compared to the control group without BFR. Hunt, Galea, Tufft, Bunce, and Furguson, (2013) saw a 14% increase in capillary density over six weeks (3 days/week) of unilateral plantar flexion training. Adaptations such as increased capillary density from the upregulation of VEGF appear to be impacted by ischemic conditions during BFR, although further investigation is still needed.

Near-Infrared Spectroscopy and BFR

The use of continuous-wave near-infrared spectroscopy (NIRS) has been shown to be a reliable tool for assessing local tissue or skeletal muscle blood flow in physically active adults (Lucero et al., 2018). The Moxy Monitor (Hutchinson, MN) uses NIRS technology to measure muscle oxygen saturation (SmO₂), total heme (tHb), oxygenated hemoglobin (OxyHb) and deoxygenated hemoglobin (DeoxyHb). A study by Crum, Connor, Loo, Valckx, and Stannard, (2017) found Moxy to produce reliable measurements during low-moderate intensity exercise. A large number of investigations have used NIRS to quantify oxygenation while performing resistance training (Biazon, et al., 2019; Downs et al., 2014; Ganesan et al., 2015; Ilett et al., 2019; Kacin and Strazar 2011; Karabulut et al., 2014; Kilgas, et al., 2018; Killinger, Lauver, Donovan, and Goetschius, 2019; Larkin, et al., 2012; Reis, et al., 2019; Yanagisawa and Sanomura, 2017), eccentric (Lauver et al., 2017), isometric (Cayot et al., 2014; Lucero et al., 2018), and aerobic exercises (Christiansen, Murphy, Bangsbo, Stathis, and Bishop, 2017; Keramidas, Kounalakis, and Geladas, 2011; Mahoney et al., 2019; Willis, Alvarez, Millet, and Borrani, 2017) with BFR.

According to Cayot et al., (2016) and Lauver et al., (2017), DeoxyHb from using NIRS may be an indicator of metabolic stress while performing resistance training with BFR. Kilgas et al., (2018) and Reis et al., (2019) looked at the influence different percentages of limb occlusion pressure (LOP) had on muscle microvascular oxygenation between handgrip and knee extension exercise protocols. Reis and colleagues, (2019) found that 60% LOP was a threshold to induce higher DeoxyHb and decreased tissue oxygenation levels. Another finding was that 80% LOP had no further effect on changing DeoxyHb compared to 60%. Relative pressures between 60-80% to maintain an anaerobic environment has been supported in prior research (Ferguson et al., 2018; Hunt, Stodart, and Ferguson, 2016; Ilett et al., 2019). These findings have not been extensively investigated while performing aerobic exercise with BFR.

Training Studies of Aerobic Exercise with BFR

A review by Silva et al., (2019) found AE with BFR to have an effect on neuromuscular, metabolic, and cardiovascular adaptations in a variety of populations. Bennett and Slattery, (2018) found light intensity AE with BFR to be effective at promoting improvements in aerobic fitness and aerobic performance in some populations, especially for populations in which high-intensity training is unfitting. Aerobic training and high-intensity endurance training helps make improvements in maximum oxygen uptake (VO_{2max}) as well as anaerobic threshold (Wenger and Bell, 1986) compared to resistance training. Further adaptations through aerobic training include increased mitochondria volume density, capillary density, glycogen content, and enzymes involved in oxidative metabolism (Park et al., 2010). A large number of investigations have looked at the impact BFR with aerobic training has compared with traditional aerobic training. While a variety of aerobic training modes have been used during investigations of aerobic training with BFR, walking and cycling have been the primary modes (Patterson et al., 2019). A two-week walking program study conducted by Park et al., (2010) demonstrated that the group that utilized BFR had significantly increased VO_{2max} (11.6%, P = 0.005) and V_{Emax} (10.6%, P = 0.003) with a walking intensity of 40% VO_{2max}. An 8-week study conducted by Conceicao and co-workers, (2019) compared groups performing traditional resistance training, endurance training, and a group doing

low-intensity cycling with BFR (power output that elicited 40% of VO₂ reserve). There was a significant increase (11%, P = 0.012) in VO_{2max} in the endurance group and cycling with BFR group while no changes (2.9%, P = 0.541) were observed in the resistance training group. Improvements in functional capacity may be due in part to central and peripheral adaptations to endurance training. BFR with AE led to improvements in aerobic capacity in several different populations. The magnitude of improvement in cardiorespiratory fitness will likely depend on initial levels of cardiorespiratory fitness and may have limited applicability to healthy and athletic individuals. Lower training intensities and training time may be of greater benefit to those who struggle to achieve the recommendations for time and intensity of cardiorespiratory exercise that are favorable to achieve favorable adaptations.

Aerobic training with BFR has shown neuromuscular adaptations such as strength and hypertrophy that are not commonly seen in traditional aerobic training programs (Silva et al., 2018). Abe et al., (2010) demonstrated that an 8-week cycling program using BFR can lead to improvements in isometric strength of the knee extensors and flexors as well as leg muscle hypertrophy. The same group that utilized BFR also had an increase in thigh cross sectional area by 5.1%. Another 8-week cycling training protocol with BFR by Conceicao et al., (2019) showed significant increase in dynamic strength demonstrated through a single repetition max leg press. Park et al., (2010) conducted a two-week walking program with BFR on college male athletes and showed no main effect on either absolute or relative peak knee extension but showed significant bilateral knee flexion in the group using BFR. These adaptations to increased hypertrophy are relatively unclear, but the mechanisms may be related to increased levels of GH (growth hormone), IGF-1 (insulin-like growth factor 1), and other myogenic regulatory factors (Loenneke, Wilson, Wilson, Pujol, and Bemben, 2012). A single bout of walking with bilateral BFR at a low training volume has shown to significantly increase serum GH concentration (Abe et al., 2006). It may be ideal for certain individuals to participate in a single mode of training (walking, cycling, etc.) that can provide benefits in strength and hypertrophy. Older adults may be fearful of performing traditional resistance exercise, even though diminished muscle mass, strength, and ability to perform activities of daily living is common with an aging population.

Methodology

Training studies involving aerobic training with BFR have either been developed to compare BFR training to conventional-unrestricted groups, or BFR training groups compared to a control group doing the same protocol without BFR. These studies have primarily included a healthy adult and athletic population, although Ozaki et al., (2011) performed a study involving sedentary women, aged 57-73 years. Renzi, Tanaka, and Sugawara, (2010) conducted a study trying to determine the impact of leg BFR during walking on cardiovascular function in young (26 ± 1 years) healthy participants. It should be important to note that the magnitude of training adaptations depend on the training stimulus, training experience of the participants, and initial fitness levels.

The training protocols in these research studies vary in different ways, including the length of the study, training and duration, what measurements were being made, and how measurements were collected. The cuff occlusion pressures on participants also tended to differ among studies. These studies ranged from two weeks (Park et al., 2010) to 10 weeks (Abe et al., 2006) long. The studies that were 2-3 weeks long had training sessions two times a day, 6 days/week while several studies lasting 8-10 weeks included one training session a day for either 3 or 4 days/week. The length of training sessions of participants in these studies using BFR were relatively short, typically less than or equal to 30 min total exercise time per training session. Shorter training durations may be more achievable for sedentary and/or less conditioned populations, whereas longer training durations may be more conductive for those who are more conditioned. As noted in the 2-week walking study conducted by Park et al., (2010), cardiorespiratory endurance functions were improved in college athletes, while no statistically significant improvement was made in anaerobic power or muscular strength. Abe et al., (2006) conducted a similar walking study over three weeks with similar training frequency and showed statistical significant increase (10.4%, P < 0.05) in muscular strength demonstrated through maximum isometric knee extension.

There where various methods used to assess neuromuscular adaptations from training studies of aerobic exercise with BFR. MRI measurements were used in multiple studies in order to measure muscle cross-sectional area and volume of the thigh and lower leg. BFR cuffs were focused on the upper most portion of the proximal thigh during these studies. Sakamaki, Bemben, and Abe, (2011) found that changes in muscle size were specific to the occluded limb and not the non-restricted trunk muscles. Muscle hypertrophy was observed only in leg muscles distal to the cuff, whereas non-blood flow restricted muscles such as the gluteus maximus and other trunk muscles did not (Sakamaki, Bemden, and Abe, 2011). Overall BFR with walk training seems to be an innovative method for improving muscle volume in older women (Ozaki et al., 2011), young healthy men (Abe et al., 2006), and trained individuals (Park et al., 2010).

