Investigating the Effects of Aerobic Exercise with Blood Flow Restriction on Vastus Lateralis Muscle Oxygenation

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Abstract

Blood flow restriction training (BFRT) is a novel adaptation to traditional forms of aerobic or resistance exercise. By restricting blood flow to the active skeletal muscles, previous research has demonstrated that it can induce similar benefits to musculoskeletal health as non-blood flow restricted (BFR) exercise, despite exercising at lower intensities and for a shorter duration of time. The mechanisms through which BFRT stimulates physiological adaptations remains uncertain however, one proposed stimulus is localized skeletal muscle hypoxia. This thesis aimed to investigate this stimulus by assessing muscle oxygenation during low-intensity aerobic exercise with BFR. Vastus lateralis oxygenation was assessed using continuous-wave near-infrared spectroscopy in 15 participants (n=15) during 20 minutes of BFR and non-BFR exercise sessions. Significant differences in muscle tissue oxygenation was observed ($P<0.001$) indicating that BFR during low-intensity walking exercise reduced muscle oxygenation more so than non-BFR exercise. Furthermore, significant differences in total hemoglobin (THb) and deoxygenated hemoglobin (HHb) were observed ($P<0.05$ and $P<0.001$, respectively), such that THb and HHb were significantly greater during BFR-exercise versus non-BFR exercise. Oxygenated hemoglobin (O_2Hb) on the other hand, was not significantly different between exercise sessions. These findings suggest that BFR during low-intensity aerobic exercise may induce a localized hypoxic response in the skeletal muscle tissues distal to the cuffs. The reduction in muscle oxygenation in the presence of increased tissue blood volume may suggest that BFRT influences oxygenation by moderating blood flow through the active skeletal muscle tissues during exercise.

KEY WORDS: Blood flow restriction training, muscle oxygenation, aerobic exercise, near-infrared spectroscopy.

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List of Abbreviations

ATP	Adenosine triphosphate
AUC	Area-under-the-curve
BF%	Body fat percentage
BFR	Blood-flow restriction
BFRT	Blood-flow restriction training
BMI	Body mass index
BP	Blood pressure
CO	Cardiac output
CO_2	Carbon dioxide
CV	Coefficient of variation
DBP	Diastolic blood pressure
FFM	Fat-free mass
FM	Fat mass
FMD	Flow-mediated dilation
GXT	Graded exercise test
Hb	Hemoglobin
Hb-O_2	Hemoglobin-oxygen
HIF	Hypoxia-inducible factor
HMb	Deoxygenated myoglobin
HR	Heart rate
HR_{Max}	Maximum heart rate
MAP	Mean arterial pressure
Mb	Myoglobin
Mb-O_2	Myoglobin-oxygen
min	Minute
mm Hg	millimeters of mercury
MVC	Maximum voluntary contraction

NO	Nitric oxide
O_2	Oxygen
O_2Hb	Oxygenated hemoglobin
O_2Mb	Oxygenated myoglobin
P_{CO2}	Partial pressure of carbon dioxide
P_{O2}	Partial pressure of oxygen
PP	Pulse pressure
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SE	Standard error
SV	Stroke volume
THb	Total hemoglobin
TOI	Tissue oxygenation index
VEGF	Vascular endothelial growth factor
VO_2	Volume of oxygen consumption
$\dot{V}O_2max$	Maximum volume of oxygen consumption
$\dot{V}O_2peak$	Peak volume of oxygen consumption

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Chapter I: Introduction

Blood flow restriction training (BFRT) is a novel adaptation to the traditional forms of training, combining aerobic or resistance exercises with externally applied compression cuffs to the active limbs (Horiuchi & Okita, 2012, Scott et al., 2014). These cuffs, either simple elastic nylon wraps/tourniquets or a more expensive inflatable version, are placed on the upper arms or legs, depending on both the type of exercise and the skeletal muscles involved in the movement (Pope et al., 2013, Scott et al., 2014). Individuals then exercise at an intensity of 20-30% their maximum voluntary contraction (MVC) for resistance training or at a light aerobic intensity (<64 % maximal heart rate (HR_{max})) (Pope et al., 2013, Pescatello, 2014). BFRT is unique because it has been shown to induce comparable benefits to cardiorespiratory, cardiovascular and musculoskeletal health as resistance and aerobic exercise programs, despite exercising at lower-intensities and for a shorter duration of time than what is thought necessary to stimulate adaptations (Loenneke et al., 2012a, Horiuchi & Okita, 2012, Scott et al., 2014).

Previous studies examining the acute physiological responses to BFRT have demonstrated that the mechanical compression of the cuffs restrict arterial blood flow entering the skeletal muscle tissues while simultaneously occluding venous blood return (Manini & Clark, 2009). Within a short period of time, this results in the congestion of blood within the systemic capillary beds distal to the cuffs as well as the accumulation of plasma within the extracellular spaces (Iida et al., 2005). During blood flow restricted (BFR) exercise, researchers hypothesize that there may be significant metabolic accumulation and changes in muscle oxygenation, and following compression release; reactive hyperemia enhances luminal blood flow through the formerly restricted arteries (Horiuchi & Okita, 2012, Pope et al., 2013). This response develops a

shear stress stimulus for endothelial dependent flow mediated dilation (FMD) thus resulting in the release of several vasodilatory and angiogenic factors (Pyke & Tschakovsky, 2005, Thijssen et al., 2011). Furthermore, there is significant evidence of a systemic hormonal response following a bout of BFRT where hormones such as growth hormone, insulin-like growth factor 1and norepinephrine are released into circulation (Takano et al., 2005, Tanimoto et al., 2005, Abe et al., 2006, Pope et al., 2013). Such physiological stimuli, both during exercise and after cuff release, may enhance the muscle and circulatory responses to exercise. However, research findings to date remain inconclusive regarding the physiological pathways through which BFRT exerts its influence, (Manini & Clark, 2009, Ganesan et al., 2015).

One proposed pathway BFRT may stimulate training adaptations is through hypoxia. Since the amount of oxygen delivered to the body's tissues is dependent on the volume flow rate of arterial blood, disruption of regional blood flow can result in tissue hypoxia during periods of high metabolic activity (Dunn et al., 2016). The mechanical compression exerted by the cuffs during BFRT may disrupt the continuous flow of oxygenated blood to the muscles distal to the cuffs (Kawada, 2005, Manini & Clark, 2009). Therefore as exercise progresses and the metabolic demands of the skeletal muscle tissues increase, a mismatch between oxygen supply and demand may develop, resulting in a hypoxic intramuscular environment. As such, there may be an augmented reduction in intramuscular oxygen content during BFRT in comparison to the degree of oxygen desaturation that occurs normally in skeletal muscles during traditional exercise (Manini & Clark, 2009, Pope et al., 2013). This localized hypoxia accompanying BFR exercise would help to explain some of the muscular adaptations observed by researchers following long-term use of BFRT, such as increased capillarization and muscular hypertrophy (Abe et al., 2010a, Abe et al., 2010b, Evans et al., 2010). For example, acute hypoxia is associated with the

upregulation of genes encoding for vascular endothelial growth factor (VEGF) and anaerobic enzymes and frequent exposure to short-duration hypoxia has been shown to stimulate angiogenesis and increase mitochondrial volume density (Hoppeler & Vogt, 2001). Considering these peripheral adaptations alone to acute hypoxia, it is important to establish whether BFR exercise affects muscle oxygen tension and with this insight it may help researchers to understand how this form of training is capable of evoking physiological change despite reducing the intensity and duration of exercise.

Evidence that BFRT significantly reduces skeletal muscle tissue oxygenation during exercise is both limiting and conflicting. To date, only a few studies have investigated this relationship and these studies have only assessed muscle oxygenation during resistance-based BFR exercise. Thus far, two studies have demonstrated greater reductions in muscle oxygen saturation during BFR exercise in comparison to non-BFR exercise (Tanimoto et al., 2005, Manini & Clark, 2009) and a single study measured no differences in muscle oxygenation between BFR and non-BFR protocols (Ganesan et al., 2015). Additional studies have assessed muscle oxygenation during BFRT; however the intent of the research was to determine the influence of cuff tightness on oxygenation as opposed to comparing and contrasting BFRT to traditional forms of training (i.e. non-restricted aerobic or resistance exercise) (Karabulut et al., 2011, Karabulut et al., 2014). Moreover, only one study to date has investigated which physical characteristics influence the degree of muscle deoxygenation that occurs in response to the application of the BFRT cuffs (Karabulut et al., 2011). Karabulut and colleagues (2011) discovered that leg lean body mass, total lean body mass and thigh circumference were significantly correlated with rectus femoris tissue oxygenation across different restrictive pressures, such that higher leg and total lean mass and larger thigh circumferences resulted in

greater reductions in oxygenation at rest. Despite these results however, no study to date has investigated whether these characteristics or other physical characteristics, such as aerobic capacity, influences the degree of oxygenation during BFR exercise.

Furthermore, in examining the literature there are substantial discrepancies in the methodologies used by researchers, as no standardized protocol for BFRT exists to date (Dankel et al., 2016, Pope at al., 2013). Across research studies, variability in cuff materials, sizes, pressures, and training regimens increase the uncertainty whether BFRT is capable of inducing local tissue hypoxia. Despite not having consistent BFRT protocols, traditionally BFRT involves the application of the restriction cuffs prior to exercise while in a seated position (Abe et al., 2006, Abe et al., 2010b, Ozaki et al., 2010, Loenneke et al., 2012b, Scott et al., 2014). Individuals using the inflatable cuffs, then undergo a warm-up cycle where the cuffs are inflated and then deflated at regular intervals while simultaneously increasing restriction pressure (Abe et al., 2006, Abe et al., 2010b, Ozaki et al., 2010, Loenneke et al., 2012b, Scott et al., 2014). Once the warm-up cycle is completed, the cuffs are then inflated to a training pressure and this is done so in a resting state immediately prior to exercise. For the purpose of this study, traditional BFRT is defined by the above cuff application procedure and any alteration to this procedure is defined as a modified BFRT protocol. In the current study, the BFR cuff application protocol was modified in attempt to augment the BFR stimulus and to compare muscle oxygenation responses to the traditional protocol. In order to increase the BFR stimulus, training pressures were held for a period of time prior to exercise in order to increase the degree of venous blood pooling and decrease muscle oxygen saturation.

Overall, investigators have only assessed skeletal muscle oxygenation during resistance exercise with BFR and it remains to be seen how BFR influences muscle oxygenation during

aerobic exercise. As well, it remains to be seen whether certain physical characteristics, such as aerobic capacity is a determining factor in BFR cuff pressure and a correlating factor in determining the degree of muscle oxygenation during exercise with BFR. Therefore, the objectives of this study were to examine the influence of BFR on vastus lateralis muscle oxygenation during low-intensity aerobic exercise in a young, healthy, adult male population, to determine which physical characteristics of this population influence the degree of muscle oxygenation during an aerobic-based BFRT regimen and to determine which physical characteristics are related to BFRT pressures when pressures are standardized to changes in muscle oxygenation.

1.1 Statement of the Problem

The primary research problem of this study was to investigate the effects of traditional and modified BFRT compared to low-intensity aerobic exercise without BFR on vastus lateralis muscle oxygenation. The secondary research problem was to determine which physical characteristics influence the amount of tissue oxygenation during low-intensity aerobic exercise with and without BFR.

1.2 Hypotheses

The following hypotheses were tested within this study:

1. BFR during walking exercise will decrease vastus lateralis tissue oxygenation compared to non-BFR walking.
2. Increasing the amount of venous blood pooling during a modified BFRT protocol will decrease vastus lateralis muscle oxygenation more so than the traditional BFR protocol.
3. Thigh circumference and aerobic capacity will be positively correlated with the BFRT pressure when training pressures are standardized to muscle oxygenation.
4. Thigh circumference and aerobic capacity will be positively correlated with the change in vastus lateralis tissue oxygenation during low-intensity aerobic exercise with BFR.

Chapter II: Literature Review

2.1 Hypoxia

Hypoxia is defined as a state of reduced oxygen partial pressure (P_{O2}) and/or oxygen concentration from normal (Solaini et al., 2010, MacIntyre, 2014). It can be classified as either systemic, affecting systems of organs, or localized, where it effects specific tissues or cells (Hoppeler et al., 2003). Hypoxia can also be classified in reference to duration of exposure. A permanent hypoxic condition designates exposure to low P_{O2} over the lifespan of a particular organism, whereas long-term hypoxia refers to hypoxic conditions from weeks to months of exposure and is associated with the acclimatization process (Hoppeler et al., 2003). Hypoxia can also be classified as short-term in nature, with exposure duration lasting from hours to minutes and these short-term exposures can be further divided into continuous or intermittent (Hoppeler et al., 2003). It is continuous short term hypoxic conditions that are associated with physical exercise and therefore it is in this context that we will discuss the acute physiological responses to hypoxia in the following literature review.

2.1.1 Tissue Hypoxia

In the body, the transportation of oxygen is a product of complex interactions between convective and diffusive processes. In particular, the active transport of oxygenated blood by the heart from the pulmonary to systemic circulation and the movement of oxygen down pressure gradients within the lungs and between systemic capillaries and the body's tissues (Leach and Treacher, 1998, Dunn et al., 2016). Tissue hypoxia can arise if there is disruptions to these processes, particularly if there is modification to (1) arterial oxygen content (2) oxygen delivery, and (3) oxygen extraction/utilization (MacIntyre, 2014, Dunn et al., 2016).

2.1.1.1 Oxygen Delivery

Oxygen delivery is the process of transporting oxygenated blood from the pulmonary circulation to the body's tissues. This process is affected by factors such as cardiac output (CO), regional blood flow, arterial blood oxygenation, hemoglobin (Hb) concentration as well as structural and functional characteristics of circulation (Heistad et al., 1980, Dunn et al., 2016). CO specifically affects global oxygen delivery, which is defined as the amount of oxygen delivered to tissues each minute (Dunn et al., 2016). Increasing CO increases the overall amount of oxygen delivery; however this is independent of blood distribution in the body (Leach and Treacher, 2002). Consequently, depending on regional metabolic demands, increasing CO may or may not increase oxygen delivery to a specific tissue (MacIntyre, 2014). For example during exercise, increased CO and increased redistribution of blood to skeletal muscle tissue, increases oxygen delivery to this particular tissue (MacIntyre, 2014). The importance of regional distribution of blood flow on oxygen delivery is therefore apparent and a key determinant of tissue hypoxia (MacIntyre, 2014). Systemic factors, such as sympathetic vascular tone, systemic inflammatory mediators (e.g. leukotrienes) and local factors, such as endothelial-derived nitric oxide (NO), redirect the flow of blood to areas of greatest metabolic need by modulating the vasculature (MacIntyre, 2014). Returning to the example of exercise, stimulation of α_1-adrenergic receptors by norepinephrine in abdominal viscera constricts vessels supplying blood to gastrointestinal organs and stimulation of β_2-adrenergic receptors results in vasodilation of vessels in skeletal muscle (Gordon et al., 2015). As a result, significant blood flow is redistributed to skeletal muscles to supply the active tissues with adequate oxygen and nutrients to meet the demands of metabolism. However, when metabolic demand remains high and there is a disruption in the distribution of blood flow to the active tissues, hypoxia within the tissue ensues (MacIntyre, 2014, Dunn et al., 2016).

Furthermore, oxygen delivery is also affected by the arterial oxygen content, which is the sum of oxygen bound to Hb and oxygen dissolved in blood plasma (Samuel & Franklin, 2008, Dunn et al., 2016). The amount of oxygen bound to Hb is affected by factors such as blood Hb concentration, oxygen carrying capacity of Hb, and arterial Hb saturation, whereas the amount of oxygen dissolved in plasma is dependent on the plasma solubility of oxygen as well as the P_{O2} in arterial blood (Dunn et al., 2016). Overall, a decrease in arterial oxygen content (i.e. hypoxemia) may prompt tissue hypoxia if the body fails to adjust accordingly. Compensation can be achieved by either increasing oxygen delivery to the active tissues (e.g. increasing CO or regional blood flow) or by decreasing tissue oxygen consumption (Samuel & Franklin, 2008). Failure to do so will result in a mismatch between oxygen supply and demand within the active tissues, progressing to hypoxia (Dunn et al., 2016).

2.1.1.2 Oxygen Extraction/Utilization

Oxygen extraction and utilization is defined as the process through which oxygen disassociates from Hb, diffuses into cells and is used by mitochondria during oxidative phosphorylation (MacIntyre, 2014). These processes are affected by the rate of oxygen delivery, the hemoglobin-oxygen (Hb-O_2) dissociation relationship, the magnitude of the arterial blood to cellular P_{O2} gradient, the diffusion distance between circulation and cells as well as metabolic rate (Leach and Treacher, 2002, MacIntyre, 2014, Dunn et al., 2016). Oxygen extraction can also be defined as the amount of oxygen delivered by the heart and the vasculature and is used by the tissues, thus it is the ratio of oxygen consumption to oxygen delivery (Dunn et al., 2016). In healthy adults, approximately 20-30% of the oxygen delivered to tissues is used at rest and during exercise the extraction ratio increases up to 50%, peaking at an extraction of 75-80% during maximal exercise (MacIntyre, 2014, Dunn et al., 2016). During exercise, the oxygen

extraction ratio of skeletal muscle increases by approximately 20-30% because the amount of oxygen delivered to these tissues increases from increased muscle perfusion (Kime et al., 2009, Dunn et al., 2016). With increasing exercise intensity the amount of oxygen delivered to the muscle concurrently increases however, it does not match the increase in oxygen consumed (Dunn et al., 2016). Therefore, oxygen consumption by muscle tissues will reach a plateau and the extraction ratio from circulation will peak, with the greatest extraction ratio being observed at maximal intensity exercise.

A significant determinant of oxygen extraction is the Hb-O_2 dissociation relationship. Approximately 98% of the oxygen transported in blood is reversibly bound to Hb, an iron-containing protein found within erythrocytes (Pittman, 2011, Hooley, 2015). The iron-containing group is a heme prosthetic group and Hb contains 4 heme groups and is therefore capable of binding to four oxygen molecules (Schechter, 2008, Pittman, 2011, Hooley, 2015). Hb and oxygen display a unique form of interaction called cooperativity, where the binding or release of one oxygen molecule increases or decreases the affinity of Hb to additional molecules of oxygen respectively (Schechter, 2008, Pittman, 2011). The oxygen dissociation curve of Hb is used to illustrate this relationship graphically (Figure 2-1):

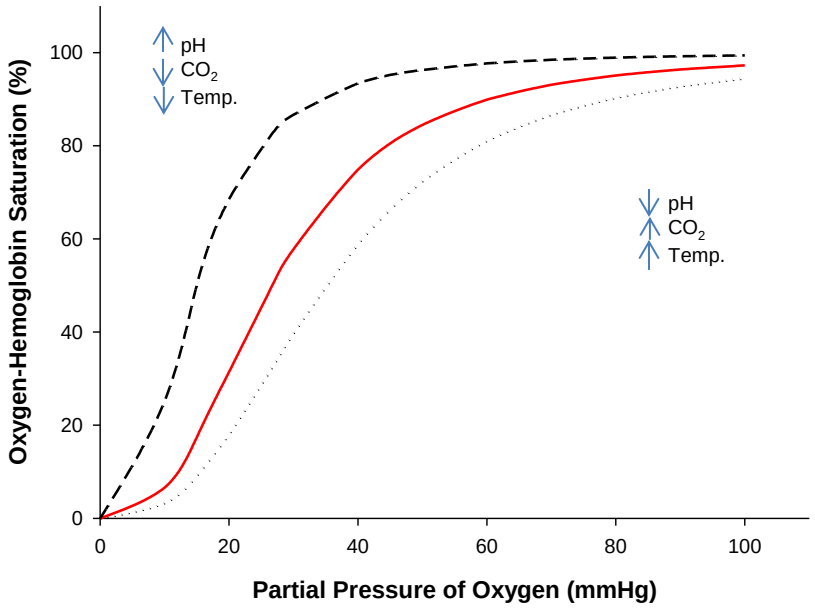

Figure 2-1. The oxygen dissociation curve of hemoglobin. Notes: The solid red line indicates the normal curve whereas the two dashed lines represent the Bohr shift. The large dash line (left) represents the leftward shift resulting in hemoglobin increasing its affinity for oxygen. The small dash line (right) represents the rightward shift resulting in hemoglobin decreasing its affinity for oxygen. Generated using the Hill Equation and using standard conditions for the normal curve (temperature = 37 °C, pH = 7.4 and P_{CO2} = 40 mmHg). 50% oxygen saturation for hemoglobin is displayed at a partial pressure of oxygen of 26.7 mmHg.

This curve demonstrates the affinity of Hb for oxygen by displaying the saturation percentage of Hb with oxygen at various P_{O2}. However, due to the influence of cooperativity, the oxygen saturation of Hb increases sigmoidally rather than exponentially or linearly (Pittman, 2011, Hooley, 2015, Dunn et al., 2016). Typical arterial P_{O2} ranges from 80 to 100 mmHg, whereas arterial oxygenated Hb saturation plateaus around 97%, therefore under normal conditions small fluctuations in arterial P_{O2} have little effect on the amount of oxygen bound to Hb (Hooley, 2015). In the body's tissues on the other hand, P_{O2} varies significantly ranging from approximately 30 to 70 mmHg depending on the tissue type (Carreau et al., 2011). Skeletal muscle for example, displays a mean P_{O2} of 29.2 ± 1.8 mmHg whereas the mean P_{O2} within the large intestine has been determined to be 57.6 ± 2.3 mmHg in a healthy population (Carreau et al

2011). As a consequence of the differential partial pressure gradients, as blood moves through systemic capillaries oxygen is released by Hb and diffuses into the surrounding cells (Dunn et al., 2016). This relationship however is significantly affected by various physiological and chemical factors relating to pressure gradients, blood pH, carbon dioxide partial pressure (P_{CO2}), as well as temperature (Pittman, 2011, Dunn et al., 2016). As blood pH becomes more acidic, and P_{CO2} and temperature increase, the Hb-O_2 curve shifts to the right, as seen in Figure 2-1. The specific relationship between Hb, oxygen, pH and P_{CO2} is referred to as the Bohr Effect and the rightward shift reflects Hb's decreased affinity for oxygen at any given P_{O2} (Pittman, 2011, Dunn et al., 2016). The purpose of the Bohr shift is to increase the availability of oxygen to meet the metabolic demands of the body's tissues (Pittman, 2011, Dunn et al., 2016). For example, when metabolic demands increase, such as during exercise, more oxygen is required by energy producing cells to convert chemical energy into mechanical energy. As exercise continues, CO_2 accumulates in the tissues as a by-product of metabolism and the pH of the blood becomes more acidic (Belardinelli et al., 1995, Pittman, 2011). Both CO_2 and pH affect the Hb-O_2 dissociation curve through allosteric regulation of deoxygenated Hb (Pittman, 2011). The resulting conformational change of the Hb protein reduces its affinity for oxygen and facilitates oxygen offloading to increase oxygen delivery to tissues (Pittman, 2011).

Another important oxygen-binding protein is myoglobin (Mb). Mb is another hemeprotein originating within the sarcoplasm of both cardiomyocytes and skeletal muscle fibres (Kelly et al., 1991, Wittenberg & Wittenberg, 1975, Wittenberg & Wittenberg, 2003). It functions to transfer and deliver oxygen from the sarcolemma of these muscle tissues to the mitochondria in order to maintain optimal intracellular oxygen tension (Kelly et al., 1991, Wittenberg & Wittenberg, 1975, Wittenberg & Wittenberg, 2003). Mb may also function as a

storage site for oxygen, buffering short term changes in oxygen concentration and stabilizing oxygen supply to active muscle tissues (Wittenberg & Wittenberg, 1975, Wittenberg & Wittenberg, 2003, Schechter, 2008). Moreover, this carrier protein is distinguishable from blood Hb due to its chemical structure and oxygen binding capacity (Ordway & Garry, 2004, Schechter, 2008). It is comprised of a single polypeptide chain and heme prosthetic group and is therefore limited in binding to a single oxygen molecule (Wittenberg & Wittenberg, 1975, Ordway & Garry, 2004, Schechter, 2008). As a result, Mb does not display cooperative binding and in contrast to the sigmoidal dissociation curve of Hb-O_2, Mb-O_2 demonstrates a hyperbolic oxygen saturation relationship (Ordway & Garry, 2004) (See Figure 2-2).

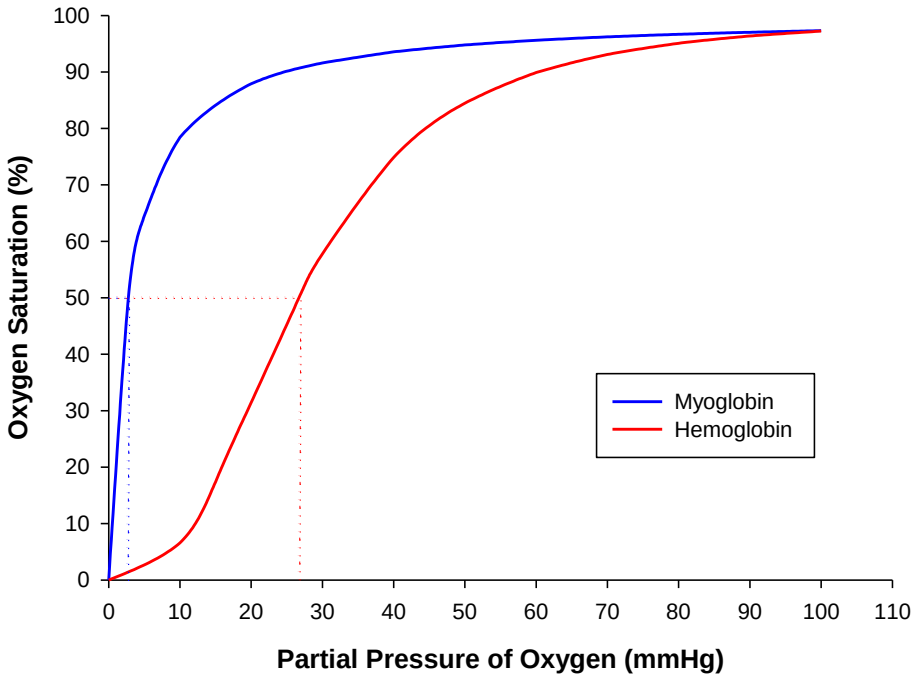

Figure 2-2. Myoglobin-oxygen dissociation curve compared to the hemoglobin-oxygen dissociation curve. Notes: Myoglobin displays a hyperbolic shaped curve whereas hemoglobin displays the sigmoidal-shaped curve. Generated using the Hill Equation and using standard conditions (temperature = 37 °C, pH = 7.4 and P_{CO2} = 40 mmHg). 50% oxygen saturation for hemoglobin and myoglobin is displayed at a partial pressure of oxygen of 26.7 mmHg and 2.75 mmHg, respectively.

The Mb-O_2 disassociation curve reveals the narrow range of P_{O2} in which Mb exists in its deoxygenated form and this illustrates the functional roles of Mb within muscle tissue. In order to be an effective intracellular carrier of oxygen, Mb must display a higher affinity for oxygen in comparison to Hb (Ordway & Garry, 2004). This ensures that the oxygen released by Hb at the capillary-tissue interface is collected by Mb and transported within cardiomyocytes and myocytes alike. As such, Mb displays 50% oxygen saturation when the P_{O2} is equal to 2.8 mmHg and in contrast, Hb displays 50% oxygen saturation at a much higher pressure of 26.7 mmHg (Kelly et al., 1991, Pittman, 2011). Although Mb demonstrates the ability to facilitate O_2 diffusion, this role also parallels that of diffusion of oxygen across the capillary membrane and therefore oxygen tension may not entirely be dependent on Mb's capacity to transport oxygen within muscle tissue (Ordway & Garry, 2004). Furthermore, the high binding affinity of Mb for oxygen also plays an important role when muscle P_{O2} becomes very low. Within the lower ranges of oxygen pressure, oxygenated Mb (O_2Mb) contributes a significant portion of oxygen supply and deoxygenated Mb (HMb) transports a significant amount of the total oxygen absorbed by muscle tissues (Wittenberg & Wittenberg, 1975, Ordway & Garry, 2004). These characteristics are related to Mb's role in oxygen storage and P_{O2} buffering and in the absence of adequate oxygen; intracellular oxygen concentration can be maintained for cellular metabolism.

