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Impacts of blood flow occlusion on the human neuromuscular system

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Abstract

Blood flow restricted or occlusion (BFO) exercise uses external pressure to impede blood flow, thus reducing the amount of oxygenated blood reaching the muscle, with greater pressure causing greater obstruction of blood flow. The result is enhanced muscle fatigability. The four studies in this thesis investigate how BFO acutely affects muscle fatigue, the neuromuscular system, muscle contractile properties and motor unit firing rates, using a combination of electrical stimulation, transcranial magnetic stimulation, transmastoid stimulation, force production, and electromyography (EMG).

Study one investigated whether BFO low-intensity isometric contractions to failure of the arm flexors elicits greater muscle fatigue compared to low-intensity contraction with free blood flow. Results showed that BFO created a greater amount of low-frequency fatigue in a significantly reduced amount of time, but recovers at a normal rate once blood flow is restored. This indicates that BFO can reduce overall time required to produce greater amounts of muscle adaptations.

Study two explored modulation of corticospinal tract excitability during low-intensity isometric arm flexion with and without BFO. The study found that BFO enhanced motoneuron excitability in a lesser amount of time to muscle failure. This likely indicates that BFO enhances excitability of the motoneuron possibly through a feedback loop activated by type III and IV muscle receptors.

Study three investigated whether BFO low-intensity dynamic arm flexion to failure produced similar or greater amounts of muscle fatigue, as well as reduced power output, compared to high-intensity normal blood flow. The particular novelty was to explore dynamic actions rather than isometric. Results showed low-intensity exercise with BFO produced greater low-frequency fatigue and greater reductions in power. Therefore likely enhancing the requirement of the muscle adaptation.

Study four investigated the modulation of motor unit firing rates (MUFRs) with BFO either distal or proximal to the tibialis anterior muscle. Results indicate when BFO was proximal

MUFRs decreased as the muscle became more fatigued, and more than when flow was occluded distally, and both being more than control. This indicates that blood flow obstruction either proximal or distal to working muscle affects muscle fatigue greater than with free blood flow.

Overall these investigations expand our knowledge of the acute effects of BFO, and can be extrapolated to factors responsible to long term training adaptations with blood flow restriction.

Keywords

blood flow restriction; human; fatigue; transcranial magnetic stimulation; transmastoid electrical stimulation; electromyography; motor unit; neuromuscular system; muscle

Summary for Lay Audience

Blood flow occlusion (BFO) or the removal of blood flow, can be used to enhance muscle and central nervous system fatigue when combined with low-intensity contractions. The use of BFO with low-intensity contractions has been observed to increase muscle growth (hypertrophy) and muscle strength when incorporated into long-term resistance training programs. The goal of the four studies presented within this thesis sought to explore the short-term effects of BFO on muscle and central nervous system fatigue.

Experiment one investigated whether BFO with a low-intensity contractions to exhaustion (unable to maintain muscle contraction) of the muscles that produce flexion of the arm cause greater muscle fatigue compared to the same contraction with normal blood flow. The results indicate that BFO caused greater fatigue in a reduced amount of time. This indicates that BFO can shorten overall time required to produce greater muscle changes during exercise.

Experiment two explored changes in the central nervous system during a low-intensity contraction of the arm flexors with and without BFO. The experiment found BFO caused greater changes within the central nervous system in a shorter amount of time. This indicates that BFO causes changes within the brain and spinal cord that are different than contractions performed with normal blood flow.

Experiment three investigated whether BFO during low-intensity arm flexor contractions to exhaustion produced greater amounts of muscle fatigue, as well as reduced the speed of contracting muscles compared to a high-intensity bout of contractions with normal blood flow. Results showed low-intensity exercise with BFO produced greater muscle fatigue and greater reductions in muscle speed.

Experiment four investigated the changes in how muscle fibers activated when BFO is applied either above or below a working muscle. Results indicate when BFO was above the working muscle (no inward blood flow) muscle fibers decreased their rate of activation as the muscle became more fatigued, and this was more than when the outflow of the working muscle (below) was occluded.

The results of these experiment indicate that occlusion of blood flow changes how muscles activated and fatigued, when compared to contractions of muscle during normal blood free flow.

Co-Authorship Statement

This thesis contains material from published, under review, and unpublished manuscripts (Chapters 2-5). On all manuscripts, David B. Copithorne was the first author and Dr. Charles Rice was co-author. Christopher J. McNeil was also co-author in Chapter 3. All experimental data presented was collected, analyzed and interpreted by David B. Copithorne.

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List of Abbreviations

1RM – one repetition max

Ag-AgCl – silver-silver chloride electrodes

ANOVA – analysis of variance

ATP - Adenosine triphosphate

CMEP – cervicomedullary motor evoked potential

CSA – cross sectional area

BFO – blood flow occlusion

BFR – blood flow restriction

E-C – excitation contraction coupling

EMG – electromyography

ES – effect size

FF – blood free flow

FFfail – blood free flow to failure

FFiso – blood free flow time matched

FP – fail point

HRT – half relaxation time

Hz - hertz

ITT – interpolated twitch technique

kHz - kilohertz

LFF – low-frequency fatigue

mA – milliamp

MEP – motor evoked potential

Mmax – M-wave max stimulation

mmHg – millimeters of mercury

MU – motor unit

mV - millivolt

MVC – maximal voluntary contraction

Nm – newton meter

PT – peak torque

R0-R30 – recovery at 0 minutes to 30 minutes

RMS – root-mean-square

s - seconds

SD – standard deviation

SE – standard error

TMS – transcranial magnetic stimulation

TPT – time to peak twitch

V - Volts

VA – voluntary activation

μs – microsecond

μV - microvolt

Chapter 1

1 General Introduction

The implementation of resistance exercise programs have been previously observed to produce adaptations in human muscle strength and have hypertrophic effects. Exercise training paradigms are manipulated in terms of intensity, repetitions, volume, and duration in order to ascertain specific muscle adaptations determined by the individual. For example, the Canadian Society for Exercise Physiology and the American College of Sports Medicine, recommend for strength and hypertrophy, that an intensity of 65-85% (maximal voluntary contraction; MVC) will provide optimal stimulus for muscle adaptations[1]. However, there is growing support that exercise performed at lower-intensities (<30% 1RM) performed to failure may provide sufficient stimulus for muscle growth that is equal to or greater than the stimulus provided from higher-intensity loads[2]. Along with providing an effective alternative to high-intensity exercise in terms of muscle adaptations, there is also support that in some populations or circumstances low-intensity exercise helps to protect joint health, or provides a training environment that is less detrimental to a previous insult. Along with low-intensity exercise the application of blood flow restriction (BFR) or blood flow occlusion (BFO), provided by an external pressure (blood pressure cuff, elastic bands, etc.) is also supported to provide adequate stimulus for muscle adaptations that is perhaps more suited for rehabilitation settings and other populations[3,4,5].

1.1 Blood flow restriction and occlusion exercise

BFR low-intensity exercise is a growing training method, that combines low load resistance training (<50% MVC), with varying levels of vascular restriction. The use of BFR with low-intensity resistance exercise is a popular training method in Japan. The first training program to adapt BFR in Japan was designed by Dr. Yoshiaki Sato and is referred to as KAATSU training which he then patented in 1994. KAATSU training is defined as a method that promotes a state of blood pooling in the capillaries within the limb musculature[6]. The original application of this method designed by Dr, Sato was

thought to not only provide a method of exercise that will produce muscle adaptation in a general healthy population, but also more specific populations such as athletes, the elderly, rehabilitation settings, and other various disease population (i.e. cardiovascular and orthopedic conditions). Although this training method has numerous names, from here on it will be referred to as either BFR (restriction that does not occlude arterial blood flow) or BFO (restriction that does occlude arterial flow). The use of chronic BFR training has been previously studied with program lengths varying from 1wk – 16wks at frequencies of 2x – 7x (days/wk). The observations from these studies report increases in cross sectional area (CSA) of the muscle[7,8,9,10,11,12,13,14], Strength[7,8,15,16,17,11,12,13,14], and muscle activation reported as increases in electromyography (EMG)[16,13]. The results of these studies are outlined in a review by Karabulut and colleagues[18], however, this review does not summarize an exhaustive list of studies that will be reported on in this thesis. Although BFR exercise has been extensively studied in a chronic setting there are very few studies that have explored the effects of BFR on the neuromuscular system in acute studies. Those studies reported that BFR causes greater relative increases in EMG, compared to exercise of the same intensity without vascular restriction[13,19], and greater increases in EMG are observed with blood flow occlusion[19]. They suggest that the likely cause of this EMG increase results from greater muscle activation, specifically larger type II motor units rather than type I motor units, caused by the restriction, and that greater nerve compression and restriction of blood flow exacerbates these changes.

1.2 The neuromuscular system

1.2.1 The corticospinal tract

The primary motor cortex in humans provides the major corticospinal output that produces voluntary movements[20]. When the primary motor cortex is excited it transmits cortical commands down the lateral corticospinal tract, which decussates within the medulla and finally synapses with the motoneuron (see Figure 1.1). This pathway can be non-invasively activated in order to study the complexities of human voluntary movement as a sum of anatomical structures rather than observing only outcome measures of the process such as force generation. These methods were first developed in 1980, by Merton and Morton[21]. This original method relied on an electrical stimulus that

was sufficient to pass through the skull and excite the motor cortex. This technique was later adapted using changing magnetic fields that were able to excite the motor cortex, called transcranial magnetic stimulation (TMS)[22]. The advancement of this technology allowed researchers to explore the major functions of this voluntary pathway, to better understand how voluntary movements can be affected by task demands, force modulation, and fatigue. TMS is not considered selective, however it is generally agreed that TMS, activates transynaptically the cortical motoneurons, which elicits a descending volley down the pathway to the muscle of interest, and is detected as a compound motor evoked potential (MEP).

However, the resulting MEP, which is elicited by the excitation of the motor cortex, only allows insights into the entire pathway. The MEP cannot explain what may be happening at the level of the cortex, without first understanding the independent contribution of the motoneuron that is included in the resulting MEP detected at the level of the muscle. Or for that matter, the stimulation of the cortex using TMS is also affected by the excitability of the target muscle, which can be effected by contraction history and task dependence. The delivery of external stimuli at differing levels along the motor pathway can address this question. That is, the responses to TMS of the motor cortex (MEP), transmastoid electrical stimulation (TMES) (cervicomedullary motor evoked potential, CMEP), and peripheral nerve stimulation (compound muscle action potential, M-wave) have been used collectively to assess cortical, spinal and peripheral excitability during fatiguing tasks[23].

In order to test for modulation of motoneurons that enables the separation of supraspinal and spinal excitability, the use of TMES is required. Stimulation of the descending axons at a subcortical level[24], provides information about the excitability of the motor pool that is void of descending input from the motor cortex. This technique has been shown to occlude the response of TMS[24], because an antidromic volley produced by TMES disrupts the volley for TMS, if the proper latency of response coincides.

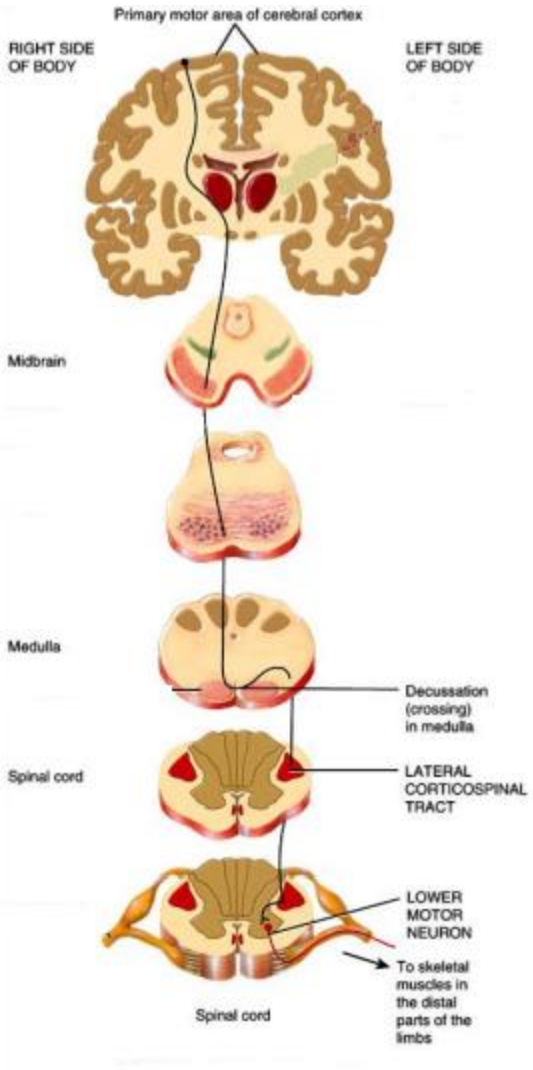


Figure 1.1 The lateral corticospinal tract the pathway responsible for transmission of voluntary control of skeletal muscle (adapted from http://ksumsc.com/download_center/Archive/2nd/434/1-CNS%20Block/Teams/Physiology/5.%20Physiology%20of%20Motor%20Tracts.pdf)

1.2.2 Transcranial magnetic stimulation

TMS is a non-invasive, commonly used technique to assess corticospinal excitability modulation in humans. This technique uses a magnetic stimulator which generates a magnetic field that is passed into the motor cortex. TMS activates the cortical motoneurons

transynaptically to elicit a MEP. According to Martin and colleagues[25] the magnetic field that is generated, activates interneurons within the cortex which synapse to large pyramidal cells and descend along the corticospinal tract (see Figure 1.2, red stimulation site). It is likely that the initial response in some neurons will result from direct stimulation of the axon or axon hillock within the corticospinal neuron called the D response, and others from indirect, trans-synaptic activation, called the I response[26]. The indirect stimulation of the cortical motoneuron produces a descending volley (multiple signals) of I waves that are separated by ~1.5ms intervals. The descending volleys temporally summate upon the motoneuron which will elicit an action potential or increase its excitability by slightly depolarizing the resting voltage threshold of the cell. The motor pathway from the motor cortex to the spinal motoneuron is thought to be primarily monosynaptic especially in the biceps brachii[27,28]. Therefore, the activation of the descending motor pathway provides a measure of corticospinal excitability. The excitability is referred to as supraspinal rather than motor cortex excitability since the MEP can be affected by intracortical facilitation and interhemispheric inhibition. TMS on its own can only assess corticospinal excitability as a whole, thus it cannot distinguish between changes that are spinal in nature. Therefore TMES (see Figure 1.2, yellow stimulation site) must be used along with TMS to indicate the location of the changes.