Maximum isometric strength was tested on a dynamometer in several studies, while maximum dynamic strength was assessed by a 1-RM on a leg press machine by Conceicao et al., (2019).

According to Patterson et al., (2019), there has been a lack of standardization of cuff pressure during BFR with aerobic exercise and should be a focus for future studies. Future research should investigate the effects different relative occlusion pressures (LOP) while performing aerobic training has on adaptations. There is inconsistency with how occlusion pressures were determined and if these pressures should be adjusted through the course of the study. A review by Loenneke et al., (2011) suggested that restrictive pressures should be based on limb circumference of the user and cuff width. External cuff or tourniquet pressure should be high enough to allow some arterial inflow while restricting venous return in working musculature during exercise (Patterson et al., 2019). According to a review by Scott, Loenneke, Slattery, and Dascombe, (2014), consideration should be taken to apply cuff pressures that are individualized and specific to cuff width.

Training sessions also varied in their frequency, duration, and intensity. The dose-response relationship between the time and intensity of occlusion with the significance of adaptations were not compared within studies. Due to the nature of BFR, training loads (intensity and duration) are lower than traditional training programs. Exercise intensity for participants was determined or estimated from a graded exercise test. While doing a maximal graded exercise test may be the gold standard of assessing cardiorespiratory fitness, determining training intensities in a real-world training program may require using alternative methods such as HRR (heart rate reserve) or RPE (rating of perceived exertion). The training session duration and exercise intervals were also

variable among studies. The BFR training group in Conceicao et al., (2018) exercised for a continuous 30 minutes, while exercise duration for a study conducted by Park et al., (2010) was of five 3-minute bouts.

Safety

Safety with the use and application of BFR with AE as how it is prescribed should be a major consideration. The risks of BFR include but not limited to venous thromboembolism (VTE), skeletal muscle damage, and abnormal exercise pressure reflex (Vanwye, Weatherholt, and Mikesky, 2017). These have been primarily investigated within studies utilizing BFR with RE (resistance exercise). Risk factors for VTE events include a history of prior VTE, obesity, immobility, physical inactivity, family history, genetic conditions that affect blood clotting, and oral contraceptives. Skeletal muscle damage either through ischemia-perfusion injury or as a result from the load greatly exceeding the fitness capability of an individual may be another risk. Abe et al., (2006) noted that from a metabolic perspective, the intensity of bilateral BFR walking is equivalent to the metabolic cost of 10-20% 1 RM. Investigators also noted that blood markers for muscle damage, such as from CPK and myoglobin were not elevated after bilateral BFR walking. Due to the potential risks of AE with BFR, unconditioned and older adult populations may be at an increased risk, although it is unclear how much more of a risk it would be in comparison with partaking in a regular exercise program (Anderson et al., 2019).

CHAPTER III

Methods

Population

This study collected data on 19 physically active healthy adult participants, 7 males, 12 females, between 19 and 46 years of age. Inclusion criteria for participants included: being physically active (\geq 3 days/week of at least 30 minutes of moderate-intensity physical activity) and being between the ages of 18-64 years old. Four participants were excluded from the final analysis. Three participants were excluded due to a body fat % > 35% and one was excluded due to multiple signal dropouts in two of the Moxy monitors. Table 1 displays descriptive characteristics for participants included in the final analysis. Participant recruitment was from January 13th – February 10th. Data collection on participants was performed from January 28th, 2021 – February 12th, 2021.

Table 1

Characteristics	Descriptive Outcomes (Mean ± SD)
Age	27.7 ± 7.3
Gender (Males/Females)	7 Males/8 Females
Height (cm)	168.6 ± 9.1
Weight (kg)	69.2 ± 13.0
Body Fat %	26.8 ± 7.5
Occluded Limb (Right/Left)	6 Right/9 Left
Resting Heart Rate (bpm)	70.9 ± 9.2
Resting Systolic Blood Pressure (mmHg)	113.9 ± 6.0
Resting Diastolic Blood Pressure (mmHg)	69.7 ± 9.2

Summary of Participants' (n = 15) Descriptive Outcomes

Note. SD: Standard Deviation; cm: Centimeter; kg: Kilogram; bpm: Beats Per

Minute; mmHg; millimeter of mercury = unit of pressure

Informed consent was obtained from all participants in accordance with the Sam Houston State University Institutional Review Board (IRB) Guidelines. A health history questionnaire (HHQ) was administered to participants after signing the informed consent. Sections 1, 4, and 5 on a self-reported HHQ were used to assess health history, physical activity levels, and medication use. This information determined participant eligibility based on both inclusion and exclusion criteria.

Equipment

A Delfi Personalized Tourniquet System for BFR unit (Delfi Medical Innovations Inc., Vancouver, BC) was used to measure participant's LOP, manipulate cuff pressure based on LOP measurement, and to perform lower-limb bilateral calibration. Four Moxy monitors (Fortiori Design LLC, Hutchinson, MN) measured local muscle oxygen saturation (SmO₂) and total heme (tHb) bilaterally on the medial head of the gastrocnemius and vastus lateralis muscles at the positions recommended by Rainoldi, Melchiorri, and Caruso, (2004). A Garmin Running Dynamics Pod (Garmin Ltd., Olathe, KS) was clipped onto the participant's waistband (on backside) prior to exercise testing. This device allowed assessors to view and analyze walking/running metrics realtime. Data regarding ground contact balance (GCTB) was collected from the Garmin Pod. Participants wrapped a Garmin chest heart rate (HR) strap (Garmin Ltd., Olathe, KS) around their chest to record HR. A Welch Allyn trigger aneroid sphygmomanometer (Welch Allyn Inc., Skaneateles Falls, NY) and 3M Littmann Classic II S.E. stethoscope (3M Littman, Saint Paul, MN) was used to manually assess blood pressure (BP). Participant's height, weight, and body fat percentage were measured using a stadiometer and SECA mBCA 514 bioelectrical impedance scale (SECA, Hamburg,

Germany). A Cosmed T170 Treadmill (Cosmed, Rome, Italy) was used for walking during exercise testing. A poster of a 10-point RPE scale was posted in front of the treadmill for participants to see.

Data from the Moxy and Garmin devices was wirelessly collected to Perfpro software (Hartware Technologies, Rockford MI) using a laptop computer during the exercise protocol. This software connects to wireless ANT+ enabled devices. This allowed the assessors to view SmO₂, tHb, HR, GCTB metrics, and other data real-time from one source.

Procedural Design

This study involved participants performing a walking protocol with a blood flow restriction cuff applied to the proximal portion of the left or right leg while changes in muscle oxygen saturation were measured using near infra-red spectroscopy (NIRS) in bilateral gastrocnemius and vastus lateralis muscles. Each participant acted as his/her own control for the study. Blood flow restriction cuff placement on the participant's right or left upper leg as well as the order of occlusion pressure was randomized. Other measurements including HR, BP, rated perceived exertion (RPE), and GCTB were also measured during testing. This study also used a self-reported health history questionnaire as an intake for health history, physical activity levels, and medication use. Participants completed testing in 1 visit that lasted approximately 1.5-1.75 hours in length.

Participants who arrived for testing had their forehead temperature taken and completed a COVID-19 symptom-related questionnaire. The reason for these procedures was to comply with Sam Houston State University COVID-19 precautions. Participants were given an opportunity to read and sign an informed consent. Participants were encouraged to ask questions and/or concerns regarding the research study or their participation in it. Participants then completed a health history questionnaire. The Principal Investigator (PI) provided instruction of the procedures and exercise testing. Participants were given a non-identifiable participant ID (i.e. BFR 001). This ID was used as a replacement for their name throughout testing. Participant's height was measured using a stadiometer. Waist circumference was taken manually using a retractable measuring tape. Weight, BMI, and body fat percentage were measured using a SECA body composition scale. Participants put on the chest HR strap/monitor and sat in a chair for several minutes while PerfPro software recorded HR. The lowest HR value over several minutes was recorded as the participant's resting HR. A seated resting BP was taken manually by the PI on the side determined to be occluded during the walking protocol.