Beyond Mb's ability to bind to and transfer oxygen, this carrier protein may also play important roles in regulating both the rate of capillary oxygen delivery to muscle tissue and the rate of oxygen utilization by the mitochondria through NO-dependent pathways (Ordway & Garry, 2004). Research suggests that O_2Mb is capable of binding to sarcoplasmic NO, preventing it from binding to cytochrome *c* oxidase of the electron transport chain in mitochondria (Ordway & Garry, 2004). Such a reaction, diminishes the inhibitory action of NO on mitochondrial

respiration, thus Mb indirectly regulates the rate of oxygen utilization within myocytes (Wittenberg & Wittenberg, 2003). Furthermore, O_2Mb may also be capable of regulating the rate of capillary oxygen delivery by binding to endogenous NO, diminishing the NO-dependent vasodilatory response (Wittenberg & Wittenberg, 2003). Ultimately, the resulting reduction in capillary circulation would decrease the amount of oxygen delivered to myocytes.

Another important factor in determining oxygen extraction and utilization is the diffusion of oxygen from arterial blood into the oxygen-consuming cells. The rate at which gases diffuse across a membrane is determined by Fick's Law, which states that the rate of diffusion is directly proportional to the capillary surface area and the partial pressure concentration gradient across the capillary membrane and is inversely proportional to the diffusion distance (Dunn et al., 2016). Thus adapting Fick's Law for capillary oxygen exchange:

$$Flux = \frac{DA(\Delta C)}{T}$$

Where flux equals the rate of oxygen diffusion across a capillary membrane, *D* is the diffusion constant (capillary permeability) which combines factors such as molecular size, charge and lipid solubility, *A* is the capillary surface area, ΔC is the concentration gradient or difference in P_{O2} between capillary blood and the gas-consuming cell, and *T* is the diffusion distance. Therefore, considering the aforementioned relationships, oxygen will rapidly diffuse from capillary circulation and into cells given a high P_{O2} gradient exists and/or the diffusion distance is small, and conversely, the rate of oxygen diffusion will decrease given the P_{O2} gradient is low and the diffusion distance is increased. This relationship is important because factors influencing diffusion have a significant effect on tissue oxygenation. Thus despite changes to oxygen

delivery (i.e. CO and regional blood flow), tissue hypoxia is also impacted by the diffusive properties of oxygen transport (Dunn et al., 2016).

Furthermore, research also suggests that oxygen diffusion and tissue oxygenation may be impaired by interstitial edema. The excessive accumulation of fluid within tissues, through increased vascular permeability and/or fluid infusions (e.g. saline infusion), increases the diffusion distance between capillary circulation and the surrounding cells (Leach & Treacher, 2002). Consequently, the rate of oxygen diffusion across the capillary membrane decreases. Although this relationship seemingly affects the rate of diffusion, its influence on oxygen extraction is contingent on factors such as arterial P_{O2}, blood flow and Hb concentration (Leach & Treacher, 2002). With a normal diffusion distance, as illustrated in Figure 2-3a, the relationship between oxygen delivered to oxygen consumed is preserved when arterial P_{O2}, Hb concentration and blood flow decreases (Leach & Treacher, 2002). However with increasing distance (i.e. tissue edema), the relationship is altered only when arterial P_{O2} decreases (i.e. hypoxemia) yet remains unchanged when blood flow or Hb concentration decreases (as illustrated in Figure 2-3b) (Leach & Treacher, 2002). Therefore, under circumstances of increased diffusion distance and low concentrations of arterial oxygen, it is necessary for greater amounts of oxygen to be delivered at lower levels of metabolic demand due to the disruption in arterial oxygen tension (Leach & Treacher, 2002). Therefore, hypoxia associated with excessive fluid accumulation within the tissue is more pronounced if the reduction in oxygen delivery is related to changes in arterial oxygen versus changes in flow or blood Hb.

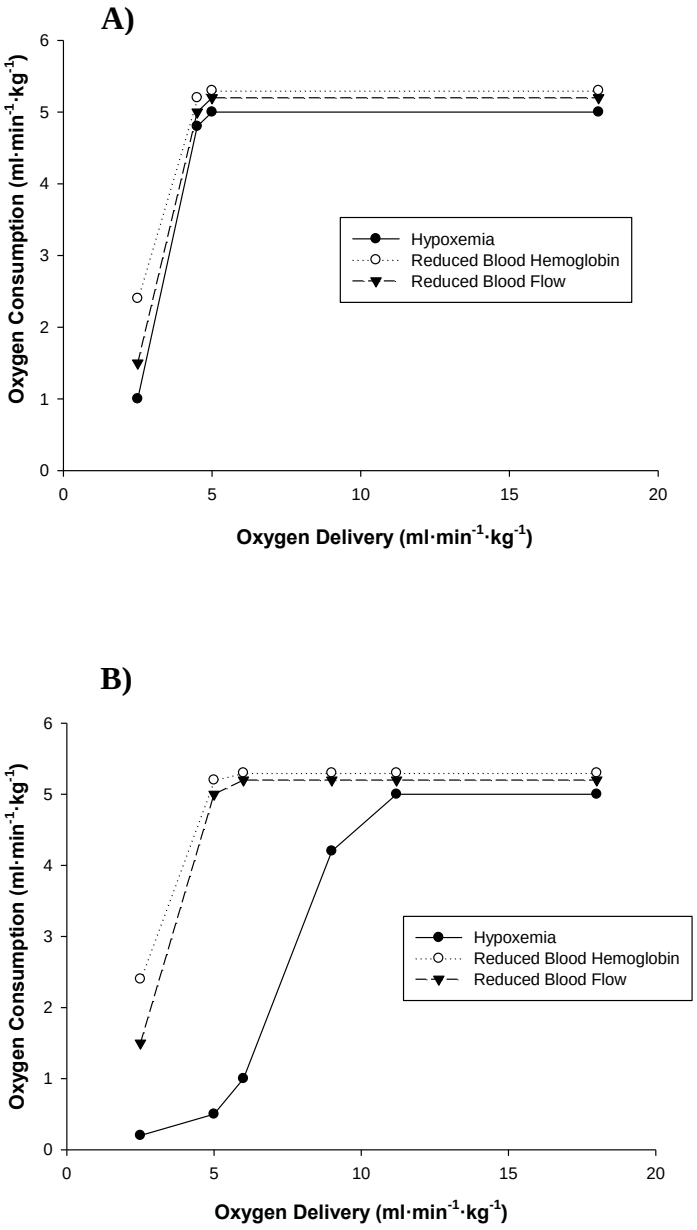

Figure 2-3. The influence of diffusion distance on the oxygen delivery/consumption relationship when muscle tissue is exposed to hypoxic blood, anemic blood and low blood flow conditions. Notes: The graph on the top represents normal diffusion distance (A) whereas the graph on the bottom demonstrates the effect of increasing diffusion distance (B). Adapted from Leach and Treacher (1991).

2.1.1.3 Arterial Oxygen Content

One of the most important determinants of tissue oxygen content is the amount of oxygen circulating in arterial blood. When low levels of oxygen are circulating in systemic arteries, this condition is referred to as hypoxemia (MacIntyre, 2014). Arterial blood is considered mildly hypoxemic if at rest P_{O2} is between 60 to 80 mmHg and it is considered severely hypoxemic if P_{O2} decreases below 60 mmHg (MacIntyre, 2014). One profound factor that influences the amount of arterial oxygen is the P_{O2} of inspired air. For example at sea level, P_{O2} is approximately 150-160 mmHg, while normal arterial P_{O2} ranges between 80 to 100 mmHg (Hoppeler et al., 2003, MacIntyre, 2014, Hooley, 2015). Conversely at low atmospheric $P_{O2,}$ such as at the summit of Mount Everest where P_{O2} is equal to approximately 43 mmHg, arterial P_{O2} can decrease to as low as 30 mmHg (West et al., 1983, Grocott et al., 2009). The reason for the difference in arterial P_{O2} is attributed to both the partial pressure gradient across the alveolar-capillary membrane and the Hb-O_2 dissociation relationship. Typically under normoxic conditions, alveolar P_{O2} ranges from 100 to 110 mmHg and deoxygenated blood contained within the pulmonary capillaries ranges between 20 to 40 mmHg (Hoppeler et al., 2003, MacIntyre, 2014). This difference in the P_{O2} promotes the diffusion of oxygen across the alveolar membrane and into the capillaries, as oxygen moves down its pressure gradient (Erecinska and Silver, 2001). Under hypoxic atmospheric conditions however, alveolar P_{O2} is significantly lower due to a lower atmospheric P_{O2}. This ultimately reduces the potential energy of oxygen's pressure gradient across the alveolar membrane therefore decreasing the rate of diffusion of oxygen into capillary circulation (MacIntyre, 2014). As a result, arterial P_{O2} significantly decreases and depending on how much oxygen diffuses into circulation, ultimately determines the amount of oxygen that diffusion into the body's tissues.

Another important factor that influences arterial oxygen content and consequently the amount of oxygen within tissues is the transit time for which erythrocytes pass through capillary circulation. In healthy populations, oxygen diffusion across the alveolar membrane and binding to Hb occurs within approximately 250 ms, and at rest, a single red blood cell passes through an alveolar capillary within 500-750 ms (MacIntyre, 2014). If the rate of pulmonary circulation increases, as demonstrated during exercise, so that it surpasses the rate for adequate oxygen transfer, the shorter transit time may result in hypoxemia and progress into tissue hypoxia (MacIntyre, 2014). An additional determinant of arterial oxygen content is the amount of Hb within the blood. In healthy adult populations, the average Hb concentration ranges from 13.5 to 18.0 $g \cdot dL^{-1}$ in men and 11.5 to 16 $g \cdot dL^{-1}$ in women (Dunn et al., 2016), however in diseased populations' Hb levels can decrease to less than 11 $g \cdot dL^{-1}$, resulting in a blood condition referred to as anemia (Beutler and Waalen, 2006, Gilbertson et al., 2009). Recall, approximately 98% of the oxygen in the blood is bound to Hb therefore lower concentrations of this particular blood protein will greatly reduce arterial P_{O2} and this will affect tissue oxygenation.

2.1.2 Physiological Responses to Hypoxia

Physiological responses to hypoxia are the result of complex interactions involving many different compensatory pathways. Often the final response observed from a particular tissue is a product of both excitatory and inhibitory signals relayed from both central and peripheral control mechanisms (Ursino & Magosso, 2000). Furthermore, hypoxia-induced physiological responses are phasic in nature. Early phase responses occur within seconds of hypoxia detection and involve an oxygen-sensitive sensory system that is capable of regulating the cardiorespiratory functions quickly through neural reflexes (Ursino & Magosso, 2000). The primary goal of the initial compensatory responses is to preserve P_{O2} of tissues that require significant metabolic

energy, such as cerebral and cardiac tissues (Ursino & Magosso, 2000). Late phase responses on the other hand involve further modification of cardiorespiratory functions and participation of extraneous reflex systems, such as the baroreflex and the lung stretch reflex (Ursino & Magosso, 2000). These responses will vary depending on the magnitude of oxygen deprivation and whether the hypoxia is systemic or localized, however similar to the early phase responses, secondary compensation mechanisms work to preserve functioning of vital tissues and to improve systemic oxygen delivery.

Prior to discussing the effects of hypoxia on the body and how the body compensates for such a stress, it is important to briefly discuss the regulation of cellular metabolism and how hypoxia affects this process. One of the most vital components to the health of all living cells is the adequate production of energy (Boutilier, 2001). Almost all intracellular processes (i.e. cellular growth, maintenance, enzymatic reactions etc.) whether directly or through indirect means, require the production of adenosine triphosphate (ATP) to fuel chemical reactions (Boutilier, 2001). For example, thermodynamically unfavourable reactions, such as the movement of ions against their electrochemical gradient via ion transports depend upon the hydrolysis of the high energy phosphate bonds of ATP to maintain ionic and osmotic equilibriums (Boutilier, 2001). During severe ATP depletion, failure to maintain these gradients can result in the extensive movement of ions across the cellular membrane, inhibition of cellular activity and the catabolism of important cellular components (i.e. enzymes, organelles) (Boutilier, 2001). In this context, the significant mismatch between ATP supply and demand will lead to an incapacitating cascade of events, where the intracellular environment deteriorates, the integrity of the plasma membrane becomes compromised and the cell ruptures. Ultimately, a sufficient store of energy is essential for maintaining a viable intracellular environment and if

cells are unable to support their metabolic requirements, this will result in debilitating consequences.

An important factor that significantly affects the ability of cells to produce sufficient energy is the availability of oxygen. Oxygen is an important component of mitochondrial oxidative phosphorylation, which is the primary metabolic pathway for energy production (Solaini et al., 2010). In fact, mitochondria use the greatest amount of oxygen out of all organelles within cells, consuming approximately 85-90% of all inspired oxygen (Solaini et al., 2010). In brief, oxidative phosphorylation is a chemical process through which high energy electron carriers are oxidized by a series of intermembrane proteins and organic molecules within mitochondria. As high energy electrons are transported along the molecular chain, energy released from redox reactions is used to generate an electrochemical gradient across the inner mitochondrial membrane and the energy stored within this gradient is used to drive the phosphorylation of adenosine diphosphate into ATP. At the end of the electron transport chain is where oxygen plays an important role in this process. Oxygen is used as the final electron acceptor, reducing from its diatomic form into water, allowing for the regeneration of the original electron carriers and the continuation of mitochondrial respiration.

Under hypoxic conditions, this electron transport chain becomes inhibited and the mitochondria are unable to regenerate the electron carriers necessary for ATP production (Connett et al., 1990, Michiels, 2004). As a result, the ATP supply becomes limiting and cells must adapt to the low concentrations of oxygen by increasing anaerobic energy production and by reducing nonessential ATP consumption (Connett et al., 1990, Michiels, 2004). An important consideration is that some cell types are more sensitive to the changes in intracellular oxygen concentration and despite compensatory pathways, they cannot withstand prolonged exposure to

hypoxia. For example, upon cessation of cerebral perfusion neuronal cells are only capable of functioning for <3 minutes during hypoxia before necrosis (Leach & Treacher, 1998). In contrast, skeletal muscle cells display significantly greater tolerance for low oxygen supply and can survive approximately 60-90 minutes before damage occurs (Leach & Treacher, 1998). Researchers believe that the difference in sensitivity to hypoxia may be related to the electrical activity of a particular cell type (Michiels, 2004). For example, up to 80% of all ATP consumption of neuronal cells is associated with the use of ATP driven ion transports (Michiels, 2004). These ion transports are vital to the generation of action potentials and therefore have a crucial role in the transmission of signals between neurons. Neuronal cells are continuously relaying information from sensory receptors in the periphery to the central nervous system for processing and interpretation; therefore they require a substantial amount of ATP to support their functional roles. On the other hand, skeletal muscle cells are more resistant to hypoxia and only 20% of their total ATP consumption is associated with the use of ATP dependent ion transports (Buttgereit & Brand, 1995). Accordingly, researchers speculate that the differential sensitivity to hypoxia may largely be affected by a cells dependence on the active transport of ions against their electrochemical gradients however further investigation is required (Semenza, 2006). Nonetheless, all cells within the body react to changing oxygen concentrations and respond to hypoxia whether the reduced P_{O2} is acute or chronic in nature (Semenza, 2006). Acutely, cells are capable of altering their total metabolic activity to support essential biochemical processes, whereas chronic exposure to hypoxia may lead to epigenetic changes to improve cellular viability within low oxygen concentrations (Semenza, 2000). The following sections will now discuss in detail the cellular and tissue physiological responses to hypoxia.

2.1.2.1 Cellular Physiological Responses to Hypoxia

At the cellular level, hypoxia results in an increase in anaerobic metabolism in order to maintain functional levels of ATP to power metabolic reactions. Recall, oxygen is a key regulator of the electron transport chain therefore reductions in intracellular P_{O2} can affect the ability of mitochondria to regenerate ATP through aerobic metabolism (i.e. oxidative phosphorylation). An important note however, is that acute exposure (seconds to minutes) to hypoxia does not reduce the flux of electrons through the electron transport chain nor does it affect the concentration of the high-energy electron carriers (Wheaton & Chandel, 2011). Therefore, only when exposure is chronic in nature, lasting hours to days, does hypoxia influence the rate of aerobic metabolism within cells. Nonetheless despite reductions in oxygen concentration and ATP supply, metabolic requirements of cells initially remain constant resulting in a mismatch between energy supply and demand. This disparity results in the activation of non-oxidative pathways to increase the amount of ATP available for cellular use, however anaerobic ATP production through glycolysis cannot sustain functional energy levels indefinitely (Boutilier, 2001).

Another cellular response to hypoxia is the reallocation of existing energy stores to support the essential ATP consuming reactions. Work from Buttgereit and Brand (1995) established a hierarchy for intracellular processes that demand energy and according to their research, the most sensitive to energy supply is the synthesis of macromolecules, namely the synthesis of proteins and polynucleotides (i.e. DNA/RNA). In other words, following a reduction in energy supply, processes that synthesize macromolecules are the first to be inhibited as they are not necessary for the immediate needs of cells. ATP-dependent sodium ion channels (i.e. sodium/potassium-ATPase) display the next highest sensitivity to intracellular energy stores

followed then by ATP-dependent calcium ion channels. Failure to support ion channel activity results in significant movement of ions across the plasma membrane and if such an ion movement is left uncontrolled it will result in the disruption of intracellular processes as well as deterioration of cellular structure. The least sensitive intracellular process to a reduction in cellular energy is mitochondrial proton leak, as Buttgereit and Brand (1995) demonstrated that the movement of protons across the inner mitochondrial membrane takes the greatest priority of all ATP-consuming processes. Proton leak is an important component in dissipating redox energy generated by high-energy electrons moving across the electron transport chain during oxidative phosphorylation (Rolfe et al., 1999). The continuous cycling of protons across the mitochondrial membrane maintains the proton electrochemical gradient that is essential to oxidative ATP production. Furthermore, it has been speculated that proton leak functions in maintaining thermoregulatory processes, reducing free radical production as well as increasing sensitivity of substrates to oxidative energy production (Rolfe & Brand, 1997). Overall when ATP supply becomes limiting, such as in the example of cellular hypoxia, reactions not essential for the immediate needs of the cell, such as biosynthesis of molecules, are first to be inhibited before processes that maintain ionic and osmotic electrochemical gradients.

Intermittent hypoxia in skeletal muscle tissue also appears to result in the formation of mitochondrial reactive oxygen species (ROS). ROS formation peaks when the skeletal muscle tissue transitions from normal P_{O2} to hypoxic P_{O2}, however the exact mechanisms through which this process occurs is not fully understood (Clanton, 2007). Researchers speculate that ROS formation during hypoxia may serve to protect skeletal muscle tissue by inhibiting its contractile function thus preserving its intracellular energy stores (Clanton, 2007). In addition, ROS formation may also contribute to the signaling of adaptive responses to hypoxia, such as the

stabilization of hypoxia-inducible factor (HIF) proteins, specifically HIF-1. These transcription factors have been associated with increasing the expression of hypoxic-inducible genes encoding for glucose transporters, erythropoietin, angiogenic factors (VEGF) as well as glycolytic enzymes (phosphofructokinase) (Hoppeler et al., 2003, Clanton, 2007, Wheaton & Chandel, 2011). ROS may also be associated with influencing intracellular skeletal muscle cell calcium ion concentrations during hypoxia (Clanton, 2007). The sarcolemmal membrane of skeletal muscle tissues depolarizes when exposed to mild hypoxia, resulting in the opening of voltage-gated calcium ion channels (Clanton, 2007). These channels allow the influx of calcium ions as they move down their electrochemical gradient, resulting in a significant rise in intracellular calcium concentration. High sarcoplasmic calcium concentration can result in membrane degradation, increased protease activity as well as a conditional state known as contracture, which is defined as the permanent shortening and hardening of muscle tissue (Cherry, 1980, Clanton, 2007). ROS may be associated with these responses by preventing the removal of calcium from the sarcoplasm as well as promoting the movement of extracellular calcium into skeletal muscle cells (Clanton, 2007). Although doing so would damage the muscle tissue, researchers believe that such a regulatory response during hypoxia could be related to an oxygen sensory role that would activate compensatory pathways, however more investigation is required (Clanton, 2007). Another potential significant role of hypoxia-induced ROS formation is the regulation of vasomotor tone of vessels within skeletal muscle tissue. Richardson and colleagues (2007) investigated the effects of free radicals on brachial arterial blood flow during exercise and discovered that upon administration of a mixture of antioxidants (vitamins C, E and alpha-lipoic acid), vasodilation significantly decreased. This suggests that oxidative stress from ROS may have an important role in regulating local blood circulation and this would prove beneficial

during hypoxia as low P_{O2} has an inhibitory influence on local NO production (Clanton, 2007). Therefore during hypoxia, despite a reduction in local NO production, ROS may compensate for the inhibited endothelial dilatory response and promote vasodilation of local vessels supplying hypoxic skeletal muscle tissue.

2.1.2.2 Cardiovascular Responses to Hypoxia

Cardiovascular responses to hypoxia are dependent on whether the hypoxic stress is localized to a particular tissue or it is affecting the entire body. Local hypoxia occurs when hypoxic blood enters the vascular bed of a tissue despite normal levels of oxygenation being preserved throughout the body (Heistad et al., 1980). Under such conditions, vessels carrying the hypoxic blood will dilate however the arterial oxygen pressure must be reduced to <40 mmHg before there is substantial local vasodilation (Daugherty et al., 1967, Heistad et al., 1980). Furthermore, researchers have also demonstrated a variable dilatory response to hypoxia amongst different tissues (Heistad et al., 1980). Daugherty and colleagues (1967) illustrated this difference examining the effects of local hypoxemia on coronary and renal arterial perfusion pressures at constant blood flow. When blood flow is constant, changes in perfusion pressure reflect changes in local vascular resistance therefore the researchers used this relationship to examine the influence of hypoxia on vessel diameter. When these researchers reduced coronary arterial P_{O2} from 114 mmHg to 21 mmHg they observed ~30 % reduction in coronary perfusion pressure (110 mmHg to 78 mmHg), whereas in comparison reducing renal arterial P_{O2} from 105 mmHg to 17 mmHg only reduced renal perfusion pressure by ~10% from 114 mmHg to 102 mmHg (Daugherty et al., 1967). Therefore, the researchers concluded that the vascular beds of tissues that are most sensitive to changes in oxygen pressure, such as cerebral and cardiac, demonstrate the greatest reductions in vascular resistance during local hypoxia.

In regards to systemic hypoxia, cardiovascular responses are regulated by neural reflexes and the ventilatory response to hypoxia (Ursino & Magosso, 2000). The neural compensatory pathways involve an oxygen-sensitive sensory system comprised of specialized chemoreceptors (Ursino & Magosso, 2000). These chemoreceptors are located within aortic and carotid bodies and they are responsible for monitoring arterial oxygen content (Ursino & Magosso, 2000). When arterial P_{O2} is reduced below 40 mmHg, these peripheral receptors initiate a neural compensatory pathway called the chemoreflex, which alters both parasympathetic and sympathetic activity of certain tissues (Leach & Treacher, 1998, Schultz & Ding, 2007, Chapleau & Sabharwal, 2011). Alternatively, ventilatory control of the chemoreflex cardiovascular responses is mediated by specialized mechanoreceptors located within the smooth muscle of bronchi and bronchioles (Ursino & Magosso, 2000). If these pulmonary stretch receptors do not detect changes in tidal lung volume, than the primary chemoreflex responses are observed. Conversely, activation of pulmonary stretch receptors triggers another reflex pathway which results in secondary chemoreflex responses.

Recall, physiological responses to hypoxia are phasic in nature. During the early phase adaptations to hypoxia ventilatory rate and volume remain constant and pulmonary stretch receptors are not activated. As a result the primary chemoreflex responses are observed and this consists of increased cardiovagal activity as well as sympathetic activity to peripheral efferents (Heistad et al., 1980, Marshall et al., 1994, Chapleau and Sabharwal, 2011, Paton et al., 2013). These alterations in autonomic output result in the lowering of HR and the constriction of vascular beds supplying blood to skeletal muscle tissue, renal and visceral organs (Vatner et al., 1980, Paton et al., 2013). An interesting observation however is that CO remains constant during the early phase responses to systemic hypoxia (Ursino & Magosso, 2000). Researchers speculate

that maintenance of CO is a protective response, as increasing HR and stroke volume would significantly increase metabolic demands of cardiac tissue during a period of oxygen deprivation (Ursino & Magosso, 2000). The reflex vasoconstriction response on the other hand compensates for the direct vasodilating effect of local hypoxia, decreasing blood flow to the peripheral tissues such as skeletal muscle and renal tissues, thereby reducing their oxygen uptake (Heistad et al., 1980). Furthermore, the primary chemoreflex response increases peripheral vascular resistance and this consequently increases systemic arterial pressure (Heistad et al., 1980, Vatner et al., 1980, Schultz & Ding, 2007).

During the later phase of systemic hypoxia responses, carotid chemoreceptors influence ventilation by increasing phrenic nerve activity via sympathetic pathways and this increases ventilatory rate and volume (Thomas, 2011). Increased ventilation triggers the pulmonary stretch reflex and this evokes the secondary chemoreflex responses which consist of reduced cardiovagal activity, increased cardiac sympathetic activity and inhibition of sympathetic outflow to peripheral efferents (Ursino & Magosso, 2000). As a result, HR and CO increases and blood vessels supplying blood to peripheral tissues such as skeletal muscle, renal and visceral organs dilate ((Ursino & Magosso, 2000, Paton et al., 2013). Coronary and cerebral vessels on the other hand exhibit a different response to systemic hypoxia. Activation of carotid chemoreceptors produces a reflex coronary vasodilatory response directly mediated through vagal efferent pathways, whereas cerebral blood vessel dilation appears to be mediated directly by autonomic activity and indirectly by arterial chemoreceptors (Hackett et al., 1972, Heistad et al., 1980). Overall, it is apparent that the initial chemoreflex cardiovascular responses to systemic hypoxia works to redistribute blood from less metabolically active tissues, such as visceral organs, to those that require continuous and significant oxygen supply such as cerebral

and cardiac tissue. In contrast, secondary chemoreflex responses attempt to improve systemic oxygen delivery but are dependent on ventilator response to hypoxia and the magnitude of the pulmonary stretch reflex.

2.1.2.3 Skeletal Muscle Tissue Responses to Hypoxia

Skeletal muscle tissue is very tolerant to hypoxia and unlike cerebral or cardiac tissues, it experiences significant local reductions in oxygen pressure under normal physiological conditions without detrimental consequences. For example, resting myocyte intracellular P_{O2} has been measured to be approximately 34 ± 6 mmHg, however during periods of high metabolic activity, such as during exercise, intracellular P_{O2} can decrease to as low as 2-5 mmHg without impairing mitochondrial respiration (Richardson et al., 2001, Richardson et al., 2006). In fact, previous studies have determined that isolated myocytes only began to experience inhibition of mitochondrial oxidative phosphorylation when intracellular P_{O2} decreased below 1.25 ± 0.22 mmHg and the absolute critical P_{O2} for mitochondrial metabolism was discovered to be between 0.1 to 0.5 mmHg (Richardson et al., 1995, Richardson et al., 2006). In comparison to normoxic conditions, when atmospheric oxygen was reduced to approximately 10%, resting myocyte P_{O2} only decreased to 23 ± 6 mmHg and during hypoxic (12% ambient oxygen) incremental exercise testing, intracellular myocyte P_{O2} only decreased to 2.3± 0.1 mmHg at maximal intensity (Richardson et al., 2006, Richardson et al., 1995). Therefore, despite significant reductions in extracellular oxygen concentration and/or increasing skeletal muscle energy demands, intracellular myocyte P_{O2} remains above the critical oxygen pressure for metabolism. Skeletal muscle tissues in reality benefit from the local reductions in oxygen pressure, as they are capable of utilizing the steep oxygen gradient created between circulation and its cells to increase oxygen extraction to support metabolism (Richardson et al., 2006, Clanton, 2007). The ability of skeletal

muscle tissue to do so, in part demonstrates its ability to compensate for changes in oxygen pressure. However to support such high influx of oxygen during periods of high metabolic demand, these tissues demand a much greater increase in oxygen delivery (Richardson et al., 2006).