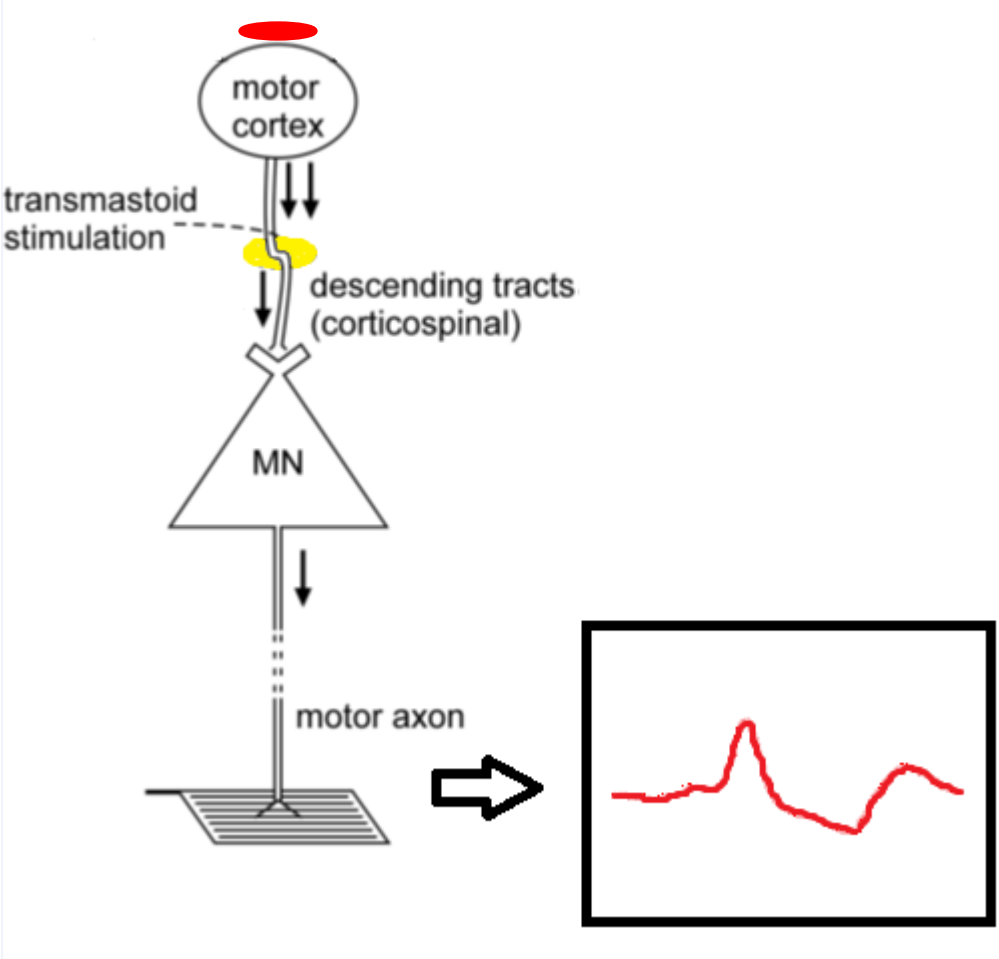


Figure 1.1 Representation of the volleys and pathways involved in production of the CMEP and MEP (adapted from McNeil, C.J., Butler, J.E., Taylor, J.L. and Gandevia, S.C., 2013. Testing the excitability of human motoneurons. Frontiers in human neuroscience, 7, p.152.)

1.2.3 Transmastoid stimulation

Electrodes are placed just below the mastoid processes at the back of the skull through which an electrical current is passed to activate the descending corticospinal tract (see Figure 1.3). Taylor[28] suggested TMES to be the most effective, and direct means available to examine motoneuron response to synaptic input in humans, non-invasively. Through collision experiments involving both TMS and TMES it is suggested that many

of the same axons sub serve the two responses[26]. The single volley evoked by TMES can largely 'occlude' the response to cortical stimulation when the interstimulus interval is appropriate[26], through the collision of the antidromic volley interacting with the descending action potential. This stimulation elicits a cervicomedullary motor evoked potential (CMEP). The resulting CMEP is a measure of the spinal excitability void of supraspinal input. The application of this technique can be used to measure motoneuron excitability during contractions of the upper limbs. Changes seen in the CMEPs are indicative of the excitability changes within the motoneuron. Since the motor pathway from the motor cortex especially in the biceps brachii is thought to be primarily monosynaptic[27,28], any increase or decrease seen in CMEP amplitude or duration can be assumed to be changes at the motoneuron void of supraspinal excitability changes as well as through sensory feedback loops.

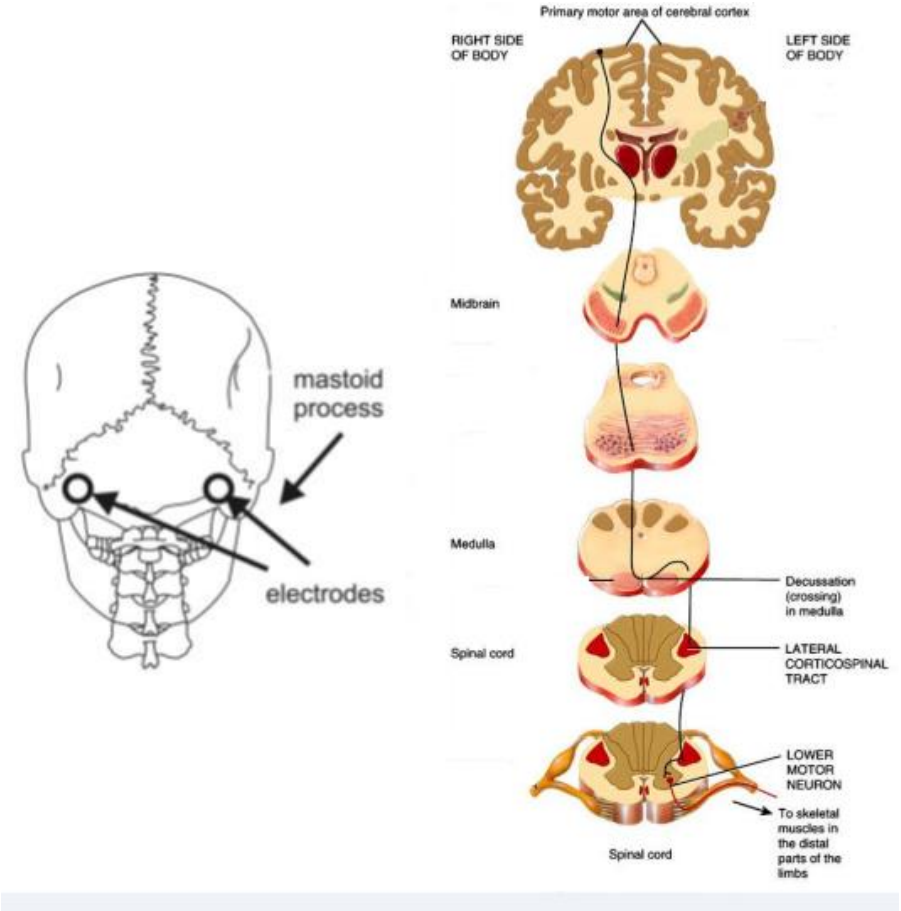


Figure 1.2 Electrode placement in order to elicit a cervicomedullary motor evoked potential (CMEP) in humans (adapted from Taylor, J.L. and Gandevia, S.C., 2004. Noninvasive stimulation of the human corticospinal tract. Journal of applied physiology, 96(4), pp.1496-1503, and http://ksumsc.com/download_center/Archive/2nd/434/1-CNS%20Block/Teams/Physiology/5.%20Physiology%20of%20Motor%20Tracts.pdf)

1.2.4 How does contraction intensity, movement complexity, and contraction duration affect supraspinal, spinal, and peripheral muscle excitability?

MEPs are generally measured as either peak-to-peak amplitudes or as overall area, the resulting analysis of a MEP will provide insights into the excitation of the corticospinal tract at the time of stimulation, which can be greatly affected by task dependency as well as contraction intensity. The interaction between contraction intensity and MEP amplitudes have been previously studied during voluntary efforts in varying muscles. In the gastrocnemii and soleus of the leg, MEP amplitude was observed to increase as contractions intensity increased, with the greatest modulation occurring at ≤60% of MVC[30]. These results were verified in the biceps brachii, brachioradialis, and the FDI[31]. However, at contractions intensities exceeding 50-60% of MVC, MEP amplitude was observed to plateau or decrease[30,31]. When normalized, CMEP area (CMEP/Mmax) increased during a submaximal isometric contraction sustained at a constant force until exhaustion[32,33], peripheral muscle excitability during a sustained elbow flexion contraction at 20% of MVC until exhaustion observed a marked reduction in Mmax area[34]. These results indicate that the contraction intensity in which studies are explored, greatly affects the resulting corticospinal excitability, and thus the MEP amplitude. The complexity of the voluntary movement can also have an effect on the excitability of the corticospinal tract. Positron emission tomography, which can be used to measures indirect localized blood flow, has shown increases in regional blood flow to the ipsilateral premotor area, bilateral posterior parietal area, and the precuneus, positively related to sequential movements[35]. Therefore a sequential or more complex task requires greater

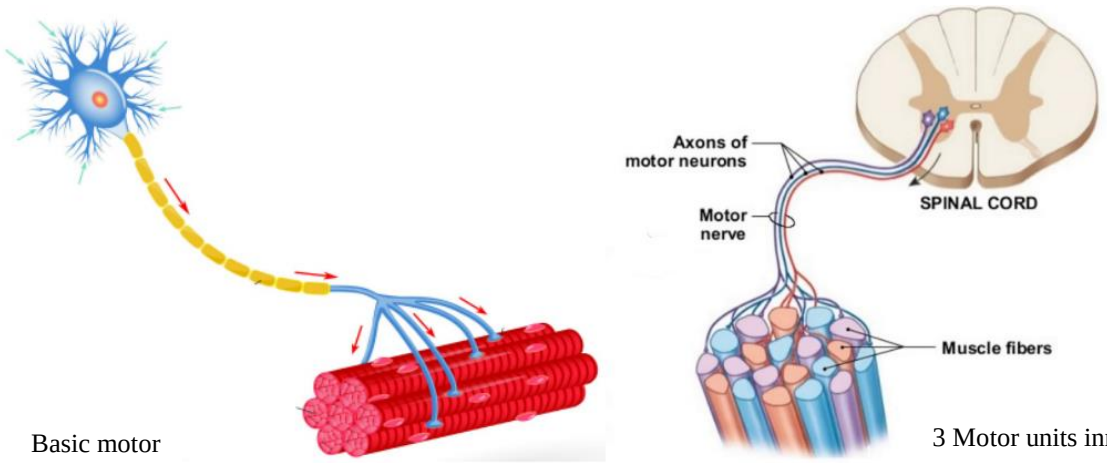
planning and a greater activation of pre-frontal cortex regions. This result has been substantiated using TMS which found that MEP amplitudes prior to a simple hand movement resulted in less excitability of the corticospinal tract (smaller MEP amplitude), compared to a more complex sequential task[36].

1.3 The motor unit and muscle fiber types

1.3.1 Motor unit physiology

The motor unit (MU) is comprised of the alpha motoneuron which exits the spinal column and into the periphery, and the muscle fibers in which it innervates (see Figure 1.4). The motor unit is considered the functional contractile unit in neuromuscular physiology. Excitatory potentials are received by the cell body of motoneuron from supraspinal structures and sensory afferents, and if the summation of the excitatory potentials received reaches the motoneurons voltage threshold, a resulting action potential is produced and propagated down the axon. When the action potential reaches the neuromuscular junction, which is the synapse between the terminal ends of the motoneuron and the muscle, the signal is passed to the muscle and continues its propagation. The muscle is then responsible for the generation of force, via the actomyosin complex.

The MU that is comprised of the alpha motoneuron and the muscle fibers it innervates, can vary throughout the human body. The larger skeletal muscle of humans, may have hundreds or perhaps thousands of MUs, whereas smaller muscles such as those in the hands would have much less. The number of muscle fibers within a single MU varies both within a particular muscle and more widely from muscle to muscle[37]. The function of the muscle likely dictates the amount of innervated muscle fibers. The MUs that contain the most muscle fibers occur in those muscles that act on the largest body mass[37]. For example, in larger muscles such as the biceps brachii, each MU can innervate hundreds of muscle fibers, designed for gross force generation; whereas, extrinsic eye muscles[37] that generate fine motor movement, each MU would innervate only a couple dozen.



Basic motor unit

3 Motor units innervated differing muscle fibers

Figure 1.3 Representation of the alpha motoneurone and the muscle fibers it innervates (adapted from http://www.muaythaischolar.com/motor-unit-2/ and http://faculty.pasadena.edu/dkwon/chapt_11/textonly/slide47.html)

1.3.2 The size principle

There is a general rule for MU recruitment within the human muscle that is referred to as the size principle[38], which states the smaller MUs are recruited before larger MUs. During most muscle contractions this rule is not violated, the cell body of the motoneuron determines whether a MU is recruited at a given force[39], and in accordance to the size of contraction they produce[40], during isometric contractions. Smaller diameter motoneuron cell bodies correspond with slower more fatigue resistant muscles fibers that can produce less force but can sustain low contraction intensities, and require less excitation in order to produce an action potential. Whereas larger diameter cells innervate greater numbers of large more fatigable muscle fibers, that can produce large forces but cannot be sustained[40,41]. Larger motoneurons also increase their rate of firing more rapidly[42] and attain higher firing rates in order to produce fused contractions[43,41]. MUs are classified into slow twitch fatigue resistant (type I) and fast twitch fatigable (type II) units[44], with differing subsets of type II fibers based upon their fatigue resistance. Therefore, it is important to understand the orderly recruitment of MUs, because the size principle will

dictate which muscle fiber types will be recruited in order to maintain task intensities across a range of individual force production.

1.3.3 Muscle fiber type and contractile properties

Muscle fiber types can be classified by their speed of contraction (slow vs. fast) (see Figure 1.5) and fatigability (fatigue resistance vs. fatigable)[45], and mixed in human muscles. Muscle fibers are predominantly influenced by the central nervous system (motoneuron), and this is evident by the homogenous composition of MUs[45]. Muscle fibers are the unit for producing contractile force, through the actomyosin complex. The properties of the actomyosin complex leads to the sliding filament theory as proposed by Huxley[46]. The contractile complex consumes ATP in order to generate force, with slow twitch fibers consuming less ATP, than fast twitch[45]. When comparing muscle fibers in terms of power output, peak power values increase orderly from slow to fast fibers[47]. Another distinction between fibers types is the speed at which they shorten. Slow type I fibers have slower shortening velocity along with production of less force, when compared to fast type II fibers[47]. Therefore, recruitment is not only based upon the development of force, but also the speed at which the desired movement requires[47]. However, the rapid force and velocity generation of type II fibers does come at a greater "cost", compared to slow type I fibers. Slow muscle fibers are able to generate nearly all required ATP by oxidative mitochondrial processes, while their consumption of ATP during contraction is relatively low[47], which contributes to their ability to maintain contractions for extended periods of time. In contrast fast type II muscle fibers rely upon glycolytic processes in order to generate ATP quickly, which limits their duration of contraction[47]. Since slow type I muscle fibers require oxygen for metabolism it is not surprising that the density of capillary beds surrounding type I fibers is greater[47]. Therefore, without the proper oxygen delivered by an oxygen rich blood supply it is likely that type I fibers would fatigue at an accelerated rate compared to normal conditions.

Figure 1.4 Force-velocity characteristics of human muscle fiber types (https://neupsykey.com/the-motor-unit-and-muscle-action/)

1.4 Purpose and Hypotheses

The broad purpose of the studies described within Chapters 2-5, was to investigate the acute physiological impacts of blood flow occlusion (BFO) on the neuromuscular system. Specifically, the purposes and hypotheses are as follows.