While lying in a prone position on a cushioned table, LOP was determined using the Delfi BFR unit. The Delfi unit is an instrument that uses calculation sensors and software alongside pneumatic surgical-grade tourniquet cuffs to determine LOP. Once LOP was determined, the base of the BFR cuff was wrapped with microspore tape and flex wrap to prevent the cuff from falling during the exercise protocol. Moxy monitor placement on the vastus lateralis muscles were positioned on a reference line 9.4 cm from the superior lateral side of the patella to the anterior superior iliac spine (Rainoldi, Melchiorri, and Caruso, 2004). Monitor placement on the gastrocnemius muscles were placed approximately 50-60% of the distance along a reference line that began from the Achilles tendon insertion to the medial side of the popliteus cavity. Sites were traced with a non-permanent marker before securing the monitors with micropore tape and flex wrap. Participants warmed-up by walking on a treadmill for 5-10 minutes at 2.0 mph. The exercise protocol immediately followed the warm-up. Exercise testing involved walking continuously on a treadmill for seven 5-minute stages at 3.0 mph while the BFR cuff pressure at the beginning of each stage was manipulated. Cuff pressures for stages 1, 3, 5, and 7 were at 0% LOP. Cuff pressure order for stages 2, 4, and 6 were randomized between 40%, 80%, and 100% LOP. Data from the Moxy monitors, HR monitor, and Dynamic Pod were continuously monitored. During exercise testing, the PI operated the Delfi unit and took BP readings. The second assessor monitored data on PerfPro, asked for RPE, and recorded data at each stage. Verbal feedback regarding GCTB was given when the L/R GCTB % deviated below 45% or above 55%. RPE, BP, and HR were recorded at 3 and 5 minutes of each stage.

Following exercise testing, bilateral physiological calibrations for the Moxy monitors were obtained. The BFR cuff was inflated to 100% LOP for 8 minutes on both legs while PerfPro collected data on SmO₂ and tHb. Moxy uses a calibration model Once data had been collected, Moxy monitors were sequentially removed and powered off along with the other equipment/sensors.

Statistics

Data compiled in PerfPro was exported along with data collected by the assessors to Microsoft Excel for organization prior to being analyzed on GraphPad Prism (version 9) (GraphPad Software, San Diego, CA). NIRS data was analyzed three separate ways: (a) area of desaturation (ADS), (b) initial change (Δ) in SmO₂ and tHb, and (c) final change (Δ) in SmO₂ and tHb. These analyses were performed on both BFR and control (CON) limbs. ADS analyses were used to approximate the total area of SmO₂

- Starting SmO₂ for each occlusion stage was determined by averaging SmO₂ five seconds prior to the start of each occlusion stage.
- 2. The starting SmO₂ was then multiplied by the time difference of the first and final time interval of each occlusion stage to approximate the total area of SmO₂.
- The trapezoidal rule was used to approximate the area of SmO₂ underneath the SmO₂ curve (AUC).
- 4. ADS was calculated by subtracting the AUC from the total SmO₂ area.

The differences in BFR and CON SmO₂ ADS were calculated by subtracting CON data from BFR values. A two-way repeated measures analysis of variance (ANOVA) test was performed using the differences in ADS data to determine the main effects of muscle and LOP%. A Tukey post hoc test was performed to compare ADS of both muscles.

We calculated both the initial and final ΔSmO_2 and ΔtHb for each occlusion stage. The purpose of these calculations was to quantify changes in NIRS data after the first minute of occlusion and during the final minute of occlusion. We calculated ΔSmO_2 and ΔtHb through the following calculations and is illustrated in Figure 2:

> The average SmO₂ and tHb one minute prior to each occlusion stage was determined.

- The average SmO₂ and tHb during the first and final minute of each occlusion stage was determined.
- 3. Initial Δ SmO₂ and Δ tHb was calculated by taking the differences in average SmO₂ and tHb during the first minute of the occlusion stage by the minute prior to that occlusion stage.
- Final ΔSmO₂ and ΔtHb was calculated by taking the differences in average SmO₂ and tHb during the final minute of the occlusion stage by the minute prior to that occlusion stage.

The differences in Δ SmO₂ and Δ tHb were calculated by subtracting CON data from BFR data. Separate two-way repeated measure ANOVA tests were performed on Δ SmO₂ and Δ tHb to determine the main effects of muscle and LOP%. A Tukey post hoc test was used to compare Δ SmO₂ and Δ tHb of both muscles.

Separate one-way repeated measures ANOVA tests were conducted on 5-minute HR, SBP, DBP, RPE, and GCTB data of each occlusion stage. Comparisons for HR, SBP, and DBP for each occlusion stage were compared to resting values using Dunnett's test and also between one another using Tukey's test. Similar post hoc analysis of RPE and GCTB means were performed with average data collected during the first stage of the walking protocol. GCTB deviation values represent the mean % deviation from the occluded limb. For example, a 52% left-ground contact balance with right leg occlusion would represent a 4% deviation (52% left – 48% right) in favor of the non-occluded leg while walking. Negative SmO₂ and tHb values in the analysis represent the % deviation in

favor of the occluded limb. Significance for all statistical comparisons was determined at an alpha level of p < 0.05.

Figure 1

Area of SmO₂ Desaturation (ADS)



Note. Representative tracing illustrating the approximation of total area of SmO₂, area under the SmO₂ curve (AUC), and area of SmO₂ desaturation (ADC) during each occlusion stage. Created with BioRender.com.

Figure 2

Initial and Final Change in SmO₂ and tHb



Note. Representative tracing illustrating the averages for the last minute of the nonocclusion stage, first minute of each occlusion stage, and last minute of the occlusion stage. These averages were calculated for both SmO₂ and tHb. The average SmO₂ and tHb during both the initial or final 1-minute interval of each occlusion stage was subtracted from the average SmO₂ and tHb 1-minute prior to each occlusion stage (Stages 1, 3, and 5). Created with BioRender.com.

CHAPTER IV

Results

Area of SmO₂ Desaturation

We observed a significant main effect of LOP% on ADS, F (1.432, 40.08) = 32.74, p < 0.0001. Tukey's multiple comparison test of ADS revealed a significant difference for VL 40% vs. 80% LOP (p = 0.0201), 40% vs 100% LOP (p = 0.0004), and 80% vs. 100% LOP (p = 0.0098). On the other hand, ADS comparisons for the GM 40% vs. 100% LOP (p = 0.0010) and 80% vs.100% LOP (p = 0.0012), but not 40% vs 80% LOP (p = 0.0821) showed a significant difference. There was no significant main effect for Muscle overall, F (1, 28) = 0.5056, p = 0.4829. The results of the two-way repeated measures ANOVA on ADS data revealed that there was a statistically significant interaction between the effects of LOP% and Muscle on SmO₂ desaturation, F (2, 56) = 4.530, p = 0.0150.

Table 2 displays mean \pm standard deviation SmO₂ desaturation measured from each limb at each occlusion stage as well as the difference between the two limbs. Based on the difference between the limbs, ADS was the greatest at 100% LOP (VL: 3804 \pm 2463.6 u.a, GM: 6607 \pm 5786.4 u.a), while being the lowest at 40% LOP (VL: 521.3 \pm 607 u.a, GM: -551.1 \pm 1068.8 u.a) for both muscles.