When resting skeletal muscle tissues are exposed to systemic hypoxia, after the initial chemoreceptor induced vasoconstriction, a vasodilatory response is observed (Casey & Joyner, 2011). This increases blood flow through skeletal muscle vascular beds in order to maintain muscle oxygen delivery (Casey & Joyner, 2011). Research has indicated that activation of β-adrenergic receptors by circulating adrenaline is responsible for nearly 50% of the augmented blood flow observed during hypoxia and that a significant portion of the β-adrenergic vasodilation is mediated by NO pathways (Weisbrod et al., 2001, Wilkins et al., 2008). Evidence also indicates that local vasodilators influence the hypoxic vasodilatory response in skeletal muscle tissue. Local vasodilators such as endothelial NO, adenosine, prostaglandins and ATP may synergistically work to regulate vasomotor tone and thus blood flow during a state of oxygen deprivation, though the magnitude of contribution from these vasodilators remains unclear (Wilkins et al., 2008, Casey & Joyner, 2011). Researchers are certain however that as metabolic activity increases, β-adrenergic induced vasodilation decreases and local vasodilatory responses prevail during hypoxic conditions (Casey & Joyner, 2011).

2.1.2.4 Respiratory System Responses to Hypoxia

Recall, activation of peripheral chemoreceptors by hypoxic blood increases phrenic nerve activity via sympathetic pathways. This increases alveolar ventilation to promote oxygen diffusion across the alveolar-capillary membranes to improve arterial $P_{O2.}$ Research has demonstrated that both sympathetic and phrenic nerve activity can remain elevated for as long as

1 hour after exposure to short-term intermittent hypoxia despite the arterial oxygen content returning to a normal level (Dick et al., 2007). Research has also indicated that acute systemic hypoxia effects pulmonary blood circulation, producing a rapid increase in pulmonary vascular resistance through vasoconstriction of the pulmonary arteries and veins (Michiels, 2004, Hainsworth et al., 2007). It remains to be determined whether this constrictive response is regulated by a pulmonary chemoreceptor reflex similar to the reflex produced by carotid and/or aortic bodies to hypoxemia or whether pulmonary vasoconstriction is a product of intrinsic P_{O2} sensing (Michiels, 2004). Nonetheless, similar to arterial chemoreceptors the lungs possess neuroepithelial bodies that respond to changes in inspired oxygen concentration. These neuronal cells uniquely synapse with both afferent and efferent vagal fibres and are capable of direct communication with respiratory control centres within the brainstem that regulate respiratory rate and bronchiole tone (Cutz & Jackson, 1999, Chang et al., 2015). Overall, regardless of the feedback pathway researchers suggest that increasing pulmonary blood pressure during hypoxia may play an important role in optimizing alveolar-capillary gas exchange through two mechanisms. Firstly, increasing pulmonary vascular resistance redistributes pulmonary circulation to match lung perfusion with ventilation and secondly, the increased vascular tone may assist in maintaining a balance between ventricular filling and ejection volumes during hypoxia (Fishman, 1976, Cutz & Jackson, 1999, Weissmann et al., 2001).

2.1.3 Measuring Tissue Oxygenation

One non-invasive method to investigate local tissue oxidative metabolism and oxygenation is near-infrared spectroscopy (NIRS). Before discussing how NIRS is capable of measuring tissue oxygenation it is important to know that there are 3 different types of NIRS instruments based on how the instrument illuminates the infrared light. There are: (i) continuous-

wave, (ii) frequency-domain, and (iii) time-domain variations of NIRS instruments. Continuous wave NIRS (CW-NIRS) is only capable of measuring relative variations in oxygenated Hb (O_2Hb) and deoxygenated Hb (HHb) saturation of tissues, whereas frequency-domain and time-domain based NIRS instruments are not only capable of measuring adsorption and scattering properties but can also measure the absolute concentrations of O_2Hb, HHb and total Hb (THb) within tissues (Ferrari et al., 2011). For the purpose of this literature review however, only CW-NIRS will be discussed.

CW-NIRS is capable of monitoring the dynamic relationship between oxygen supply and demand within tissues by measuring O_2Hb and HHb saturation (Ferrari et al., 2011). The ability to do so is grounded on the principle that near-infrared light can easily pass through biological tissues (Hampson & Piantadosi, 1988, Ferrari et al., 2011, Pagano et al., 2016). When light strikes a boundary between two different media, its path of travel will depend on three factors: (i) absorption, (ii) dispersion and (iii) reflection (Lima & Bakker, 2011). Reflection is primarily dependent on the angle in which the light strikes the surface of the second medium, whereas dispersion and absorption properties are influenced by the wavelength of the light being emitted (Lima & Bakker, 2011). Wavelengths of near-infrared light within the range of 700 to 1000nm, display the greatest ability to pass through biological tissues (Ferrari et al., 2004). As near-infrared photons pass through the surface of the skin, they are scattered by the underlying tissues and absorbed by various molecular species called chromophores (Ferrari et al., 2004, Lima & Bakker, 2011). As a result of the scattering, light emitted into tissues follows a curved path known as the optical pathlength, rather than travelling through the tissue in a straight line (See Figure 2-4). As chromophores absorb near-infrared photons, the light is attenuated thus decreasing its intensity (Lima & Bakker, 2011). A detector probe then recovers part of the

diffused-scattered light, for which the NIRS instrument measures the attenuation for specific wavelengths of near-infrared light. This relationship in biological tissues is described by the modified Beer-Lambert equation, which calculates the change in concentration of chromophores:

$$\Delta A = \sum \varepsilon \times \Delta c \times (d \times DPF)$$

Where A represents light attenuation (log scale), ε is the extinction coefficient (molar absorptivity), c represents the concentration of the measured compound in solution (chromophore), d is the distance between the light emitter and the detector, and DPF is the differential pathlength factor (Lima & Bakker, 2011).

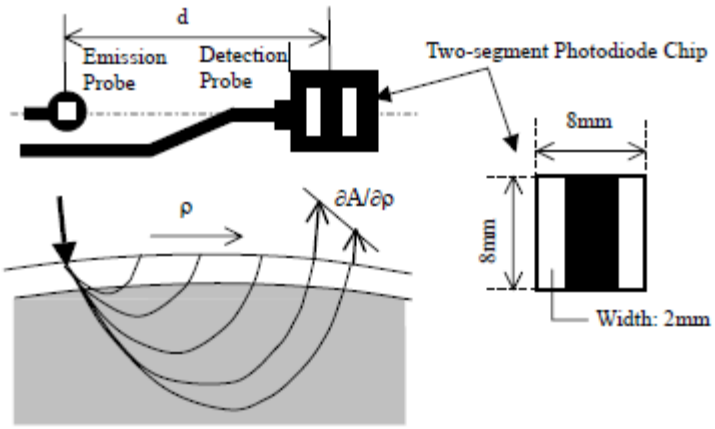

Figure 2-4. Graphical representation of the NIRS optical pathlength as observed in biological tissues. Taken from NIRO-200 manual.

The two main chromophores within our body that absorb penetrating near-infrared light are O_2Hb and HHb, however muscle Mb and cytochrome oxidase aa_3 of the mitochondrial electron transport chain are also capable of absorbing it (Hampson & Piantadosi, 1988, Ferrari et al., 2011). Therefore, in order to differentiate each molecule within the NIRS signal, specific near-infrared wavelengths of light associated with each chromophore are used by NIRS

instruments. For example, O_2Hb and HHb have peak near-infrared absorption wavelengths of 920nm and 760nm, respectively, and their isosbestic point (i.e. the wavelength in which their absorbance of near-infrared light are equal) is at approximately 800nm (Marcinek et al., 2007, Scholkmann et al., 2014). Cytochrome oxidase aa_3 on the other hand, has a peak absorption range between 820-840nm (Cooper et al., 1997). In skeletal muscle tissues, Mb and Hb absorb similar wavelengths of near infrared light, therefore the NIRS signal is unable to separate the oxygenated and deoxygenated forms of each (Hampson & Piantadosi, 1988). Researchers speculate that approximately 20% of the interpreted Hb signal from skeletal muscle tissue is contributed to by Mb however, this percent may change with exercise therefore specific estimates of the relative contributions are conflicting (Marcinek et al., 2007, Jones et al., 2016). Therefore, when considering the total oxygen supply within skeletal muscle tissue, this value is the sum of both O_2Hb and oxygenated Mb (Hampson & Piantadosi, 1988). Nonetheless, the simplest CW-NIRS devices emit continuous near-infrared light at two or three known wavelengths corresponding with the absorption spectra of the tissue chromophores (Jones et al., 2016, Pagano et al., 2016).

Regardless, illuminating a specific tissue with known wavelengths of near-infrared light and by measuring the change in attenuation at these wavelengths, the CW-NIRS instruments are capable of measuring the relative change in the concentration of O_2Hb and HHb from a baseline value (Lima & Bakker, 2011). Moreover using spatially resolved spectroscopy, which calculates chromophore concentrations by measuring the change in light attenuation over a distance, allows for the calculation of another measure called tissue oxygenation index (TOI). TOI is calculated from the following equation (Ferrari et al., 2004):

$$TOI\ (\%) = \frac{kO_2\text{Hb}}{kO_2Hb + kHHb} \times 100$$

TOI is a percentage ratio of O_2Hb to total Hb (THb), which is the sum of O_2Hb and HHb, and is a measure of blood oxygen saturation of the tissue being assessed (Lima & Bakker, 2011). TOI reflects the dynamic relationship between oxygen supply and demand within the underlying tissue whereas THb reflects changes in tissue blood volume (Ferrari et al., 2011). In regards to the NIRS signal, researchers have determined that the projecting near-infrared light does not pass through superficial blood vessels of greater than 1mm due to absorbance (Mancini, et al., 1994, Jones et al., 2016). For this reason, it is assumed that changes in Hb measurements are primarily from the small vessels such as arterioles, capillaries and venules, however the specific contribution of each of these components to the Hb signal remains unknown (Mancini et al., 1994, Jones et al., 2016).

Overall, although CW-NIRS can be an effective and non-invasive tool to assess local tissue oxidative metabolism, a degree of uncertainty exists regarding its measurements. Depending on where the emitter and detector probes are placed, factors such as the spatial distribution of blood flow and O_2Hb, Mb content, microvascular composition and adipose tissue thickness significantly affect NIRS measures (Quaresima et al., 2004, Lai et al., 2009, Jones et al., 2016). For example, adipose tissue has been shown to decrease the degree of near-infrared light absorption by tissue chromophores (McCully & Hamaoka, 2000, van Beekvelt et al., 2001). A study by van Beekvelt and colleagues (2001) discovered that muscle oxygen consumption decreases with increasing subcutaneous adipose tissue, however this observation was a product of metabolically inactive adipose tissue having lower Hb concentrations than skeletal muscle tissue (McCully & Hamaoka, 2000). As a result, changes in the NIRS signal may not truly reflect

changes in muscle tissue oxygenation due to additional scattering of light by adipose tissue and such is the case when adipose tissue thickness is high. Furthermore, reliability also depends on elements such as proper optode positioning and preparation, as well as if dynamic measurements of oxygenation are being investigated (e.g. exercise) versus static measurements (e.g. resting skeletal muscle tissue) (Ferrari et al., 2011, Pagano et al., 2016). Nonetheless previous studies have demonstrated the validity of CW-NIRS as an appropriate tool for measuring relative changes in tissue oxygen saturation. One study that aimed to determine whether changes in absorption of near-infrared light (760-800 nm) correlated with changes in venous Hb saturation in humans was conducted by Mancini and colleagues (1994). The researchers inserted a catheter into a forearm vein and placed the NIRS optodes over the flexor forearm muscles while monitoring both venous oxygen saturation and muscle oxygenation during progressive forearm flexion exercise (Mancini et al., 1994). They discovered that venous oxygen saturation and absorption of near-infrared light between 760-800 nm were significantly correlated and exhibit a strong positive relationship ($r=0.92 \pm 0.07$, $P<0.05$).

Another study determined that the absolute reliability of vastus lateralis TOI and THb NIRS measurements, as indicated by coefficients of variation (CV), were 2.6% and 5.0%, respectively during resting conditions, and 2.7% and 7.4%, respectively during dynamic resistance exercise (Tew et al., 2010). Tew and colleagues (2010) also reported test-retest reliability using intraclass correlation coefficients (ICC) and reported ICCs 0.93 and 0.96 for TOI measures and 0.86 and 0.88 for THb during resting and exercise periods, respectively. Furthermore, another study examining the reliability of NIRS for measuring gastrocnemius and vastus lateralis tissue oxygenation during maximal exercise reported ICCs of 0.88 and 0.99 (Austin et al., 2005). Ihsan and colleagues (2013) also demonstrated reliable NIRS

measurements of vastus lateralis TOI and THb during continuous aerobic exercise on a treadmill. Comparing the results from two treadmill training sessions, the researchers calculated a 3.5% (2.6-5.9%) CV and a 0.87(0.64-0.96) ICC for mean TOI and a 12.0% (8.7%-20.6%) CV and a 0.83 (0.54-0.94) ICC for THb during exercise (P-values >0.05) (Ihsan et al., 2013). Overall, CW-NIRS is a reliable and valid non-invasive instrument for measuring local skeletal muscle oxygenation and blood volume changes. Its functionality allows researchers to assess the dynamic relationship between oxygen supply and demand in specific tissues during both resting and exercising conditions and thus ultimately providing insight on peripheral regulation of oxygen consumption (Lai et al., 2009).

2.2 Blood Flow Restriction Training

BFRT combines low-intensity aerobic exercise or resistance training with an externally applied compression device (Horiuchi & Okita, 2012, Scott et al., 2014). These cuffs can either be placed on the arms or legs, depending on the type of exercise and the working skeletal muscles involved in the movement. The prescribed exercise intensity is set to 20-30% MVC for resistance training or at a light aerobic intensity (<64% HR_{Max}) with the restricted exercise making up ≤20-minutes within a ≤30-minutes training session, including a warm-up and cooldown (Pope et al., 2013, Pescatello, 2014, Scott et al., 2014). In examining the literature there are substantial discrepancies in the BFRT methodologies used by researchers, as no standardized protocol for BFRT exists to date (Dankel et al., 2016, Pope at al., 2013). Across research studies, variability in cuff sizes, pressures, and training regimens increase the uncertainty of the physiological responses to BFRT. Despite not having consistent BFRT protocols, traditionally BFRT involves the application of the restriction cuffs prior to exercise while in a seated position (Abe et al., 2006, Abe et al., 2010b, Ozaki et al., 2010, Loenneke et al.,

2012b, Scott et al., 2014). Individuals using the inflatable cuffs, then undergo a warm-up cycle where the cuffs are inflated and then deflated at regular intervals while simultaneously increasing restriction pressure (Abe et al., 2006, Abe et al., 2010b, Ozaki et al., 2010, Loenneke et al., 2012b, Scott et al., 2014). Once the warm-up cycle is completed, the cuffs are then inflated to a training pressure and this is done so in a resting state immediately prior to exercise. As mentioned above, this protocol is defined as traditional BFRT and any modification to this cuff application procedure is referred to as a modified BFRT protocol.

In any case, once the BFR cuffs are inflated to a specific training pressure, this pressure should be capable of restricting arterial blood flow through the working skeletal muscle while simultaneously impeding venous return to the heart, consequently increasing tissue blood volume (Kawada, 2005, Horiuchi & Okita, 2012, Scott et al., 2014). Such an effect may increase tissue perfusion pressure resulting in the accumulation of fluid within the intramyocellular compartment and it may decrease local skeletal muscle oxygenation by disrupting arterial oxygen delivery (Pope et al., 2013). Following cuff release reactive hyperemia ensues and the significant increase in luminal flow elicits a shear stress stimulus for endothelial-dependent FMD. In fact, specific to the current thesis study a modified BFRT protocol was implemented with the purpose to augment the above physiological responses by holding restrictive pressures for an extended period of time after the warmup procedure, thereby increasing venous blood pooling and tissue blood volume prior to exercise. The following sections will investigate these physiological responses further.

2.2.1 Physiology of Reactive Hyperemia and FMD

Local regulation of blood flow throughout the cardiovascular system is achieved by altering arteriolar resistance in tissues. This is accomplished by the release of paracrine factors

such as NO (Dakak et al., 1998), prostaglandins (Engelke et al., 1996), adenosine (Costa et al., 1999), O_2 (Daugherty et al., 1967), CO_2 (Daugherty et al., 1967), H^+ (Scott et al., 1970, Toth et al., 2007), and K^+ (Scott et al., 1970) secreted by the vascular endothelium or by the cells in which the arterioles are supplying blood.

Reactive hyperemia refers to a locally mediated temporary increase in blood flow following a period of low perfusion (Toth et al., 2007). When blood flow to a tissue is occluded, oxygen levels decrease and metabolic paracrines such as H^+ and CO_2 accumulate in the interstitial fluid (Segal, 2005). Local hypoxia of the tissue ensues and stimulates the vascular endothelial cells to synthesize and release NO (Toth et al., 2007). The increased concentrations of NO and metabolic vasodilators in the extracellular fluid triggers dilation of the arterioles however, the obstruction prevents reperfusion (Toth et al., 2007). Upon the removal of the occlusion, the decreased arteriolar resistance causes a significant increase in blood flow through the vasculature and in the surrounding tissue. As the vasodilating paracrines are either displaced or metabolized (Engelke et al., 1996) and the increased transmural pressure stretches the smooth muscle fibres in the arteriole wall, the arterioles constrict and blood flow begins to normalize (Koller & Bagi, 2002). Overall, the longer the duration of the occlusion, the greater the metabolic stimulus for vasodilation, thus leading to increases in peak reactive hyperemia and duration of hyperemia (Koller & Bagi, 2002). Research has also demonstrated that additional mechanisms may be responsible for flow regulation during reactive hyperemia. Deformation of the endothelium (Sun et al., 2001), deoxygenation of Hb (Allen et al., 2009), adenosine release (Costa et al., 1999) as well as local sensory nerve reflexes (Larkin & Williams, 1993) appear to mediate post-occlusion blood flow in conjunction with tissue hypoxia and the accumulation of metabolic products. Furthermore, in a lab setting, reactive hyperemia can be utilized as a non-

invasive method to assess endothelial-dependent FMD by transiently increasing shear stress and luminal flow within an artery (Thijssen et al., 2011).

Following reactive hyperemia, the significant increase in luminal blood flow elicits a shear stress stimulus for FMD (Kelm, 2002, Koller & Bagi, 2002, Pyke et al., 2004, Thijssen et al., 2011). The blood flow-mediated shear stress is detected by mechanosensitive structures originating in the vascular smooth muscle cells in the arterial wall (Thijssen et al., 2011). These structures initiate a signaling cascade, which modifies the release of several vasodilatory factors such as NO, prostaglandins (prostacyclin), and endothelium-derived hyperpolarizing factor (Koller & Bagi et al., 2002, Pyke et al., 2004). The production of these vasodilators depends primarily on the duration of the stimulus duration. Researchers propose that the mechanisms responsible for FMD change as the shear stress stimulus is prolonged (Pyke & Tschakovsky, 2005). The precise vasodilatory pathways for FMD remains unclear however, NO release appears to be the primary mechanism responsible for initiating the response (Pyke & Tschakovsky, 2005).

Within the vascular smooth muscle, the vasodilators trigger an additional cascade resulting in a reduction in sarcoplasmic Ca^{2+} concentrations and a relaxation of the smooth muscle (Rush et al., 2005). As such, the vessels dilate resulting in decreased vascular resistance and increased flow. The relative diameter change of the vessel is dependent on the intrinsic wall properties (Thijssen et al., 2011). Specifically, the ratio of elastin to collagen will significantly determine the ability of the arterial wall to distend in response to FMD (Thijssen et al., 2011). More compliant arteries, as signified by a greater proportion of elastin, will display greater FMD than non-compliant arteries independent of normal endothelial NO production (Lind, 2006). Overall, peak FMD is quantified as the percent change in vessel diameter from baseline

measurements following introduction of a flow stimulus and this is used as an index of endothelial function and NO bioavailability (Pyke & Tschakovsky, 2007, Thijssen et al., 2011).

2.2.2 Physiological Responses to BFRT

In examining the literature, investigators have proposed numerous BFRT response pathways albeit with conflicting conclusions. It appears that without a standardized methodology for BFRT application, factors such as cuff type, training pressure, and training protocol have significantly varied across research studies, and this has had a profound impact on the observed responses to this form of training. Nonetheless, with respect to local vascular responses, some researchers believe that during BFRT the external cuff pressure occludes venous return to the heart and creates turbulent flow in the arterial vasculature proximal to the occlusion (Horiuchi & Okita, 2012, Pope et al., 2013, Scott et al., 2014). The occlusion of venous circulation may result in the accumulation of venous blood within the vascular beds of skeletal muscle tissues distal to the cuffs and this could increase the perfusion pressure gradient across the vascular/muscle fibre interface (Pope et al., 2013). Following cuff release, ischemic reperfusion might elicit a hyperemia response and this could enhance vasodilation through endothelial-dependent FMD as significant mechanical stress is placed on downstream vascular walls (Horiuchi & Okita, 2012, Pope et al., 2013).

With regards to the specific responses within the active skeletal muscle tissues, some researchers have proposed that BFRT may induce local tissue hypoxia, increase the production of ROS and endogenous hormones, recruit fast-twitch muscle fibres, increase metabolic by-product accumulation as well as activate the mammalian target of rapamycin (mTOR) pathway (Fujita et al., 2007, Loenneke et al., 2010, Nielsen et al., 2012). Although these responses have been observed in some studies, it is important to note that a considerable amount of the research

has been directed towards resistance training exercise with BFR as opposed to an aerobic-based BFRT regimen. Therefore, some physiological responses to BFRT may not occur during different exercise modalities. For example, during aerobic BFRT, metabolite accumulation and fast-twitch recruitment are significantly less than what is observed during resistance-based exercises (Loenneke et al., 2012c, Pope et al., 2013). However, vascular responses such as venous blood pooling and reactive hyperemia following cuff release may occur regardless of the training modality, although differences in cuff application protocols and exercise prescription may influence these responses (Loenneke et al., 2012c, Pope et al., 2013).

2.2.2.1 Physiological Responses to Resistance-based BFRT

Evidence of BFRT significantly increasing blood flow post exercise was observed by Gundermann and colleagues (2012). These researchers aimed to determine whether reactive hyperemia following low-intensity resistance exercise with BFR influenced muscle protein synthesis and they did so by comparing femoral artery blood flow after a BFRT session to an exercise trial where the hyperemia response was pharmacologically induced (Gundermann et al., 2012). Although the researchers determined that the reactive hyperemia response does not increase muscle protein synthesis, the initial peak blood flow response (first 10-minutes) following the BFR low-intensity exercise was significantly greater than the pharmacological trial. Resting femoral artery flow was determined to be ~100 mL min^{-1} for both trials, increasing to >300 ml·min^{-1} and ~200 mL·min^{-1} during the BFRT and pharmacological sessions, respectively ($P<0.05$) (Gundermann et al., 2012). After the initial 10-minute hyperemia response however, the researchers observed no significant differences between conditions 15-minutes post-exercise. Furthermore, using an area-under-the-curve (AUC) analysis for the blood flow response at one and two hours post-exercise, no differences in blood flow were observed when

compared to resting values (Gundermann et al., 2012). Another finding by Gundermann and colleagues (2012) was that BFRT induced a 7.3-fold increase in lactic acid production and this response, as stated by the researchers, may in part be responsible for increasing muscle fibre recruitment via muscular fatigue pathways. Overall, the results from this study clearly indicate the effect BFRT has on post-exercise blood flow. When low-intensity resistance exercise is paired with BFR, blood flow responses are significantly augmented up to 15-minutes post-exercise and this may acutely improve the delivery of nutrients to the skeletal muscles distal to the cuff during this period of time.

A study conducted by Ganesan and colleagues (2015) investigated muscle hemodynamics and oxygenation during low-intensity resistance exercise with BFR, to gain insight on physiological mechanisms responsible for BFRT responses. Using time-resolved NIRS, the investigators were able to quantify absolute Hb concentrations of the vastus medialis muscle of six young healthy males during isokinetic knee extension exercise (Ganesan et al., 2015). During non-restricted resistance exercise, the researchers described a typical pattern for NIRS measurements as follows: HHb increases and O_2Hb decreases with both trends reversing during recovery (Ganesan et al., 2015). They also stated that the recovery period is associated with a hyperemic response where both O_2Hb and TOI increase due to an increased oxygen demand of the working skeletal muscle and local vasodilation (Ganesan et al., 2015). During the BFRT session, the researchers observed a similar pattern of oxygenation however, recovery TOI was significantly lower than the control group during rest periods (7.5 to 11.2% lower) and THb was much greater than the control group during both the exercise and recovery periods ($P<0.05$ for all) (Ganesan et al., 2015). Furthermore, Ganesan et al. (2015) also observed a much higher concentration of HHb in the BFRT group during the resting periods versus the control group,

with the average resting HHb concentration measuring 49% higher than the non-restricted training group at rest. O_2Hb concentration on the other hand, measured significantly higher for the BFRT group when the vastus medialis muscle was active; however no differences were observed between groups during recovery (Ganesan et al., 2015). Overall, the researchers concluded that while low-intensity resistance training with BFR increases tissue blood volume, it does not limit oxygen delivery during exercise or rest but it may slow down the efflux of deoxygenated blood resulting in greater oxygen extraction (Ganesan et al., 2015). Therefore, while BFRT reduces tissue oxygenation, during recovery periods when the cuffs remain on, it remains to be seen whether this duration of reduced oxygenation, indicated by TOI, activates hypoxic compensatory pathways. An alternative hypothesis proposed by Ganesan and colleagues (2015) is that the reduced oxygenation might be a consequence of greater oxygen availability and extraction. The evidence for this stems from the fact that reductions in TOI occur despite significantly higher THb concentrations. Thus, while the restriction cuffs increase tissue blood volume by slowing down the clearance of blood from the muscle, this could facilitate increased oxygen extraction during the post-exercise oxygen deficit.

Another study that examined the effects of resistance exercise with BFR on skeletal muscle oxygenation observed significantly lower oxygen levels during exercise and hyperoxygenation post-exercise (Tanimoto et al., 2005). Using CW-NIRS, Tanimoto and colleagues (2005) monitored oxygenation of the vastus lateralis muscle during knee extension exercise in six young males (ages 20-22 years). The participants each performed four different exercise regimens using knee extension exercise: (1) low-intensity (30% one-rep max) with BFRT application, (2) low-intensity (50% one-rep max) with slow eccentric and concentric movement (~three seconds each), (3) low-intensity (50% one-rep max) isometric hold (~56

seconds), and (4) high-intensity (80% one-rep max). Recall CW-NIRS is unable to measure absolute concentrations of Hb, therefore to demonstrate the changes in muscle oxygenation during exercise the researchers expressed changes to a relative scale where 0% was the minimum TOI achieved during arterial occlusion and 100% was the resting baseline (Tanimoto et al., 2005). Using this relative scale, the researchers illustrated that the BFRT regimen reduced muscle oxygenation to approximately 20% of the resting TOI values, however this level of oxygenation was also similar to the regimens (2) and (3) ($P>0.05$) (Tanimoto et al., 2005). Post-BFR exercise, the researchers observed a state of hyperoxia once the cuffs were released, as the relative oxygenation increased to approximately 140% of resting baseline. This change however, was not significantly different than the change in oxygenation observed after regimens (2) and (4) but was significantly greater than regimen (3) ($P<0.05$). Tanimoto et al. (2005) also obtained venous blood samples to measure blood lactate, plasma growth hormone and plasma norepinephrine concentrations pre- and post-exercise (0, 15 and 30-minutes post) and despite these concentrations being higher than pre-exercise levels for the BFRT regimen, similar results were observed after regimens (2) and (4). Overall, the researchers concluded that low-intensity exercise with BFR induces a hypoxic intramuscular environment and increases metabolic by-product production during exercise whereas post-exercise, BFRT elicits a hyper-oxygenated state within the active muscles upon cuff release.