Chapter 2. Purpose: To compare neuromuscular fatigue of a low-intensity (20% of MVC) sustained isometric elbow flexion with blood flow occlusion to failure vs. an intensity-matched control protocol to failure. The hypotheses were:

a) that BFO exercise will cause exercise-induced voluntary failure in a shorter absolute time

b) that BFO will cause greater peripheral fatigue compared to the unrestricted exercise

c) that the recovery following a greater amount of peripheral fatigue caused by BFO will be attenuated compared to the intensity-matched protocol

Chapter 3. Purpose: To compare corticospinal excitability changes during a low-intensity (20% of MVC) sustained isometric contractions with blood flow occlusion until failure

vs. an intensity matched contraction without blood flow occlusion and with a time-matched control to the BFO protocol. The hypothesis was:

a) the impact of blood flow occlusion will cause greater modulation of corticospinal excitability compared to the intensity matched contraction

Chapter 4. Purpose: To examine whether low-intensity (25% of MVC) dynamic elbow flexion until failure with blood flow occlusion is a suitable alternative to traditional high-intensity dynamic elbow flexion exercise, with respect to power impairments following an acute bout. The hypotheses were:

a) that acute BFO exercise will induce greater decreases in velocity, and therefore power impairments, to a similar or greater amount than high-intensity (HI) exercise, for the same number of repetitions to failure

b) that acute low-intensity BFO will cause greater peripheral fatigue compared to the unrestricted blood flow during high-intensity exercise

Chapter 5. Purpose: To explore the impacts of blood flow occlusion on tibialis anterior (TA) motor unit firing rates (MUFRs), and how occlusion distal vs. proximal to a working muscle effects firing rates and fatigue endurance. The hypotheses were:

a) BFO proximal to the TA will cause the greatest reduction in MUFR

b) BFO distal to the TA will cause greater MUFR decreases than control, but less the BFO proximal

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Chapter 2

2 The effect of blood flow occlusion during acute low-intensity isometric elbow flexion exercise

2.1 Introduction

It is generally accepted that resistance training with contractile intensities of ~70% of maximal voluntary contraction (MVC), are required to stimulate muscle hypertrophy and strength gains[1,2]. However, evidence supports the use of blood flow restricted (BFR) exercise, sometimes referred to as occlusion exercise, as a training technique that elicits hypertrophy and strength gains by incorporating low-intensity muscular contraction (~20-30% of MVC) in combination with BFR[3,4]. Although it is commonly reported that strength and hypertrophy gains are maximized at relatively high-intensity, other studies report low levels of intensity (<50% of MVC) performed to failure that will elicit similar gains in strength and hypertrophy[5,6]. These results would indicate that sufficient protein synthesis, and therefore, muscle hypertrophy can occur following lower exercise intensities than previously suggested[2]. BFR is most often achieved through the application of external pressure around the limb segment proximal too the working muscle. External pressure can be applied using a sphygmomanometer that applies an adjustable and quantifiable pressure (mmHg), or by using elastic bands that cannot provide an exact measure of restriction. Whereas the physiological mechanisms are not fully understood, BFR training has been observed to facilitate positive muscular adaptations in both rehabilitation[7] settings and athletes[8].

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Specifically, blood flow restricted exercise has been explored from a chronic adaptation perspective over four or more weeks of exercise training in both young and elderly populations, as well as in healthy and rehabilitation settings[9,10]. These studies observed increases in hypertrophy, strength[11,10,9], and muscle endurance[11]. Hypertrophy has been assessed by changes in muscle girth, and through magnetic resonance imaging to evaluate CSA (cross sectional area)[11,9,10]. Restricting or occluding blood flow essentially attenuates oxygenated blood and blood borne substrates (glucose and free fatty acids) from reaching the working muscle[12], with greater levels of restriction causing greater attenuation. This localized hypoxic stimulus may be an important mechanism that in combination with low-load exercise contributes to positive strength adaptations[13]. In addition, the effect of BFR distal to the restriction, likely causes a larger accumulation of metabolic products due to the greater hypoxic state[13]. This presumably causes aerobic fibers (type I slow motor units), to metabolize ATP in a low oxygen anaerobic environment rather than a preferred oxygen rich environment, and therefore recruitment of anaerobic fibers (type II fast motor units) is enhanced in order to offset and maintain a given contraction intensity. Type II fibers produce more force per fiber than type I, but are far less fatigue resistant[14]. Although this concept has not been directly assessed, indirect measures would manifest as shorter time to fatigue, and relatively greater neural activation of muscle reflected in the electromyographic (EMG) signal[15,4]. A study by Karabulut et al. 2010 reported in the thigh muscle (vastus lateralis) an increase in EMG amplitude across isotonic submaximal repetitions with blood restriction set at 44% greater than systolic blood pressure. Towards the end of the intervention contractions EMG declined indicating some neural drive failure at the muscle level, although it must be recognized there are surface EMG limitations during dynamic contractions[16]. Also, they did not go to failure, or explore the recovery profile. In another study a continuous increase in EMG indicated contractile output during complete blood flow occlusion was maintained by greater neural output[12], Furthermore, Yasuda et al, 2009 noted that under complete blood flow occlusion (BFO) with low level fatiguing contractions there was an extreme mismatch in energy demand (increasing muscle activation, or neural compression) and energy supply (no blood flow) that resulted in feelings of maximal exertion and complete mechanical failure.

Neuromuscular fatigue has both voluntary and involuntary components. The involuntary (peripheral) component can be assessed from electrically evoked contractions of the muscle, which bypasses the central nervous system. During prolonged contraction of a moderate to high intensity, the muscle fibers become weaker and contract slower, often due to muscle damage[17] or excitation-contraction (E-C) failure[18]. Excitation-contraction coupling failure is one of a number possible causes of fatigue[19], and can be evaluated indirectly following fatiguing contractions by comparing the response of the muscle at lower frequencies of tetanic stimulation (i.e., <20Hz) to the response from maximal frequencies of excitation (i.e. 50 Hz)[19]. An increase in this ratio (10Hz to 50Hz) after fatiguing contractions is an indication of peripheral fatigue and is referred to as low frequency fatigue (LFF). Central components of fatigue are more difficult to evaluate because they include assessing the degree of voluntary drive generated from spinal and supraspinal factors. Voluntary activation (VA) of the system can be indirectly assessed using the interpolated twitch technique (ITT)[20]. Following fatiguing low-intensity blood flow restricted exercise VA was decreased[21] and EMG, as a measure of voluntary neural activation, was increased. These changes were interpreted as an increase in central fatigue following low-intensity blood flow restricted exercise[21].

Thus, assessing changes in neural activation and muscle properties using an acute bout of fatiguing exercise with a high level of occlusion should provide further understanding of relevant peripheral factors affected during chronic BFR training paradigms. Therefore, the purpose of this experiment was to quantify the degree of fatigability in response to prolonged steady-state contraction of the elbow flexors at low-intensity (20% MVC) with a high level of external pressure (250mmHg) to failure, compared with the responses to the same exercise at 20% MVC during unrestricted blood flow to failure (FF). We hypothesized, H1: that BFO exercise will cause exercise induced voluntary failure in a shorter absolute time, and H2: that BFO will cause greater peripheral fatigue compared to the unrestricted exercise. H3: that the recovery following a greater amount of peripheral fatigue caused by BFO, will be attenuated compared to the FF protocol. Electrically evoked tetanic responses were used to specifically target intrinsic (peripheral) changes in the muscle and voluntary activation in combination with time to fatigue and EMG were used as measures to assess overall fatigability of the system.

2.2 Methods

2.2.1 Subjects

Ten healthy male subjects (see Table 2-1 for characteristics) participated in two testing trials, administered in a random order, and each was separated by at least 48 hours, with both completed within 7 days. All procedures were approved by the local institutional ethics committee (REB# 107212, WREM at The University of Western Ontario) and conformed to the declaration of Helsinki. Written and verbal consent were obtained from each participant. All subjects were right hand dominant and therefore to minimize limb dominance effects, the non-dominant left arm was tested in each participant.

Parameters	Participants (n = 10 males)
Age (years)	**27 ± 4**
Height (cm)	**178 ± 7**
Mass (kg)	**84.9 ± 10.2**
BFO Time to Failure (s)	**234 ± 44 †**
FF Time to Failure (s)	**1026 ± 752**
BFO MVC (Nm)	**337.1 ± 76.2**
FFfail MVC (Nm)	**322.6 ± 66.2**

Table 2-1 Values are means ± SD. BFO, blood flow occlusion at 20% intensity. FF, no blood flow occlusion at 20% intensity. MVC, maximal voluntary isometric contraction. †denotes significant difference between BFO and FF time to failure in seconds.

2.2.2 Experimental set-up

Participants were supine on an examination plinth with their legs supported by a large wooden box (to minimize extraneous movement of the lower limbs) placing their hip and knee joints at ~90 degrees (see appendix C). To record elbow flexion, the left arm was in the dependent position and the elbow joint was flexed to ~90 degrees with the supinated wrist secured to a linear force transducer (SST-700-100A; AS Technology, Haliburton, ON, Canada) using a velcro strap. The force transducer was adjusted so that force was measured at the wrist in a standardized fashion for all participants. A brace was adjusted to press down firmly on the left shoulder to minimize any extraneous shoulder or torso movements, as well as in inelastic strap was fastened securely across the chest. EMG of the flexors were recorded, via adhesive Ag-AgCl electrodes (Kendall, H59P cloth electrodes) arranged in a monopolar fashion. Recording electrodes were placed on the skin over the mid belly of the muscle and reference electrodes were secured over the ulna on the posterior forearm. For elbow flexor muscle stimulation custom made aluminum foil electrode pads (~2x5cm) covered in damp paper towel were placed over the distal and proximal 1/3 of the arm flexors.

In all sessions, torque and EMG data were recorded using an A/D converter (CED 1401; Cambridge Electronic Design Ltd, Cambridge, UK) in conjunction with Spike2 software (v. 7.02; Cambridge Electronic Design). The torque and EMG data were sampled at 500 and 5000Hz, respectively. EMG data were amplified (x1000) and bandpass filtered (10Hz – 20KHz, with a 60Hz notch filter) using Neurolog; NL844, Digitimer, Welwyn Garden City, UK.

2.2.3 Experimental Protocol

Participants were randomly assigned to complete either first the low-intensity BFO to failure protocol or the low-intensity unrestricted blood flow (FF) to failure protocol (see Appendix E for details). Except for the addition of the blood pressure cuff to restrict blood flow all procedures were identical in both trials. The blood pressure cuff (adult sized sphygmomanometer, 10cm in width), was applied over the most proximal portion of the biceps muscle belly and pressure was maintained at 250mmHg during the BFO protocol

for all participants. This pressure was sufficient to abolish the radial pulse during the resting state for all participants. To determine MVC strength and voluntary activation of the elbow flexors maximal electrical stimulation doublets (200μs pulse width; 400V; 100Hz doublet; range 90-168mA) were applied using a constant current stimulator (DS7AH; Digitimer, Hertfordshire, UK) by increasing current intensity until the torque response no longer increased with an increase in current intensity, or coactivation of other muscles impeded the elbow flexor torque. For the interpolated twitch technique (ITT)[20] doublets were used to assess voluntary activation in the elbow flexors. Participants were instructed to perform two to three brief (~3-5 seconds) elbow flexion MVCs, which included a superimposed doublet at the peak torque, and a potentiated doublet was applied at rest immediately following the MVC. If variability in maximal torque was 5% or more between MVCs then a third MVC was performed. It has been reported that inexperienced participants may produce less than maximal MVCs when expecting electrical stimuli[22], thus multiple MVCs were conducted for familiarization. Two to three minutes of rest was given between each MVC. Strong verbal encouragement and visual feedback were provided during all voluntary contractions. The greatest elbow flexion MVC was selected as the baseline value. Tetanic 50Hz (200μs pulse width; 400V; 1s duration; range 35-90mA) stimulation was applied for 1 second to elicit 30% of elbow flexor MVC torque which from pilot testing was tolerable and did not activate antagonist muscles of the arm. Stimulations at 1Hz (twitch) and 10Hz (same stimulation parameters as 50Hz) were applied using this same intensity. After baseline measures were acquired (at the beginning of each testing day), participants completed one of the two intervention protocols. Participants were instructed to maintain the 20% of MVC constant isometric contraction, while elbow flexor MVCs (~3-5 seconds) with ITT were assessed every minute during the fatiguing intervention until failure. Failure was defined as the point at which participants were unable to maintain the 20% testing contraction after two consecutive attempts. During a 30 minute recovery period at each time point a 1Hz, 10Hz and 50Hz response was elicited followed by an MVC with ITT. Parameters were assessed immediately following the fatiguing task (at failure point, FP; and ~2-3 seconds following failure (R0), which accounted for the time needed to remove the blood pressure cuff) and at 2, 5, 10, 20, and 30mins of recovery.

2.2.4 Data and statistical analyses

Off-line quantification of measures consisted of time-to-peak torque (TPT), peak torque (PT), and half-relaxation-time (HRT) for the 1Hz stimulation, and PT and HRT for the 50- and 10Hz stimulations. Peak torque (Nm) was measured in the MVCs. Voluntary activation was calculated using the interpolated twitch equation: [1 – (superimposed/potentiated twitch)*100][23,24]. The superimposed twitch refers to the doublet stimulation applied at the MVC peak, and the potentiated twitch refers to the doublet stimulation applied following the MVC at rest. Data are described as mean ± SD, while displayed as means ± SE in all figures. A Two-way repeated measures ANOVA with a modified Bonferroni was performed in order to determine between-group differences of time and protocol. Cohen's d effect sizes (ES) were also calculated for main findings. A *post-hoc* power analysis was completed for the 10Hz tetanic stimulation and found that the sample size of 10 was sufficient. When only a main effect of time was observed, paired sample t-tests were used in conjunction with a Dunnet's table test for multiple comparisons. Paired t-tests were used to compare group differences in time-to-failure as well as MVC strength. All statistical analyses were performed using SPSS version 25. Statistical significance accepted at $\alpha < 0.05$.

2.3 Results

2.3.1 Voluntary Characteristics

Time to failure point (FP) was 80% longer for FF compared with BFO (see table 2-1). There was a main effect of time for MVC ($P < 0.01$), which decreased similarly in both protocols by ~60% at FP (see Figure 2.1) Following 30mins of recovery MVC rebounded at similar rates to ~87% of baseline following both protocols but was significantly lower than baseline. During both protocols voluntary activation decreased to ~89% of baseline at FP. There was a main effect of time ($P < 0.01$) and protocol ($P = 0.012$) and FF was ~5% less than BFO at the mid-point of task failure ($P = 0.02$, ES = 0.41) (see Figure 2.2). The FF protocol remained significantly lower compared to BFO at recovery time points R0-R10. At the completion of recovery (30 mins) voluntary activation for both protocols was not different from baseline at ~94% and ~91%, respectively (see Figure 2.).

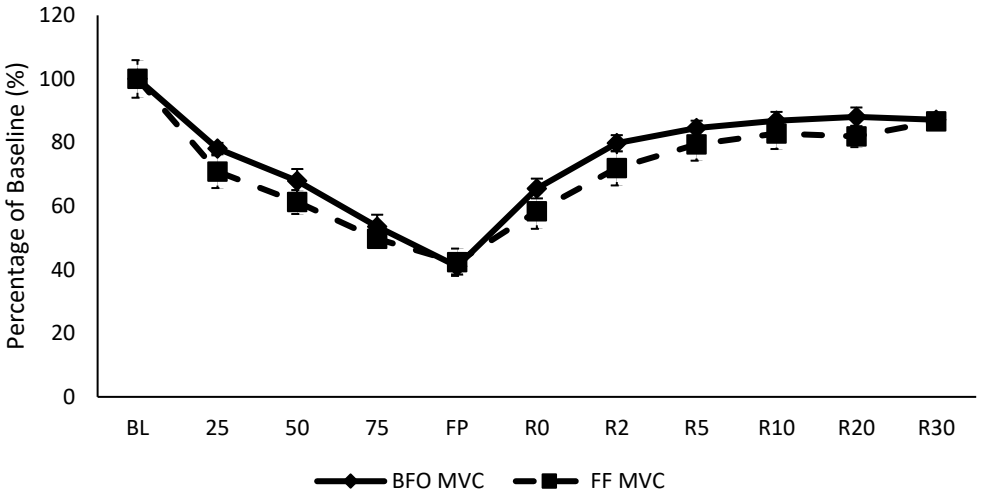

Figure 2.1 Maximal voluntary contraction normalized to time displayed as 25% - failure point (FP) for both BFO and FF trials. Recovery time points from R0 – R30. Values represented as percent change from baseline and displayed as means ± SE.