Table 2

	BFR				CON				Difference			
-	VL		G	M	V	L	G	M	V	\mathbf{L}	GN	4
LOP	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
40%	261.7	516.6	-325.7	653.2	-259.5	410.4	225.3	1193.8	521.3	607.0	-551.1	106 8.8
80%	1540.3	1776.9	2840.6	3465.6	73.9	431.4	1385.1	1650.1	1466.4	1471.3	1455.5	354 0.9
100%	4070.4	2587.4	8739.5	6290.7	266.1	937.5	2132.9	1829.1	3804.4	2463.6	6606.6	578 6.4

Mean and Standard Deviation for Area of Desaturation (ADS)

Note. LOP: Limb Occlusion Pressure; BFR: Blood Flow Restriction; CON: Control; VL: Vastus lateralis; GM: Gastrocnemius; M: Mean; SD: Standard Deviation

Initial Δ SmO₂ and Δ tHb

The analysis of initial Δ SmO₂ found a statistically significant LOP% main effect, F (1.8, 52) = 28, *p* < 0.001. Post hoc comparisons showed a non-statistically significant difference between VL 40% vs 80% (*p* = 0.555) but revealed a significant difference between VL 40% vs 100% (*p* = 0.004) and 80% vs 100% (*p* = 0.010). There was a significant difference for the GM at 40% and 80% (*p* = 0.004), 40% vs 100% (*p* < 0.001), and 80% vs 100% (*p* = 0.020). There was a statistically significant main effect for Muscle, F (1, 28) = 6.7, *p* = 0.015, with mean Δ SmO₂ for the GM (12%) being significantly higher than the VL (5.0%). There was a statistically significant LOP%*Muscle interaction, F (2, 56) = 10, *p* < 0.001. There was no significant main effect of LOP%, F (1.1, 31) = 4.0, *p* = 0.051 or Muscle, F (1, 28) = 1.7, *p* = 0.205 on initial Δ tHb. There was however a statistically significant LOP%*Muscle interaction for initial Δ tHb, F (2, 56) = 3.5, *p* = 0.037.
Final Δ SmO₂ and Δ tHb

There was a significant main effect for LOP%, F (1.359, 38.04) = 9.631, p = 0.0016 on final Δ SmO₂. Post hoc analysis for final Δ SmO₂ revealed only a significant difference for VL 40% vs. 100% (p = 0.0029) and 80% vs 100% (p =0.0079). There was no significant difference between VL 40% vs 80% (p = 0.1388). The comparison of GM means showed no significant difference between 40% and 80% (p = 0.9944), 40% vs 100% (p = 0.3663), or 80% vs 100% (p = 0.2651). There was a significant main effect for Muscle, F (1, 28) = 4.285, p = 0.0478 on final Δ SmO₂, with mean Δ SmO₂ in the VL (8.6%) being significantly greater than the GM (1.8%). There was no statistically significant interaction between the effects of LOP% and Muscle on final Δ SmO₂, F (2, 56) = 0.5507, p = 0.5796. The results for final Δ tHb showed no significant main effects for LOP%, F (1.062, 29.73) = 0.4475, p = 0.5202 and Muscle, F (1, 28) = 0.8177, p = 0.3736. There was also no significant interaction between the effects of LOP% and Muscle on final Δ tHb, F (2, 56) = 0.7350, p = 0.4841. Table 3 displays the mean \pm SD for both the initial as well as final change in SmO₂ and tHb.

Table 3

Mean and Standard Deviation of Δ *SmO*² *and* Δ *tHb for Each Occlusion Stage*

	Initial ΔSmO ₂						Initial A	tHb	
	V	L	G	M		VL	<u>.</u>	<u>G</u>	M
LOP	M	SD	М	SD		M	SD	M	SD
40%	2.06	4.81	-1.96	5.32		-0.25	0.88	< 0.00	0.05
80%	3.94	4.97	13.64	14.82		-0.02	0.08	0.02	0.10
100%	9.13	7.39	25.60	18.24		1.45	3.11	0.06	0.23
	Final ΔSmO ₂						Final A	tHb	
40%	2.43	3.09	-1.33	3.66		-0.01	0.10	-0.03	0.06
80%	5.46	6.35	-1.00	13.57		-0.07	0.13	-0.02	0.09
100%	17.81	13.87	7.73	24.25		-0.06	0.34	0.14	0.84

Note. Note. LOP: Limb Occlusion Pressure; ΔSmO₂: Change in Muscle Oxygen Saturation; ΔtHb: Change in Total Hemoglobin; VL: Vastus lateralis; GM: Gastrocnemius; M: Mean; SD: Standard Deviation

HR, RPE, SBP, DBP, and GCTB

The results of the repeated measures ANOVA determined that there was a statistically significant main effect of LOP% on HR, F (1.454, 20.36) = 69.75, p < 0.0001, RPE, F (1.962, 27.47) = 57.93, p < 0.0001, SBP, F (2.467, 34.54) = 49.46, p < 0.0001, and DBP, F (2.133, 29.86) = 7.342, p = 0.0022. Tukey's post hoc test for HR revealed a significant difference between 40% vs 100% (p = 0.0482) but not for 40% vs 80% (p = 0.0805) or 80% vs 100% (p = 0.1535). Multiple comparisons of SBP and RPE were significant for 40% vs. 80% (p = 0.0106, p = 0.0060, respectively), 40% vs 100% (p = 0.0001, respectively), and 80% vs 100% (p = 0.0421, p < 0.0001, respectively). Table 4 displays the outcomes for HR, SBP, and DBP at rest and at the 5-minute point of each occlusion stage. Comparison of mean DBP showed 40% vs. 80% (p = 0.0056), 40% vs 100% (p = 0.0014), but not 80% vs 100% (p = 0.170) to be

significantly significant. We observed no statistically significant difference (p = 0.1264) in GCTB between stages. Table 5 displays the outcomes regarding average RPE and GCTB deviation for the first stage (CON) and each occlusion stage.

Table 4

Descriptive Statistics for HR, SBP, and DBP

		HR (bpm)			SBP (n	nmHg)			DBP (r	nmHg)	
Measure	Rest	<u>40%</u>	<u>80%</u>	<u>100%</u>	<u>Rest</u>	<u>40%</u>	<u>80%</u>	<u>100%</u>	Rest	<u>40%</u>	<u>80%</u>	<u>100%</u>
Mean	70.9	103.8	109.9	114.9	113.9	124.5	131.1	136.8	69.73	67.47	71.73	74.40
SD	9.24	13.68	18.76	21.23	6.02	8.09	9.65	10.19	9.13	8.34	9.32	8.59

Note. HR: Heart Rate; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SD: Standard Deviation

Table 5

Descriptive Statistics for RPE and GCTB Deviation

		RPE	(1-10)		GCTB Deviation (%)			
Measure	CON	40%	80%	100%	CON	40%	80%	100%
Mean	4.20	4.93	5.67	6.67	0.13	0.08	-1.88	-1.82
SD	1.01	1.16	1.23	1.18	3.73	5.18	6.49	5.81

Note. Rating of Perceived Exertion; GCTB: Ground Contact Time Balance; SD: Standard Deviation.



Area of SmO₂ Desaturation (ADS) Between LOP% and Muscles

Note. Comparison of the area of SmO₂ desaturation (ADS) for each LOP% stage and muscles. Data shown as means ± standard deviation. * Significant difference from 40% LOP; [#] Significant difference from 80% LOP; [¥] Significant difference from 100% LOP; ⁺ Significant difference from all comparisons.

Initial and Final Δ SmO₂ for Each Occlusion Stage



Initial Δ SmO₂

Note. Comparison of the initial and final Δ SmO₂ (%) for each LOP% stage. Data shown as means ± standard deviation. * Significant difference from 40% LOP; # Significant difference from 80% LOP; * Significant difference from 100% LOP; * Significant difference from all comparisons.

Comparisons of HR, RPE, SBP, and DBP





Note. Stage comparison of HR, RPE, SBP, and DBP. Means ± standard deviation. * Significant difference from 40% LOP; [#] Significant difference from 80% LOP; [¥] Significant difference from 100% LOP; ⁺ Significant difference from all comparisons.

GCTB Deviation



Note. Comparison of the GCTB deviation (%) during stage one and at each occlusion stage. Means \pm standard deviation.

CHAPTER V

Discussion

Main Findings

The hypothesis that there would be no significant differences in SmO₂ between 40%, 80%, and 100% LOP while walking at 3.0 mph was rejected. Our analysis of ADS data revealed a statistically significant main effect for LOP% on SmO₂ desaturation. Post hoc analysis revealed a significant difference for each comparison of LOP except 40% vs. 80% LOP for the GM. We observed an increase in mean SmO₂ desaturation with each incremental increase in LOP% (-15 u.a, 1461 u.a, and 5205 u.a for 40, 80, and 100% respectively). These finding suggest that incremental increases in LOP will bring about greater SmO₂ desaturation and may therefore instigate a different burden on the muscles. The non-significant main effect for muscle on ADS suggests that both muscles responded similarly to occlusion during our study protocol. We therefore suspect both muscles experienced non-significant differences in muscle strain during occlusion.