Larkin and colleagues (2012) also examined the effects of BFRT on the vastus lateralis muscle oxygenation during low-intensity resistance exercise as well as post-exercise expression of growth factors such as VEGF, HIF-1 and nitric oxide synthase (NOS) isoforms (neuronal, inducible and endothelial). Muscle biopsies and blood draws were taken at baseline prior to exercise as well as four hours and 24 hours post-exercise. Performing 10 sets of 12 knee

extensions at 40 % one repetition maximum, the researchers observed similar results to the study conducted by Ganesan et al (2015). BFRT significantly increased THb throughout the duration of the exercise, and this increase was largely attributed to higher relative concentrations of HHb. Furthermore, these concentrations were significantly different than the control group who performed the same training protocol without the restriction of blood ($P<0.05$, for HHb and THb) (Larkin et al., 2012). With respect to mRNA expression of the aforementioned factors, four hours after training the researchers observed a 4-fold increase in the VEGF expression and approximately a 2-fold increase in the primary VEGF receptor (VEGF-R2) expression however, relative to the control group and considering absolute concentrations of both serum and muscle VEGF this change was not significantly different (time x condition interaction, $P>0.05$). On the other hand, HIF-1 and neuronal NOS gene expression exhibited an approximate 1.5-fold increase from baseline values four hours post-exercise, and this change was significantly different from the control group ($P<0.05$ for both). After 24 hours, relative to the control group VEGF,VEGF-R2, neuronal NOS and inducible NOS gene expressions remained elevated in the BFRT group ($P<0.05$ for all) however similar to the four hours post-exercise measures of absolute VEGF concentrations, these concentrations were not significantly different than the control group. Overall, the results from this study further support the notion that BFRT creates a hypoxic intramuscular environment and increases total blood volume during resistance exercise. Moreover, this study also demonstrated that a resistance based-BFRT regimen alters gene-expression of some angiogenic-growth factors although changes in VEGF in particular, were not reflected in venous blood samples or a biopsy of vastus lateralis tissue four hours or 24 hours post-BFR exercise.

2.2.2.2 Physiological Responses to Aerobic-based BFRT

Studies examining the acute impact of BFRT on cardiovascular responses have observed significant increases in systolic blood pressure (SBP),diastolic blood pressure (DBP), and mean arterial pressure (MAP) during low-intensity aerobic BFRT sessions in healthy young adults, although these responses diminished following a 20-minute post-exercise rest period (Renzi et al., 2010). Renzi and colleagues (2010) recruited 17 adults to participate in two different experimental conditions, a BFRT session and a non-BFR control session. Participants were required to walk on a treadmill at a speed of two miles per hour, for five bouts of two minutes of walking with one minute rest periods between each bout. Comparing blood pressure (BP) measurements from baseline values to peak exercise values, SBP increased from ~105 mmHg to ~140 mmHg, MAP increased from ~75 mmHg to ~100 mmHg, and DBP increased from ~65 mmHg to ~80 mmHg during the BFRT session ($P<0.05$ vs. baseline for all) (Renzi et al., 2010). Examining the BP responses during the non-BFR control session on the other hand, SBP only increased from ~105 mmHg to ~115 mmHg, DBP decreased from ~65 mmHg to ~60 mmHg and MAP did not significantly change (Renzi et al., 2010). Furthermore, despite observing a significantly greater HR and reduced stroke volumes (SV) in the BFRT condition (vs. control), no differences in CO between the two conditions were observed (Renzi et al., 2010). With greater elevations in BP and HR, double product (SBP x HR), an index of myocardial oxygen demand, was significantly greater in the BFRT condition than in the control session (Renzi et al., 2010). Moreover, SV/pulse pressure ratio, a surrogate measure of systemic arterial compliance, was significantly lower in the BFRT group in comparison to the control group (Renzi et al., 2010). Overall, these findings suggest that training with a compression device reduces venous return and elevates total peripheral resistance, thus reducing SV and increasing cardiac afterload (Renzi et al., 2010). As such, in order to maintain adequate CO

during exercise, cardiac sympathetic activity increases and a corresponding increase in HR and cardiac contractility are observed (Renzi et al., 2010). Other research studies have also confirmed these cardiovascular responses to BFRT (Takano et al., 2005, Iida et al., 2005, Fahs et al., 2012). In addition, endothelial function appears to be affected by BFRT post-exercise as these same researchers (Renzi et al., 2010) observed a significant decrease in popliteal arterial FMD 20-minutes post walking exercise. The researchers believe this is related to the impairment of endothelial-mediated vasodilation (FMD) upon occlusion release, although this contradicts the results published by Gundermann and colleagues (2012) who observed significant post-exercise hyperemia up to 15-minutes post-BFR exercise. It is possible that the significant reperfusion of blood following the cuff release may affect endothelial function after the hyperemic response; however additional research is warranted to investigate this relationship as FMD was not a measure of Gundermann and colleagues (2012) study. Furthermore, Renzi and colleagues (2010) only assessed FMD 20-minutes after a bout of low-intensity walking exercise with BFR and applied a restriction pressure of 160 mmHg to the thigh, whereas Gundermann and colleagues (2012) assessed blood flow every minute during the first 15-minutes following a resistance-based BFRT exercise and then every 15-minutes thereafter until one hour post-exercise and they applied a restriction pressure of 200 mmHg. Therefore, it is possible that the differences in exercise modality, restriction stimulus and post-exercise assessment period contributed to the differences in results and so future research should address these gaps in the literature.

A study conducted by Loenneke and colleagues (2012d) examined whether aerobic exercise with BFR acutely increases metabolite accumulation in skeletal muscle tissue, specifically measuring whole blood lactate concentrations. Using elastic knee wraps to restrict blood flow through the legs, nine participants walked on a treadmill performing five, two minute

bouts of walking at 75 metres per second separated by one minute rest intervals (Loenneke et al., 2012d). After the walking session, three-minute post-exercise venous blood fingertip samples were collected and the researchers saw no significant changes in whole blood lactate concentrations from pre-exercise levels. The researchers concluded that low-intensity walk training with practical BFR does not result in the accumulation of metabolites, and this may explain why aerobic-based BFRT results in smaller changes in muscle strength and hypertrophy in comparison to BFR resistance based exercises (Loenneke et al., 2012d). Overall despite these results, the researchers failed to measure changes in blood flow following application of the elastic wraps therefore it remains to be seen whether an adequate pressure was applied. Furthermore, the researchers only measured changes in lactate concentration to determine the impact of aerobic-BFRT on metabolism. Metabolites such inorganic phosphate, hydrogen ions, and intramuscular phosphocreatine are also important to consider as they collectively contribute to metabolic stress and impact pathways such as hormone release (i.e. growth hormone), tissue hypoxia and the production of ROS (Conrado de Freitas et al., 2017).

With respect to muscle oxygenation changes during BFR-aerobic exercise, no research to data has investigated this response however, oxygenation has been extensively reviewed during traditional forms of non-restricted aerobic exercise. Previous research has demonstrated that muscle oxygen saturation will increase with the onset of aerobic exercise and then subsequently decrease (Wilson et al., 1989, Costes et al., 1996). Oxygenation will either return to or surpass pre-exercise oxygenation levels during steady state intensities or progressively decrease with increasing workload (Wilson et al., 1989, Costes et al., 1996). The initial rise in oxygenation is likely attributed to the rapid increase in muscle blood flow during the first few seconds of exercise and this hyperemic response is followed by a secondary regulatory response around 15-

20 seconds which may normalize blood flow within one to three minutes of exercise initiation if workload is maintained (Wilson et al., 1989, Tschakovsky et al., 2004, Stöcker et al., 2016). The immediate exercise-induced hyperemia appears to be a product of both the mechanical compression by surrounding skeletal muscle tissue and vasodilation of the systemic vasculature, although the exact physiological mechanisms remain uncertain (Shoemaker et al., 1998, Tschakovsky et al., 2004). According to Hughson and colleagues (2001), one theory is that the significant increase in blood flow, and thus oxygenation, may function as an anticipatory response for metabolic demands, supplying oxygenated blood to both active and inactive muscle fibres so that feedback mechanisms can accurately match muscle oxygen content. The secondary circulatory response then works to adjust blood flow through feedback pathways, which will subsequently match oxygen delivery to the oxygen demands of the exercise and adapt accordingly if workload/intensity changes (Hughson et al., 2001).

In summary, in collectively examining the literature regarding the physiological responses to BFRT it appears that such a method of training may influence muscle oxygenation and increase tissue blood volume although to the best of our knowledge, no studies to date have examined these responses during aerobic-based BFRT regimens despite being assessed in non-BFRT studies. Furthermore, there are inherent differences regarding the methodologies utilized by researchers to study BFRT. For example, investigating the effects of resistance training with BFRT in comparison to aerobic BFRT may lead to dissimilar outcomes, as well as employing different exercise intensities, occlusion times, cuff types or intervention durations can influence the training responses. Therefore, additional research is required to develop a standardized protocol to maximize training responses and thus adaptations to such a novel form of training. Overall, although the vascular and muscular responses to resistance exercise BFRT have been

well documented and support its applicability, there are limited studies exploring the physiological responses to aerobic exercise with BFR. Therefore, extensive research is still required to enhance the knowledge in this particular area.

2.2.3 BFRT Pressures

As it stands, no optimal method has been developed for the application of BFRT. In order to ensure appropriate pressures are being used, there are numerous factors to consider that are not only dependent on specific characteristics of the user but also the type of cuff used in the BFRT technique. Previous research has indicated that some of the most important individual variables to consider are blood pressure and thigh circumference; however these factors are only applicable to the use of BFR on the legs (Loenneke et al., 2012b). These results were presented by Loenneke and colleagues (2012b), who recruited 116 young healthy adults to determine how cuff width affects leg cuff pressure and to determine which factors should be accounted for when prescribing training pressures. The researchers used two cuff widths; one was classified as wide (13.5cm x 83cm) and the other as narrow (5cm x 135cm) and using Doppler instrumentation to measure pulse pressure at the posterior tibial artery, they attempted to find the cuff pressure that completely occlude arterial blood flow through the leg. They discovered that restrictive pressures were significantly less when using the wider cuff diameter, achieving arterial occlusion with a pressure of 144 mmHg (SD= 17 mmHg) in comparison to the narrow cuff occlusion pressure of 235 mmHg (SD= 42 mmHg) ($P=0.001$, Cohen's $D= 2.52$). They also compared the use of thigh circumference to thigh composition to determine the best measure to explain the variance in arterial occlusion pressures. Thigh composition was represented by muscle cross-sectional area and fat cross-sectional area as measured by peripheral quantitative computed tomography. Using hierarchical regression models, the researchers determined that thigh circumference and thigh

composition explained similar degrees of the variance in the arterial occlusion pressure. Thigh circumference explained approximately 49.5% of the variation in pressure when the wide cuffs were used in comparison to the 47.5 % explained by thigh composition. For the narrow cuffs models, thigh circumference also explained 14.8% of the variation in pressure in comparison to explaining approximately 12.9% of occlusion pressure when thigh composition was used. However, the addition of ankle BP and brachial DBP to the thigh circumference model collectively increased the explanation to 63.5% for the wide cuff pressure (Adj. R^2= 0.635, Sig. F change= 0.001) and 30.5% for the narrow cuffs/thigh circumference model (Adj. R^2= 0.305, Sig. F change= 0.005). Overall, it is evident that measuring either thigh circumference or composition best predicts appropriate restriction pressures regardless of the cuff size used. Loenneke and colleagues (2012b) highlight that measuring thigh circumference is relatively easy and more practical than thigh composition and therefore such a measure may be one of the most important characteristics to consider when prescribing training pressures as those with larger legs appear to require higher pressures to restrict arterial blood flow versus an individual with smaller legs (Loenneke et al., 2012b).

Loenneke et al (2012b) also discovered that brachial DBP was a complementary factor for predicting restriction pressures for both the narrow and wide cuffs and they believe that this is related to the relationship between DBP and peripheral vascular resistance. It is well accepted that DBP reflects the amount of vascular resistance the heart needs to overcome during the ventricular contractions, hence representing measures of arteriolar tone (O'Rourke, 1990). Therefore, it is speculated that higher cuff pressures are needed to restrict individuals with high DBP and in contrast, lower pressures are required for those with low DBP. Taken together, the results presented by Loenneke et al (2012b) indicates that thigh circumference or composition,

ankle BP, and brachial DBP are the best overall predictors of pressures when using wide cuffs and thigh circumference or composition and brachial DBP best predict restriction pressures of the narrow cuff.

2.3 Rationale for BFRT Study

Although BFRT results in significant muscular and cardiorespiratory health benefits (i.e. hypertrophy, strength, and improved aerobic capacity) the exact mechanisms through which this adaptation occurs remain uncertain (Pope et al., 2013, Scott et al., 2014). One plausible explanation is that BFRT induces a hypoxia intramuscular environment and through this effect, this form of training exerts influence on numerous signaling pathways. Supporting this theory, previous studies have determined that low-intensity resistance exercise with BFR reduces intramuscular oxygen saturation however; currently no study to date has determined if the same response is achieved through an aerobic-based BFRT regimen. Therefore, this study addressed this gap in the literature.

2.4 Statement of the Problem

The primary research problem of this study was to investigate the effects of traditional and modified BFRT compared to low-intensity aerobic exercise without BFR on vastus lateralis muscle oxygenation. The secondary research problem was to determine which physical characteristics influence the amount of tissue oxygenation during low-intensity aerobic exercise with BFR.

2.5 Hypotheses

The following hypotheses were tested within this study:

1. BFR during walking exercise will decrease vastus lateralis tissue oxygenation compared to non-BFR walking.

2. Increasing the amount of venous blood pooling during the modified BFRT protocol will decrease vastus lateralis muscle oxygenation more so than the traditional BFR protocol.

3. Thigh circumference and aerobic capacity will be positively correlated with the BFRT pressure when training pressures are standardized to muscle oxygenation.

4. Thigh circumference and aerobic capacity will be positively correlated with the change in vastus lateralis tissue oxygenation during low-intensity aerobic exercise with BFR.

Chapter III: Methodology

3.1 Participants

Fifteen young, healthy, normotensive (brachial BP ≤120/80 mmHg) adult males were recruited for this study. Pre-experimental power and sample size calculations were determined from pilot study data and were based off of the mean difference in vastus lateralis AUC for TOI ($TOI_{AUC)}$ between the traditional BFRT and the non-BFRT protocols (See Appendix A). With an effect size of -2.51, a sample size of four participants per training protocol (n=4) would have provided a statistical power of 0.82 at a *P*-value < 0.05. Furthermore, prospective power for a sample size of 15 (*n*=15) was determined to be 99%, given the pilot study results.

Participants met the inclusion criteria if they were male between the ages of 18 to 30 years and if they were currently not partaking in any rigourous resistance or aerobic training programs defined as training more than three times per week at high intensity. Participants were excluded from the study if they smoked, or have used or were currently using BFR as part of their training regimen. Furthermore, participants were excluded if they were taking any medications or supplements that altered their cardiovascular or skeletal muscle functioning. They were also excluded if they had a body mass index (BMI) of less than 18.5 $kg \cdot m^{-2}$ or greater than 29.9 $kg \cdot m^{-2}$, were under medical supervision, and/or had a medical history of cardiovascular disease. If participants had been diagnosed with or had a history of hypertension, hypotension, coronary artery disease, varicose veins, heart failure, valve stenosis, chronic obstructive pulmonary disease, peripheral arterial disease, deep vein thrombosis, unstable angina, controlled and/or uncontrolled hypertension as well as controlled and/or uncontrolled atrial arrhythmia or ventricular dysrhythmia, they were excluded from the study. Furthermore, if participants had chronic diseases such as diabetes, malignant tumors/cancer, orthopedic and/or musculoskeletal

disorders (i.e. fractures, dislocations, injuries) or diseases and/or conditions of the skin they were excluded from the study.

All participants who met the inclusion criteria were informed of the experimental methods, protocol, scientific merits of the study, any potential risks associated with BFRT as well as their rights and remuneration. They were also informed that they were able to withdraw from the training study at any point throughout the study. If the participant accepted these terms and conditions, they were asked to sign a consent form (Appendix B) as well as complete a Medical History and Screening document (Appendix C) .

3.2 Experimental Protocol

Session 1 (~1.5 hour): Familiarization

To begin the study, the participants were scheduled for a one and a half-hour appointment at the Human Hemodynamics Laboratory (Welch Hall, room #22) at Brock University. Prior to this meeting, they were instructed to bring a set of tight and loose-fitting clothing, refrain from any form of aerobic or resistance training, consuming alcohol and caffeine for at least 24 hours before the initial visit and they were also instructed to refrain from eating at least three to four hours prior. Participants were also reminded to consume at least 500 mL of water at least 30 minutes before the session to ensure adequate hydration before exercising. Doing so limited the potential effects of food, drink and exercise on the measured parameters thus standardizing participant conditions.

During the first meeting, the participants were informed of the experimental protocol and the potential risks associated with using the KAATSU compression cuffs (Refer to BREB 16-247 Section C: 15). After the consent form and the Medical History and Screening questionnaire

were signed and completed (See BREB 16-247 Appendix B), all anthropometric and body composition measures were collected. Anthropometric measurements included body mass (kg), height (cm), thigh circumference (cm) and body composition testing was determined using air displacement plethysmography (BODPOD) (Life Measurement Inc, Concord, CA). After these measures were taken, a NIRS probe (NIRO-200, Hamamatsu Photonics, Hamamatsu, Japan) was placed on the distal portion of the right vastus lateralis muscle and the participants sat resting for 10-15 minutes to achieve a stable baseline measurement of tissue oxygenation. The KAATSU cuffs were then placed on the uppermost portion of the thigh, less than five cm from the inguinal crease, and the participants underwent the BFRT warm-up procedure. The warm-up cycle involved inflating and deflating the cuffs at regular intervals while simultaneously increasing cuff pressure. As such, the cuffs were inflated to 90-100 mmHg and held for 20 seconds and then deflated for five seconds. The pressure was then inflated to 120 mmHg for 20 seconds and then deflated for five seconds. The pressure incrementally increased by 20 mmHg using the method of 20 seconds on, followed by five seconds off until 230 mm Hg was reached. After this warm-up cycle, the participant's training pressure was determined using the following protocol:

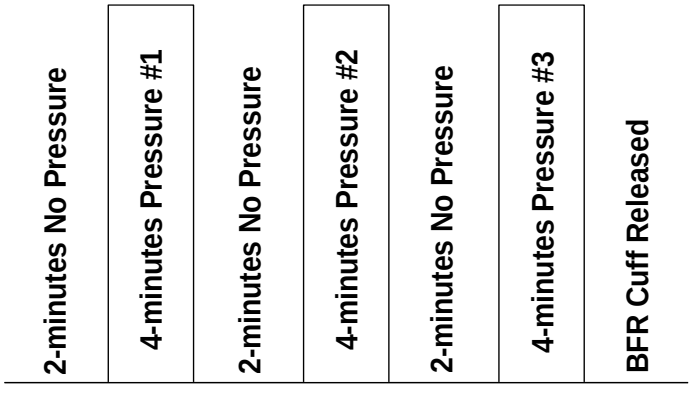

Figure 3-1. Blood Flow Restriction Training (BFRT) pressure determination protocol.

At the very least each participant underwent three rounds of holding a restriction pressure for four minutes followed by two minutes of no pressure. The training pressure was defined as the pressure that decreased vastus lateralis TOI by 10% from the resting seated baseline average that was recorded prior to the warm-up cycle. An absolute reduction of 10% TOI was used for this study because it could be achieved with comfortable BFR pressures. Pilot testing demonstrated that a 10% reduction could be achieved with cuff pressures ranging from 170 to 190 mmHg and such pressures were more comfortable and tolerable for BFRT. In comparison, previous aerobic-based BFRT studies using the same cuffs (KAATSU: width: 5cm x length: 135cm) utilized starting pressures ranging from 140 to 160 mmHg (Abe et al., 2006, Abe et al., 2010a, Abe et al., 2010b, Ozaki et al., 2010, Renzi et al., 2010, Iida et al., 2011). These studies however, did not implement a standardized protocol for determining individual-based training pressures and this merits a limitation of their research. Every participant began with the same pressure regardless of variance in physical characteristics; therefore in order to account for individual differences, the current study standardized training pressures to absolute changes in muscle oxygenation at rest.

After these measurements were taken, the cuffs were removed and skin fold thickness was measured with a caliper at the placement site of the near-infrared spectroscopy probe. Finally, after a brief resting period of 10-minutes, the participants underwent a maximal graded exercise test (GXT) on a motorized treadmill to determine $\dot{V}O_2$ peak. Participants warmed up for five minutes at a self-selected pace, then they performed the maximal aerobic power test. The treadmill speed was set to 7.24 $km \cdot h^{-1}$with an initial grade of 0%. After the first minute, treadmill grade was increased by 3% to a maximum of 9% every other minute and treadmill

speed was increased by 1.61 $km \cdot h^{-1}$ on alternating minutes until eight minutes then 0.80 $mi \cdot h^{-1}$.every minute thereafter.

Session 2 (~ 1.0 hours): Non-BFR Exercise

During this session we assessed vastus lateralis tissue Hb oxygenation saturation using NIRS during low-intensity aerobic exercise without BFR. Tissue oxygenation was first collected during a 10-15-minute seated baseline measurement followed by a three minute standing period on the treadmill to achieve baseline values. During the 10-15 minute seated baseline, three BP and HR readings were recorded using an automated brachial oscillometric device (Omron Blood Pressure Monitor, Omron Healthcare Co., Ltd, Kyoto, Japan). Following the three minute standing baseline measurement, tissue oxygenation was assessed during a 20-minute bout of treadmill exercise at an intensity of ~64% HR_{Max}. Furthermore, the participants were given a HR sensor chest strap to wear and HR was recorded at one minute intervals during the exercise to ensure that the participants were achieving their target exercise intensity. During each minute we also recorded a rating of perceived exertion (RPE; Borg 6-20 point scale, See Appendix H) and treadmill speed and grade were also recorded by the researcher within the Walk Training Log sheet (See Appendix E- Walk Training sheet/optimal pressure determination). Overall, the testing protocol for this session ran as follows:

1) NIRS Preparation: ~5 minutes

2) Seated Baseline: 10-15 minutes

- Sat quietly to achieve stable resting baseline muscle oxygenation.

3) Standing Baseline: 3 minutes

- Stood on the treadmill to achieve stable muscle oxygenation values prior to exercise.

4) Training: 20 minutes

- Walked on the treadmill at ~64% HR_{Max}

Session 3 (~1 hour): Traditional BFRT

During this session we assessed vastus lateralis tissue Hb oxygenation saturation using NIRS during low-intensity aerobic exercise with traditional BFR. Initially tissue oxygenation was collected during a 10-15-minute seated baseline measurement and was followed by measures of BP and HR using the automated method. After BP and HR were recorded, the KAATSU cuffs were placed on the participant's upper thighs and then began the BFR warm-up procedure. This procedure involved inflating/deflating the cuffs at 20-second intervals while simultaneously increasing pressure to their target training pressure determined in Session #1. Once the warm-up was completed and while still seated, the cuffs were inflated to the participant's training pressure. The participants then stood for three minutes while on the treadmill to achieve standing baseline values. Following the standing baseline measurement, tissue oxygenation was assessed during a 20-minute bout of treadmill exercise at an intensity of ~64% HR_{Max}. Similar to Session #2, HR, RPE, treadmill speed and grade were recorded at one minute intervals within the Training Session Exercise Log sheet (See Appendix F- BFRT sheet). Overall, the testing protocol for this session ran as follows:

1) NIRS Preparation: ~5 minutes

2) Seated Baseline: 10-15 minutes

- Sat quietly to achieve stable resting baseline muscle oxygenation.

3) BFR Warmup: 5 minutes

- KAATSU cuffs were inflated and deflated at 20 second intervals while pressure simultaneously increased to training pressure.

4) Standing Baseline: 3 minutes

- Stood on the treadmill to achieve stable muscle oxygenation values prior to exercise.

5) Training: 20 minutes

- Walked on the treadmill at ~64% HR_{Max}

Session 4 (~1.0 hour): Modified BRFT

During this session we assessed vastus lateralis tissue Hb oxygenation saturation using NIRS during low-intensity aerobic exercise with a modified BFR protocol. Initially tissue oxygenation was collected during a 10-15-minute seated baseline measurement followed by measures of BP and HR using the automated method. After BP and HR were recorded, the KAATSU cuffs were placed on the participant's upper thighs and they began the blood flow restriction warm up procedure. This procedure involved inflating/deflating the cuffs at 20-second intervals while simultaneously increasing pressure to their target training pressure. Once the warm-up was completed and while still seated, the cuffs were inflated to the participant's training pressure and this pressure was held for five minutes. After the five minute pressure hold, the participants then stood for three minutes on the treadmill to achieve baseline values. Following the standing baseline measurement, tissue oxygenation was assessed during a 20-minute bout of treadmill exercise at an intensity of ~64% HR_{Max}. Similar to previous sessions, HR, RPE, treadmill speed and grade were recorded at one minute intervals within the Training Session Exercise Log sheet (See Appendix G- BFRT sheet-modified protocol). Overall, the testing protocol for this session ran as follows:

1) NIRS Preparation: ~5 minutes

2) Seated Baseline: 10-15 minutes

- Sat quietly to achieve stable resting baseline muscle oxygenation.

3) BFR Warmup: 5 minutes

- KAATSU cuffs were inflated and deflated at 20 second intervals while pressure simultaneously increased to training pressure.

4) Training Pressure Hold: 5 minutes

5) Standing Baseline: 3 minutes

- Stood on the treadmill to achieve stable muscle oxygenation values prior to exercise.

6) Training: 20 minutes

- Walked on the treadmill at ~64% HR_{Max}

Overall, each session occurred at the same time of day (±2 hours) and participants completed all four sessions within a two week period.

3.3 KAATSU Air Cuff Protocol

Participants wore pneumatic cuffs (KAATSU-Nano, Sato Sports Plaza, Tokyo, Japan) (KAATSU: width: 5cm x length: 135cm) on both legs during aerobic training and proper placement of the cuffs was in accordance with the safety standards developed by KAATSU Global. According to KAATSU Global protocol, leg cuffs should be placed less than five cm from the inguinal crease/folds. Once the participants were seated, the cuffs were placed in the correct position and the initial manual tightness pressure was recorded. This base pressure was assessed using the KAATSU Nano auxiliary device, which relayed cuff pressures on the devices' digital screen, and as recommended by KAATSU for young adults was between 20-25 mmHg. After the base pressure was measured, the cuffs were inflated to 90-100 mmHg for 20 seconds and then deflated for five seconds. The pressure was then inflated to 120 mmHg for 20 seconds and then deflated for five seconds. The pressure incrementally increased by 20 mmHg using the

method of 20 seconds on, followed by five seconds off until the final training pressure specific to each participant was achieved (See Section 3.2 Session 1: Familiarization). According to previous research, this process of inflating/deflating the KAATSU cuffs to reach training pressure effectively stimulates peripheral and central circulation of both arterial and venous blood and is considered a part of the warm-up process (Abe et al., 2010b). Overall, preparation time for cuff application took approximately five minutes and once the correct pressure was achieved, the participants began their standing baseline and then their 20-minutes of low-intensity treadmill exercise maintaining an intensity of ~64% HR_{Max}. Furthermore, in order to maintain target training intensity, the researchers adjusted both treadmill grade and speed to achieve an intensity of ~64% HR_{Max} and these training variables were recorded by the principal investigator. Once the 20-minute training was completed, the cuffs' pressure was immediately released.