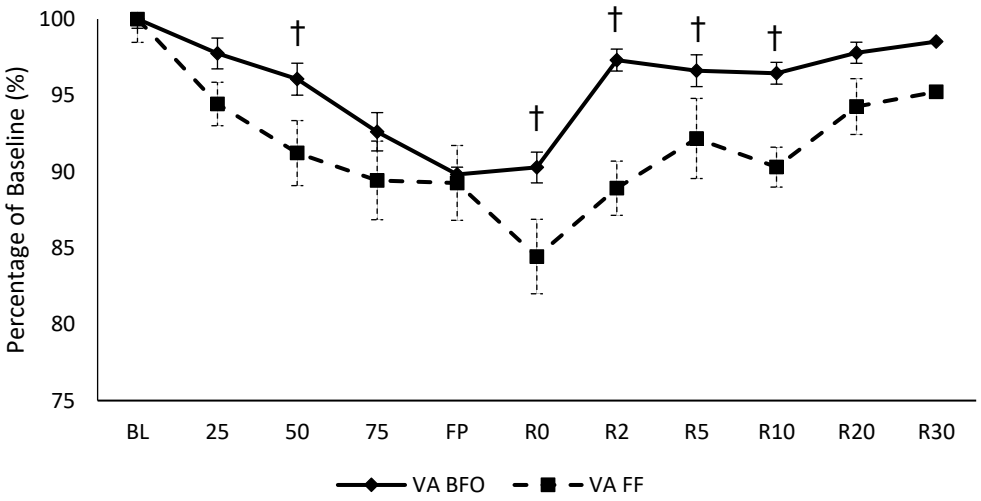

Figure 2.2 Voluntary activation values normalized to time displayed as 25% - fail point (FP) for both BFO and FF trials. Recovery time points from R0 – R30. Values represented as percent change from baseline and displayed as means ± SE. †denotes significant difference between BFO and FF conditions.

2.3.2 Twitch properties

For the twitch there was a significant decrease in peak torque following BFO compared to FF at R0 of ~75% and ~31% from baseline, respectively ($P < 0.01$, ES = 0.88), as well as a main effect of time ($P < 0.01$). However, no statistical difference was observed between trials at R2 or for the remainder of the 30min recovery period (see Figure 2.3). The twitch (1Hz) following the 30min recovery period, remained depressed for both protocols at ~55% and ~64%, respectively of baseline. TPT and HRT of the twitch (1Hz) response were unchanged following the task and throughout recovery (data not displayed).

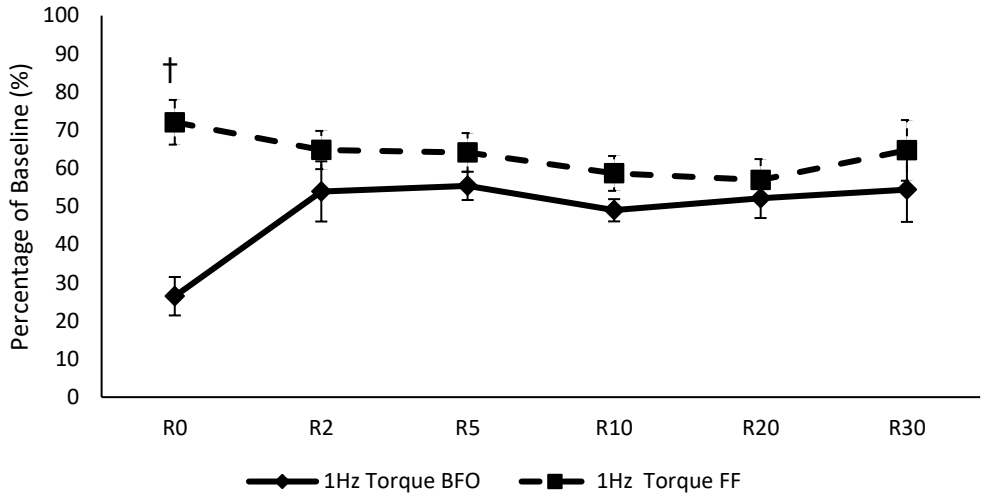

Figure 2.3 Represents values of 1Hz torque percent changes during R0 – R30 minutes of recovery compared to baseline. †denotes significant difference between BFO and FF conditions. Values displayed as means ± SE.

2.3.3 Tetanic Properties

The 10Hz tetanic stimulation had a greater decrease during BFO (~80% decrease from baseline) compared to FF (~45% from baseline) at R0 ($P < 0.01$, ES = 0.56). Both the BFO and FF protocols had a main effect of time ($P = 0.01$) and 10Hz remained depressed by ~40% from baseline following 30mins of recovery (see Figure 2.4). The 10Hz HRT was unchanged throughout both protocols and recovery with no difference between protocols (data not displayed). The 50Hz peak torque was not significantly different between groups but was significantly reduced from baseline following both protocols and throughout the

duration of recovery (see Figure 2.5A). The changes in 50Hz torques were similar to changes observed for MVC torques. However, the 50Hz HRT had a main effect of time ($P < 0.01$) and was significantly longer following the BFO at R0 and R2, by ~107% ($P < 0.01$, ES = 0.95) and ~18% ($P < 0.01$, ES = 0.74), respectively, compared to baseline. In contrast, 50Hz HRT during FF remained unchanged from baseline at R0 (~96%) and R2 (~91%). Following 5mins of recovery 50Hz HRT was similar to baseline for both protocols (see Figure 2.5B). The 10Hz to 50Hz peak torque ratio between protocols was significantly different ($P < 0.01$, ES = 0.51) immediately following both protocols (at R0), but was reduced more following BFO (to ~32% of baseline) compared to the FF (~79% of baseline) (see figure 2.6).

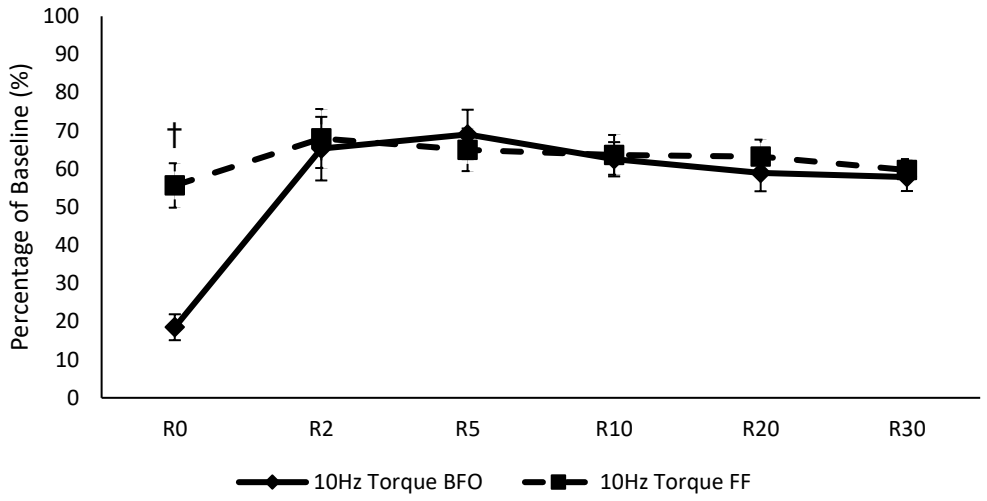

Figure 2.4 Represents values of 10Hz torque percent changes during R0 – R30 minutes of recovery compared to baseline. †denotes significant difference between BFO and FF conditions. Values displayed as means ± SE.

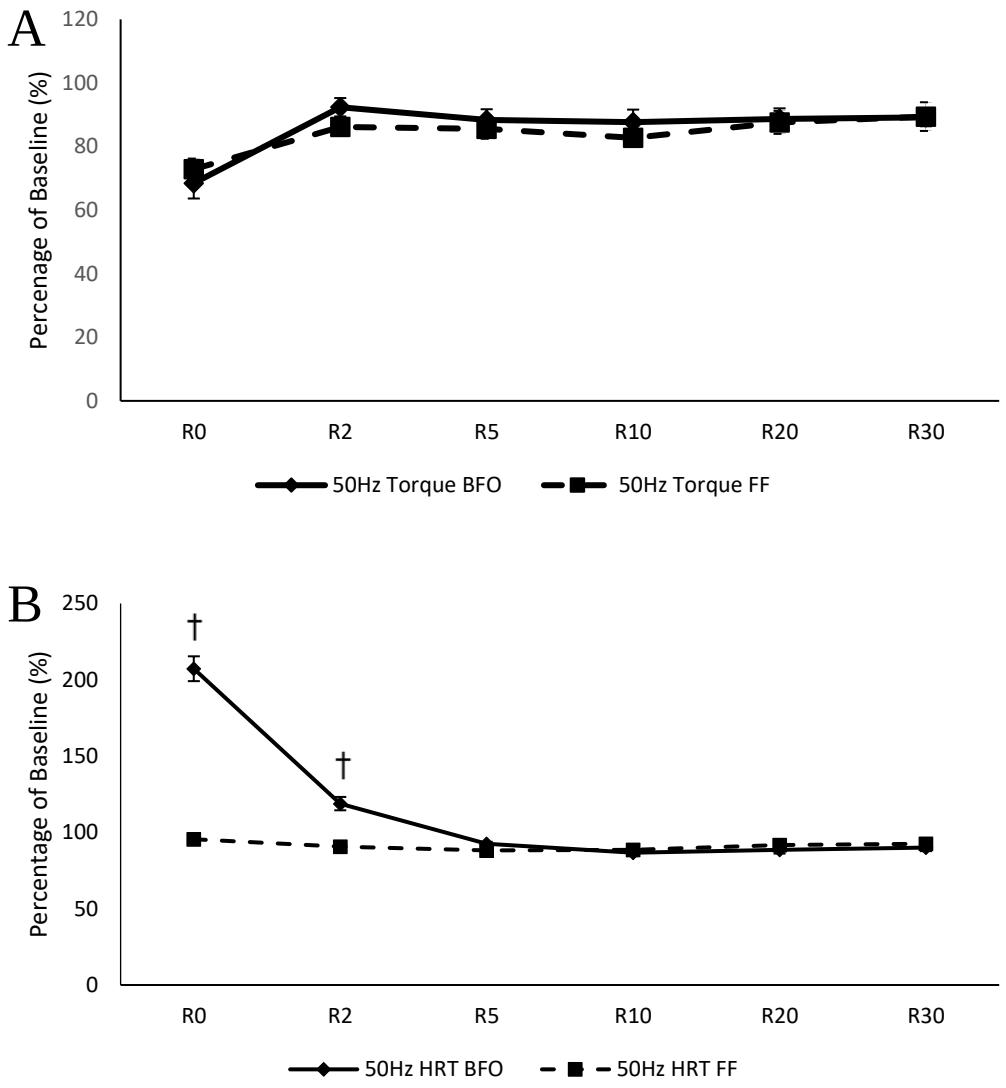

Figure 2.5 (A) Represents values of 50Hz torque percent changes during R0 – R30 minutes of recovery compared to baseline. (B) Represents values of 50Hz half-relaxation-time percent change during R0 – R30 minutes of recovery compared to baseline. †denotes significant difference between BFO and FF conditions. Values displayed as means ± SE.

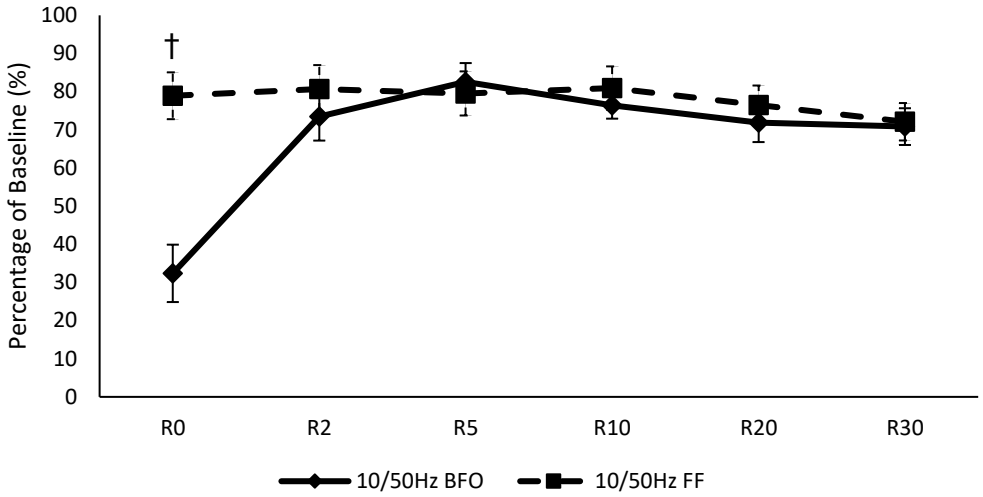

Figure 2.6 Represents values of the 10/50Hz torque ratio as percent changes during R0 – R30 minutes of recovery compared to baseline. †denotes significant difference between BFO and FF conditions. Values displayed as means ± SE.

2.3.4 EMG

With BFO maximal EMG at the 25% normalized time-to-fatigue value was statistically greater than baseline, exhibiting an increase in muscle activation before declining, while the FF protocol did not increase before declining. Maximal elbow flexor EMG following both protocols showed similar declines between protocols that were not significant at FP but were at R0 (11-30%). However, by R5 maximal EMG had recovered to baseline values in both protocols. Submaximal flexor EMG following each protocol showed a strong trend ($P = 0.06$) with BFO increasing from ~27% at 25% of normalized time to 65% at task failure, and FF increasing from ~25% to 40% at task failure (data not displayed). Submaximal EMG was only recorded during the fatiguing task and was not assessed during the recovery period.

2.4 Discussion

The current study assessed the effects of a single bout of blood flow occlusion during low-intensity elbow flexor contraction compared with the same protocol performed with an unrestricted blood flow. This BFO paradigm was used to explore relevant factors responsible for results found from established chronic training BFR protocols known to

cause hypertrophy and strength improvements[5,6]. The main findings of the current study were: 1) time to task failure (FP) was shorter during blood flow occlusion compared with the unrestricted bout, (234 ± 44 vs. 1026 ± 752 seconds, respectively); 2) MVC strength (voluntary fatigue) was reduced to a similar degree in both protocols with no differences between protocols in voluntary activation; 3) although the 50Hz torque responses were similar to the changes in MVC, in contrast the intrinsic contractile torque measures at 1Hz and 10Hz were depressed to a greater amount following BFO than those during the FF protocol (see Figures 2.3 and 2.4), and 50Hz HRT was longer following BFO. During the 30 min recovery period, MVC and VA recovered at a similar rate between protocols, but MVC remained significantly depressed. The more attenuated 1Hz and 10Hz torques following BFO compared to FF were no longer different between protocols by R2, but remained lower than baseline values. The 50Hz torque responses were however recovered in both protocols by 30mins. These changes are indicative of a greater amount of peripheral fatigue induced during BFO than FF.

When normalized for time, the rates of decline and recovery in the MVC were similar between the two protocols as previously observed when comparing low-intensity bouts of contraction with and without BFR[25], however, voluntary activation was impaired more in the FF protocol in the current study. This was presumably due to the greater time in the FF protocol and therefore more contractile work required to reach task failure of ~60% of MVC. The response from the 50Hz stimulation likely paralleled the MVC decline in both protocols during the fatiguing task, but was not assessed until R0 at which time the loss was significant and similar to the MVC loss. Recovery of voluntary activation was more impaired following the longer task (FF) for the first 10 minutes of recovery, which is likely due to greater time under tension (80% longer) during FF. These results are in agreement with Behm and St. Pierre[26] who found similar results in VA following a long term fatiguing task when compared with a short task. The accumulation of metabolic by-products would be countered upon release of the restriction[27], and a return to “normal” blood flow shown by the rapid recovery of half-relaxation-time observed within 5mins of rest following the fatiguing task. Therefore, the similar decrease in MVC between protocols indicates that blood flow occlusion caused the same amount of voluntary fatigue but in a significantly shorter amount of time than with FF (see Figure 2.1).