During our investigation we observed changes in SmO₂ from start to finish of occlusion stages. Our analyses of initial and final Δ SmO₂ were used to represent the change in SmO₂ during the first as well as final minute of the occlusion stages respectively. In each analysis we observed higher LOP were associated with greater decreases in SmO₂. The analyses of initial and final Δ SmO₂ revealed that muscles demonstrated significant differences in the change of SmO₂. Our results particularly revealed a change in significance for the GM between initial and final Δ SmO₂ analyses. Multiple comparisons of initial Δ SmO₂ showed the GM had significant differences at each comparison of LOP. Final Δ SmO₂ however revealed a non-significant difference at

each comparison of LOP. These findings suggest that average SmO₂ desaturation for the GM was not consistent from start to finish during occlusion periods. Both initial and final Δ SmO₂ for the VL showed significant differences for each comparison except at 40% vs. 80% LOP.

We found an increase in perceptual and physiological variables of physical exertion alongside increased relative BFR pressures. Our results of RPE appear to be consistent with prior research (Wei et al., 2020) that measured perceived exertion at different relative pressures. Prior to testing, participants were instructed to report their RPE based on perception of effort rather than pain and/or discomfort. While there was no increase in exercise intensity, participants in our study reported greater perception of effort with increased levels of occlusion. Increases in HR, SBP, and DBP were congruent with findings reported in a review by Silva et al., (2019) which covered acute responses of aerobic exercise with BFR. Displayed in Figure 5, these measurements, with the exception of DBP also increased from resting/control values. Caution should be taken when interpreting these blood pressure responses because we used physically active healthy adult participants in our investigation.

We observed non-significant changes in walking symmetry to different LOP% based on average GCTB deviation, represented in Figure 6. These results indicate that GCTB did not significantly differ with increased LOP%. The non-significant changes in walking symmetry indicate we were effective in preventing participants from significantly favoring one limb over the other during occlusion stages.

Muscle Oxygen Saturation

We sought to quantify and compare SmO₂ at several different LOP% while walking on a treadmill. To our knowledge, no previous investigation had explored changes in SmO₂ during BFR-walking at recommended occlusion pressures for aerobic exercise (Patterson et al., 2019). Several studies have investigated the impact different relative pressures of BFR had on microvascular oxygenation using NIRS technology (Ilett et al., 2019; Kilgas et al., 2018; Reis et al., 2019; Wei et al., 2020). These investigations are important because relative muscle oxygen desaturation has provided indirect insight on localized tissue perfusion and has been used as an indicator of intramuscular metabolic stress (Ganesan et al., 2015; Larkin et al., 2012; Pearson and Hussain, 2015). Given that BFR exercise commonly uses low exercise intensities and loads, optimizing metabolic stress in order to promote positive muscle adaptations should be a priority. The results of our study suggest that different LOP% can generate varying magnitudes of SmO₂ desaturation. Increased SmO₂ desaturation as a result of higher LOP% may necessitate lower training workloads. However, our results also suggest that higher LOP% may intensify perception of effort and physiological variables. Despite the potentially heightened stimulus of using higher LOP%, practitioners and exercise professionals should program such levels of LOP% with caution.

Comparison of our results with other studies is difficult due to differences in study methodology. An investigation by Wei et al., (2020) analyzed relative tissue oxygenation on participants while they performed seven 5-minute continuous stages of cycling. Five of the stages were with BFR at 40%, 50%, 60%, and 80% of an estimated LOP. They analyzed the changes in tissue oxygen saturation by subtracting the average thirty seconds at the end of the occlusion stage from the minute prior to their exercise protocol. Their comparisons did not find significant differences in the reduction of relative tissue oxygenation between 40% - 80% LOP of the VL. We observed a nonsignificant reduction in SmO₂ for the VL between 40% and 80% LOP. Our study design similarly used an aerobic exercise modality in contrast to investigations by Ilett et al., (2019), Kilgas et al., (2018), and Reis et al., (2019) who either used knee extension or hand grip exercises. Despite these non-significant changes in measurements of tissue oxygen saturation, our study demonstrated that changes in SmO₂ were not consistent during a 5-minute occlusion period. We therefore believe associating change in tissue muscle oxygen saturation during a brief portion of BFR exercise with total muscle stress to be misleading and a matter of conjecture.

A study by Reis et al., (2019) compared total area of deoxygenated heme in the VL during unilateral knee extensions at 40%, 60%, and 80% LOP. In parallel to their findings, we observed an increase in accumulated oxygen deoxygenation with increased relative pressures of BFR. Their results also revealed that there were significant differences in accumulated oxygen extraction between 40% and 80% LOP. Our findings go along with there being significant differences in total SmO₂ desaturation between 40% and 80% LOP. We did not analyze differences in lower-limb SmO₂ desaturation between 0% and 40%. The study by Reis et al., (2019) found that 40% LOP had significantly greater total desaturation than no BFR. Our findings showed there was minimal lower-limb SmO₂ desaturation at 40% while walking at 3.0 mph and may suggest the futility of conducting BFR walking at 40% LOP if lower-limb SmO₂ desaturation is the goal. Reverting back to recommendations by Patterson et al., (2019), we found 80% LOP to be

more meaningful than 40%. We acknowledge that we did not investigate LOP between 40-80% and the effect these LOP had on other physiological variables or adaptations. Our findings suggest using LOP < 80% for BFR with aerobic exercise may not be optimal to enhance muscle adaptations. One major finding of their study was there were non-significant differences in oxygen extraction past using 60% LOP during low-intensity knee extension exercise. Reis and colleagues did not investigate the effects full arterial occlusion may have had on deoxygenated heme. Our findings suggest 100% LOP will give rise to significantly greater total SmO₂ desaturation than 80% LOP.

Application

In application, higher relative pressures for BFR walking may be beneficial to produce greater skeletal muscle deoxygenation and aid in causing stress to targeted musculature. However, these higher pressures may be accompanied by greater perceived exertion and hemodynamic variables that could potentially make BFR walking at such pressures uncomfortable for individuals. Based on our results, programming BFR walking at 3.0 mph using 40% LOP may not be an adequate stimulus for SmO₂ desaturation in the lower-limb. The calculation of personalized tourniquet pressures using the Delfi BFR device appeared to be practical and a tool for objective measurements which could be easily taken prior to each BFR session. Overall walking with BFR may be a valuable exercise modality in a rehabilitative setting due to its advantage of being easy to perform and familiarity.

Limitations and Future Research

As represented by standard deviation for SmO₂, we detected a spread in data points at each stage of occlusion and as LOP% increased. We suspect such variability at greater LOP% transpired due to individual differences within our sample that we did not account for. We speculate thickness of adipose tissue was one potential reason, considering this is a known limitation of NIRS technology. While we excluded participants who met a body fat % > 35%, we did not have exclusion for adiposity at measurement sites. Despite such limitations of NIRS, we believe the utility of the MOXY sensors allowed for ease of use in our study. Another reason may have been due to slight differences in MOXY sensor placement. As previously mentioned, original reference for monitor placement on the gastrocnemius muscles was assumed to be too low and would need to be placed slightly higher according to each individual participant.

We did not observe earlier occlusion stages having an impact on SmO₂ for later stages. We are unsure if our study design had any impact on the accuracy of SmO₂ measurements in later stages. While our study incorporated randomized LOP% order and a non-occluded stage between each occlusion stage, future research may benefit from measuring muscle oxygen saturation changes during isolated stages of occlusion. Our study could have benefited from using relative exercise intensities from maximal exercise testing rather than a set speed of 3.0 mph. Additional research is needed to investigate the effect different relative pressures outside of 40%, 80%, and 100% have on SmO₂ while walking. Future investigations of SmO₂ using NIRS technology should apply more strict criteria of adiposity in participants, especially at the measurement sites where muscle oxygen sensors are used.

Conclusion

The findings of our study revealed there are some significant effects of BFR walking at 40%, 80%, and 100% LOP on SmO₂ desaturation. We sought to determine if lower relative occlusion pressures would induce comparable SmO₂ desaturation as higher occlusion pressures. Additionally, if perceived exertion and hemodynamic variables at lower occlusion levels were significantly less than higher pressures it may not be necessary to use higher pressures in practice. Based on comparisons of LOP%, there was a non-significant difference in SmO₂ desaturation between 40% and 80% LOP for the GM. Heart rate was also found to be non-significant between these stages. While we saw significant changes in SmO2 during the initial and final minute of certain occlusion stages, we don't believe these measurements provided an extensive outlook of the total demand placed on the VL and GM within each occlusion stage. Some of our results for change in SmO₂ appear to be consistent with prior research that has investigated changes in oxygen saturation at different relative pressures. However, comparisons with results of other investigations are difficult due to discrepancy of methods used. Further research is needed to investigate the differences in SmO₂ at additional LOP beyond what was included in our study.