3.4 Experimental measurements

3.4.1 Anthropometric and Body Composition Measurements

All anthropometric measurements were taken in the Human Hemodynamic Laboratory at Brock University. Standing height was measured using a stadiometer (Stat 7X, Ellard Instrumentation 50 Ltd Monroe, WA, USA) and was recorded to the nearest 0.1 cm. Body mass was measured using an electronic scale (BWB-800S, Tanita Digital Scale, Tokyo, Japan) and this value was recorded to the nearest 0.1 kg. BMI was calculated using the height and body mass values ($kg \cdot m^{-2}$). Thigh circumference was also recorded to the nearest 0.1 cm. Thigh circumference was measured at 33% of the distance from the inguinal crease to base of the patellar bone. Adipose tissue thickness was measured with a skin fold caliper (Harpenden

Skinfold Caliper, Baty International, Burgess Hill, England) at the placement site of the NIRS probe and was recorded to the nearest 0.1 mm.

Body fat percentage (BF%), fat mass (FM), and FFM (fat free mass) were determined using air-displacement plethysmography. These body composition measures were collected using the BOD POD (Life Measurement Inc., Concord, California, USA). The BOD POD is a valid and reliable method of measuring body composition (Vescovi et al., 2001). Research has shown that it has a test-retest reliability of 99%, as well as a reported coefficient of variation of 3.4% for body fat (Vescovi et al., 2001). During BOD POD testing, participants were asked to wear tight fitting clothing, such as spandex, and were provided a head cap. This was done to ensure that the most accurate measurements were taken, as the surface area of clothes and hair can impact air volume in the chamber (Fields et al., 2000).

3.4.2 Blood Pressure and Heart Rate Measurements

During sessions #2, #3, and #4 BP and HR were recorded using an automated oscillometric device (Omron Blood Pressure Monitor, Omron Healthcare Co., Ltd, Kyoto, Japan). During the 10-15 minute seated resting baseline, the participants sat with their feet flat on the floor where three BP and HR readings were taken on the right arm and the average of the 2nd and 3rd readings were used to determine SBP, DBP and MAP. MAP was calculated using the following formula:

$$MAP = (1/3) * SBP + (2/3) * DBP$$

Furthermore, during all training sessions, HR was continuously monitored using a chest strap HR sensor as well as a corresponding HR wrist watch (Polar S810i, Polar Electro Inc.,

Woodbury, New York, USA). This ensured that the aerobic training intensity was in accordance with the predetermined ~64% HR_{Max} intensity for each participant.

3.4.3 $\dot{V}O_2$max Testing

During the first testing session participants performed a maximal GXT using a motorized treadmill. This test determined the participant's $\dot{V}O_2$max, a well-accepted surrogate measure of cardiorespiratory fitness. During $\dot{V}O_2$max testing, both HR and ventilation was continuously monitored. Oxygen and carbon dioxide concentrations of expired air were measured by a calibrated breath-by-breath electronic gas analyzer (MOXUS Metabolic Cart, AEI Technologies, New Orleans, Louisiana, USA) in order to calculate carbon dioxide output, the amount of oxygen consumed and the respiratory gas exchange ratio (RER). The following American College of Sports Medicine (ACSM) criteria was used to determine that a maximal effort had been achieved during the GXT (Pescatello, 2014):

1) $\dot{V}O_2$ plateau despite increase in workload (or failure to increase $\dot{V}O_2$ by 150 $ml \cdot min^{-1}$)
2) Maximum RER ≥1.10
3) Failure of HR to increase with further increases in exercise intensity

Of these conditions, a $\dot{V}O_2$ plateau was the primary criterion for determining a maximal effort however, if a $\dot{V}O_2$ plateau was not attained the subsequent criteria are secondary conditions to validate a $\dot{V}O_2$max. Although $\dot{V}O_2$max is considered the gold-standard measurement for the integrated functioning of the cardiovascular, respiratory and skeletal muscle systems, some participants may fail to produce an apparent $\dot{V}O_2$ plateau (Howley et al., 1995, Poole & Jones, 2017). Therefore, $\dot{V}O_2$peak, which is defined as the highest $\dot{V}O_2$ attained during a maximal test, was recorded (Howley et al., 1995, Poole & Jones, 2017).

GXT ran as follows:

1) Warmup: 5 minutes

- Participants walked and/or jogged at a self-selected pace (~70% age-predicted HR_{Max}) on level grade (0%)

2) Rest: 1-2 minutes

- The Hans Rudolph headgear and facemask were fastened to the participant's head

3) Test:

- Participants began treadmill jogging at a constant rate of 7.24 $km \cdot h^{-1}$ at 0% incline
- Treadmill speed and grade were increased during alternate minutes such that grade increased by 3% during the 2^{nd} minute of the test and speed increased by 1.61 $km \cdot h^{-1}$ during the 3^{rd} minute.
- This process continued until the treadmill incline reached 9%. Upon this final incline adjustment, an increase in speed by 0.80 $km \cdot h^{-1}$ occurred every minute.

4) Cooldown: 5 minutes

- Participants walked at level grade for 5-minutes at a speed of less than 6.44 $km \cdot h^{-1}$

Overall, the values obtained during the GXT determined the physical conditioning status of each participant as well as the individualized treadmill exercise intensity. Recall, exercise training intensity was ~64% of the participant's HR_{Max}, and this HR_{Max} was the maximal HR achieved during the GXT.

3.4.4 Tissue Oxygenation

Vastus lateralis TOI (tissue oxygenation) was assessed using NIRS (NIRO-200, Hamamatsu Photonics, Shizuoka, Japan). The probe was attached to the right vastus lateralis approximately 70% the distance between the greater trochanter and the lateral condyle of the femur. Prior to the first visit, participants were asked to shave the hair on their thigh to decrease interference between the light emitted and the light detected by the NIRS probe. During each session both the vastus lateralis and the NIRS probe were thoroughly cleaned using alcohol wipes prior to its attachment to the thigh. The probe was secured to the vastus lateralis using both double-sided adhesive tape applied directly to the rubber housing and by an adhesive non-woven fabric sheet (Hypafix, BSN Medical, United States) that covered the entire NIRS apparatus on the thigh to limit probe movement artifact during exercise. Furthermore, the NIRS emission and detection probes were housed in an opaque rubber holder to minimize extraneous light interference and to maintain the distance between the emitter and the detector at four cm (Probe Holder T-3/4 (A9785), Hamamatsu Photonics, Shizuoka, Japan) and a piece of aluminum foil was tapped directly over the rubber housing to further minimize light interference. In addition an indelible marker was used to indicate the exact position of the rubber holder to ensure absolute consistency in the placement of the NIRS probe between testing sessions.

In order to measure the absolute concentration changes in both O_2Hb and HHb, a Differential Pathlength Factor (DPF) of 4.95 was used for the vastus lateralis skeletal muscle tissue (Duncan et al., 1995). Using this DPF and the distance between the emitter and detector probes (d=4cm), the optical pathlength (L) was equal to 19.8 cm (L= DPF*d). In addition, TOI was measured using spatially resolved spectroscopy. Spatially resolved spectroscopy calculated concentrations of O_2Hb and HHb by measuring the change in light attenuation along the distance

for which the light travelled in tissue. Therefore using these relative concentrations, TOI was calculated from O_2Hb and HHb as follows:

$$TOI\ (\%) = \frac{kO_2Hb}{kO_2Hb + kHHb} \times 100$$

Where k represents a constant, which is determined by the light scattering property measured in the tissue and is treated as an unknown constant by the NIRO-200.

3.5 Statistical Analysis

All values were expressed as mean ± standard error (SE) for continuous variables and as a frequency (%) for ratio variables. NIRS measures (TOI, THb, HHb and O_2Hb) were calculated as the difference from their respective resting seated baseline average, which consisted of five minutes of continuous data recording. Furthermore, all NIRS measures were averaged at one minute intervals for the seated and standing baseline periods as well as for the exercise portion of the testing sessions. Shapiro-Wilk tests of normality were used to assess distribution of values for each TOI, THb, HHb, and O_2Hb. The AUC was calculated to quantify the individual degree of variation in TOI, HHb, O_2Hb and THb during non-BFR exercise, traditional BFR exercise and the modified BFR exercise protocols, however only the data from the 20-minute exercise portion was used in the calculation (TOI_{AUC}, THb_{AUC}, HHb_{AUC}, and O_2Hb_{AUC}). The AUC between each one minute data point of the 20-minute exercise portion was calculated using Riemann sums, where the total area is equal to:

$$Area = \sum_{i=0}^{n-1} f(x_i)(\Delta x)$$

Where n is the number of subintervals by i = 0, 1, 2, … n-1 and $f(x_i)$ is the absolute change in oxygenation from seated baseline average and Δx is equal to the time between intervals.

Furthermore, positive area was defined as an increase in absolute oxygenation from the seated baseline average and negative area was defined as a reduction in absolute oxygenation from the seated baseline average.

One-way repeated measures ANOVA tests were performed to determine AUC differences in vastus lateralis TOI, THb, HHb, and O_2Hb across the three different training protocols and post-hoc Tukey tests were performed to make pairwise comparisons of the means. Recall, AUC NIRS measures reflected the absolute changes from their respective seated baseline average, and it was this data assessed with the one-way repeated measures ANOVA analysis. In addition, Pearson tests of correlation were employed to assess univariate demographic, anthropometric and hemodynamic correlates of TOI_{AUC}, stratified by training protocol and a Pearson test of correlation was used to assess which demographic, anthropometric and hemodynamic variables correlate with BFRT pressures. Graphs were generated using SigmaPlot and all statistical analyses were made using SPSS software (Version 20, SPSS Inc., Chicago, IL, USA). Tests were two-tailed with the level of significance $\alpha=0.05$.

Chapter IV: Results

4.1 Descriptive Statistics

Fifteen young adult males (*n*= 15) were recruited to participate in the study, with only a single participant not completing all three training sessions. The participant withdrew from the study having not completed the modified BFRT protocol due to muscle soreness and discomfort 48 hours after the traditional BFRT session. This participant's demographic data was included within the sample characteristics and well as the correlational analyses and the NIRS data from their first three training sessions were included in the one-way ANOVA analyses. Furthermore, the current study does not have data for three participant's $\dot{V}O_2$ peaks. The participants underwent a GXT and gases were collected, however after the tests were completed it was evident that the data overestimated $\dot{V}O_2$. The metabolic cart was then sent for repairs and fixed but the three participants that were tested, did not want to repeat their GXTs. In addition, RHR and BP data are an average from all four days of testing. Overall, the participant demographics (mean ± standard error) are highlighted in Table 4-1.

Table 4-1. *Sample demographics, anthropometrics, and hemodynamics*

	All Participants (*n*=15)
Age (years)	22.5 ± 0.4
Height (cm)	176.4 ± 1.5
Body Mass (kg)	76.1 ± 1.7
BMI ($kg \cdot m^{-2}$)	24.5 ± 0.6
BF (%)	12.5 ± 1.3
FFM (kg)	66.5 ± 1.7
FM (kg)	9.5 ± 1.0
Thigh Circumference (cm)	57.7 ± 0.6
Thigh Thickness (mm)	8.3 ± 0.7
RHR (beats per minute)	64 ± 1.4
HR_{Max} (beats per minute)	194 ± 1.7
SBP (mmHg)	117 ± 1.8
DBP (mmHg)	66 ± 1.4
MAP (mmHg)	83 ± 1.4
Relative $\dot{V}O_2$ Peak ($ml \cdot kg^{-1} \cdot min^{-1}$)*	53.5 ± 1.4
BFRT Pressures (mmHg)	178 ± 4.7

Values are Means ± SE. BMI, body mass index; BF%, body fat percentage; FFM, fat free mass; FM, fat mass; RHR, resting heart rate; HR_{Max}, maximal heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ; VO_2, volume of oxygen consumption; BFRT, blood flow restriction training.
* Indicates *n*=12

4.2 Pattern of Changes in Vastus Lateralis TOI

Figure 4-1 illustrates the pattern of changes in vastus lateralis TOI during the three different testing sessions. The absolute change in vastus lateralis oxygenation was calculated as the TOI percentage change from the resting seated baseline average. TOI is expressed as the percentage ratio of O_2Hb to THb; therefore the absolute change represents this percentage ratio referenced to the average seated baseline value. As illustrated in the diagram below, vastus lateralis TOI decreased during the standing baseline period across all three protocols from the seated baseline value. TOI then progressively increased during the 20-minutes of walking.

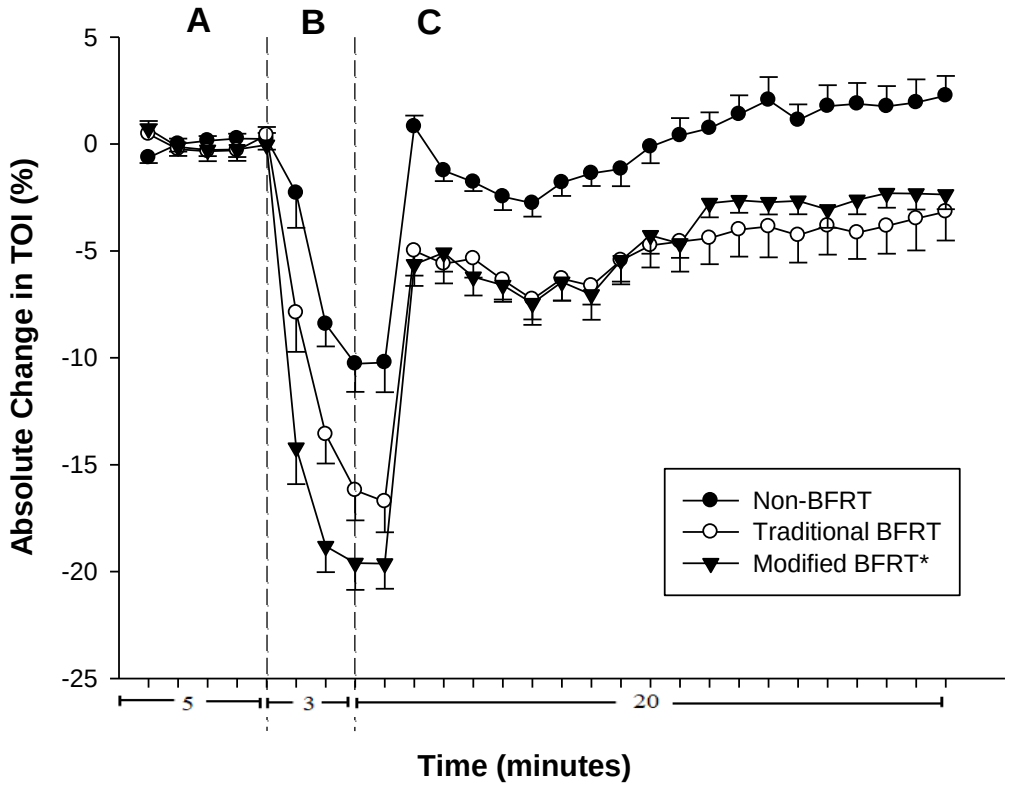

Figure 4-1. Absolute changes in vastus lateralis tissue oxygenation index (TOI) from the seated baseline average during the non-blood flow restricted walk training, the traditional blood flow restriction training and the modified blood flow restriction training sessions. Diagram includes the 5-minute seated baseline (A), 3-minute standing baseline (B) and 20-minute walking protocol (C), displaying 1-minute averages. Values reported as Mean ± SE (*n*=15).
* indicates *n*=14.

4.3 Pattern of Changes in Vastus Lateralis THb

Figure 4-2 represents the pattern of changes in vastus lateralis THb levels during the three different testing sessions. The absolute change in THb was calculated as the micromolar (μM) change from the resting seated baseline average. As you can see, vastus lateralis THb increased during the standing baseline period from the average seated baseline value and this was consistent across all three protocols. THb then decreased during all three protocols with the onset of walking, and then there was a progressive increase in THb during the 20-minute walking period.

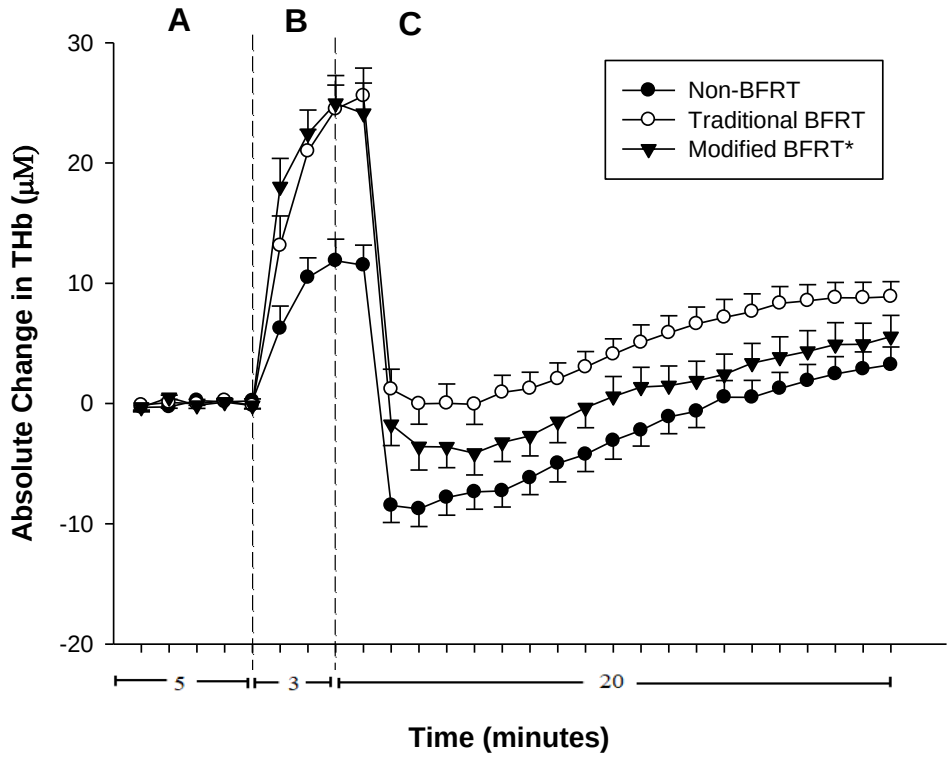

Figure 4-2. Absolute changes in vastus lateralis total hemoglobin (THb) from the seated baseline average during the non-blood flow restricted walk training, the traditional blood flow restriction and the modified blood flow restriction training sessions. Diagram includes the 5-minute seated baseline (A), 3-minute standing baseline (B) and 20-minute walking protocol (C), displaying as 1-minute averages. Values reported as Mean ± SE (*n*=15).
* indicates *n*=14.

4.4 Pattern of Changes in Vastus Lateralis O_2Hb

Figure 4-3 represents the pattern of changes in vastus lateralis O_2Hb levels during the three different testing sessions. The absolute change in O_2Hb was calculated as the micromolar (µM) change from the resting seated baseline average. As illustrated in the figure below, O_2Hb remained unchanged from the seated baseline value during the standing baseline period for the non-BFRT and traditional BFRT protocols, however O_2Hb decreased for the modified protocol. At the onset of exercise, O_2Hb decreased from the standing baseline value then there was a progressive increase during the walking exercise and this pattern was evident for all three protocols.

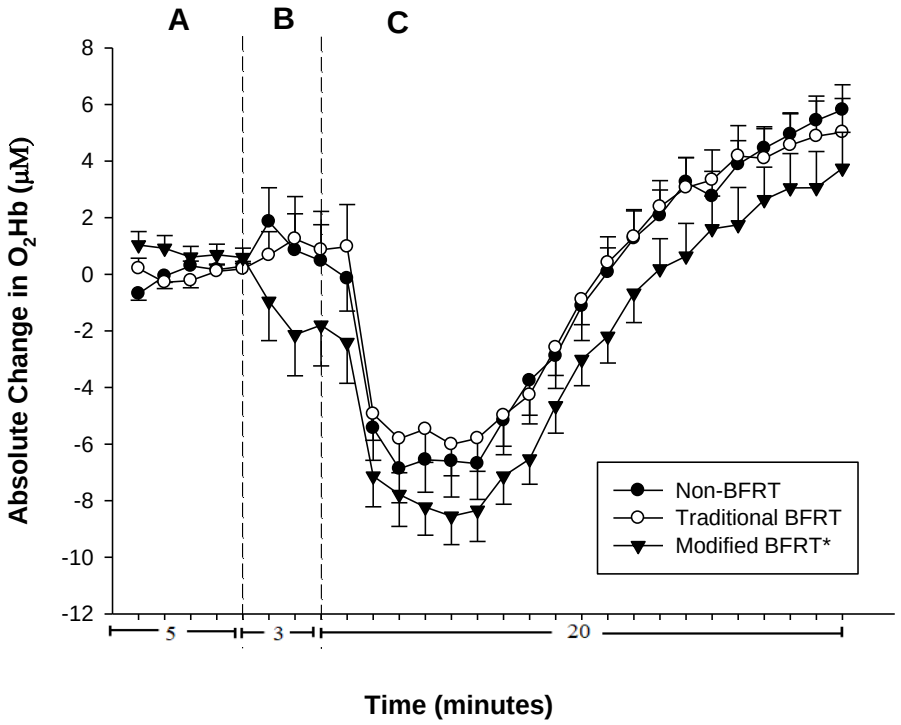

Figure 4-3. Absolute changes in vastus lateralis oxygenated hemoglobin (O_2Hb) from the seated baseline average during the non-blood flow restricted walk training, the traditional blood flow restriction training and the modified blood flow restriction training sessions. Diagram includes the 5-minute seated baseline (A), 3-minute standing baseline (B) and 20-minute walking protocol (C), displaying as 1-minute averages. Values reported as Mean ± SE (*n*=15).
* indicates *n*=14.

4.5 Pattern of Changes in Vastus Lateralis HHb

Figure 4-4 represents the pattern of changes in vastus lateralis HHb levels during the three different testing sessions. The absolute change in HHb was calculated as the micromolar (µM) change from the resting seated baseline average. As you can see, HHb increased from the seated baseline value during the standing baseline period and this was consistent across all three protocols. HHb then decreased within the first minute of exercise and then remained constant over the 20-minute duration of the walking exercise.

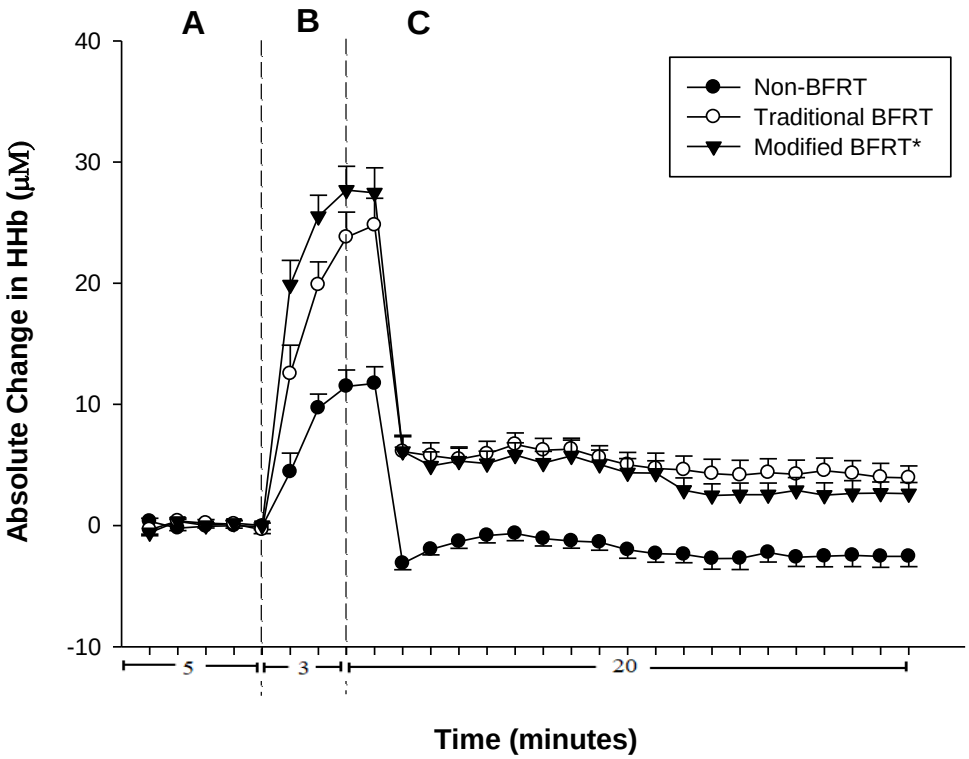

Figure 4-4. Absolute changes in vastus lateralis deoxygenated hemoglobin (HHb) from the seated baseline average during the non-blood flow restricted walk training, the traditional blood flow restriction training and the modified blood flow restriction training sessions. Diagram includes the 5-minute seated baseline (A), 3-minute standing baseline (B) and 20-minute walking protocol (C), displaying as 1-minute averages. Values reported as Mean ± SE (*n*=15).
* indicates *n*=14.

4.6 The Effect of BFRT on Vastus Lateralis TOI

Comparing the mean TOI_{AUC} among the training protocols, a repeated measures one-way ANOVA indicated that there were statistically significant differences in oxygenation levels between the conditions (Power= 1.00, F=25.74, P= <0.001, Figure 4-5). The post-hoc Tukey Test revealed that the mean TOI_{AUC} for the traditional BFRT protocol was significantly greater than the mean TOI_{AUC} for the non-BFRT protocol (traditional BFRT: -99.07 ± 20.08 Vs. Non-BFRT: -2.85 ± 11.53, P= <0.001). As well, the mean TOI_{AUC} for the modified BFRT was significantly greater than the non-BFRT protocol (modified BFRT: -90.97 ± 11.59 Vs. Non-BFRT: -2.85 ± 11.53, P= <0.001). The test also revealed that there was no difference in vastus lateralis TOI between the traditional BFRT and the modified BFRT protocols (P= 0.914).

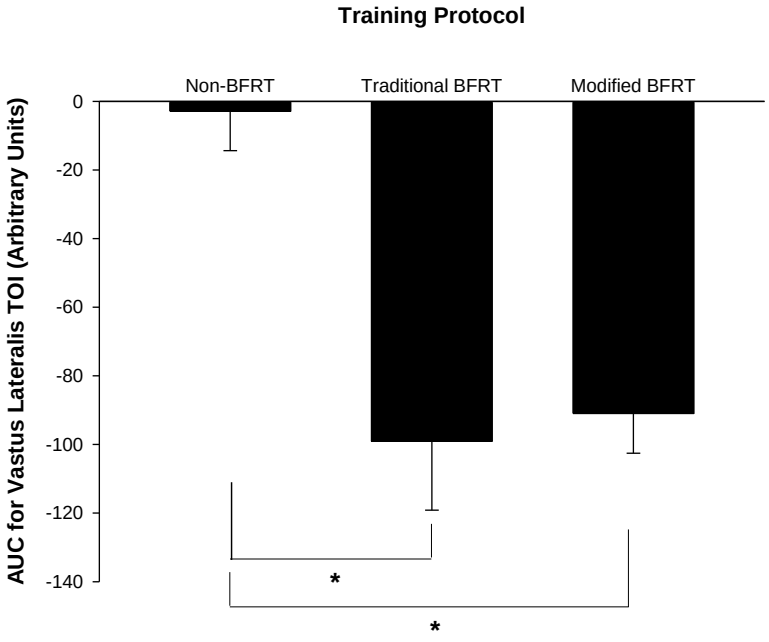

Figure 4-5. Vastus lateralis area-under-the curve for tissue oxygenation index (TOI) by training protocol. Repeated measures one-way ANOVA was employed. *n*= 15 non-blood flow restricted training, *n*= 15 traditional blood flow restriction training, *n*= 14 modified blood flow restriction training. Bars represent group means. Error bars represent SE.
*indicates P < 0.05.