However, the MVC recovered at similar rates between the two protocols, but neither returned to baseline (~90% of baseline) following 30min (see Figure 2.1). The similar change in MVC and the 50Hz responses have been observed previously in other fatiguing protocols[18] and supports that voluntary activation was maximal and that these recover rapidly (within 30mins) as compared with force responses induced by lower frequencies of activation.

The depression of torques at lower frequencies of stimulation compared with high frequencies is reflected in the 10/50Hz ratio (see figure 2.6). For the BFO protocol this ratio was depressed by ~79% from baseline compared with ~20% for FF at R0, and did not recover by the end of 30 min in either protocol. The inability of the muscle to produce force at low-frequencies (10Hz), compared to the relatively small reduction in force elicited from high-frequencies (50Hz) is indicative of fatigue-induced muscle impairment referred to as low-frequency fatigue[18,28]. In this experiment immediately following the BFO protocol we observed greater reductions in both 1Hz and 10Hz torques compared with the FF protocol (see figures 2.3 and 2.4). The reduction of the 10/50Hz ratio is indicative of failure to produce force due to mechanisms beyond the point of the neuromuscular junction[19]. This low frequency fatigue has been observed extensively and used as an indicator of peripheral fatigue following bouts of exercise or fatiguing contractions[18,29,30]. Low-frequency fatigue features a more severely affected loss of force that may take days to recover fully[19]. In our study, two minutes after the task, the 10Hz tetanic contraction following BFO had recovered to the same amount as FF (~60% of baseline), however, both protocols remained depressed from baseline for the remainder of recovery. This low-frequency fatigue may be indicative of structural damage to the muscle fiber or impairments in excitation-contraction coupling mechanisms caused by Ca2+ disruption following prolonged exercise[31,32]. Perhaps the greater amount of peripheral fatigue induced by BFO is indicative of an alteration in the preferred metabolic energy production of the type I oxidative fibers, caused by the ischemic environment. The early (0-2mins) rapid recovery may be due to reperfusion and a hyperemic response following restriction providing a renewed supply of oxygen for type I fiber metabolism.

It has been reported that contractile output during low-intensity contractions (20% MVC) with complete occlusion of blood flow results in greater neural activation (EMG) to maintain task force[12]. Yasudo et al[33], also noted greater increases in EMG following BFO compared to moderate blood flow restriction, and attributed this to increased neural compression and blood flow impairment resulting in greater muscle activation and greater energetic demand at the same external load. Although the current study does not have a direct measure of arterial perfusion, we believe that the pressure applied (250mmHg) likely created near complete occlusion, and therefore the observed increase in submaximal RMS EMG during each protocol indicates greater neural drive was required to maintain force during the fatiguing task. However, the BFO and FF protocols did not differ in the increased amount of submaximal RMS EMG at task failure likely because both protocols ended with each participants volitional failure point (FP), when task force (20% MVC) was no longer able to be maintained. However importantly the BFO protocol was ~80% shorter in absolute time than the FF protocol. Therefore, the change in submaximal RMS EMG indicates a relatively greater modulation in muscle activation pattern and supports what has been shown in other similar studies[12,13,4]. Compared to the (Karabulut et al, 2010[21]) who observed a greater reduction in maximal EMG amplitude (at pre- and post-MVCs) following blood flow restriction, the current study observed no difference in maximal RMS EMG values between protocols at task termination. The difference is likely due to the current study terminating the intervention at failure, compared with completion of a set number of repetitions in the former study.

Therefore we propose, that although both protocols reached a similar level of voluntary fatigue (MVC), low-intensity exercise in combination with blood flow occlusion causes a greater amount of peripheral fatigue than low-intensity exercise alone, and that BFO therefore recovers at an accelerated rate compared to FF despite remaining lower than baseline after 30 minutes. Based on other studies[12,13,4], in an ischemic environment fatigue will be greater and result in an earlier or greater amount of activation of type II muscle fibers compared with an environment without BFO. Fatigue following BFO recovers at a faster rate when unrestricted blood flow is restored, causing a reperfusion of oxygenated blood flow. This is likely due to anaerobically (due to the ischemic environment caused by BFO) fatigued type I muscle fibers reverting to aerobic energy

production, which allows voluntary activation of the muscle to be restored despite a greater amount of peripheral impairments.

This study provides important insights about how BFO may alter the neuromuscular system to enhance strength and hypertrophy gains with chronic training as used in previous studies[9,10,11]. Although training regimes at near complete blood flow occlusion are not likely to be used chronically, the results of this study provide evidence that blood flow occlusion results in a greater level of peripheral fatigue, observed through intrinsic changes of the muscle properties at low-frequency.

2.5 References

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Chapter 3

3 The acute modulation of corticospinal excitability following isometric arm flexor contractions to failure with and without blood flow occlusion

3.1 Introduction

Although high-intensity resistance training (i.e., a load of ≥ 70% of the 1 repetition maximum, 1-RM) is recommended for muscle hypertrophy[1], muscle growth can also be achieved by low-intensity resistance training performed with blood flow restriction (see Hwang and Willoughby 2017 for review[2]). This form of low-intensity resistance exercise could be valuable for individuals whose circumstances make traditional (i.e., high-intensity) resistance training impractical or unsafe; however underlying mechanisms are not well-understood, particularly factors related to corticospinal excitability.

With low-intensity intermittent isometric[3] or dynamic[4] contractions, muscle activation, as assessed by surface electromyography (EMG), was markedly higher during protocols with blood flow occlusion (BFO) compared to a control protocol without external compression. These observed increases in muscle activation have been attributed to an altered state of energy supply reaching the working muscle[3] as well as nerve impairment caused by the external compressive force used to occlude blood flow[4].

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Moritani and colleagues[3] also analyzed intramuscular EMG and noted increases in motor unit (MU) spike amplitude and firing frequency during the period of occlusion, which suggested oxygen availability influences recruitment and discharge rate of relatively high-threshold units. As neither of the aforementioned restricted blood flow studies[3,4] assessed cortical excitability, it is unclear whether the greater muscle activation found with occlusion resulted from increased excitability of the motoneurons at the spinal level, or whether restriction of blood flow also increased motor cortical excitability.

The delivery of external stimuli at different points along the motor pathway can address this question. That is, the responses to transcranial magnetic stimulation (TMS) of the motor cortex (motor evoked potential, MEP), transmastoid electrical stimulation (TMES) (cervicomedullary motor evoked potential, CMEP), and peripheral nerve stimulation (compound muscle action potential, M-wave) have been used collectively to assess cortical, spinal and peripheral excitability during fatiguing tasks[5]. These responses (MEP, CMEP and M-wave) reflect the excitability of all elements between the site of stimulation and the recording electrodes over the target muscle; hence, interpretation of a change in MEP or CMEP size depends on the availability of measures of excitability downstream to the stimulus site. For example, a change in MEP size can be interpreted as a change in: 1) motor pathway excitability (if only the MEP is recorded); 2) corticospinal excitability (if the MEP is normalized to the maximal M-wave, M_{max}); 3) cortical excitability (if the MEP is normalized to the CMEP). Therefore, assessment of the normalized MEP (MEP/CMEP), normalized CMEP (CMEP/ M_{max}) and absolute M_{max} provide insight into fatigue-induced changes at cortical, spinal and peripheral levels, respectively.

A recent study by Brandner et al[6], examined the effect of continuous (80% of resting systolic blood pressure) and intermittent blood flow restriction (130% of resting systolic blood pressure) on corticospinal excitability (MEP normalized to M_{max}) following an acute bout of intermittent, dynamic elbow flexion exercise at 20% of 1-RM. They observed a ~25% increase in normalized MEP amplitude at 5min post-exercise that remained elevated for 60min post-exercise. This finding indicates that there is an increase in corticospinal excitability following an acute bout of blood flow restricted exercise; however, the

experimental design did not allow for separation of cortical and spinal excitability (i.e., no CMEPs were recorded), nor did it offer insight into changes in neural excitability *during* blood flow restricted exercise. Thus, the purpose of this study was to determine the influence of BFO on cortical and spinal excitability during and after a low-intensity, fatiguing task. Full occlusion of blood flow was chosen to mimic the conditions of the pioneering work in this field[3,4].

3.2 Methods

3.2.1 Subjects

Nine healthy male subjects (age: 25.7 ± 4.2yr; height: 1.79 ± 0.1m; body mass: 87.7 ± 11.0kg) participated in three protocols performed on separate days (each separated by at least 48h). All experimental protocols were approved by the local institutional ethics review board and conformed to the declaration of Helsinki, except for registration in a database. Written and verbal consent were obtained from each participant prior to testing.

3.2.2 Experimental set-up

Participants were supine on a testing plinth (see appendix D), with legs resting on a box to eliminate extraneous lower body movement (hips and knees at ~90° flexion). To record elbow flexion force, the left upper limb was secured to a custom-made dynamometer[7]. The shoulder was slightly abducted and the elbow was at ~90° of flexion. The supinated wrist was secured to a linear force transducer (SST-700-100A; AS Technology, Haliburton, ON, Canada) using a Velcro strap. The shoulder was braced and the arm strapped to minimize any extraneous movement. To record evoked and voluntary EMG signals from the biceps brachii, a pair of adhesive Ag-AgCl electrodes (Kendall, H59P cloth electrodes) were arranged in a bipolar fashion over the muscle belly with an inter-electrode distance of 2.5cm. The subject was grounded with an electrode over the posterior aspect of the forearm. A blood pressure cuff (12cm wide), was applied proximal to the biceps muscle belly and pressure was maintained at 250mmHg during the BFO protocol for all participants.

In all sessions, force and EMG data were recorded using a A/D converter (CED 1401; Cambridge Electronic Design Ltd, Cambridge, UK) in conjunction with Spike2 software

(v. 7.02; Cambridge Electronic Design). The force and EMG data were sampled at 500 and 5000Hz, respectively. EMG data were pre-amplified (×1000) and bandpass filtered (10-10000Hz, with a 60Hz notch filter) using the NeuroLog system (modules NL136, 820 and 844; Digitimer, Welwyn Garden City, UK).

Peripheral nerve stimulation. To elicit M_{max} in the biceps brachii, a constant-current stimulator (DS7AH; Digitimer, Hertfordshire, UK) was used to deliver single electrical stimuli (200μs pulse width at ≤400V) to the brachial plexus at Erb's point. The cathode and anode were placed in the supraclavicular fossa and over the acromion, respectively. Stimulus current was increased incrementally for successive stimuli until the peak-to-peak amplitude of the resting M-wave reached a plateau (M_{max}). Stimulus intensity was then set to 120% of the current required to produce a maximal response (range 90-250mA). Three responses were then collected during a brief (~12s) elbow flexor contraction at 20% of MVC.

Transmastoid electrical stimulation. Stimulation of the corticospinal tract at the cervicomedullary level was accomplished using a second constant-current stimulator (DS7AH). The stimulus (100μs pulse width at ≤400V) was passed between adhesive Ag-AgCl electrodes (Kendall, H59P cloth electrodes) fixed to the skin ~1cm superior and medial to the mastoid processes, with the anode on the left[8,9] . Stimulation intensity (190-325mA) was set to evoke a CMEP with an amplitude of ~20% M_{max} during brief elbow flexor contractions at 20% MVC.

Transcranial magnetic stimulation. The motor cortex was stimulated over the vertex of the skull using a circular coil (13.5cm outside diameter) attached to a Magstim 200^2 stimulator (Magstim, Dyfed, UK). Stimuli were delivered during brief elbow flexor contractions at 20% MVC. The stimulus intensity (42-62% stimulator output) was set to evoke an MEP with an amplitude equivalent to that of the CMEP; i.e., ~20% M_{max}.

3.2.3 Experimental procedures

Participants began each protocol with at least two brief (~2s) elbow flexor MVCs (see Appendix F for details), separated by ≥2min of rest. A third MVC was performed if the

first two differed by >5%. Visual feedback and strong verbal encouragement were provided during each MVC. The peak force achieved was used to set the 20% MVC target used for the remainder of the experiment. Following the MVCs, the stimulus intensity required to elicit M_{max} was established and three responses were collected during a 12s contraction at 20% MVC force (stimuli were applied at 1, 6 and 11s). Next, the stimulation intensities for TMES was determined by the delivery of single stimuli during brief (~2s) contractions at 20% MVC force. When the peak-to-peak amplitude of the CMEP was ~20% of that for M_{max}, three responses were collected during a 12s contraction. The same procedures were followed to set the TMS intensity and collect baseline MEP data. Participants then performed one of three fatigue protocols in a pseudo-randomized order, followed by 5min of recovery measures. The BFO protocol was always completed first in order to establish time-to-failure; however, the order of the remaining protocols was randomized.

BFO protocol. In this protocol, blood flow was occluded (250mmHg) during a sustained isometric elbow flexion contraction at 20% MVC force until failure (i.e., when the target force could not be maintained for 2s). The blood pressure cuff was inflated 2s before the start of the sustained contraction. Visual feedback and verbal encouragement were provided throughout the fatiguing task. Beginning at 30s, MEP, CMEP, and M_{max} (each stimulus separated by 2s) were elicited every 30s until task failure. Five seconds after failure (the time required to remove the cuff), MEP, CMEP, and M_{max} were elicited (each stimulus separated by 2s) during a brief (~6s) test contraction. This contraction was repeated at 1, 2, 3, 4 and 5min post-failure.

FFfail protocol. Participants performed a sustained isometric elbow flexion contraction at 20% MVC force until failure, without occlusion of blood flow (i.e., free flow; FF). All other aspects were the same as those outlined for the BFO protocol.

FFiso protocol. With free flow, participants performed a sustained isometric elbow flexion contraction at 20% MVC force until the time of failure during the BFO protocol (i.e., iso-time). All other aspects were the same as those outlined for the BFO protocol.

3.2.4 Data analyses and statistics

Offline, Spike2 software (v. 7.02; Cambridge Electronic Design) and Signal software (v. 5.08; Cambridge Electronic Design) were used to analyse all data. Peak force was recorded as the greatest value generated during the brief MVCs prior to the fatiguing contraction. Maximal voluntary EMG (root mean square, RMS amplitude) was measured over a 200ms epoch centered about the peak force. During the fatigue protocol and the recovery contractions, mean RMS EMG was calculated over a 200ms epoch (−210 to −10ms) prior to each stimulus and expressed as a percentage of the value obtained during the MVC. Evoked potential (M_{max}, CMEP and MEP) areas were measured between the initial deflection from the baseline to the second crossing of the horizontal axis (Martin et al., 2006a). To assess spinal excitability at each time point during fatigue and recovery, the CMEP was normalized to M_{max} (i.e., CMEP area / M_{max} area) and expressed as a percentage of the ratio of these two potentials at baseline. Similarly, to assess cortical excitability during fatigue and recovery, the MEP was normalized to the CMEP (MEP area / CMEP area) and expressed as a percentage of the ratio obtained at baseline.