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APPENDIX A

IRB Approval

IRB Approval: IRB #: IRB-2020-256 Title: Effects Different Percentages of

Blood Flow Restriction While Walking Have on Muscle Oxygen Saturation. Creation

Date: 11-10-2020 End Date: 5-15-2021. Status: Approved Principal Investigator: Clayton

Shriver Review Board: SHSU IRB

Date: 4-21-2021
IRB #: IRB-2020-256 Title: Effects Different Percentages of Blood Flow Restriction While Walking Have on Muscle Oxygen Saturation
Creation Date: 9-13-2020 End Date: Status: Approved
Principal Investigator: Clayton Shriver Review Board: SHSU IRB
Sponsor:

Study History

Submission Type Initial	Review Type Expedited	Decision Approved
Submission Type Renewal	Review Type Unassigned	Decision
Submission Type Modification	Review Type Unassigned	Decision

Key Study Contacts

Member	Patrick Davis	Role	Co-Principal Investigator	Contact	davisp@shsu.edu
Member	Clayton Shriver	Role	Principal Investigator	Contact	cts039@shsu.edu
Member	Clayton Shriver	Role	Primary Contact	Contact	cts039@shsu.edu
Member	Sydney Beverly	Role	Investigator	Contact	smb081@shsu.edu
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Member	Yvette Figueroa	Role	Investigator	Contact	ylf001@shsu.edu

APPENDIX B

Informed Consent



Sam Houston State University Consent for Participation in Research

KEY INFORMATION FOR Comparison of Different Percentages of Blood Flow Restriction on Muscle Oxygen Saturation during Aerobic Exercise

You are being asked to be a participant in a research study about the effects that different percentages of blood flow restriction (BFR) have on the supply and demand of oxygen to leg muscles while walking on a treadmill. You have been asked to participate in the research because you are a physically active healthy adult without any known cardiovascular disease and may be eligible to participate.

WHAT IS THE PURPOSE, PROCEDURES, AND DURATION OF THE STUDY?

The purpose of this study is to determine if circulation at low pressures, which are more tolerable, provide a similar reduction of blood oxygen levels compared to higher pressures in your lower extremities.

The study will involve you walking on a treadmill with a blood flow restriction cuff applied to either your right or left upper-leg while changes in blood oxygen levels in active muscles are measured using muscle oxygen monitors. During this study your heart rate (HR), blood pressure (BP), walking symmetry between your right and left foot, and how hard your body is working using a scale from 6 to 20 will also be analyzed.

Your participation in this research will last about 1 hour and 45 minutes long.

For a complete description of testing procedures, refer to the Detailed Consent

WHAT ARE REASONS YOU MIGHT CHOOSE TO VOLUNTEER FOR THIS STUDY?

You may choose to volunteer for this study to help provide insight on how blood oxygen levels to muscles change with different percentages of blood flow restriction training. Your participation may help us learn more about the acute effects to your cardiovascular system while performing aerobic exercise with blood flow restriction. The information learned from this study may be useful to help guide decision-making for practitioners and clinicians that utilize blood flow restriction training. For a complete description of benefits, refer to the Detailed Consent.

WHAT ARE REASONS YOU MIGHT CHOOSE NOT TO VOLUNTEER FOR THIS STUDY?

Risks for this study include likely muscle fatigue and perceived discomfort/pain, and a slight chance of delayed onset muscle soreness (DOMS). Blood pressure and heart rate may increase with increases in blood flow restriction pressures.

For a complete description of risks, refer to the Detailed Consent.

DO YOU HAVE TO TAKE PART IN THE STUDY?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any services, benefits, or rights you would normally have if you choose not to volunteer.

WHAT IF YOU HAVE QUESTIONS, SUGGESTIONS OR CONCERNS?

The person in charge of this study is Clayton Shriver, PI of the Sam Houston State University Department of Kinesiology who is working under the supervision of Dr. Patrick Davis. If you have questions, suggestions, or concerns regarding this study or you want to withdraw from the study his contact information is: Clayton Shriver, PI: email – <u>cts039@shsu.edu</u>, cell – 281-528-3635, Dr. Patrick Davis: email – <u>davisp@shsu.edu</u>, phone – 936-294-2645. If you have any questions, suggestions or concerns about your rights as a volunteer in this research, contact the Office of Research and Sponsored Programs – Sharla Miles at 936-294-4875 or e-mail ORSP at <u>sharla_miles@shsu.edu</u>.

Sam Houston State University

Consent for Participation in Research

DETAILED CONSENT Comparison of Different Percentages of Blood Flow Restriction on Muscle Oxygen Saturation during Aerobic Exercise

Why am I being asked?

You are being asked to be a participant in a research study about the effects that different percentages of blood flow restriction have on blood oxygen levels in working leg muscles while walking on a treadmill conducted by Clayton Shriver, PI of Sam Houston State University Department of Kinesiology. I am conducting this research under the direction of Dr. Patrick Davis. You have been asked to participate in the research because you are a physically active healthy adult with no known cardiovascular disease and may be eligible to participate. We ask that you read this form and ask any questions you may have before agreeing to be in the research.

Your participation in this research is voluntary. Your decision whether or not to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Why is this research being done?

There are several acute metabolic responses to aerobic exercise with blood flow restriction training that have been investigated but none have investigated changes in blood oxygen levels to working muscle while performing BFR. This study will help us to learn more about the supply and demand of oxygen to working muscles while being under the application of blood flow restriction. Current exercise prescription recommendations for aerobic exercise with blood flow restriction are to apply cuff pressures between 40-80% arterial occlusion pressures. Research is needed to investigate the acute impact the recommended occlusion pressures have on individuals.

By doing this study, we hope to gain insight on how blood oxygen levels in working leg muscles changes to different percentages of blood flow restriction training while also observing changes in acute cardiovascular effects and perceived exertion. Your participation in this research will last about 1 hour and 45 minutes long.

Risks for this study include likely muscle fatigue and perceived discomfort/pain, and a slight chance of delayed onset muscle soreness (DOMS). Blood pressure and heart rate may increase with increases in blood flow restriction pressures.

What is the purpose of this research?

The purpose of this study is to determine if circulation at low pressures, which are more tolerable, provide a similar reduced blood oxygen levels to muscles compared to higher pressures in your lower extremities. Muscle oxygen monitors will analyze data between each stage of walking as well as between BFR and non-BFR legs.

Here are the following research questions for this study:

- 1. How will higher limb occlusion pressure (LOP) percentages effect muscle oxygen saturation (SmO₂) compared to lower percentages while walking?
- 2. How will higher limb occlusion pressure (LOP) percentages effect HR, BP, and RPE compared to lower percentages while walking?

What procedures are involved?

Each participant of this study will walk on a treadmill for 7-5 minute stages at 3.0 mph while a medical device designed to measure blood pressures is applied to either their right or left upper-leg. The BFR unit is a device that inflates a cuff to specific pressures and restricts blood flow in a specific limb while performing exercise. This cuff will be placed on either your right or left leg. Four Moxy monitors will be placed on both legs while performing the test. This device is similar to how a pulse oximeter works, except these monitors will measure blood oxygen levels in the working muscles. A health history questionnaire (HHQ) will be given to you to determine your level of physical activity and personal medical history.

If you agree to be in this research, we would ask you to do the following things in chronological order:

- 1. You will complete a Health History Questionnaire (HHQ) that will contain questions asking about your level of physical activity and health history. (4 minutes)
- 2. We will give you instructions on the exercises you will be doing (10 minutes)
- 3. Your body composition will be calculated. (2 minutes)
- 4. Your resting blood pressure and heart rate will be measured. (2 minutes)
- 5. Prior to exercise testing, you will have several devices/equipment hooked up to you. (10-15 minutes)
- 6. You will warm up by walking on a treadmill for 5-10 minutes at 2.0 mph. (5-10 minutes)
- 7. You will conduct an exercise test that will involve walking on a treadmill for 7-5 minute stages at 3.0 mph while we manipulate the BFR cuff pressure at the beginning of each stage. (35 minutes)
- 8. You will rest in a chair for 16 minutes following the final stage of exercise testing. The BFR cuff will be inflated to 100% LOP on both limbs for 8 minutes while data is collected. After this period, you will sit for 5 additional minutes while the assessors take the equipment/devices off and take your final blood pressure and heart rate. (30 minutes)

Your entire involvement will be about 1 hour and 45 minutes in duration.