4.7 The Effect of BFRT on Vastus Lateralis THb

Comparing the mean THb_{AUC} among the training protocols, there was a statistically significant difference in THb levels within the vastus lateralis tissue (Power= 0.999, F=16.34, P= <0.001, Figure 4-6). The Tukey post-hoc test revealed that the traditional BFRT protocol was associated with a significantly greater AUC for THb in comparison to the non-BFRT condition during the exercise component of the testing protocol (traditional BFRT: 96.53 ± 24.66 Vs. Non-BFRT: -45.27 ± 25.16, P= <0.001). Furthermore, the post-hoc test also revealed that the mean THb_{AUC} for the modified BFRT protocol was significantly different from both the non-BFRT and the traditional BFRT protocols (modified BFRT: 23.13 ± 30.45 Vs. Non-BFRT, P= 0.02 and modified BFRT Vs. Traditional BFRT, P= 0.033).

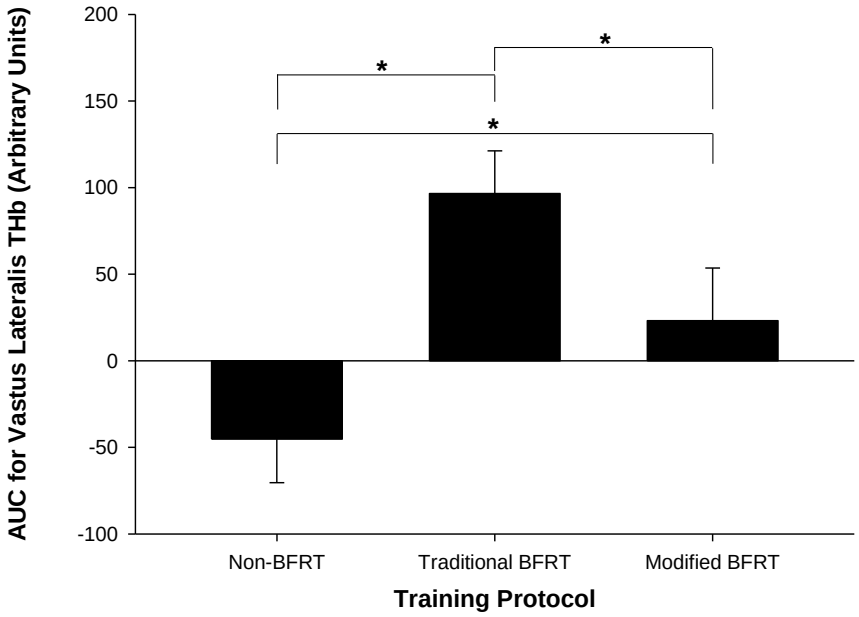

Figure 4-6. Vastus lateralis area-under the curve for total hemoglobin (THb) by training protocol. Repeated measures one-way ANOVA was employed. *n*= 15 non-blood flow restricted training, *n*= 15 traditional blood flow restriction training, *n*= 14 modified blood flow restriction training. Bars represent group means. Error bars represent SE.
*indicates P < 0.05.

4.8 The Effect of BFRT on Vastus Lateralis O_2Hb

Comparing the mean O_2Hb_{AUC} among the three different training protocols, the repeated measures one-way ANOVA indicated that there was a significant differences in O_2Hb levels within the vastus lateralis tissue (Power= 0.451, F=3.513, P= 0.04, Figure 4-7). However, the pairwise comparison analysis indicated that there were no significant differences between means (traditional BFRT: -9.52 ± 17.37 Vs. non-BFRT: -14.09 ± 17.22, P= 0.951; traditional BFRT: -9.52 ± 17.37 Vs. modified BFRT: -50.59 ± 17.95, P= 0.054; modified BFRT: -50.59 ± 17.95 Vs. non-BFRT: -14.09 ± 17.22, P= 0.098).

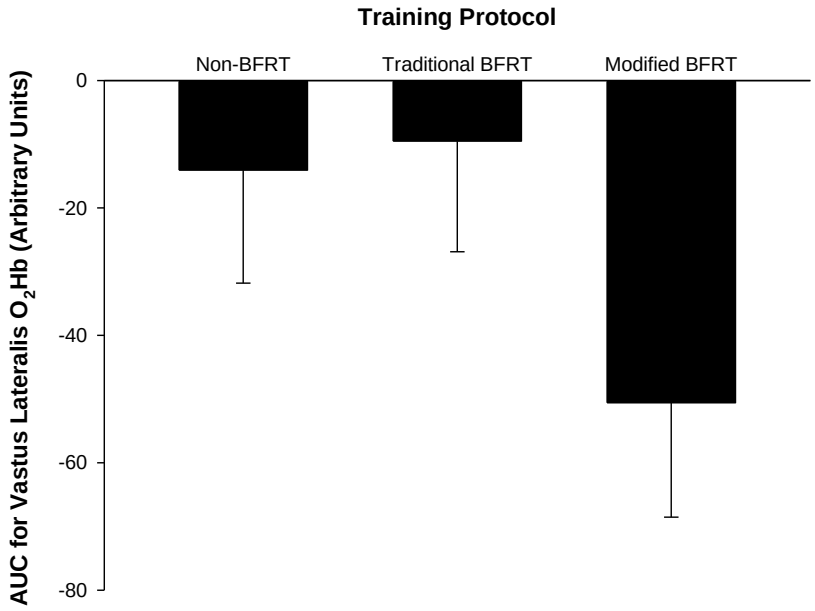

Figure 4-7. Vastus lateralis area-under-the-curve for oxygenated hemoglobin (O_2Hb) by training protocol. Repeated measures one-way ANOVA was employed. *n*= 15 non-blood flow restricted training, *n*= 15 traditional blood flow restriction training, *n*= 14 modified blood flow restriction training. Bars represent group means. Error bars represent SE.

4.9 The Effect of BFRT on Vastus Lateralis HHb

Comparing the mean HHb_{AUC} among the training protocols, there were statistically significant differences in HHb levels within the vastus lateralis tissue during the low-intensity walking exercise (Power= 1.00, F=33.66, P= <0.001, Figure 4-8). The Tukey post-hoc test revealed that both the traditional BFRT and the modified BFRT protocols were associated with significantly greater HHb levels than the non-BFRT condition (traditional BFRT: 106.71 ± 19.81, modified BFRT: 88.28 ± 19.13 Vs. Non-BFRT: -31.48 ± 11.96, P=<0.001 for both pairwise comparisons), while there was no differences in HHb levels between the two BFRT protocols (P= 0.668)

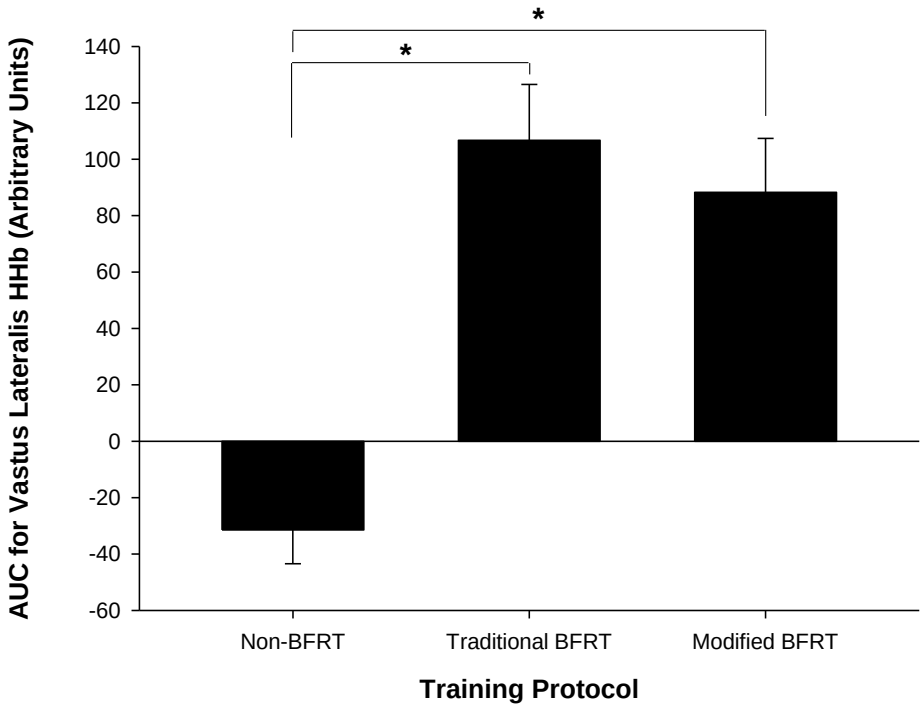

Figure 4-8. Vastus lateralis area-under-the-curve for deoxygenated hemoglobin (HHb) by training protocol. Repeated measures one-way ANOVA was employed. *n*= 15 non-blood flow restricted training, *n*= 15 traditional blood flow restriction training, *n*= 14 modified blood flow restriction training. Bars represent group means. Error bars represent SE.
*indicates P < 0.05.

4.10 Univariate Correlates of Vastus Lateralis Tissue Oxygenation Index

Table 4-2 displays the Pearson correlation coefficients associating changes in vastus lateralis TOI during each training protocol to various demographic, anthropometric and hemodynamic variables of the study sample. Vastus lateralis TOI demonstrated significant positive and moderate strength correlations with age (r= 0.601, P= 0.018), body mass (r= 0.668, P= 0.007), and FFM (r= 0.768, P= 0.001) and a significant inverse relationship with SBP (r= -0.515, P= 0.049) during the traditional BFRT protocol. Similarly, age (r= 0.591, P= 0.026), body mass (r= 0.769, P= 0.001) and FFM (r= 0.671, P= 0.009) displayed positive correlations with moderate strength for the modified BFRT protocol, however a significant positive correlation with HR_{Max} was also detected (r= 0.595, P= 0.025). No variable displayed significant correlations with vastus lateralis TOI changes during the non-BFRT protocol.

Table 4-2. *Univariate correlates of TOI_{AUC}*

	Non-BFRT			Traditional BFRT			Modified BFRT		
	n	r	P	*n*	r	P	*n*	r	P
Age (years)	15	0.341	0.213	15	0.601	0.018*	14	0.591	0.026*
Height (cm)	15	-0.038	0.892	15	0.405	0.135	14	0.420	0.135
Body Mass (kg)	15	0.322	0.243	15	0.668	0.007*	14	0.769	0.001*
BMI ($kg \cdot m^{-2}$)	15	0.328	0.233	15	0.346	0.207	14	0.529	0.052
BF (%)	15	-0.048	0.865	15	-0.347	0.205	14	-0.043	0.885
FFM (kg)	15	0.304	0.271	15	0.768	0.001*	14	0.671	0.009*
FM (kg)	15	0.018	0.947	15	-0.190	0.497	14	0.138	0.638
Thigh Circumference (cm)	15	0.149	0.597	15	0.275	0.321	14	0.151	0.607
Thigh Thickness (mm)	15	0.187	0.505	15	0.061	0.830	14	0.376	0.185
RHR (beats per minute)	15	0.310	0.261	15	0.366	0.179	14	0.440	0.115
HR_{Max} (beats per minute)	15	-0.008	0.978	15	0.417	0.122	14	0.595	0.025*
SBP (mmHg)	15	-0.227	0.415	15	-0.515	0.049*	14	-0.364	0.201
DBP (mmHg)	15	0.136	0.629	15	0.210	0.453	14	0.187	0.521
MAP (mmHg)	15	0.040	0.888	15	-0.004	0.989	14	0.033	0.911
Relative $\dot{V}O_2$peak ($ml \cdot kg^{-1} \cdot min^{-1}$) ¥	12	-0.483	0.112	12	-0.439	0.154	11	-0.412	0.208
BFRT Pressure (mmHg)	NA	NA	NA	15	-0.042	0.882	14	0.003	0.991

Notes: Pearson tests of correlation were employed. TOI_{AUC}, area-under-the-curve for tissue oxygenation index; BFRT, blood flow restriction training; BMI, body mass index; BF%, body fat percentage; FFM, fat free mass; FM, fat mass; RHR, resting heart rate; HR_{Max}, maximal heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ; VO_2, volume of oxygen consumption.

* indicates $P < 0.05$.
¥ indicated $n = 12$.

4.11 Correlates of BFRT Pressure

In order to determine which physical characteristics influence the BFRT pressure, univariate correlations between training pressure and various factors were made (Table 4-3). Recall, the training pressure applied during the BFRT sessions was the pressure that resulted in a 10% reduction in vastus lateralis TOI from the seated baseline average, therefore the following correlations are referenced to the training pressure that achieved such change in vastus lateralis oxygenation. Overall, this analysis revealed that both RHR and thigh circumference were significantly correlated with participant training pressures. In particular, RHR displayed an inverse relationship of moderate strength with cuff pressure ($r= -0.537$, $P= 0.039$) and thigh circumference a positive correlation of moderate strength with training pressure ($r= 0.733$, $P= 0.002$).

Table 4-3. *Univariate correlates of BFRT Pressure*

	n	r	P
Age (years)	15	-0.299	0.279
Height (cm)	15	-0.295	0.286
Body Mass (kg)	15	0.253	0.362
BMI ($kg \cdot m^{-2}$)	15	0.447	0.095
BF (%)	15	-0.052	0.854
FFM (kg)	15	0.264	0.342
FM (kg)	15	-0.026	0.927
Thigh Circumference (cm)	15	0.733	0.002*
Thigh Thickness (mm)	15	0.289	0.296
RHR (beats per minute)	15	-0.537	0.039*
HR_{Max} (beats per minute)	15	0.285	0.303
SBP (mmHg)	15	0.132	0.640
DBP (mmHg)	15	-0.265	0.340
MAP (mmHg)	15	-0.202	0.470
Relative $\dot{V}O_2$ Peak ($ml \cdot kg^{-1} \cdot min^{-1}$)¥	12	-0.081	0.803

Notes: Pearson tests of correlation were employed. BFRT, blood flow restriction training; BMI, body mass index; BF%, body fat percentage; FFM, fat free mass; FM, fat mass; RHR, resting heart rate; HR_{Max}, maximal heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ; VO_2, volume of oxygen consumption.

* indicates $P < 0.05$.
¥ indicated $n = 12$.

Chapter V: Discussion

5.1 Introduction

The purpose of this study was to investigate the effects of low-intensity aerobic exercise with BFR on vastus lateralis muscle oxygenation in a young adult male population. Previous research had demonstrated that BFR exercise can induce muscular hypertrophy and improvements in cardiorespiratory fitness, despite exercising at a much lower-intensity and for a shorter duration of time than what was thought necessary to stimulate adaptations (Dankel et al., 2016, Horiuchi & Okita, 2012, Scott et al., 2014). These findings have provoked further research into how this form of training exerts an influence on both the muscular and cardiorespiratory systems however, such an area of research still remains incomplete. One proposed pathway is local tissue hypoxia, as BFR is believed to decrease the rate of oxygen delivery by disrupting the continuous flow of arterial blood. Using NIRS instrumentation, researchers are capable of determining whether muscle oxygenation is affected by this unique form of training, yet conflicting results have been published thus far regarding the influence of resistance exercise with BFR on oxygenation, and to date no study has examined whether muscle oxygenation is affected during aerobic exercise with BFR. Furthermore, without a standardized cuff application protocol, it is still unclear as to which variables should be considered when determining appropriate BFR training pressures, therefore this study also aimed to investigate this uncertainty in the literature.

In order to explore these objectives, this study tested four hypotheses. Firstly, BFR during walking exercise will decrease vastus lateralis oxygenation more so than a protocol of non-BFR walk training. Secondly, increasing the amount of venous blood pooling during a modified BFR protocol will decrease vastus lateralis muscle oxygenation more so than the traditional BFR

protocol. Thirdly, thigh circumference and aerobic capacity will demonstrate positive, linear correlations with the changes in vastus lateralis oxygenation during BFRT and lastly thigh circumference and aerobic capacity will be positively correlated with the BFRT pressure when training pressures are standardized to muscle oxygenation.

Overall, we discovered that BFRT reduced muscle oxygenation more so than non-BFR exercise; however increasing venous blood pooling prior to exercise had no additional effect on vastus lateralis oxygenation when walking, but had a significant effect reducing THb levels when compared to the traditional BFRT protocol. We also discovered that regardless of the BFRT protocol, such a style of training results in significantly greater THb levels during aerobic exercise, albeit this elevation was a product of increased HHb as opposed to O_2Hb. In addition, the current study demonstrated that age, body mass and FFM were positively correlated with vastus lateralis TOI_{AUC} during both the traditional and modified BFRT protocols whereas, HR_{Max} was only positively correlated with the oxygenation changes during the modified protocol and SBP was only negatively correlated with the oxygenation changes during traditional BFRT. Lastly, the current study discovered that thigh circumference was the strongest determinant of training pressure when pressures were standardized to changes in muscle oxygenation.

5.2 Aerobic Exercise with BFR and Muscle Oxygenation

Previous research has demonstrated that muscle oxygen saturation will increase with the onset of aerobic exercise, then subsequently decrease and then return to or surpass pre-exercise oxygenation levels during steady state intensities (Wilson et al., 1989, Costes et al., 1996). The initial increase in oxygenation is believed to be attributed to the rapid increase in muscle blood flow during the first few seconds of exercise and this is followed by a secondary regulatory response shortly after to normalize flow (Wilson et al., 1989, Tschakovsky et al., 2004, Stöcker

et al., 2016). Considering the responses observed in the current study, at the onset of exercise, it appears that a similar oxygenation response occurred during both the non-BFR and the BFR exercise protocols, albeit the changes from seated baseline were more negative for the BFRT protocols. Interestingly, the mean change in TOI from standing baseline values to the onset of walking for both protocols were similar (non-BFR= Δ11.09± 0.91%, traditional BFRT= Δ 11.20± 1.28%, modified BFRT= Δ13.96± 1.42%). Therefore, while BFRT may induce blood pooling and restrict arterial inflow, it appears to not influence the immediate exercise-induced hyperemic and oxygenation responses, as the pattern of muscle oxygenation change remains similar to that of traditional exercise. Future research should investigate the hemodynamic response using Doppler ultrasound to assess changes in blood flow, as the basis of this statement is under the assumption blood flow directly reflects the changes in muscle oxygenation during BFR-exercise.

Overall, our results concerning the pattern of changes in muscle oxygenation during steady state non-BFRT aerobic exercise was consistent with previous research. Costes and colleagues (1996) demonstrated that vastus lateralis oxygenation during 30-minutes of steady state aerobic exercise on a cycle ergometer (80% of $\dot{V}O_{2Max}$) slightly decreases within the first five minutes of exercise after the initial hyperemic response, then gradually increases to or exceeds pre-exercise values as the exercise progresses. We observed a similar trend with our 20-minute non-restricted walking protocol at an intensity of ~64% HR_{Max}, where TOI decreased after the initial hyperemia response until five minutes was reached, and then oxygenation progressively increased thereafter. With respect to the BFRT sessions, vastus lateralis TOI also decreased initially within the first five minutes of exercise after the immediate exercise induced hyperemia, than increased to a plateau. Since this is the first study to investigate changes in

muscle oxygenation during an aerobic-based BFRT protocol, we are unable to compare our results to those previously published. However, because BFRT is speculated to induce a localized hypoxic response, comparing our BFRT results to studies that have investigated oxygenation patterns observed during steady state exercise in hypoxic conditions, there was a notable difference. Research conducted by Costes and colleagues (1996) investigated the relationship between NIRS measures and femoral venous oxygen saturation during steady-state aerobic cycling exercise in both hypoxic and normoxic environmental conditions. Measuring vastus lateralis oxygenation within hypoxic conditions, theses researchers demonstrated that TOI significantly decreased within the first five minutes of exercise reaching a plateau, whereas we observed a decrease than increase to a plateau (Costes et al., 1996).

The difference in TOI between these two sessions may be explained by the fact that BFRT is not similar to hypoxic environmental training. In the study conducted by Costes et al (1996), participants inspired hypoxic air (O_2%= 10.5%) whereas our participants were in normoxic conditions (O_2%=21%). Inspiring hypoxic air decreases arterial oxygen content, therefore less oxygen will be delivered to the skeletal muscle tissues consequently reducing their oxygen content (Hoppeler et al., 2003, MacIntyre, 2014). In contrast, although we did not measure arterial oxygen saturation, it is presumed to remain unchanged between BFRT and non-BFRT exercise protocols because participants inspired normoxic air. Such differences in arterial oxygen content between hypoxia training and BFRT may explain the different patterns of muscle oxygenation observed during exercise, though further investigation is required.

Furthermore, there may be a different muscle oxygenation response depending on different exercise intensities. Costes et al (1996) participants exercised at a much higher intensity, 80% of $\dot{V}O_2$max for 30-minutes, whereas our participants exercised at an intensity of

~64% HR_{Max}, and this corresponds with a $\dot{V}O_2$max of ~45% (Pescatello, 2014). Bhambhani and colleagues (1999) demonstrated that vastus lateralis muscle oxygenation decreased systematically with increasing intensity when measured during constant workload aerobic exercise, therefore it is possible that by exercising at higher intensities, adaptive circulatory responses are only capable of maintaining the ratio of oxygen delivery to demand resulting in the maintenance of the initial decrease in oxygenation at the onset of exercise. Bhambhani et al (1999) however only utilized five minute training intervals at constant work rates; thus it is unknown how vastus lateralis oxygenation changes beyond this time frame within this context. Additional research is then necessary to investigate how muscle oxygenation changes across different intensities in hypoxic conditions as well as during different BFRT protocols, as the pattern of changes in oxygenation may significantly vary across training conditions.

In addition to aerobic exercise intensity, according to Tschakovsky and Sheriff (2004), the frequency of muscle contraction and the force of contraction have a significant influence on the effectiveness of the skeletal muscle pump and the degree of vasodilation during exercise. Mechanical compression of blood vessels by surrounding skeletal muscle tissues influences the rate of blood flow through the systemic circulatory network and such an influence on blood flow may affect muscle oxygen content (Gotshall et al., 1996). Muscle contraction frequency is determined by the treadmill speed or cycling cadence such that increasing the speed or cadence increases the frequency of muscle contraction (Tschakovsky & Sheriff, 2004). Furthermore, the period of time between contractions dictates the amount of venous filling in the muscle tissue and venous pressure (Tschakovsky & Sheriff, 2004). Therefore, increasing the treadmill speed, and consequently muscle contraction frequency, reduces the duration of time for venous filling, decrease venous pressure and the rate at which arterial blood enters the muscular capillary bed.

Gotshall and colleagues (1996) demonstrated this phenomena determining that higher contraction rates, as reflected by increased cycling cadence, increased both muscle blood flow and venous return at constant workloads. They concluded that the skeletal muscle pump is directly affected by contraction frequency such that higher cadences result in significantly greater perfusion of peripheral circulation (Gotshall et al., 1996). Therefore, if you consider the different intensities between Costes et al (1996) study and ours and the effect it may have on muscle blood flow, it may be unreasonable to assume that the pattern of muscle oxygenation change during exercise will be similar between studies. As well, future research should investigate as to whether the changes in oxygenation during BFR exercise differ across different exercise modalities, such as during low-intensity cycle training with BFR.

5.2.1 BFRT and Pattern of Muscle Oxygenation: Mechanisms

Considering the overall change in muscle oxygenation, as reflected by the AUC analysis, both BFRT protocols significantly reduced vastus lateralis oxygenation more so than the non-BFR exercise. Once again, to our knowledge no studies have examined how BFR during low-intensity aerobic exercise influences muscle oxygenation but under the assumption that muscle blood flow is attenuated with BFR, comparing the oxygenation response to individuals with heart failure may provide an insight on how BFR influences muscle oxygen content. In the literature it is well established that heart failure patients have compromised CO and this consequently reduces muscle blood flow during exercise (Fu et al., 2013). In order to compensate for the reduction in flow, there is an increase in oxygen extraction to maintain oxygen uptake by the muscle during periods of activity and this increased oxygen extraction decreases capillary and venous Hb and Mb oxygen saturation (Wilson et al., 1989). In fact, Wilson and colleagues (1989) observed that during incremental exercise in heart failure patients, muscle oxygenation

was significantly less than matched healthy individuals at all workloads, such that at peak exercise muscle oxygenation was approximately 26±4 % lower (Wilson et al., 1989). Therefore, considering the reduction in vastus lateralis TOI observed during our study, it is possible that the decreased oxygenation was product of increased oxygen extraction from the peripheral circulation. Ganesan et al (2015), who investigated the effects of BFR on vastus medialis oxygenation during knee extension exercise, observed that decreased muscle oxygenation occurred in the presence of significantly greater THb levels. They hypothesized that the mechanical compression exerted by the BFR cuffs may have slowed the return of deoxygenated blood to the heart thus facilitating additional oxygen extraction and an increase in HHb levels. Interestingly, some researchers have speculated that BFRT may in fact lead to hyperoxia through the pooling of blood within the muscle capillary beds despite the observed reduction in TOI as measured by NIRS instruments (Kawada, 2005).

Another theoretical explanation for the increased muscle deoxygenation observed during the BFR exercise, would be lactic acid accumulation. According to Bhambhani et al (1999), the presence of lactate within the peripheral circulation would decrease capillary pH, facilitating oxygen release by O_2Hb. The release of oxygen can be explained by the Bohr Effect, which results in the rightward shift of the Hb-O_2 dissociation curve as discussed in Section 2.1.1. The Bohr Effect and the rightward shift reflect Hb's decreased affinity for oxygen at any given P_{O2}. The purpose of the Bohr shift is to increase the availability of oxygen to meet the metabolic demands of the body's tissues, but such a response would further reduce the degree of oxygenation within the muscle during exercise (Bhambhani et al., 1999). Low intensity BFR exercise has been shown to increase blood lactate levels more so than non-restricted exercise however the significant increase may be the product of blood pooling versus increased anaerobic

metabolism and dependent on training modality (Takarada et al., 2000, Takano et al., 2005, Fujita et al., 2007, Leonneke et al., 2010, Leonneke et al., 2012d, Wilson et al., 2013). Blood pooling distal to the cuffs, would seemingly increase lactic acid concentration as the cuffs would slow the metabolic clearance rate from peripheral circulation. Nonetheless, the presence of lactic acid would facilitate oxygen offloading as previously mentioned, contributing to muscle deoxygenation. With respect to training modality, a significant increase in blood lactate has only been demonstrated during resistance exercise with BFR as opposed to aerobic BFRT (Takarada et al., 2000, Takano et al., 2005, Fujita et al., 2007, Leonneke et al., 2010, Wilson et al., 2013). To the best of our knowledge Leonneke and colleagues (2012d) are the only researchers to have investigated the blood lactate response during aerobic-based BFRT. They observed no changes in whole blood lactate following a bout of low-intensity walking exercise with BFR, although it should be noted that they used elastic knee wraps in contrast to the pneumatic cuffs used in our study and their participants only exercised for a total duration of 10 minutes (five two-minute bouts of walking). Therefore, it is possible that the combination of cuff type and training duration were not capable of inducing lactate accumulation and for these reasons additional research should address the influence of cuff type and training protocol on the metabolic responses to aerobic exercise with BFR.

Regardless of the muscle oxygen patterns we observed in our study, it remains to be seen whether the oxygenation changes observed during exercise with BFR invoked intracellular hypoxic or hyperoxic signaling. No differences in O_2Hb between non-BFR and BFR exercise were detected therefore it is unlikely that oxygen delivery to the skeletal muscle was impaired over the duration of the exercise. Tissue hypoxia is product of both the arterial oxygen content and oxygen delivery, and under the assumption arterial PO_2 was reflective of normoxic

environmental conditions in our study and oxygen delivery was not limited, there is no evidence to suggest that the vastus lateralis was subjected to hypoxia during the low-intensity walking protocol. Nonetheless, future studies need to investigate the cellular responses to the change in muscle oxygenation during aerobic BFR exercise and this can be achieved by obtaining muscle tissue biopsies before and after exercise.