A one-way repeated measures ANOVA with a Bonferroni correction was performed in order to test for differences among protocols for baseline values of MVC force, M_{max} amplitude, normalized MEP and CMEP area, as well as time-to-task failure. The statistical analyses described below for voluntary and evoked EMG data were performed separately for the fatigue and recovery periods. A three-way repeated-measures ANOVA was used to assess the effects of protocol, stimulus type and time on mean pre-stimulus RMS EMG. Two-way repeated measures ANOVAs were performed in order to determine the influence of protocol and time on normalized MEP, CMEP and M_{max} area. In cases of a main effect of protocol or a protocol × time interaction, paired-sample t-tests (with a correction for multiple comparisons, when necessary) were used at each time point to determine differences among protocols. Further, a one-way ANOVA was for each protocol to test for a main effect of time. If this was significant, paired sample t-tests were used in conjunction with a Dunnett's table to determine time points different from baseline. When only a main effect of time was observed for a two-way repeated measures ANOVA, data were pooled across sessions and paired sample t-tests were used in conjunction with a Dunnett's table

to determine time points different from baseline. All statistical analyses were performed using SPSS version 25. All data are reported in the text as the mean ± standard deviation (SD) and displayed in figures as the mean ± standard error of the mean (SE). The significance level was $P < 0.05$.

3.3 Results

3.3.1 Baseline measures and time-to-task failure

Pre-fatigue MVC force was not different among the three protocols (BFO: 406.1 ± 95.2N; FFfail: 373.8 ± 61.8N; FFiso: 397.3 ± 95.2N; $P = 0.848$). Similarly, M_{max} area was not different among protocols (BFO: 0.053 ± 0.017mV·s; FFfail: 0.047 ± 0.018mV·s; FFiso: 0.052 ± 0.012mV·s; $P = 0.968$). There was no main effect of protocol for the baseline normalized CMEP area (BFO: 19.1 ± 4.7% M_{max}; FFfail: 19.2 ± 5.1% M_{max}; FFiso: 17.1 ± 3.7% M_{max}; $P = 0.822$). Likewise, there was no main effect of protocol for the baseline normalized MEP area (BFO: 97.1 ± 14.9% CMEP; FFfail: 96.3 ± 17.3% CMEP; FFiso: 91.3 ± 24.6% CMEP; $P = 0.782$). The time-to-task failure for the time-matched BFO and FFiso protocols (183 ± 63s) was significantly shorter than that of the FFfail protocol (907 ± 445s; $P < 0.01$).

3.3.2 Voluntary EMG during fatigue and recovery

During both fatigue and recovery, the results of the three-way ANOVA for EMG RMS values prior to MEP, CMEP, and Mmax identified that there was neither a main effect of stimulus (fatigue, $P = 0.547$; recovery, $P = 0.244$) nor a protocol × stimulus interaction (fatigue, $P = 0.376$; recovery, $P = 0.560$) so data were pooled into a single value at each time point. With these data, the two-way repeated measures ANOVAs revealed a protocol × time interaction as well as main effects of protocol and time (all $P \leq 0.003$). The RMS amplitude was not different for BFO and FFfail protocols (fatigue, $P = 1.000$; recovery, $P = 0.075$) and both were greater than the FFiso protocol from 25-100% of time-to-failure ($P \leq 0.020$) and during recovery (R0-R2 for BFO and R0-R5 for FFfail; $P \leq 0.025$). One-way ANOVAs to assess the effect of time were significant for BFO and FFfail (fatigue, $P \leq 0.001$; recovery, $P \leq 0.004$) but not FFiso (fatigue, $P = 0.761$; recovery, $P = 0.460$). RMS

amplitude was greater than baseline at all fatigue time points and R0 for BFO but from 50% time-to-failure to R4 for FFfail (see Figure 3.1).

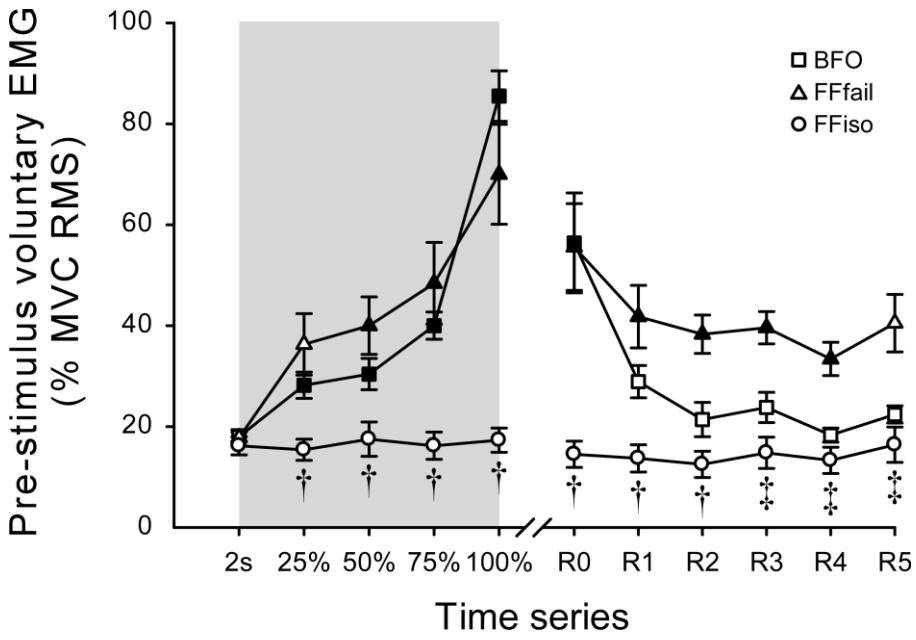


Figure 3.1 Data are mean values from 9 participants (± SE) and expressed as a percentage of the RMS amplitude recorded about the peak force achieved prior to each fatigue protocol. The time scale includes a value 2s into the sustained contraction (indicated by the shaded box), values at 25, 50, 75 and 100% of time-to-failure as well as data collected immediately (~5s) after task failure (R0) and then at 1min intervals (R1-R5). A single dagger (†) denotes a time point when FFiso is lower than both BFO and FFfail, whereas a double dagger (‡) denotes a time point when FFiso is lower than FFfail alone. A filled symbol denotes a time point that is greater than the control (2s) value.

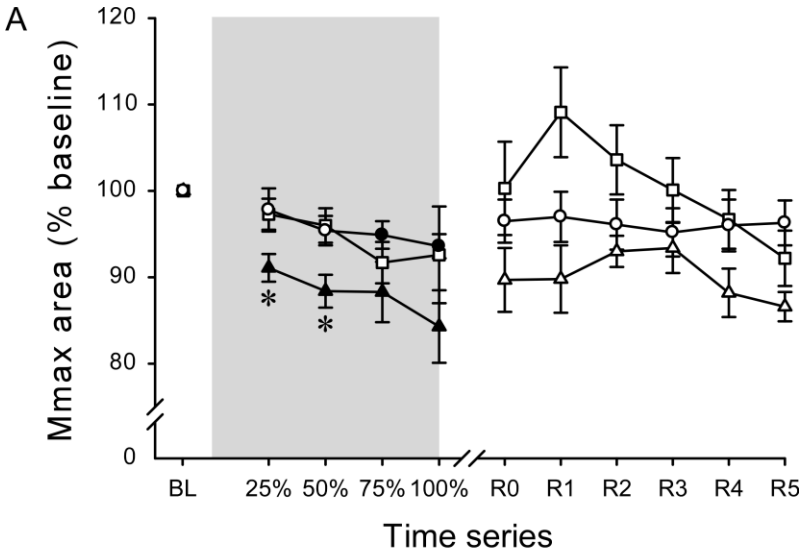
3.3.3 Mmax area

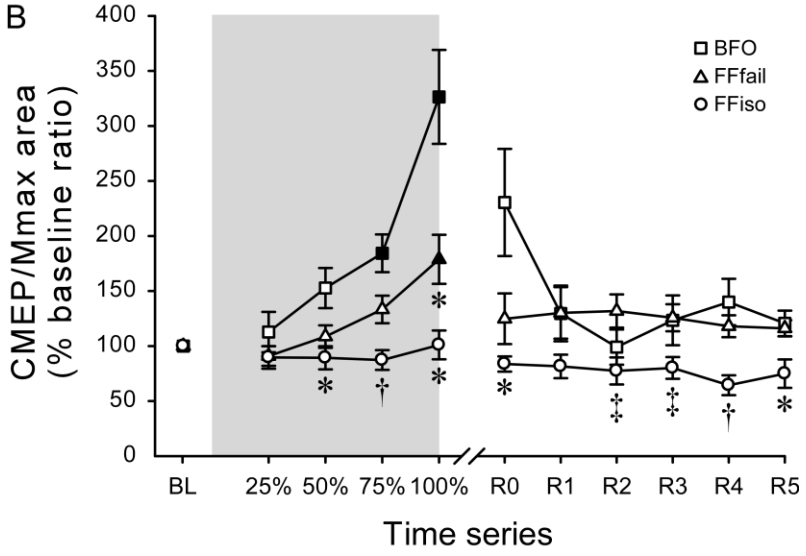
During fatigue, normalized Mmax area (expressed relative to baseline; Figure 3.2A) had main effects of protocol ($P = 0.017$) and time ($P = 0.004$) but not a protocol × time interaction ($P = 0.122$). *Post-hoc* testing indicated that Mmax area was smaller during FFfail than BFO ($P = 0.043$), with no other differences among protocols. FFfail Mmax area was smaller than BFO at 25 and 50% of time-to-failure ($P \leq 0.015$). The effect of time was not significant for BFO ($P = 0.15$) but was significant for both FFfail ($P = 0.008$) and FFiso

(P = 0.018). Mmax area was smaller than baseline at all time points for FFfail but only at 75 and 100% time-to-failure for FFiso. During recovery, there was a main effect of time (P = 0.011) but neither a main effect of protocol (P = 0.471) nor a protocol × time interaction (P = 0.120). Post-hoc testing of data pooled across protocols revealed that Mmax area was smaller than baseline at R4 and R5.

3.3.4 Cervicomedullary motor evoked potentials

During fatigue, normalized CMEP area (CMEP/Mmax and expressed relative to the baseline ratio; Figure 3.2B) had main effects of both protocol and time as well as a protocol × time interaction (all P ≤ 0.004). *Post-hoc* testing revealed that CMEP area was larger for BFO than FFfail and FFiso (P < 0.01, and P = 0.046, respectively) and also for FFfail compared to FFiso (P = 0.033). BFO CMEP area was greater than FFail at 100% (P = 0.002) and FFiso at 50-100% time-to-failure (P ≤ 0.002), whereas FFfail was greater than FFiso at 75% time-to-failure (P < 0.013). The effect of time was significant for BFO (P < 0.001) and FFfail (P = 0.002) but not FFiso (P = 0.639). CMEP area was larger than baseline at 75% and 100% of time-to-failure for BFO but only at 100% for FFfail. The normalized CMEP area during recovery had main effects of protocol (P = 0.013) and time (P = 0.047) in addition to a protocol × time interaction (P = 0.003). Specifically, CMEP area was larger during BFO and FFfail than FFiso (P = 0.026 and P = 0.003, respectively), with no difference between BFO and FFfail (P = 1.0). BFO CMEP area was greater than FFiso at R0 and R4-R5 (P ≤ 0.024), whereas FFfail was greater than FFiso at R2-R4 (P ≤ 0.023). Only the BFO protocol had a main effect of time during recovery (P < 0.01); however, after post-hoc testing, no specific time points were different from baseline.


A
Mmax area (% baseline)
120
110
100
90
80
0
BL
25%
50%
75%
100%
R0
R1
R2
R3
R4
R5
Time series


B
CMEP/Mmax area
(% baseline ratio)
400
350
300
250
200
150
100
50
0
BFO
FFfail
FFiso
BL
25%
50%
75%
100%
R0
R1
R2
R3
R4
R5
Time series

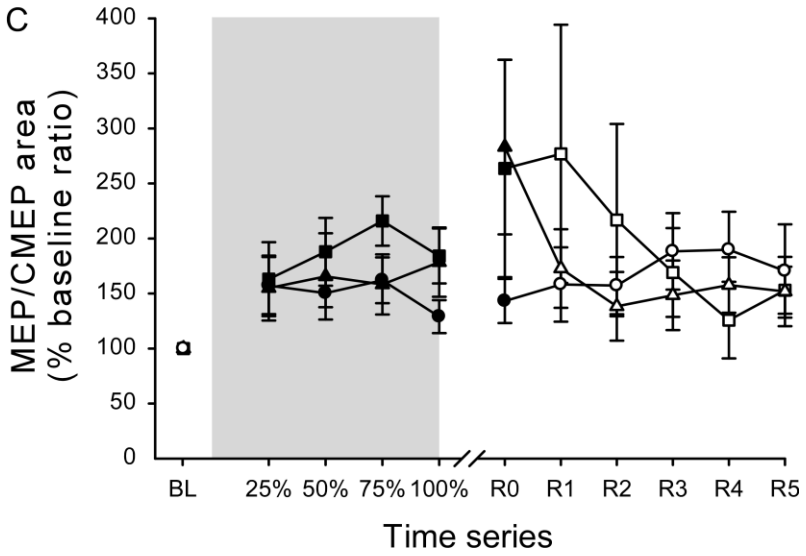

C
MEP/CMEP area
(% baseline ratio)
400
350
300
250
200
150
100
50
0
BL
25%
50%
75%
100%
R0
R1
R2
R3
R4
R5
Time series

Figure 3.2 Data are mean values from 9 participants (± SE) and expressed as a percentage of the baseline Mmax area (A), the baseline ratio of CMEP/Mmax area (B), and the baseline ratio of MEP/CMEP area (C). The time scale includes a baseline (BL) value collected prior to fatigue, values at 25, 50, 75 and 100% of time-to-failure of the sustained contraction (shaded box) and data collected immediately (~5s) after task failure (R0) and then at 1min intervals (R1-R5). An asterisk (*) denotes a time point when FFfail or FFiso is lower than BFO alone. A single dagger (†) denotes a time point when FFiso is lower than both BFO and FFfail, whereas a double dagger (‡) denotes a time point when FFiso is lower than FFfail alone. A filled symbol denotes a time point that is greater than the 2s value.

3.3.5 Motor evoked Potentials

During fatigue, normalized MEP area (MEP/CMEP and expressed relative to the baseline ratio; see figure 3.2C) showed neither a main effect of protocol ($P = 0.446$) nor a protocol × time interaction ($P = 0.580$); however, there was a main effect of time ($P < 0.01$). With data pooled across protocols, the normalized MEP area was larger than baseline at all time points. Similar to the fatigue data, normalized MEP area during recovery revealed a main effect of time ($P = 0.019$) but neither a main effect of protocol ($P = 0.870$) nor a protocol × time interaction ($P = 0.113$). *Post-hoc* testing indicated that MEP area was larger than baseline at R0.

3.4 Discussion

It was the aim of this study to determine the acute effects of BFO on neural excitability, at multiple levels of the corticospinal tract, during a fatiguing contraction. The main findings were that, compared to a sustained isometric contraction to exhaustion with normal blood flow, the occlusion of blood flow led to a faster and greater increase in motoneuron excitability but had no effect on motor cortical excitability.

Despite a time-to-task failure that was ~80% shorter for BFO compared to FFfail, the increase in RMS EMG was not different during the two protocols. This faster rate of increase in EMG for BFO supports the findings of several previous studies[3,10,4,11]. A more rapid increase in EMG during BFO suggests that increases in motor unit firing rates or

recruitment are accelerated compared to a sustained isometric task to failure without externally imposed occlusion. In the absence of a supply of oxygen-rich blood, the type I fibers recruited at the onset of the weak contraction would fatigue faster than under non-ischemic conditions[12]. This would necessitate the recruitment of additional motoneurons and possibly a greater discharge rate of active motor units in order to maintain the target.