Approximately 25 subjects may be involved in this research.

What are the potential risks and discomforts?

Physical risks associated with this research include muscle fatigue, perceived discomfort, and delayed onset muscle soreness (DOMS). Factors that may influence discomfort during BFR training include high applied pressure, long application periods, and training intensity. Blood pressure, heart rate, and perceived exertion may increase with increases in blood flow restriction pressures. These are all common risks associated with physical exercise and utilizing blood flow restriction and may not be necessary to stop exercise.

There is a risk for possible exposure to SARS CoV-2, an agent that causes COVID-19. The risk for exposure to this virus as part of this in-person research project could result in a positive development of COVID-19. The consequences of COVID infection include extended quarantine/self-isolation, additional tests, hospitalization that may require intensive care treatment, and the risk of death.

You may voluntarily stop participating at any point during testing. You will be immediately reassessed by the PI to determine test continuation. The PI will do so by asking if you wish to either continue with testing or to stop. Testing will continue if you verbally ask to continue. In the event that you wish to stop testing, the PI will immediately stop the treadmill and release any BFR cuff pressure that is applied. Other indications for terminating the test will include severe symptoms (i.e. chest pain, shortness of breath, and/or fatigue) and severe hypertension (SBP greater than 200 mmHg, DBP greater than 110 mmHg, or both). At this point you will be asked to get off of the treadmill and sit in a chair to rest for 5 minutes. After this period, you will sit for 5 additional minutes while the assessors take the equipment/devices off and collect a final blood pressure and heart rate.

For any adverse events immediate care will be provided by the PI. If necessary, emergency services/paramedics will be called. For non-emergency adverse events, you will be asked to see your primary care provider for treatment. All adverse events will be reported to the IRB.

The researchers have taken steps to minimize the risks of this study.

Please tell the researchers in the contact section about any injuries, side effects, or other problems that you have during this study. You should also tell your regular doctors.

Are there benefits to taking part in the research?

You may choose to volunteer for this study to help provide insight on how muscle oxygen saturation responds to different percentages of blood flow restriction training. Your participation may also help us learn more about the acute effects to your cardiovascular system while performing aerobic exercise with blood flow restriction. The information learned from this study is adding valuable knowledge to the research community and may be useful to help guide decision-making for practitioners and clinicians that utilize blood flow restriction training. There may be no direct benefit to you other than receiving a body composition analysis.

What other options are there?

There are no alternative for this research. The reason for not including alternative procedures to this research is to keep methodology similar across all participants and therefore improve reliability of data collected.

What about privacy and confidentiality?

The only people who will know that you are a research participant are members of the research team. No information about you, or provided by you during the research will be disclosed to others without your written permission, except:

- if necessary to protect your rights or welfare (for example, if you are injured and need emergency care or when the SHSU Protection of Human Subjects monitors the research or consent process); or
- if required by law.

When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity. If photographs, videos, or audiotape recordings of you will be used for educational purposes, your identity will be protected or disguised.

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law.

Participant's names will be coded (i.e. BP001). This code will be used in replacement of their first and last name. This code will be used in place of their name in the Perfpro software and when collecting additional data (height, weight, age, BP, and HR)

All personal information, research data, and related records will be stored on a password protected computer at the Sport and Human Performance Lab in the Health and Kinesiology Center at SHSU. Only authorized research personnel will have access to the lab and computer.

What if I am injured as a result of my participation?

In the event of injury related to this research study, you should contact your physician or the University Health Center. However, you or your third party payer, if any, will be responsible for payment of this treatment. There is no compensation and/or payment for medical treatment from Sam Houston State University for any injury you have from participating in this research, except as may by required of the University by law. If you feel you have been injured, you may contact the researcher, Clayton Shriver at 281-528-3635 or Dr. Patrick Davis at 936-242-2645.

What are the costs for participating in this research?

There are no additional research costs for which the subject will be responsible.

Will I be reimbursed for any of my expenses or paid for my participation in this research?

Participants will not receive any compensation or inducements (i.e. free care, money, gifts, gift certificates) before, during, or after participation in this study.

Can I withdraw or be removed from the study?

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may also refuse to answer any questions you don't want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise which warrant doing so.

Who should I contact if I have questions?

The researchers conducting this study are Clayton Shriver and Dr. Patrick Davis. You may ask any questions you have now. If you have questions later, you may contact the researchers at: Phone: Clayton Shriver (Student) at 281-528-3635 or Dr. Patrick Davis (Adviser) at 936-242-2645.

What are my rights as a research subject?

If you feel you have not been treated according to the descriptions in this form, or you have any questions about your rights as a research participant, you may call the Office of Research and Sponsored Programs – Sharla Miles at 936-294-4875 or e-mail ORSP at sharla_miles@shsu.edu.

You may choose not to participate or to stop your participation in this research at any time. Your decision whether or not to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

You will not be offered or receive any special consideration if you participate in this research.

Agreement to Participate

I have read (*or someone has read to me*) the above information. I have been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this research.

I understand that by participating in a research project in-person, I am at risk for possible exposure to SARS CoV-2, an agent that causes COVID-19. The risk for exposure to this virus as part of this research project could result in a positive development of COVID-19. The consequences of COVID infection include extended quarantine/self-isolation, additional tests, hospitalization that may require intensive care treatment, and the risk of death.

Your Responsibility to Minimize Your Exposure

If you decide to participate, you agree to take certain precautions that will contain a risk for exposure.

- You will only participate if you are symptom-free.
- You will take your temperature before participating. If it is elevated (100 Fahrenheit or more), or if you have other symptoms described for COVID-19 stay home.
- You will wash your hands or use an alcohol-based hand sanitizer upon arrival.
- You will wear a mask.
- You will keep a distance of 6 feet and there will be no physical contact (e.g. no shaking hands).
- You will try not to touch your face or eyes with your hands. If you do, you will immediately wash or sanitize your hands.

Consent: I have read and understand the above information, and I willingly consent to participate in this study. I understand that if I should have any questions about my rights as a research subject, I can contact Clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by email at cts/consent.com clayton Shriver, PI at 281-528-3635 or by em

Your name (printed):_____

APPENDIX C

Health History Questionnaire

All information gi appropriate testing	ven is personal 3 and exercise p	HI and confident rescription.	EALTH HIST ial. It will enat	ORY QUESTION! ble us to better under	NAIRE rstand you and y	COD our health and	E#: fitness history for
CLASSIFICATIO	N: Fresh	Soph	Jr	Sr.	Grad	Faculty	NA
OCCUPATION:	1343			AGE:	BIRTHE	DATE:/	/
RACE: Amer	ican Indian or A	Alaskan Native	:	Asian:	Black or J	African Ameri	can:
Native	e Hawaiian or (Other Pacific Is	slander:	White			
ETHNICITY:	Hispanic or I	atino:	No	t Hispanic or Latino			
GENDER:	Male	F	emale				
Charle and an it a			1. <u>PE</u>	RSONAL HISTOR	Y		
Have you ever had	ppnes to you:						
Allergies	yes	no un	sure				
Heart Attack	yes	no un	sure	Convulsions	yes	no u	insure
EKG Abnormalitie	es yes	no un	sure	Headaches	yes	no u	insure
Depression	ves	no un	sure	Emphysema	ves	no u	insure
Chest Pain	ves	no un	sure	Arm Pain	ves	no	unsure
High Blood Pressu	are ves	no un	sure	Back Pain	ves	no	unsure
Low Blood Pressu	tre ves	no un	sure	Surgerv	ves	no	insure
Shortness of Breat	th ves	no un	sure	Hernia	ves	no	insure
Diabetes	ves	no un	sure	Indigestion	ves	no	insure
Stroke	ves	no un	sure	Ulcers	ves	no i	insure
Severe Illness	ves	no un	sure	Overweight	ves	no u	insure
Hospitalized	ves	no un	sure	Insomnia	ves	no u	unsure
Joint Problems	ves	no un	sure	Black Outs	ves	no	unsure
Leg Cramps	yes	no un	sure	Vertigo	yes	no u	insure
Please explain all	had surgery, ho	w long has it b	een since the s	urgery and how did	the injury occur	- 12 	
If you previously l	had surgery, ha	ve you been cl	eared by a phy	sician to resume phy	ysical activity, ar	nd are there an	y limitations?
Do you currently e How long have yo How many days d How many minute How intensely do Where do you usu What is your head	exercise? yes_ u been exercise o you exercise es per day durin you exercise?_ ally exercise?_ ally exercise?_	no Wi ng on a consis in a typical we g your exercis	4. EXEI hat activities? tent basis? ek? e session?	RCISE HISTORY			
Approximately ho	w much of each	h of these actin	ities to you cut	rrently do in a week	2	102 52	<u>an 10 k</u>
Jog/run	Lift w	eights	Swim	Collegiate	sports	Club	Sports
Intramura Remarks:	al Sports	Cr	oss fit	Yoga/Pilat	tes	Aerobics/Zum	iba