5.3 Muscle Oxygenation during the Modified BFRT Protocol

Another interesting discovery was that increasing tissue blood volume during the modified BFRT protocol, by holding the cuff pressure at rest for five minutes prior to exercise, did not result in further reductions in muscle oxygenation during exercise, although such a modification significantly reduced THb levels when compared to the traditional BFRT protocol ($P= 0.033$). Recall, our second hypothesis was that increasing the amount of venous blood pooling during a modified BFR protocol would decrease vastus lateralis muscle oxygenation more so than the original BFR protocol. We anticipated greater reductions in oxygenation from the baseline value because the volume of deoxygenated blood within the muscle tissue would increase. Consequently, we suspected that a greater proportion of deoxygenated blood would remain within the tissue during exercise, restricted to return to venous circulation by the pressure exerted by the BFR cuffs. If BFR slowed the return of blood and facilitated increased oxygen extraction, then we theoretically would have observed a significant increase in HHb and a greater decrease in TOI during the modified protocol. It is possible that the compression of the leg muscles on the peripheral vessels completely negated the increased tissue blood volume with the onset of exercise. In other words, the skeletal muscle pump may have forced the additional accumulated blood past the restriction cuffs as soon as the participants began walking thus reducing tissue blood volume. Interestingly, we theoretically would have also observed this

similar reduction in accumulated tissue blood volume during the traditional BFRT session as well, however, THb remained elevated throughout the traditional BFR protocol.

An alternative explanation for the difference in THb between the traditional and modified BFRT protocols pairs the skeletal muscle pump with vasodilation of peripheral circulation. Restricting arterial blood flow through the leg and increasing tissue blood volume by holding restriction pressures for five minutes, may have activated vasodilatory response mechanisms in the vessels distal to the cuffs resulting in maximal dilation of peripheral vessels. Recall, reactive hyperemia is a circulatory response that occurs following a period of low perfusion (Toth et al., 2007). When blood flow to a tissue is occluded, oxygen levels decrease and metabolic paracrines such as H^+ and CO_2 accumulate in the interstitial fluid (Segal, 2005). Local hypoxia of the tissue ensues and stimulates the vascular endothelial cells to synthesize and release NO (Toth et al., 2007). The increased concentrations of NO and metabolic vasodilators in the extracellular fluid triggers dilation of the arterioles and the decreased arteriolar resistance significantly increases blood flow through the vasculature (Toth et al., 2007). Therefore vasodilation of the peripheral vessels, product of holding BFR pressures prior to exercise, may have augmented blood flow more so than the traditional BFR protocol and in conjunction with the skeletal muscle pump, may have decreased the accumulation of blood within the tissues distal to the cuffs during exercise. Future research could investigate this explanation by utilizing Doppler ultrasound technology to assess femoral artery blood flow as well as diameter during the restriction pressure hold or utilizing catheters to assess blood flow through the leg during the pressure hold and through the exercise protocol.

5.4 Correlates of Vastus Lateralis Oxygenation during Exercise

Pearson tests of correlation were employed to determine which demographic or anthropometric variables (i.e. $\dot{V}O_2$peak, BF%, thigh circumference) were correlated with TOI_{AUC}. Recall, TOI was calculated as the absolute change from the seated baseline average and AUC was calculated from the entire 20-minute walking component of the testing protocol. From our analysis we determined that age, body mass and FFM were positively correlated with the changes in vastus lateralis TOI during both BFR-exercise protocols. A positive TOI_{AUC} refers to an increase in oxygenation from baseline whereas a negative TOI_{AUC} reflects deoxygenation from the baseline mean. Consequently, a positive correlation between vastus lateralis oxygenation and age, body mass and FFM suggests that younger participants, those with lower body mass or FFM demonstrated greater deoxygenation from their baseline values during the walking exercise. Despite having a relatively homogenous sample with the average age of 22.5 ± 0.4 years, which is also very similar to the reported ages of participants of other BFRT studies (Abe et al., 2006, Abe et al., 2010a, Leonneke et al., 2012b, Karabulut et al., 2014, Hunt et al., 2016), it is possible that the positive correlation between TOI and age may reflect past history of training. Older participants may have more training experience than younger participants and so the response to the BFRT may in part be different across ages, although we cannot confirm this relationship. We did not assess physical activity or history of training, therefore future research studies may want to include an assessment of these variables such as including the International Physical Activity Questionnaire to better understand the demographics of their respective sample. With respect to FFM and changes in TOI, the significant correlation indicated that participants with less FFM experienced greater reductions in TOI from baseline during BFR-exercise. Previous research by Karabulut et al. (2011) demonstrated that both leg lean mass and lean body mass were negatively associated with changes in muscle oxygenation, however their

measurements were taken at rest and this raises the question whether or not different factors affect the BFR response from rest to exercise conditions. Nonetheless, to investigate the relationship between FFM and TOI further, an assessment of limb composition using duel energy x-ray absorptiometry would be beneficial to determine if the lower FFM was related to the leg specifically or whole body FFM. Since thigh circumference and thickness were not correlated with oxygenation changes but body mass was, measuring limb composition would provide a better understanding of the relationship between FFM and oxygenation during BFR-exercise.

Furthermore, from our analysis it was evident that no demographic or anthropometric variables were related to the changes in vastus lateralis oxygenation during the non-BFRT exercise. This may be due to similar patterns of oxygenation during low-intensity steady-state walking exercise across healthy participants regardless of differences in demographic or anthropometric factors or a product of the homogenous sample of young healthy adult males recruited for this study. Previous research has indicated that muscle oxygenation trends during exercise differ when comparing diseased, healthy and athletic populations, although intergroup oxygenation patterns appear similar in nature (Wilson et al., 1989, Miura et al., 2000, Ding et al., 2001, Bauer et al., 2007). Furthermore, some evidence suggests that the response to BFRT differs among athletes with different training backgrounds (i.e. aerobic vs. anaerobic). Takada and colleagues (2012) observed a different metabolic response between a group of sprint trained athletes and a group of endurance athletes, such that aerobically trained athletes displayed significantly higher levels of metabolic stress during BFR exercise, as indicated by significant decreases in intramuscular pH and phosphocreatine levels. Results from our study demonstrated that BFR applied during exercise resulted in a greater reduction in vastus lateralis oxygenation and so based on these results, an inverse relationship between TOI and cardiorespiratory fitness

would be expected, as those with higher aerobic fitness would experience greater metabolic stress during BFRT exercise. Although this was observed in our study (traditional BFRT, $\dot{V}O_2$ peak vs. $TOI_{AUC:}$ r= -0.439, P= 0.154; modified BFRT, $\dot{V}O_2$ peak vs. TOI_{AUC}: r= -0.412, P= 0.208), the findings were not significant. Therefore, future research should investigate this relationship further by comparing endurance athletes with recreationally active or inactive controls as different oxygenation responses to BFRT may result in different muscular or functional adaptations.

5.5 Correlates of Training Pressure

Pearson tests of correlation were also used to determine which demographic or anthropometric variables (i.e. $\dot{V}O_2$peak, BF%, thigh circumference) were correlated with training pressures. The current study proposed the hypothesis that aerobic capacity and thigh circumference would be associated with BFR training pressures when training pressure prescription was standardized to changes in muscle oxygenation. Training pressures were determined by applying a particular pressure at rest for four minutes and observing which pressure resulted in an approximate 10% reduction in TOI from their baseline average. We hypothesized that cardiorespiratory fitness would be related to training pressures because $\dot{V}O_2$peak reflects, in part, the ability of the cardiovascular system to deliver oxygen to the muscle tissues (Bassett & Howley, 2000). Previous research has demonstrated that when oxygen delivery is reduced, such as during hypoxia, aerobic capacity diminishes (Martin & O'Kroy, 1993). Similarly, treatment of β-adrenergic blockers prior to exercise reduces muscle blood flow, also reducing $\dot{V}O_2$pPeak (Pawelczyk et al., 1992). Under the assumption that the application of the BFR cuffs altered these factors; we anticipated that training pressures would be correlated with one's index of cardiorespiratory fitness. Furthermore, research has indicated that highly

trained individuals (i.e. those with greater cardiorespiratory fitness) have significant peripheral adaptations that improve muscle oxygen extraction, in comparison to sedentary individuals. These adaptations include increased muscle capillarization, increased fast twitch (type IIa) muscle fibres and mitochondrial density as well as improved mircovasuclar blood flow (Murias et al., 2010). These adaptations increase the P_{O2} gradient across arterial and venous blood and this will result in better matching of oxygen delivery and utilization by skeletal muscle tissues (Murias et al., 2010). Therefore considering the peripheral adaptations of highly trained individuals; we speculated a linear relationship between cardiorespiratory fitness and training pressures. As such, we were expecting to see higher aerobic capacities correlating with higher training pressures, however this was not evident in our analysis (r= -0.081, p-value= 0.803). It is possible that the small sample size of our study, in particular the number of participants we have cardiorespiratory fitness data for (*n*=12), may have contributed to the non-significant result between cardiorespiratory fitness and BFRT pressure. Despite this, our study was the first to examine the relationship between BFRT pressures and cardiorespiratory fitness.

We also hypothesized that training pressures would be correlated with thigh circumferences and this was based on the results published by Leonneke and colleagues (2012b), who investigated the factors that influence the prescription of BFR pressures. With respect to their study, they examined which factors would predict the minimum pressure required to completely occlude the femoral artery, in contrast to our study which based pressures off of the degree of deoxygenation within the vastus lateralis muscle (Leonneke et al., 2012b). Using hierarchical regression models, Leonneke et al (2012b) determined that thigh circumference or limb composition (proportion of muscle to adipose tissue) in conjunction with brachial DBP predicted arterial occlusion pressure when using the same restriction cuffs that were used in the

current study (KAATSU: 5cm x 135cm). Researchers have indicated that limb circumference has a profound effect on restriction pressures due to its influence on blood flow (Shaw & Murray, 1982). Larger limbs require greater tourniquet pressures to achieve the same degree of tissue pressure as smaller limbs (Shaw & Murray, 1982). Furthermore, because the mechanical pressure exerted by the cuffs decrease from the surface of the skin to the underlying bone, limbs of larger circumference require higher pressures to ensure adequate pressure within the deeper tissues (Shaw & Murray, 1982). Therefore, considering the aforementioned results we hypothesized and found that our BFRT pressures would be significantly correlated with thigh circumference when the pressures are standardized to muscle oxygenation and that this relationship would be positive in nature ($r= 0.733$, p-value= 0.002).

Interestingly, our analysis demonstrated that RHR had a significant negative correlation of moderate strength with training pressures ($r=-0.537$, p-values= 0.039). In other words, lower RHRs were related to higher cuff pressures and this was a unique finding of our study. Despite observing this correlation, it is with intrigue that aerobic capacity was not also correlated with restriction pressures, as aerobic capacity is related to RHR (Kenney, 1985, Fox et al., 2007). It is well known that parasympathetic tone suppresses sinoatrial node automaticity and higher parasympathetic input results in lower RHR (Kenney, 1985). Those with high aerobic capacities tend to have lower RHRs mediated through increased parasympathetic input and high RHRs are associated with decreased parasympathetic tone and poorer cardiorespiratory fitness scores (Kenny, 1985, Fox et al., 2007). Once again we reiterate the notion that the current study's small sample size influenced this relationship and so future studies examining the variables that effect the degree of deoxygenation when BFRT cuffs are applied should investigate these factors in a larger sample.

5.6 Strengths and Limitations

The current study had several strengths. For one, this was the first study to examine the effects of low-intensity aerobic exercise with BFR on muscle oxygenation. A significant portion of research on BFRT focuses on responses to resistance based exercises; therefore we are able to provide some insight on how BFR influences the active skeletal muscles during aerobic exercise. Furthermore, the placement of the NIRS probe was consistent across testing sessions and the probe was highly secured to the leg to prevent movements from influencing the signal. An indelible marker was used indicate the exact position of the NIRS probe enabling us to collect consistent data, as previous research has indicated that there are regional differences in muscle oxygenation therefore it is important to have the probe placement at the same location each testing session (Casavola et al., 2000, Ferrari et al., 2004). Furthermore, we assessed cardiorespiratory fitness to determine training intensity prescription. This was a strength because previous studies investigating the effects of walking with BFR had every participant walk at the same pace and this may have resulted in the participants exercising at different relative intensities, resulting in different physiological responses to BFRT (Abe et al., 2006, Abe et al., 2010b, Renzi et al., 2010, Iida et al., 2011, Leonneke et al., 2012d).

There were also several limitations to our study. First, the sample size was small (n=15), although it should be mentioned that the muscle oxygenation results (TOI_{AUC}) had high statistical power to detect significant differences between training protocols (Power= 0.999). With the small sample size, NIRS data at individual time points (i.e. the data presented in Figures 4-1 to 4-4) could not be assessed with adequate statistical power. This is a limitation because analysis of these time point data may have provided a better understanding of temporal differences in muscle oxygenation as well as tissue hemoglobin saturation across protocols. Furthermore, the

current study did not randomize the order of the trials. Adaptation to BFR exercise was not anticipated after a single session; however this can only be speculated. It would have been beneficial to reassess the training pressures used by each participant after the traditional BFR exercise, and this would provide us insight as to whether the original pressure is capable of achieving the 10% reduction in TOI following one BFR session.

Moreover, we only assessed oxygenation at a single site on the vastus lateralis muscle and at a single tissue depth. As previously mentioned, there are regional differences in oxygen saturation within a specific muscle; therefore NIRS units capable of assessing multiple sites would provide a more complete assessment of muscle oxygenation and oxidative function during exercise. With respect to tissue depth, multi-distance NIRS instruments are capable of assessing the scattering coefficient of the infrared light by measuring light attenuation at different tissue depths (Ferrari et al., 2004, Jones et al., 2016). The capability to do so would account for the influence of superficial tissues on the NIRS signal thus improving the quantification of the NIRS signal (Jones et al., 2016).

Another limitation of the current study was the standardization of the BFR stimulus. Although this was the first BFRT study to have standardized cuff pressures relative to the change in muscle oxygenation we did not account for relative differences in oxygen desaturation across participants. It is well established that CW-NIRS values reflect the balance between oxygen supply and demand in contrast to muscle oxygen consumption (Miura et al., 2000, Ferrari et al., 2004, Jones et al., 2016). Consequently, we should have assessed relative changes in O2Hb/HHb by using complete arterial occlusion to determine the physiological minimum value for deoxygenation. Doing so we would have normalized the NIRS signal such that complete deoxygenation would be 0% oxygenation and the peak hyperemic response following cuff

release would be 100% oxygenation. Thus, the prescription should have been based off of this scale as opposed to only from seated baseline values. Another limitation, of NIRS devices, is adipose tissue thickness however the current study had a relatively homogenous fit sample of young men (Niwayama et al., 2000, Ding et al., 2001, Ferrari et al., 2004, Ryan et al., 2012, Jones et al., 2016). Adipose tissue thickness was assessed directly at the site of the NIRS optode using skin fold calipers and for our particular sample the average adipose tissue thickness of the vastus lateralis was 8.3 ± 0.7 mm. Previous research by Niwayama and colleagues (2000) determined that the sensitivity of the NIRS measures decreases by approximately 50% with a 2-fold increase in adipose tissue thickness. Therefore, in order to correct for such a factor when using CW-NIRS devices, researchers recommend using the ischemic calibration method previously mentioned (Ryan et al., 2012). Once again, this method normalizes the NIRS signal to the physiological range of each participant, thus allowing for more accurate comparisons between individuals and this would be important if the current study were to be conducted in less fit populations (Ryan et al., 2012).

5.7 Future Directions

Given the results of our study and the limitations, future studies should examine the following topics. The hemodynamic responses to aerobic-based BFRT in conjunction with NIRS assessment of muscle oxygenation would provide more insight on the relationship between oxygen delivery by the circulatory system and the balance between oxygen supply and demand at the muscular level during BFR exercise. Future research could investigate this relationship by utilizing Doppler ultrasound technology to assess femoral artery blood flow at rest and post-exercise or utilize catheters to assess blood flow through the leg at rest and during the exercise component of the testing protocol. Furthermore, researchers should also assess the patterns of

oxygenation during BFR exercise across different exercise modalities, such as during low-intensity cycle training with BFR or elliptical training. Elliptical training has been shown to have greater quadriceps activation and greater quadriceps/hamstrings muscular co-activation than treadmill or cycling exercise (Prosser et al., 2011), therefore it would be interesting to investigate whether the oxygenation response to BFRT differs depending on the electromyographic patterns during exercise. In addition, although we detected significant differences in vastus lateralis TOI_{AUC} between BFR and non-BFR protocols, future studies need to assess intracellular hypoxic responses following aerobic-based BFRT by obtaining muscle tissue biopsies throughout the testing session. This would expand the knowledge on whether the reductions in muscle oxygenation observed during BFR exercise activated hypoxic signaling pathways. Moreover, because BFRT is believed to exert its influence on the muscular system through hypoxia, researchers should compare and contrast the acute muscle oxygenation response and cellular responses to aerobic exercise between BFR and hypoxic environmental training conditions.

Future studies should also compare different training protocols for aerobic-based BFR exercise. In the current study, we used a single bout of 20-minute exercise to assess how BFR influences muscle oxygenation, however such a protocol may not be the most effective for BFRT. On the premise that this form of training induces significant blood pooling in the tissues distal to the cuff and the skeletal muscle pump may reduce the amount of blood pooled during exercise, a protocol that maximizes the amount of pooling may increase the blood volume stimulus. In order to achieve this, exercise sessions should be divided into multiple periods of activity and rest so that during the resting periods, blood will re-accumulate in the muscle tissues increasing metabolic stress. In other words, a protocol of low-intensity interval training for BFR

may be more effective than continuous steady state aerobic exercise and so future studies should determine the best training protocol for aerobic-based BFR exercise.

Lastly, future research should also investigate the kinetics of muscle oxygenation during aerobic BFRT, particularly examining the recovery period following a bout of exercise with BFR. Previous research has demonstrated that the ability of the muscle to recover following a bout of exercise is dependent on the relative intensity of the exercise as opposed to the individual's cardiorespiratory fitness level (Bhambhani et al., 1999). Recovery is associated with hyperemia and local vasodilation in order to replenish oxygen stores (Ganesan et al., 2015). BFRT restricts arterial inflow and occludes venous return; therefore it is possible BFRT exerts a profound influence on recovery periods following bouts of exercise. Ganesan and colleagues (2015) have investigated this relationship with BFR resistance exercise, however to date no study has examined this relationship with aerobic-based BFRT protocols.

Chapter VI: Conclusions

This study aimed to examine the effects of low-intensity aerobic exercise with BFR on vastus lateralis muscle oxygenation. Previous research had demonstrated that BFR exercise can induce muscular hypertrophy and improvements in cardiorespiratory fitness, despite exercising at a much lower-intensity and for a shorter duration of time than what was thought necessary to stimulate adaptations (Dankel et al., 2016, Loenneke et al., 2012a, Horiuchi & Okita, 2012, Scott et al., 2014). These findings have provoked further investigation as to how this form of training exerts an influence on both the muscular and cardiorespiratory systems however, such an area of research still remains incomplete. One proposed pathway is local tissue hypoxia, as BFR is believed to decrease the rate of oxygen delivery by disrupting the continuous flow of arterial blood. As such, researchers have investigated changes in muscle oxygenation during resistance exercise with BFR, however to date no study has examined the response during aerobic-based exercise protocols. Thus, this study is novel as it is the first to examine the influence of BFRT on muscle oxygenation during aerobic exercise.

In summary, from our analysis it was evident that the application of BFR cuffs during low-intensity aerobic exercise resulted in significantly greater reductions in vastus lateralis oxygenation compared to non-BFR exercise. Despite not examining myocellular responses, future studies can assess whether the deoxygenation observed during the BFR exercise resulted in the activation of intracellular hypoxic signaling pathways within the active muscle tissues. Furthermore, this study was also the first to assess which demographic and/or anthropometric variables were correlated with aerobic-based BFRT pressures when the prescription was standardized to muscle deoxygenation and the first study to assess which demographic and/or anthropometric variables were correlated to the changes in muscle oxygenation during a bout of

BFRT. From this analysis we determined that both thigh circumference and RHR were significantly related to training pressures, such that participants with larger circumferences required higher cuff pressures to decrease resting vastus lateralis oxygenation and those with higher RHRs required lower cuff pressures. In view of these results, it is therefore recommended that these variables be considered in future studies when prescribing cuff restriction pressures based off of muscle oxygenation. Furthermore, the current study demonstrated that age, body mass, FFM and SBP were significantly correlated with the changes in muscle oxygenation during traditional BFR-aerobic exercise. Age, body mass and FFM were positively related to the changes in muscle oxygenation such that older participants or those with higher BMI's or FFM had higher levels of muscle oxygenation during BFRT, whereas participants with lower SBP had higher levels of deoxygenation during BFR exercise.

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Appendix A

7.1 Pre-Experimental Power and Sample Size Estimations

Pre-experimental power and sample size estimations were based on results of a pilot study we conducted in a young, healthy adult male sample (*n*=3). These estimations were based on the mean difference in TOI_{AUC} of the vastus lateralis between the non-BFRT training session and the BFRT training session. The means and standard errors of the mean (SEM) from this pilot were as follows: $TOI_{AUC\ Non\text{-}BFRT}$ (m_A) = 8.39, $SEM_{AUC\ Non\text{-}BFRT}$ (S_A) = 40.15, $TOI_{AUC\ BFRT}$ (m_B) = -45.18, $SEM_{AUC\ BFRT}$ (S_B) = 26.98, mean TOI_{AUC} difference= -53.57. Furthermore, we used the reliability coefficient for CW-NIRS from the study conducted by Ihsan and colleagues (2013) who calculated a reliability of 0.87 (r_{AB}=0.87) for mean TOI during treadmill training sessions. In addition, the pooled SEM was determined to be 21.33 (S_{AB}= 21.33). The following formulas were used to determine the pooled SEM (Cohen, 1988):

$$S_{AB} = \sqrt{S_A^2 + S_B^2 - 2 \times r_{AB} \times S_A \times S_B}$$

Where S_A represents the $SEM_{AUC\ Non\text{-}BFRT,}$ S_B represents the $SEM_{AUC\ BFRT}$, and r_{AB} is the reliability coefficient for the CW-NIRS instrument. The following formula was used to determine the effect size (ES) (Cohen, 1988):

$$Effect\ Size\ (ES) = \frac{(m_B - m_A) - (\mu_B - \mu_B)}{S_{AB}}$$

Where m_A represents the mean $TOI_{AUC\ Non\text{-}BFRT}$, m_B represents the mean $TOI_{AUC\ BFRT}$, μ_A represents the hypothesized mean $TOI_{AUC\ Non\text{-}BFRT}$, μ_B equals the hypothesized mean $TOI_{AUC\ BFRT}$, and S_{AB} is the pooled SEM.

The following table outlines the demographic, anthropometric, and hemodynamic characteristics of the pilot study sample:

Table 7.1. *Pilot sample demographics, anthropometrics, and hemodynamics*

	All Participants (*n*=3)
Age (years)	23.7 ± 0.9
Height (cm)	182.5 ± 2.8
Body Mass (kg)	81.1 ± 1.9
BMI ($kg \cdot m^{-2}$)	24.4 ± 1.3
BF (%)	12.1 ± 2.5
FFM (kg)	71.3 ± 2.5
FM (kg)	9.8 ± 2.1
Thigh Circumference (cm)	58.5 ± 1.3
ATT (mm)	7.2 ± 1.6
RHR (beats per minute)	62 ± 1.5
HR_{Max} (beats per minute)	199 ± 1.3
SBP (mmHg)	115 ± 2.6
DBP (mmHg)	67 ± 2.9
MAP (mmHg)	83 ± 2.6
Brachial PP (mmHg)	47 ± 2.4
Relative $\dot{V}O_2$ Peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	54.2 ± 3.6
BFRT Pressure (mmHg)	183 ± 6.7

Values are means ± SE. BMI, body mass index; BF%, body fat percentage; FFM, fat free mass; FM, fat mass; ATT, adipose tissue thickness; RHR, resting heart rate; HR_{Max}, maximal heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; VO_2, volume of oxygen consumption.

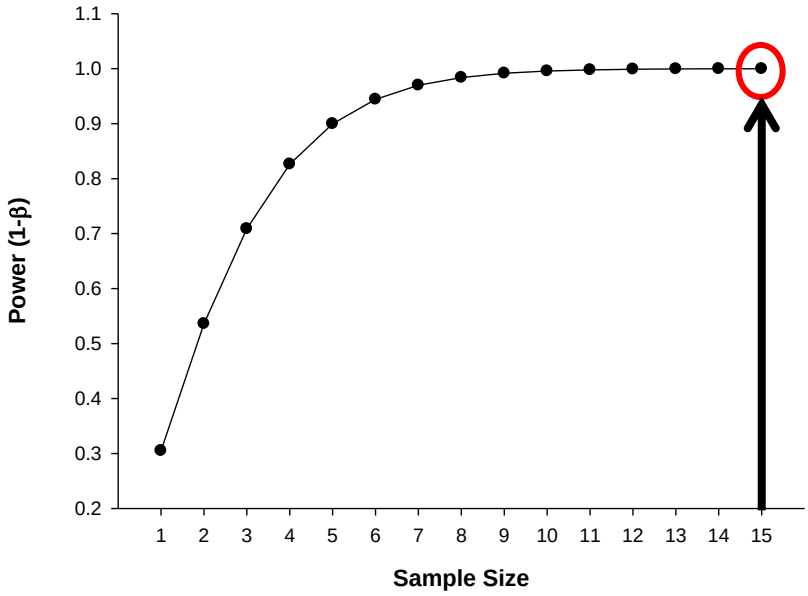

Figure 7.1. Power analysis curve

Using an paired sample t-test to compare the mean TOI_{AUC} for the non-BFRT and BFRT protocols, the effect size for the pilot study was determined to be -2.51 (ES= -2.51). Thus, based on the large observed effect size, the pilot study means, standard errors, and NIRS reliability coefficient, a sample size of 4 participants per training protocol (n=4) provides a statistical power of 0.82 at a α of *P*-value < 0.05. Furthermore, prospective power for a sample size of 15 (*n*=15) was determined to be 99%, given the pilot study results.

Table 7.2. Power Analysis

Sample Size=3 Effect Size= -2.51	**H_o True**	**H_o False**
Accept H_o	Correct Acceptance (1-α) = 0.95	Type II Error β=0.1736
Reject H_o	Type I Error α=0.05	Correct Rejection (1- β) =0.8264

Appendix B
INFORMATION AND CONSENT TO PARTICIPATE IN RESEARCH FORM

Project Title:

Investigating the Effects of Aerobic Exercise with Blood Flow Restriction on Thigh Muscle Oxygenation

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INVITATION
You are invited to participate in a research study. This letter will outline the purpose of the project, describe the procedures that are required, tell you about potential risks and benefits to yourself, and discuss your rights and confidentiality issues. If you wish to participate, you will be asked to sign a consent form at the end of this letter. Feel free to ask any questions you might have at any time.

STUDY PURPOSE
Cardiovascular disease (CVD) is one of the leading causes of mortality, contributing to approximately 30% of all deaths annually. Although research has indicated there are uncontrollable risk factors associated with CVD, for instance age and sex, adopting healthy behaviours such as regular physical activity, has been shown to significantly reduce our risk to

developing CVD. Therefore, investigating the effectiveness of various forms of physical activity on markers of cardiovascular health may provide valuable knowledge to help mitigate future risk development for CVD.

An unconventional form of exercise, known as blood flow restriction training (BFRT), may be a new therapeutic strategy to support cardiovascular health. BFRT combines low-intensity aerobic exercise with externally applied compression cuffs. Such a style of training is prescribed for a maximum of 20 minutes per session and preliminary research has demonstrated comparable benefits to cardiovascular and musculoskeletal health as traditional forms of training (i.e. resistance and aerobic exercise), however a standardization protocol for training pressures does not exist. Therefore, this study will look at which variables influence appropriate training pressures and how it relates to the oxygen saturation of your leg muscles.

AM I ELIGIBLE?