Similar to a previous study that involved a sustained isometric elbow flexor contraction at 20% of MVC force until exhaustion[13], peripheral excitability (M_{max} area) was reduced from baseline by ~17% for the FFfail protocol. In contrast, M_{max} area was not significantly lower than baseline during the BFO protocol. This discrepancy between trials to failure suggests that the magnitude of the reduction in peripheral excitability during a sustained submaximal contraction is dependent on the contraction duration, possibly related to the total number of action potentials. Alternatively, some consequence of BFO mitigates the activity-dependent reduction in peripheral excitability. During recovery, the Mmax was not different among protocols and not different from baseline. This finding is similar to that seen in the recovery period following dynamic elbow flexor contractions with and without restricted blood flow[6].

Without BFO (i.e., the FFfail trial), as seen previously[14,11], normalized CMEP area (CMEP/Mmax) increased during a submaximal isometric contraction sustained at a constant force until exhaustion. This enhanced excitability of the motoneuron pool reflects the balance of competing factors, including descending drive, afferent feedback and intrinsic motoneuronal properties. To counteract the effects of fatigue and maintain the target force, it is necessary to increase motoneuron discharge rates of active motoneurons and recruit additional motoneurons (via greater descending drive). Initially, both of these outcomes would increase the size of the CMEP; however, at rapid discharge rates, more motoneurons are likely to be in a hyperpolarized state at the time of the stimulus, which means the CMEP will decrease in size when descending drive is very high[15]. In terms of afferent feedback, muscle spindle discharge frequency decreases during a sustained submaximal contraction, which would disfacilitate motoneurons[16]. Conversely, sustained contraction will lead to increased firing of group III/IV muscle afferents, which has been shown to facilitate elbow flexor motoneurons[17]. Irrespective of

descending or afferent input, the repetitive discharge of a motoneuron during a sustained fatiguing contraction will lead to a reduced intrinsic excitability and a smaller CMEP[5]. As the normalized CMEP increases in size during a weak sustained contraction to exhaustion (FFail), it is clear that the facilitatory effects described above outpace those that make the motoneurons less responsive. With BFO, the normalized CMEP increased sooner and to a much greater extent compared to the FFfail trial (Figure 3.2B), which suggests that group III/IV afferent feedback is the primary cause of the enhanced motoneuron excitability seen with fatigue. The normalized CMEP data during recovery provide additional support to the importance of group III/IV afferent feedback because the marked difference between BFO and FFail at failure (326 vs. 179% of baseline) is abolished after only 1min of rest (both 130% of baseline).

Unlike the normalized CMEP, the normalized MEP was not different among the three protocols. Based on the pooled data, the normalized MEP (MEP/CMEP) at the end of the contraction was ~60% larger than the baseline value, which indicates an increase in cortical excitability. This finding is in contrast to the work of Lévénez and colleagues[14] who reported equivalent increases for CMEP and MEP size, which indicated the absence of an increase in cortical excitability during a sustained elbow flexor contraction at 50% MVC torque. Hoffman and colleagues[11] collected CMEP and MEP data during a sustained plantar flexion contraction at 30% MVC torque and, like us, observed an increase in cortical excitability with fatigue. The authors proposed that the discrepancy between their findings and those of Lévénez and colleagues[14] may relate to differences in the neural control of upper and lower limb muscles. However, the present data suggest that this is not the source of the discrepant findings. An alternative explanation for the conflicting results is the intensity of the sustained contraction; i.e., an increase in cortical excitability was observed at 20% MVC force (present study) and 30% MVC torque[11] but not at 50% MVC torque[14].

Brandner and colleagues[6] observed a greater increase in MEP amplitude 5-60min after resistance exercise performed with restricted compared to unrestricted blood flow. The authors proposed that group III/IV afferent feedback was responsible for the increased corticospinal excitability but were unable to distinguish between cortical and spinal sites.

Collection of the CMEP in the current study not only allowed insight into the impact of BFO on motoneuron excitability but it also enabled isolation of the cortical component of the MEP. Our results indicate that BFO does not strongly influence cortical excitability during fatigue or recovery, implying that spinal mechanisms are responsible for the observed growth of the MEP with BFO. The lack of a link between BFO and MEP size supports previous work in the elbow flexors that showed both the CMEP[18] and MEP[19] returned quickly to baseline values after a 2min MVC, even in the presence of muscle ischemia. Although muscle ischemia was not maintained into the recovery period of the present study, we observed a similarly rapid return to baseline for the CMEP and MEP. These findings contrast markedly with the prolonged (≥60min) increase in MEP size reported by Brandner and colleagues[6]. This disparity in the recovery period presumably relates to degree of blood flow restriction (complete vs. incomplete), the contraction type (isometric vs. dynamic), or the task design (exhaustion vs. a fixed number of contractions).

In summary, the current study found that BFO in combination with a low-intensity sustained isometric contraction caused a more rapid and greater increase in motoneuron excitability compared to the same task without BFO. This enhanced growth of the CMEP is likely the result of increased group III/IV afferent feedback in response to the muscle ischemia. Notably, BFO (and the presumed accompanying increase of group III/IV afferent feedback) did not influence cortical excitability.

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Chapter 4

4 Effect of blood flow on neuromuscular properties during dynamic elbow contractions

4.1 Introduction

It is generally accepted that resistance training with contractile intensities of ≥70% of maximal voluntary contraction (MVC) are required to stimulate muscle hypertrophy and strength gains[1,2]. However, chronic training with blood flow restricted (BFR) exercise, sometimes referred to as occlusion training, has shown strength gains can be accomplished at low-intensities[3,4,5]. Although BFR exercise has been studied in relation to chronic adaptations of the muscle, the acute effects of BFR that may promote longer-term muscular adaptations are not well-understood. The benefit of low-intensity exercise is that it can be performed at high velocity, and the addition of BFR is unlikely to reduce the peak velocity of non-fatiguing contractions.

The number of repetitions completed within a training protocol is less important to induce hypertrophy and strength chronically than doing repetitions to failure which likely causes greater metabolic stress[6,7]. Furthermore, repetitions to failure (fatigue) induce significant decreases in contractile velocity[6]. Thus, because power output of a muscle fiber is determined by the product of force and velocity[8], it is important to explore protocols that induce volitional failure but with higher velocities and therefore generate more power.

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Indeed, velocity and power are improved to a greater amount following chronic exercise training for those that use a faster cadence compared with training at a slow cadence[9,10].

Low-intensity contractions with BFR performed at greater velocities than those possible with higher loads may therefore be more beneficial at increasing muscular adaptations. Neuromuscular fatigue has both voluntary and involuntary components, the involuntary (peripheral) or muscle component of the neuromuscular system can be assessed from electrically evoked tetanic contractions of the muscle, which bypasses the central nervous system. During prolonged exercise that involves a high number of repetitions of a moderate to high intensity, the muscle fibers become weaker and contract slower (fatigue), often due to muscle damage[11] or excitation-contraction (E-C) failure[12]. The lack of oxygenated blood flow caused by BFR, likely facilitates the recruitment of type II fibers that are not oxygen dependent[8], causing greater type II fatigue, damage, and E-C coupling failure. Excitation-contraction coupling failure is one of a number of possible causes of fatigue[13, 14] and can be evaluated indirectly following fatiguing contractions by comparing the response of the muscle at lower frequencies of tetanic stimulation (i.e., ≤20Hz) to the response from maximal frequencies of excitation (i.e. 50Hz)[14,15]. The main features of low-frequency fatigue are that forces at low-frequency of stimulation are more impaired than those generated by higher stimulation frequencies, and impairment may last hours or days[13]. A greater 20/50Hz ratio after fatiguing contractions is an indication of peripheral fatigue and is referred to as low frequency fatigue (LFF). Central components of fatigue are more difficult to evaluate because they include assessing the degree of voluntary drive generated from spinal and supraspinal factors. Voluntary activation (VA) of the system can be indirectly assessed using the interpolated twitch technique (ITT)[16]. However, very few studies with BFR exercise have explored central and peripheral factors. In one study, following five sets of twenty dynamic fatiguing submaximal contractions at 20% of one-repetition-max with BFR, VA was decreased by 13%[17] and maximal surface EMG, as a measure of voluntary neural activation, was reduced by 12%, compared to no change in VA and maximal EMG amplitude during an MVC for a control group that performed the same protocol without BFR. In this same study the submaximal EMG increased during each consecutive set[17] in agreement with other studies reported in both chronic and acute use of BFR[18,5,19]. Overall, these findings with BFR were interpreted as an increase in

central and peripheral fatigue following exercise[17], however, these findings are based on protocols that did not reach failure, which would likely cause similar decreases in VA and increases in submaximal EMG.

The purpose of the current study was to use an acute dynamic fatiguing task to compare the effects on neuromuscular properties (velocity and power) of a low-intensity exercise with blood flow occlusion (BFO) with a high-intensity exercise bout without any imposed blood flow resistance or occlusion. A study by Yasuda et al. (2009) performed bouts of exercise with moderate and complete occlusion, and found that complete occlusion prevents arterial supply of metabolic substrates and venous return. Although the use of BFO is unlikely used chronically as an exercise intervention, it is a good model to explore BFR by enhancing fatigue during dynamic contractions to provide functional insight into the effects of restricted blood flow rather than with isometric contractions previously studied[20, 21]. Thus as a first comparison using an acute exercise bout and with specific peripheral and central measures, we chose to contrast free blood flow with an occluded rather than a restricted state. From this, insights may be gained about sites of adaptations that are affected during chronic BFR training and whether low-intensity BFR exercise is a viable training modality for muscular improvements, especially for those with functional limitations such as due to disease, rehabilitation or aging, as compared with the usual type of high-intensity exercise training.

Therefore we hypothesized, H1) that acute BFO exercise at low-intensity will induce greater decreases in velocity, and therefore power impairments to a similar or greater amount than high-intensity (HI) exercise, for the same number of repetitions, and H2) that acute low-intensity BFO will cause greater peripheral fatigue compared to the unrestricted blood flow during high-intensity exercise. Maximal velocity contractions were used to assess peak velocity and power, while electrically evoked tetanic responses were used to specifically target intrinsic (peripheral) changes in the muscle. Voluntary activation in combination with repetitions-to-failure and recording surface EMG were used as measures to assess central neural drive of the neuromuscular system.

4.2 Methods

4.2.1 Subjects

Nine healthy male subjects (see table 4-1 for characteristics) participated in two testing protocols (HI and BFO) administered in a random order and each was separated by at least 48 hours, with both protocols completed within 7 days. All procedures were approved by the local institutional ethics committee (REB# 107212, WREM at The University of Western Ontario) and conformed to the declaration of Helsinki. Written and verbal consent were obtained from each participant. All subjects were right hand dominant and therefore to minimize limb dominance effects, the non-dominant left arm was tested in each participant.

Parameters		Participants (n = 9 males)
Age (years)		27 ± 1
Height (cm)		176 ± 2
Mass (kg)		85.7 ± 9.4
MVC (Nm)		73.7 ± 23.0
Protocol	BFO	HI
Repetitions to Failure	21.3 ± 3.3 †	15.8 ± 2.9
Work (J)	3946.9 ± 1637.4	8199.02 ± 2275.6 †

Table 4-1 Values are means ± SD. BFO, blood flow occlusion at 25% of MVC intensity. HI, no blood flow restriction at 80% of MVC intensity. MVC, maximal voluntary contraction. † denotes significant difference between BFO and HI repetitions-to-failure.

4.2.2 Experimental set-up

Participants were seated upright in a Humac Norm dynamometer (Computer Sports Medicine Inc., Stoughton, MA) with feet resting on an adjustable foot rest placing their hip and knee joints at ~90 degrees. To record elbow flexion, the left arm was in the dependent position and the elbow joint allowed to move freely between 0-90º with the supinated wrist holding a hand grip in a comfortable position (wrist pronated ~5º). The axis of rotation of

the Humac norm was adjusted to the elbow joint center of rotation and lever arm with hand grip was adjusted for all participants. A snug three-point harness was adjusted around the torso for each participant to eliminate extraneous body movement, as well as a large inelastic strap was fastened securely across the chest to secure the participant to the chair. Surface EMG of the elbow flexors was recorded via adhesive Ag-AgCl electrodes (Kendall, H59P cloth electrodes) arranged in a monopolar fashion. One electrode was placed on the skin over the mid belly of the muscle and a reference electrode was secured over the ulna on the posterior forearm. For elbow flexor muscle electrical stimulation custom made aluminum foil electrode pads (~2x5cm) covered in damp paper towel were placed over the distal and proximal portions of the elbow flexors. A Digitimer (model DS7AH) was used to induce stimulation.

In all sessions, torque and EMG data were recorded using an A/D converter (CED 1401; Cambridge Electronic Design Ltd, Cambridge, UK) in conjunction with Spike2 software (v. 7.02; Cambridge Electronic Design). The torque and EMG data were sampled at 500 and 5000Hz, respectively. EMG data were amplified (x1000) and bandpass filtered (10Hz – 20KHz, with a 60Hz notch filter) using Neurolog; NL844, Digitimer, Welwyn Garden City, UK.

4.2.3 Experimental protocol

Participants were randomly assigned to complete either the low-intensity (25% of MVC) with BFO to repetition failure protocol, or the high-intensity (80% of MVC) unrestricted blood flow (HI) to repetition failure protocol (see Appendix G for details). Except for the addition of the blood pressure cuff to restrict blood flow, all procedures were identical in both protocols. The blood pressure cuff (adult sized, sphygmomanometer), was applied over the proximal portion of the biceps brachii and pressure was maintained at 300mmHg during the BFO protocol for all participants. This pressure was sufficient to abolish the forearm radial pulse during the resting state for all participants. To elicit an M-wave in the biceps brachii, a single electrical stimuli (200μs pulse width at ≤400V) was delivered to the brachial plexus at Erb's point. The cathode and anode were placed in the supraclavicular fossa and over the acromion, respectively. Stimulus current was increased incrementally for successive stimuli until the peak-to-peak amplitude of the resting M-

wave reached a plateau (M_{max}). Stimulus intensity was then set to 120% of the current required to produce M_{max}. The stimulation parameters remained constant pre- and post-intervention protocol. To determine voluntary activation (VA) during the MVC of the elbow flexors maximal electrical stimulation doublets (200μs pulse width; 400V; 100Hz doublet; range 90-168mA) by increasing current intensity until the torque response no longer increased with an increase in current intensity, or coactivation of other muscles impeded the elbow flexor torque. For the interpolated twitch technique (ITT) doublets were used to assess VA[16] in the elbow flexors. Participants were instructed to perform two to three brief (~3-5 seconds) elbow flexor MVCs, which included a superimposed doublet at the peak torque, and a doublet was applied at rest immediately following the MVC. If variability in maximal torque was 5% or more between MVCs then a third MVC was performed. Two minutes of rest was given between each MVC. Strong verbal encouragement and visual feedback were provided during all voluntary contractions. The greatest elbow flexion MVC was selected as the baseline value. Tetanic 50Hz (200μs pulse width; 400V; 1s duration; range 35-90mA) stimulation was applied for 1 second to elicit 30% of elbow flexor MVC torque which from pilot testing was tolerable and did not activate antagonist muscles of the arm. Stimulations at 1Hz (twitch) and 20Hz were applied using this same intensity as for the 50Hz. Three dynamic maximum velocity contractions were performed in order to calculate peak velocity. For the BFO protocol a 25% MVC load was used to measure velocity, while during the HI protocols, both a 25% load and 80% load were used to calculate velocity reductions during HI repetitions. For the HI protocol 80% MVC load was used to normalize the changes in velocity throughout the protocol, however, following both protocols (HI and BFO) during recovery a 25% MVC load was used in order to compare the recovery of velocity and power.