HEALTH HISTORY QUESTIONNAIRE CONT. What sports have you participated in, what was the highest level you competed, and for how many years?

CODE #_____

Highest Level Competed	Number of years 15yrs	
club team in college		
	12 - 12 - 12 - 12 - 12 - 12 - 12 - 12 -	
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	2	
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र्यय अने किंग्रे	2	
1 <u>21 26 42 6</u> 0	13 <u>1</u> 15	
	S 	
	Highest Level Competed <pre>club team in college</pre>	

5. HEALTH HISTORY

FRESENT.	Height		Weig	ht	10-10-	
Weight at:	Age 15	, Age 20	, Age 30	, Age 40		
	One year ago	, mo:	st weight	, least adult weight		
Do you take vitam	in pills?	Yes	No	Tech Scheler		
Are you currently	taking medicati	ons? Yes	No			
Over the Counter	Medications (O'	TC): Yes	No			
If Yes, Please indi	cate what medie	cation and what	for:	<u>- 11 - 11 - 11 - 11 - 11 - 11 - 11 - 1</u>		
- M. Mill	25 - 1 <u>2</u> 9	10 M	55 - 54		0	
1. 10 1022	10. MI	540 MB	र्थ और अंग्रे	2.23	(01	10 (N
1.26 (222	310 BS	26 22	<u>19 36 38 36 </u>	2-23	281	-c; - 0;
Prescribed Medica	tions: Yes	No				
If Yes, Please indi	cate what medie	cation and what	for:			
			S1945-94-5			
				1.10.10		1
	01 (0)					
		518 - 143	का स्था का स्थान इस संस्था असे अस	2.20	200	11 (P)
		10 - 10 58 - 48 26 - 48			28 28	이가 있다. 이가 있는
Approximate you	daily caffeine i	ntake:	· · · · · · · · · · · · · · · · · · ·			
Approximate your Coffee	daily caffeine i	ntake:	uks	Red bull	Monster	beer
Approximate your Coffee wine	daily caffeine i tea liquor	ntake:	ıks	Red buli	Monster	beer
Approximate your Coffee wine Do you smoke?	daily caffeine i 	ntake:	ks How many years?	Red bul1	Monster How much p	beer per day?
Approximate your Coffee Wine Do you smoke? Did you ever smol	daily caffeine i tea liquor Yes ce? Yes	ntake:	iks How many years? How many years?	Red buli	Monster How much p	beer per day?
Approximate your Coffee Wine Do you smoke? Did you ever smol Cigarettes Do you live with a	daily caffeine i tea liquor Yes ce? Yes cigars	ntake:	lks How many years? How many years? chewing t	Red bull	Monster How much p Other	beer per day?
Approximate your Coffee wine Do you smoke? Did you ever smol Cigarettes Do you live with s	daily caffeine i tea liquor Yes ce? Yes cigars omeone who sn	ntake:	uks How many years? How many years? chewing t Yes No	Red bull obacco How ma	Monster How much p Other ny years?	beer per day?
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Approximate your Coffee Do you smoke? Did you ever smol Cigarettes Do you live with s Approximate hour Vacation weeks pr Home Status: Work Status: Do you feel you a	daily caffeine i tea Yes ce? Yes cigars omeone who sn s you work per er year: very happ; very happ; r stressed?	ntake:	Iks How many years? How many years? chewing t Yes No_ ant difficult ant difficult_ no	Red bull obacco How ma problem problem unsure	Monster How much p Other ny years?	beer per day?
Approximate your Coffee Do you smoke? Did you ever smol Cigarettes Do you live with s Approximate hour Vacation weeks pr Home Status: Work Status: Do you feel you as Are you worried a	daily caffeine i liquor Yes cigars cigars omeone who sn s you work per rr year very happy very happy te stressed?	ntake:	Iks How many years? How many years? chewing t Yes chewing t Yes No mt difficult_ mt difficult_ no po	Red bull obacco How ma problem unsure unsure	Monster How much p Other my years?	beer per day?

HEALTH HISTORY QUESTIONNAIRE CONT.

CODE	#

6. APPROXIMATE A TYPICAL 24 HOUR DAY FOR YOU

Work:	Driving/Riding in a vehicle:
School Work:	Eating:
Studying/Homework:	Exercising:
TV:	TOTAL:
Relaxation:	

Please provide any information that you feel may be necessary, or would like the investigators to be aware of related to this study or

physical activity

Please check ($\sqrt{}$) below to verify that to the best of your knowledge, all of the information you have provided above is correct.

Date:	

Witness:_____ Date:_____

APPENDIX D

COVID-19 Screening Questionnaire

COVID-19 Screening Questionnaire

I. Date & Time: ______Temp:_____

Directions: Please let us know if you have experienced any of the following:

Q1: Fever of 99.6 or higher within the last 3 days Yes □ No Q2: Cough, sore throat, chills, fatigue, or shortness of breath Yes Q3: Nausea, vomiting, or diarrhea Yes □ No Q4: Dysgeusia (loss of sense of taste) Yes □ No Q5: Anosmia (loss of sense of smell) Yes ON Q6: Pneumonia and/or flu within the last 14 days Yes 🗆 No Q7: Have you had contact with anyone who has lab-confirmed Coronavirus Disease 2019 (COVID-19) within 14 days of symptom onset? Yes □ No Q8: Have you tested positive for the SARS CoV-2 virus? Yes 🗆 No If yes, date of positive test:
VITA

Clayton Shriver

Education

Sam Houston State University, Huntsville, TX

Master of Science in Kinesiology with an emphasis in Sport and Human Performance -

May 2021

Texas A&M University, College Station, TX

Bachelor of Science in Applied Exercise Physiology - August 2017

Certifications:

- CPR/AED certified through the American Heart Association Basic Life Support Program – Current
- ACLS certified through the American Heart Association Advanced Cardiac Life Support Program – Current
- CPT through the American College of Sports Medicine Current
- CSCS through the National Strength and Conditioning Association Current
- Texas A&M's Hollingsworth Certificate in Leadership Study and Development

Work Experience

Houston Methodist The Woodlands Hospital - The Woodlands, Texas – March 2018 –

Present

Exercise Physiologist

• Plans, coordinates, implements, and evaluates education and all exercise components of a Phase II Cardiac Rehabilitation program.

Fit Club 24 - The Woodlands, Texas – April 2018 – October 2019

Personal Trainer

Help clients reach their health and fitness goals through individualized coaching
1-3 times per week.

CG Arena - Austin, Texas – August 2017 – March 2018

Personal Trainer/Group Fitness Instructor

- Help individuals reach their health and fitness goals through individualized coaching.
- Prepare and conduct group exercise classes, safely administering to a wide variety of individuals.

Camp Gladiator - Austin, Texas - August 2017 - January 2018

Affiliate Trainer

- Led over 50 groups in personally developed boot-camp style workouts for a variety of fitness levels and ages.
- Generated a personal business plan and was involved with over a dozen marketing events.

Professional Affiliations

Student Member, American College of Sport Medicine - Current

Student Member, Texas Chapter of the American College of Sports Medicine - Current

Member, Clinical Exercise Physiology Association - Current

Professional Membership, National Strength and Conditioning Association - Current