STUDY INCLUSION CRITERIA:
- Male
- Age 18-30
- Healthy (Healthy participant is one that is free of CVD and the associated risk factors (i.e. high blood pressure, diabetes, high cholesterol and obesity)
- Normotensive (brachial blood pressure ≤120/80 mmHg)
- Recreationally active

STUDY EXCLUSION CRITERIA:
- Partaking in any rigorous resistance or aerobic training programs defined as training more than three times per week at high intensity
- Smoker
- Currently taking any medications or supplements that alter cardiovascular or skeletal muscle functioning (Includes chronic use of aspirin)
- Have used or are currently using blood flow restriction as part of their training regimen
- BMI of less than 18.5 kg·m-2 or greater than 29.9 kg·m-2
- Medical history or familial history of cardiovascular conditions
- Diagnosed with or have a history of hypotension, bleeding disorder, coronary artery disease, varicose veins, heart failure, valve stenosis, chronic obstructive pulmonary disease, peripheral arterial disease, unstable angina, controlled and/or uncontrolled hypertension as well as controlled and/or uncontrolled atrial arrhythmia or ventricular dysrhythmia
- Diagnosed with chronic diseases such as diabetes, cancer, orthopedic and/or musculoskeletal disorders, or a history of deep venous thrombosis

WHAT'S INVOLVED
As a participant, this study will require approximately 1-2 weeks of your time and includes 3 in-laboratory testing sessions. You will also be required to bring both loose fitting athletic clothing and tight fitting clothing. If you do not have tight-fitted clothing, it will be provided for you. Appropriate change rooms will be provided for you to change into the required clothing.

Session 1 (~45 minutes):
During this initial appointment at the **Human Hemodynamics Laboratory (Welch Hall, room #22)** you will receive information and consent forms regarding the study. At this time, one of the study investigators will explain all parts of the study and if you are still interested in participating, you will be asked to sign the consent form and fill out a medical questionnaire. After signing the necessary documents you will change into tight-fitted clothing and we will perform the following measurements:

Blood Pressure and Heart Rate will be measured using an automated blood pressure cuff.

Body Measurements which include your height, body mass, and waist, hip and thigh circumferences and thigh skinfold measurements using a skinfold caliper.

Body Composition Measurements such as body fat percentage and lean mass percentage will be taken. In order to determine these measurements, you will be required to sit within a chamber that will measure your body density. If you are uncomfortable being within confined spaces, please let one of the investigators know as we have an alternative method to assess these measures of body composition.

Peak Oxygen Uptake (VO_2peak) will be measured and used to assess your cardiorespiratory fitness. A soft silicone facemask will be worn to collect expired gases during a maximal exercise test on a treadmill. As exercise intensity is progressively increased (i.e. increased speed and grade), oxygen and carbon dioxide concentrations in the inhaled and exhaled air are measured.

Session 2 (~2 hours):
Upon arrival to the laboratory, you will be instructed to change into your loose fitting athletic clothing. During this session we will be measuring quadriceps muscle oxygenation during a 20-minute bout of low-intensity treadmill exercise and during a resting period with inflatable compression cuffs on your legs. When the compression cuffs are on your legs we will be inflating the cuffs to pressures between 100- 220 mmHg. A certain pressure will be maintained for 4 minutes before deflating and resting for 2 minutes. After the 2 minutes of rest we will inflate the cuffs to a higher pressure and again hold for 4 minutes before deflating.

Muscle oxygenation will be measured with a near infrared spectroscopy (NIRS) device secured with adhesive tape to your leg. A probe will be attached to the right vastus-lateralis approximately 70% the distance between the middle of the greater trochanter to the lateral condyle on the femur.

Session 3 (~ 1 hour):
Upon arrival to the laboratory, you will be instructed to change into your loose fitting athletic clothing. During this session we will again be measuring quadriceps muscle oxygenation during a 20-minute bout of low-intensity treadmill exercise with the compression cuffs on your leg.

Session 4 (~ 1 hour):
During this session we will again be measuring quadriceps muscle oxygenation during a 20-minute bout of low-intensity treadmill exercise with the compression cuffs on your leg. However, during this session we will be using a modified cuff inflation protocol. We will hold your training pressure for 5-minutes prior to exercising on the treadmill.

Session 5 (~1 hour):
First, this session is optional and incorporates, a 20-minute low-intensity training session while measuring muscle oxygenation of the quadriceps once again. During this session, elastic training bands will be used in the same manner as that of the compression cuffs from previous sessions. The bands will be tightened to the same pressure as the cuffs in the previous sessions and the protocol used will be the same as that in session 4.

Are there any special instructions I should follow when I come in to the lab?
Yes. On each day of **laboratory testing**, you are asked to:

- Not perform any ***strenuous*** physical activity for ***24 hours*** prior to the laboratory sessions
- Avoid caffeine or alcohol ***on the day of*** testing
- Eat only a light meal at least ***3 hours prior*** to the start of testing
- Drink at least 500ml of water at least ***30 minutes prior*** to each session
- Bring with you both loose-fitting short sleeve shirt and shorts and tight-fitting short sleeve shirt and shorts

You will be reminded before each laboratory testing of the requirements listed above.

POTENTIAL BENEFITS AND RISKS
Blood flow restriction training may be a viable form of training for those contraindicated for higher intensity training or for those looking for an effective low-intensity alternative to traditional forms of aerobic exercise, however many uncertainties are evident with proper training pressures. With your participation in this study, we hope to develop a standardized protocol for blood flow restriction training and ultimately increase the effectiveness of this form of exercise.

In addition, benefits of participation include a fitness test (VO_2peak) and body composition measurements (body fat percentage, lean mass percentage, BMI) and interpretation of these tests, typically valued at $100-$150, at no charge. As well, participation will allow you to become exposed to an interdisciplinary exercise physiology research protocol, contribute to the advancement of science, and gain knowledge about the function of your own body. Furthermore, **you will receive compensation for your time.** More details are provided under

REMUNERATION.

There also may be risks associated with participation. While no studies have reported severe adverse reactions to acute or chronic bouts of blood flow restriction training, you may develop bruises on your legs following exercise with the cuffs. These bruises are generally painless and go away 1-2 days after the training session. There is also a small but potential risk of developing deep vein thrombosis. Furthermore, you may experience skeletal muscle soreness during and/or after exercise. Delayed onset muscle soreness is a natural response to exercise and may develop 24-48 hours after exercising. In rare cases you may experience dizziness as well as temporary numbness or a feeling of coldness in your legs however, should you experience any adverse reaction to the training, emergency action plans are set in place and all researchers and assistants will be trained to handle the situation safely. Furthermore, extensive medical screening will be conducted to ensure that you are not contraindicated for blood flow restriction training.

CONFIDENTIALITY

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will not be disclosed at any point in time without your expressed permission.

To ensure full confidentiality, following your consent, you will be assigned an identification code that can only be linked back to your data by an assigned study investigator. The master list matching participants to the data will be password-protected and kept by the student PI. When the data are published in scientific journals or presented at research conferences, they will be expressed as group averages. In the event that individual data will be highlighted, the identification of that person will not be revealed. Data collected during this study will be stored in a locked laboratory cabinet in the Human Hemodynamics Lab at Brock University only accessible by study investigators. Digital information will be password protected. All information including medical questionnaires which contain personal identifiers will be destroyed if you choose to withdraw from the study.

Following publication of the data, all personal identifiers will be confidentially destroyed, as well data will be destroyed 5 years following publication, the allotted time required to keep scientific data post-publication.

Access to this data will be restricted to the PI and student PI and members of the Human Hemodynamics Laboratory.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. **You may withdraw from the study without penalty or any consequences at any time** by making the researchers aware of your decision. To withdraw, please contact either Dr. O'Leary or Jonathan Vaantaja in person, by telephone or email (indicated in the information and consent forms). If you wish, you may decline to answer any questions however, participation may be questionable if some questions are not answered (i.e. any cardiovascular-related questions in the Human Hemodynamics Laboratory Participant Screening and Medical History Questionnaire. We reserve the right to withdraw you from the study if we believe that it is necessary. As well, you may wish to withdraw from participating in

any component of the study, at any time and may do so without penalty. However, in the event of withdrawal from the study, compensation will still be given for any laboratory visits you are present for, as described under the Remuneration section below.

Your participation, or lack thereof, in this study will in no way influence your ability to participate in future studies at Brock University. The investigators may withdraw you from this research if circumstances arise which warrant doing so. Typically, this may occur due to a change in medication, nutrition, or physical activity status.

REMUNERATION

To compensate you for your time, a $40 honorarium will be provided upon completion of the study. Compensation will be prorated to percentage-based attendance. For example, if you complete 1 session, you will receive (1/4)*$40 = $10. If you so decide to participate in Session 5, your compensation will total $50.

PUBLICATION OF RESULTS

Results of this study may be published in professional journals and presented at conferences. Feedback about this study will be available approximately 1 year following study completion and will be available upon request. If you would like to receive feedback about this study please contact Dr. Deborah O'Leary (email: doleary@brocku.ca, phone: (905) 688-5550 Ext. 4339).

CONTACT INFORMATION AND ETHICS CLEARANCE

If you have any questions about this study or require further information, please contact Jonathan Vaantaja or Dr. Deborah O'Leary using the contact information provided above. This study has been reviewed and received ethics clearance through the Research Ethics Board at Brock University **#16-247**. If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

Thank you for your assistance in this project. Please keep a copy of this form for your records.

CONSENT FORM

I agree to participate in this study described above. I have made this decision based on the information I have read in the Information-Consent Letter. I have had the opportunity to receive any additional details I wanted about the study and understand that I may ask questions in the future. I understand that I may withdraw this consent at any time.

Name: _____

Signature: _____ Date: _____

Appendix C
HUMAN HEMODYNAMICS LABORATORY PARTICIPANT SCREENING AND MEDICAL HISTORY QUESTIONNAIRE

Name: _____ Date: _____

Age: _____

Please review the following questions below*.Your responses to this questionnaire are confidential. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions; however, participants may be subject to exclusion from the study based on their responses.

1. Are you currently taking any medication (including aspirin) or have you taken any medication in the last two days?	**YES**	**NO**
2. Have you taken any medication in the past six months?	**YES**	**NO**
3. Is there any medical conditions with which you have been diagnosed and are under the care of a physician (e.g. asthma, diabetes, hypertension, and anorexia)?	**YES**	**NO**
4. Have you in the past, or are currently, experiencing chest pains, dizziness or light headedness during exercise or when you are not doing physical activity?	**YES**	**NO**
5. In the past month, have you had chest pain when you were not doing physical activity?	**YES**	**NO**
6. Have you in the past, or are currently, experiencing head injury, concussion, mild "bell ringers", burns or stings, numbness in your arms or legs, convulsions, seizures, or epilepsy, neurological disease or problems, or severe headaches?	**YES**	**NO**
7. Have you in the past, or are currently, experiencing irregular heartbeats, a heart murmur or defect, high blood pressure or hypertension, low blood pressure or hypotension?	**YES**	**NO**
8. Has your doctor ever said that you have a heart or cardiovascular condition?	**YES**	**NO**
9. Have you ever been diagnosed with deep vein thrombosis or varicose veins?	**YES**	**NO**
10. Have you in the past, or are currently, experiencing high cholesterol, anaemia, blood clotting problems, or other blood-related problems?	**YES**	**NO**
11. Have you in the past, or are currently, experiencing broken bones, stress fractures or hairline cracks, dislocated joints, sprained joints, torn ligaments and/or cartilage, muscle/tendon injury, arthritis, painful or swollen joints, neck/upper back/lower back pain or injury?	**YES**	**NO**

12. Has your doctor ever said to you that you have a sleeping disorder?	**YES**	**NO**
13. Have you in the past, or are currently, experiencing hearing problems, eye injuries, vision problems?	**YES**	**NO**
14. Have you in the past, or are currently, experiencing thyroid problems, other endocrine or hormonal problems?	**YES**	**NO**
15. Have you in the past, or are currently, experiencing kidney stones, blood in your urine, other kidney problems?	**YES**	**NO**
16. Have you in the past, or are currently, experiencing oral or dental problems or injuries?	**YES**	**NO**
17. Have you in the past, or are currently, experiencing any skin problems/conditions?	**YES**	**NO**
18. Have you in the past, or are currently, experiencing a hernia?	**YES**	**NO**
19. Have you in the past, or are currently, experiencing difficulty breathing, asthma or bronchitis, pneumonia or tuberculosis?	**YES**	**NO**
20. Do you have diabetes, cancer or tumours?	**YES**	**NO**
21. Do you have a tendency for, or ever been diagnosed with, claustrophobia (a very strong fear of confined spaces)?	**YES**	**NO**
22. Have you recently undergone a surgery or operation (within the past six months)?	**YES**	**NO**
23. If "yes" to question 22, please describe: _____ _____ _____ _____		
24. Do you have a family history of any of the following: death before the age of 50, blood disorders or problems, sudden death during physical activity, arthritis, cardiovascular disease/conditions, diabetes, high blood pressure or hypertension, high cholesterol, chronic obstructive pulmonary disease, another other respiratory-related problems, other major medical problems?	**YES**	**NO**
25. If "yes" to question 24, please describe: _____ _____ _____ _____ _____		
26. Are you, or have you in the past, engaged in any extreme diet?	**YES**	**NO**
27. Do you, or have you in the past, consumed any alcohol on a regular basis (i.e. daily, males ≤2 drinks/day, females ≤1 drink/day)?	**YES**	**NO**

28. Do you, or have you in the past, smoked on a regular basis (i.e. daily, ≤ 20 cigarettes/day)?	**YES**	**NO**
29. Do you, or have you in the past, consumed any nutritional supplements (e.g. calcium, multi-vitamin, protein powders) on a regular basis (i.e. daily)?	**YES**	**NO**
30. Has your doctor ever said to you that you should only do physical activity recommended by a doctor?	**YES**	**NO**
31. Do you know of any other reason why you should not do physical activity?	**YES**	**NO**
32. Do you, or have you in the past, engaged in physical activity on a regular basis (i.e. 3-5 times per week)?	**YES**	**NO**
33. Have you ever used blood flow restriction as part of your exercise program?	**YES**	**NO**

Appendix D
GXT SHEET

Date:_____

Participant ID: _____ Height:_____

Weight:_____

Time till exhaustion: _____

HR_{max}: _____

Time (minutes)	Speed (mph)	Grade (%)	HR	RPE
0-1	4.5	0		
1-2	4.5	3		
2-3	5.5	3		
3-4	5.5	6		
4-5	6.5	6		
5-6	6.5	9		
6-7	7.5	9		
7-8	7.5	9		
8-9	8	9		
9-10	8.5	9		
10-11	9	9		
11-12	9.5	9		
12-13	10	9		

Notes:

_____
_____
_____
_____
_____
_____

Appendix E

NON-BFRT/TRAINING PRESSURE DETERMINATION SHEET

Dates:_____/_____ Thigh Circumference:_____

Participant: _____ Adipose Tissue Thickness:_____

Resting HR: _____ Initial Pressure: L:_____ R:_____

Training HR: _____ Cuff overlap: L:_____R:_____

NIRS Placement on VL: _____ Training Pressure: _____

BP: 1)_____2)_____3)_____

Time (minutes)	Speed (mph)	Grade (%)	HR	RPE
0-1				
1-2				
2-3				
3-4				
4-5				
5-6				
6-7				
7-8				
8-9				
9-10				
10-11				
11-12				
12-13				
13-14				
14-15				
15-16				
16-17				
17-18				
18-19				
19-20				

Appendix F

BFRT SHEET- TRADITIONAL PROTOCOL

Date:_____

Participant ID: _____ NIRS Placement on VL: _____

Resting HR: _____ Cuff Overlap: L: _____R:_____

Training HR: _____ Initial Pressure: L:_____R:_____

Training Pressure: _____ BP: 1)_____2)_____3)_____

Time (minutes)	**Speed (mph)**	**Grade (%)**	**HR**	**RPE**
0-1				
1-2				
2-3				
3-4				
4-5				
5-6				
6-7				
7-8				
8-9				
9-10				
10-11				
11-12				
12-13				
13-14				
14-15				
15-16				
16-17				
17-18				
18-19				
19-20				

Appendix G

BFRT SHEET- MODIFIED PROTOCOL

Date:_____

Participant ID: _____ NIRS Placement on VL: _____

Resting HR: _____ Cuff Overlap: L: _____R:_____

Training HR: _____ Initial Pressure: L:_____R:_____

Training Pressure: _____ BP: 1)_____2)_____3)_____

Time (minutes)	**Speed (mph)**	**Grade (%)**	**HR**	**RPE**
0-1				
1-2				
2-3				
3-4				
4-5				
5-6				
6-7				
7-8				
8-9				
9-10				
10-11				
11-12				
12-13				
13-14				
14-15				
15-16				
16-17				
17-18				
18-19				
19-20				

Appendix H
BORG SCALE

Rating of Perceived Exertion (RPE) Scale

Borg Scale 6-20)	Intensity	Breathing Scale	Distance Scale
6	No exertion at all		
7		Can sing full songs	Could continue all day
8	Extremely light		Could continue 4–6 hours
9	Very light	Can sing partial verses	Could continue 3–4 hours
10			Could continue 2–3 hours
11	Light	Can talk in full sentences	Could continue 1–2 hours
12			Could continue 45–60 minutes
13	Somewhat hard	Can talk in short sentences	Could continue 30–45 minutes
14			Could continue 20–30 minutes
15	Hard (heavy)	Breathing hard, thinking clearly	Could continue 15–20 minutes
16			Could continue 10–15 minutes
17	Very hard	Breakaway ventilation	Could continue 5–10 minutes
18			Could continue 2–5 minutes
19	Extremely hard		Could continue 1–2 minutes
20	Maximal exertion		Could continue <1 minute

Appendix I
EMERGENCY ACTION PLAN

For Medical Emergencies during Exercise Testing

Step 1: Remain Calm

- **CONTROL** and **ASSESS** the situation
- **CONTACT** personnel trained in emergency situations:

Campus Security Day-time extension 4200 (night-time ext 3200) OR **911**

Our address:

Human Hemodynamic Laboratory

1812 Sir Isaac Brock Way, St Catharines

Welch Hall Room 22 (basement level)

Step 2: **Perform** all measures (First aid/CPR) to ensure the safety of the subject

- **Attend** to victim until emergency personnel arrive.

Step 3: **CREATE** an incident report

Appendix J
KAATSU CERTIFICATION

KAATSU GLOBAL, INC.

Hereby congratulates

Jonathan Vaantaja

Upon having successfully passed the Certification KAATSU Specialist Course and Examination and is henceforth qualified to be a practicing

KAATSU SPECIALIST

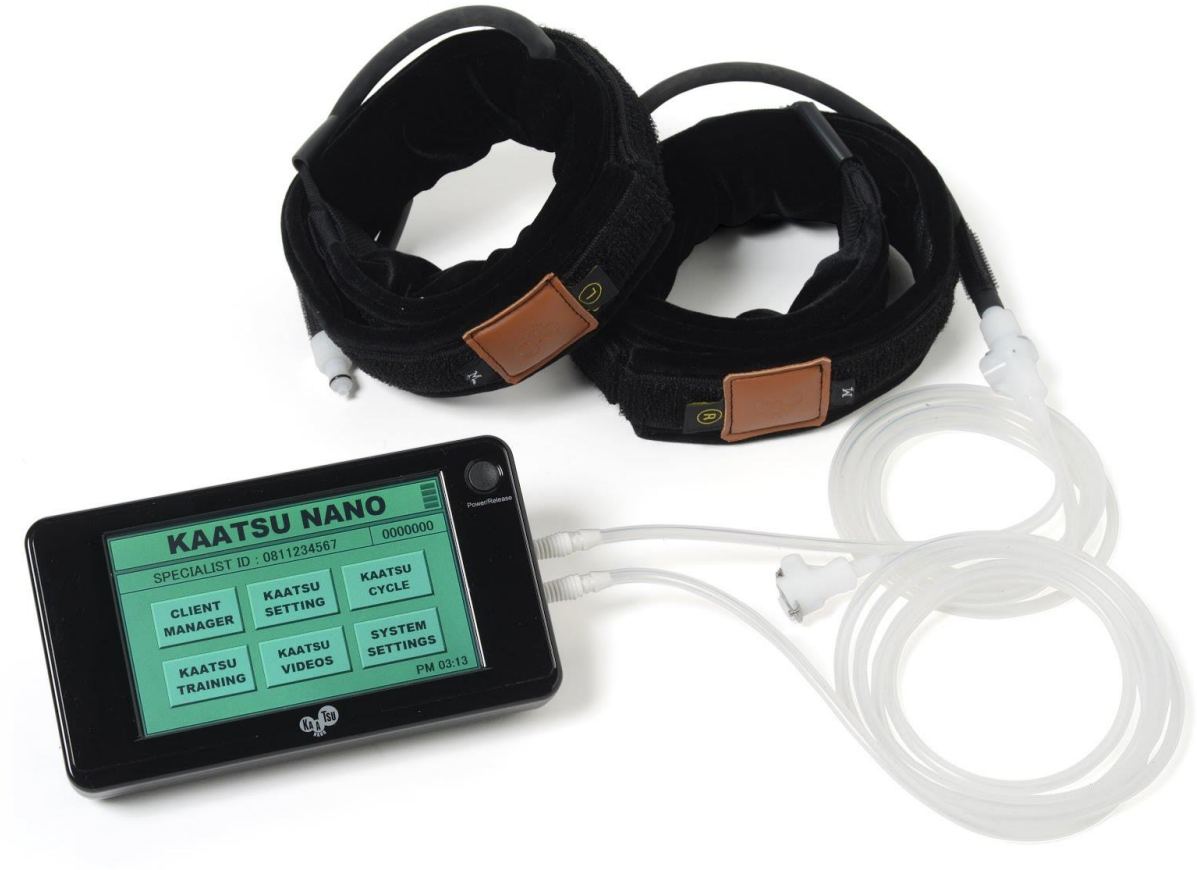
February 27, 2017

Yoshiaki Sato, M.D., Ph.D.,
Chairman
KAATSU Global, Inc.

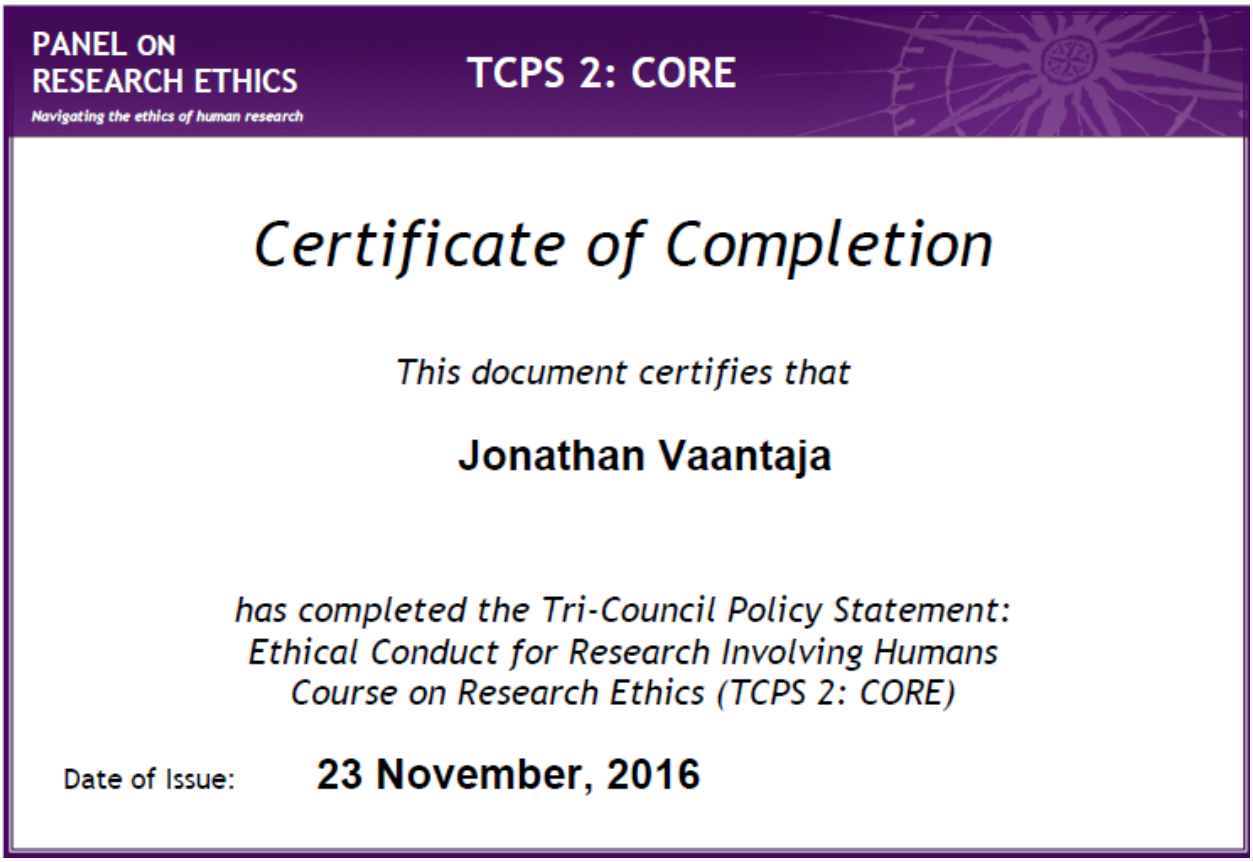
KAATSU TRAINING

Steven Munatones
President
KAATSU Global, Inc.

Appendix K
KAATSU NANO AND BLOOD FLOW RESTRICTION CUFFS



Appendix L
TCPS 2: CORE CERTIFICATE OF COMPLETION



PANEL ON RESEARCH ETHICS
Navigating the ethics of human research

TCPS 2: CORE

Certificate of Completion

This document certifies that

Jonathan Vaantaja

has completed the Tri-Council Policy Statement:
Ethical Conduct for Research Involving Humans
Course on Research Ethics (TCPS 2: CORE)

Date of Issue: **23 November, 2016**

Appendix M

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca
Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	4/19/2017		
PRINCIPAL INVESTIGATOR:	O'LEARY, Deborah - Health Sciences		
CO-INVESTIGATOR(S):	Stephen Cheung (scheung@brocku.ca); Brian Roy (broy@brocku.ca)		
FILE:	16-247 - O'LEARY		
TYPE:	Masters Thesis/Project	STUDENT:	Jonathan Vaantaja
		SUPERVISOR:	Deborah O'Leary
TITLE:	Investigating the Effects of Aerobic Exercise with Blood Flow Restriction on Arterial Stiffness		

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 4/30/2018

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **4/19/2017** to **4/30/2018**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **4/30/2018**. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with vour research.

Approved: Sandra Peters

Sandra Peters, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Appendix N

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca
Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	July 14, 2017		
PRINCIPAL INVESTIGATOR:	O'LEARY, Deborah - Health Sciences		
FILE:	16-247 - O'LEARY		
TYPE:	Masters Thesis/Project	STUDENT:	Jonathan Vaantaja
		SUPERVISOR:	Deborah O'Leary
TITLE:	Investigating the Effects of Aerobic Exercise with Blood Flow Restriction on Arterial Stiffness		

ETHICS CLEARANCE GRANTED

Type of Clearance: MODIFICATION Expiry Date: 4/30/2018

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

Modification: Extension of pilot study

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **4/30/2018**. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: Sandra Peters

Sandra Peters, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Appendix O

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca
Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	December 6, 2017		
PRINCIPAL INVESTIGATOR:	O'LEARY, Deborah - Health Sciences		
FILE:	16-247 - O'LEARY		
TYPE:	Masters Thesis/Project	STUDENT:	Jonathan Vaantaja
		SUPERVISOR:	Deborah O'Leary
TITLE:	Investigating the Effects of Aerobic Exercise with Blood Flow Restriction on Arterial Stiffness		

ETHICS CLEARANCE GRANTED

Type of Clearance: MODIFICATION Expiry Date: 4/30/2018

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

Modification: Include an additional day of testing (a 4th day) to compare the effect of a new cuff inflation protocol on thigh muscle oxygenation during walk training with BFR.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **4/30/2018**. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: Sandra Peters

Sandra Peters, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.