After baseline measures were acquired (at the beginning of each testing day), participants completed one of the two intervention protocols. Participants were instructed to complete as many dynamic repetitions as possible for both protocols, while elbow flexor MVCs (~3-5 seconds) were assessed at the completion of every 2 repetitions, and the ITT was assessed at the estimated (from pilot testing) 50% of repetition-to-failure point. Failure was defined as the point at which participants were unable to complete a repetition through the full range of motion (0-90º). During a 20min recovery period at each time point (0, 2, 5, 10,

and 20 minutes) participants completed in order three maximal velocity contractions (25% load for both protocols), the isometric M_{max}, 1Hz, 20Hz and 50Hz responses followed by an MVC with ITT. Parameters were assessed immediately following the fatiguing task (at failure point, FP; and ~2-3 seconds following failure (R0), which accounted for the time needed to remove the blood pressure cuff and during the recovery period.

4.2.4 Data and statistical analyses

Off-line quantification of measures consisted of peak MVC torque, M_{max} area, peak dynamic velocity at 25% MVC load (for HI protocol group velocity at 80% MVC load was used to normalize reductions in velocity and power), peak power (peak dynamic torque*peak velocity), peak twitch torque (PT), time-to-peak torque (TPT), and half-relaxation-time (HRT) for the 1Hz stimulation, and PT and HRT for the 50- and 20Hz stimulations. Voluntary activation was calculated using the interpolated twitch equation: [1 – (superimposed/potentiated twitch)*100][11]. The superimposed twitch refers to the doublet stimulation applied at the MVC peak, and the potentiated twitch refers to the doublet stimulation applied following the MVC at rest. The average amount of Work performed by each group in each protocol was calculated as W = T * D, where T is the torque in Nm performed in each protocol, and D is the distance the moment arm travelled degrees. Data are described in text as mean ± SD and in all figures as the mean ± SE. A two-way repeated measures ANOVA with a modified Bonferroni correction was performed to determine between-group differences of time and protocol for MVC torque, VA, Mmax area, peak dynamic velocity at 25% and 80% MVC load, peak power, PT, TPT, and HRT for the 1Hz stimulation, and PT and HRT for the 50- and 20Hz stimulations. When only a main effect of time was observed, paired sample t-tests were used in conjunction with a Dunnet's table test for multiple comparisons. A power analysis was completed for the peak power results and found that the sample size of 9 was sufficient. Paired t-tests were used to compare group differences in repetitions-to-failure, Work and MVC. All statistical analyses were performed using SPSS version 25. Statistical significance accepted at $\alpha < 0.05$.

4.3 Results

4.3.1 Voluntary characteristics

The number of repetitions required to reach failure (see Table 4-1.) during BFO (21.3 ± 3.3) was greater than the number during HI (15.8 ± 2.9; $P < 0.01$). Baseline MVC was not different between protocols (BFO: 73.7 ± 23.0N.m; HI: 60.5 ± 26.3N.m) ($P > 0.05$). There was a main effect of time ($P < 0.01$) and protocol for MVC ($P < 0.01$) which decreased to a greater amount during BFO by ~77% at FP compared to a reduction of ~23% during HI (see Figure 4.1). BFO MVC was significantly reduced compared to HI throughout the normalized repetitions-to-failure, however, immediately following the termination of the BFO protocol, the MVC between the two protocols was not statistically different (R0) (see Figure 4.1). Despite the reduction of MVC (~77%) at the end of the BFO protocol, when the blood pressure cuff was removed (~3s) and blood flow restored, MVC recovered to values that were no longer different between protocols (refer to RO in Figure 1.) However, BFO MVC still remained reduced from baseline for the recovery period (20mins), whereas HI MVC recovered by R2 compared to baseline values. Voluntary activation at baseline was not statistically different ($P > 0.05$) but was reduced at FP equally in both protocols to ~88% of baseline for the BFO protocol and ~92% for the HI protocol. At the completion of recovery (20 mins) voluntary activation for both BFO and HI was not different from baseline at ~~99% and ~98%, respectively, or to each other (see Figure 4.2).

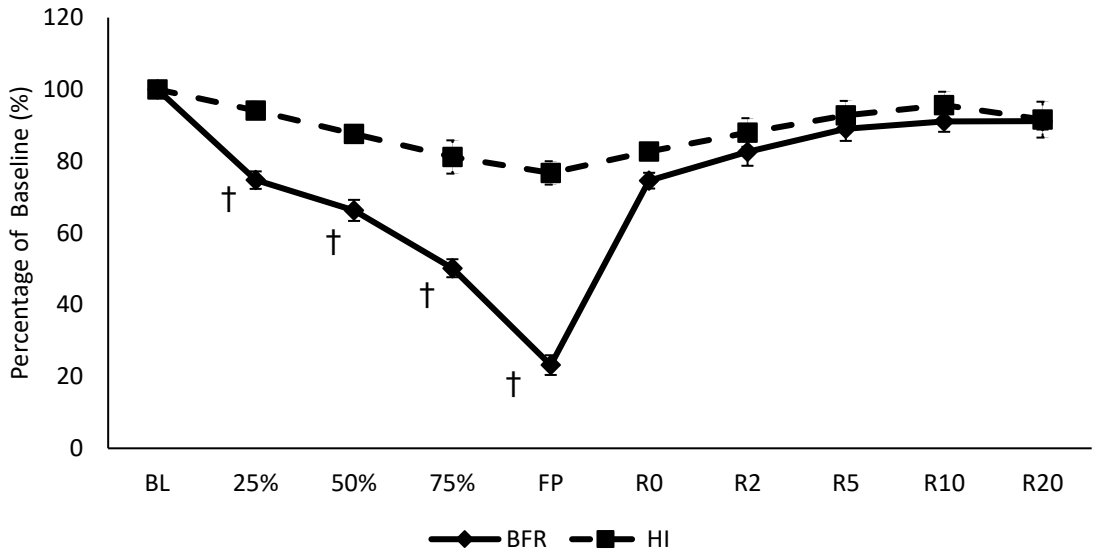

Figure 4.1 Maximal voluntary contraction normalized as repetitions-to-failure displayed as 25% - failure point (FP) for both BFO and HI trials. Recovery time points from R0 – R20 are in real time. Values represented as percent change from baseline and displayed as means ± SE. † denotes significant difference between BFO and HI.

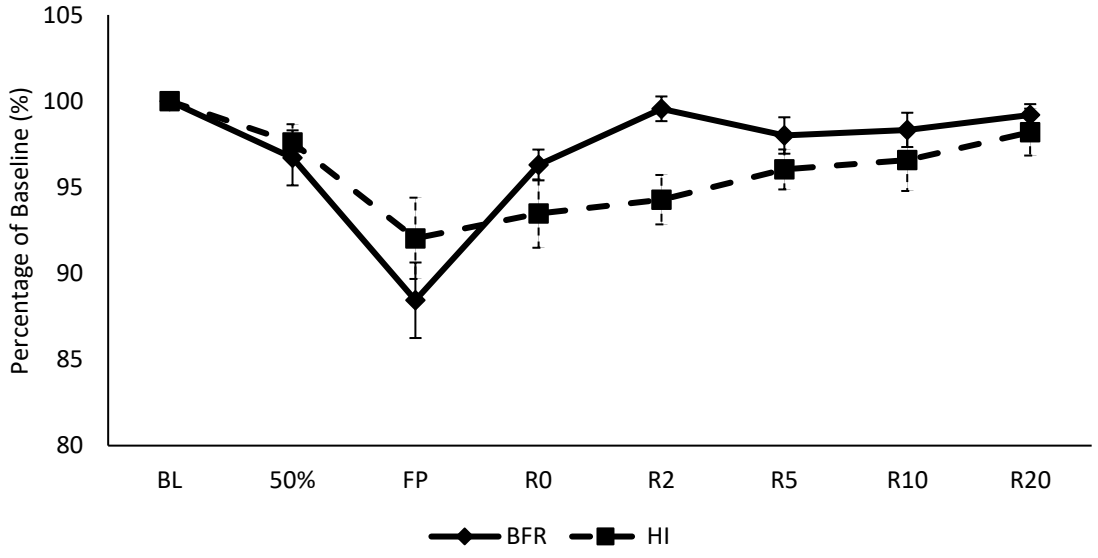

Figure 4.2 Voluntary activation normalized as repetitions-to-failure for both BFO and HI trials. Recovery time points from R0 – R20 are in real time. Values represented as percent change from baseline and displayed as means ± SE.

4.3.2 Velocity, power, and work

Baseline velocity at a 25% load was not different between groups ($P > 0.05$). Velocity showed a main effect of time ($P < 0.01$), with both protocols becoming significantly reduced by 25% of normalized repetitions-to-failure, and recovered by 5mins (see Figure 4.3). Baseline absolute power at low-intensity (25% of MVC load) was not statistically different between protocols ($P > 0.05$). There was a main effect for time ($P < 0.01$) and protocol ($P < 0.01$) for power and an interaction of protocol and time ($P < 0.01$) such that by 25% of normalized repetitions-to-failure until FP, BFO was reduced by 90% compared with 67% for HI (see Figure 4.4.). Power in each protocol recovered at a similar rate but HI recovered by 2 minutes, whereas BFO was not recovered until 20 minutes. The amount of work ($W = T * D$) completed during the BFO protocol (180.8 ± 56.5 J / repetition) was significantly lower than the HI protocol (527.5 ± 129.5 J / repetition), with an overall amount of work of 3946.9 ± 1637.4 J for BFO and 8199.02 ± 2275.6 J for HI ($P < 0.01$).

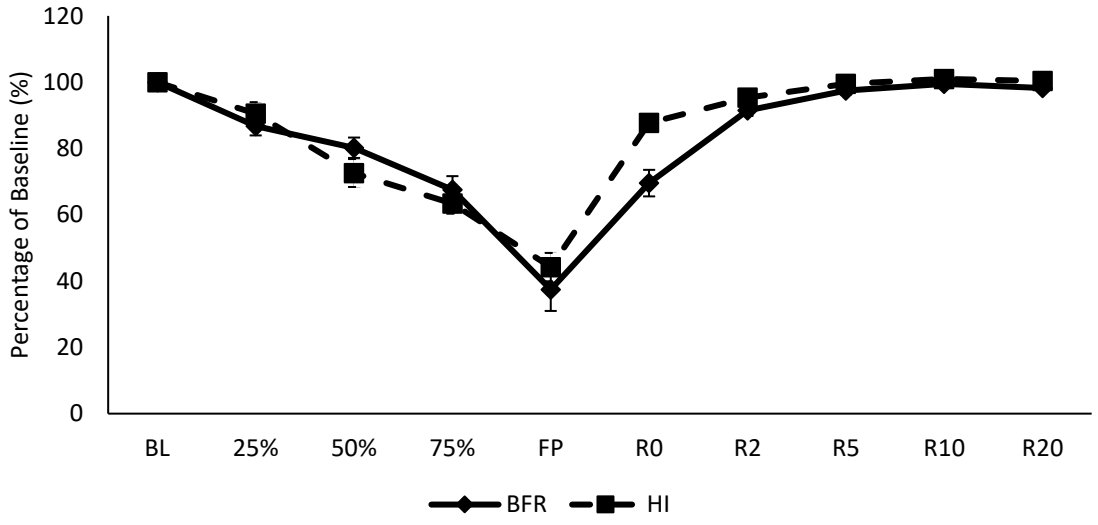

Figure 4.3 Represents peak velocity values normalized as repetition-to-failure displayed as 25% - fail point (FP) for both BFO and HI protocols. Recovery time points from R0 – R20. Values represented as percent change from baseline and displayed as means ± SE.

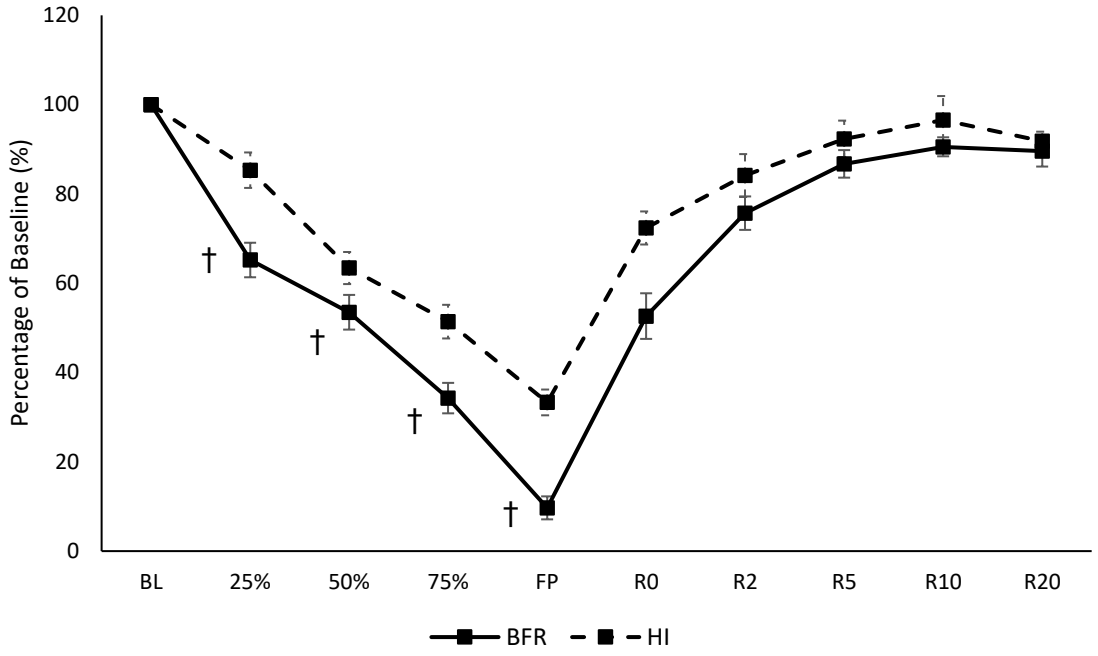

Figure 4.4 Represents relative peak power values normalized as repetitions-to-failure displayed as 25% - fail point (FP) for both BFO and HI protocols. Recovery time points from R0 – R20. Values represented as percent change from baseline and displayed as means ± SE. † denotes significant difference between BFO and HI.

4.3.3 Twitch and tetanic properties

Baseline M_{max} area was not different between protocols ($P > 0.05$), and remained unchanged throughout both protocols compared to baseline (data not displayed)**.** Baseline twitch torque was not different between protocols ($P > 0.05$), but showed a main effect of time ($P < 0.01$) and protocol ($P = 0.023$). The twitch amplitude was significantly decreased following BFO compared to HI at R0 of ~88% and ~51% from baseline, respectively ($P < 0.01$). However, no statistical difference was observed between protocols at R2 or for the remainder of the recovery (data not displayed). Twitch torque at 20mins of recovery remained depressed for BFO at ~57% of baseline, but HI was recovered by R2. The 20Hz tetanic stimulation had a greater decrease at R0 following BFO (~78% of baseline) compared to HI (~48% of baseline) ($P < 0.01$). Both protocols had a main effect of time (P

< 0.01) and protocol (P = 0.01) and 20Hz remained depressed from baseline following 20mins of recovery (see Figure 4.5A). The 20Hz HRT showed a main effect of time (P < 0.01) and protocol (P = 0.02), with BFO having a greater increase in HRT at R0 (P = 0.03), compared to HI. The 20Hz HRT recovered by 5 minutes (see Figure 4.5B). The 50Hz peak torque was not significantly different between groups but did show a main effect of time (P < 0.01) remaining significantly reduced from baseline by ~20% until 10mins of recovery (data not displayed). The 20Hz to 50Hz peak torque ratio had a main effect of time (P < 0.01) and protocol (P < 0.01) with the ratio following BFO reduced to ~70% of baseline at R0, compared to a reduction in HI of ~9% from baseline (P < 0.01) (see Figure 4.6).

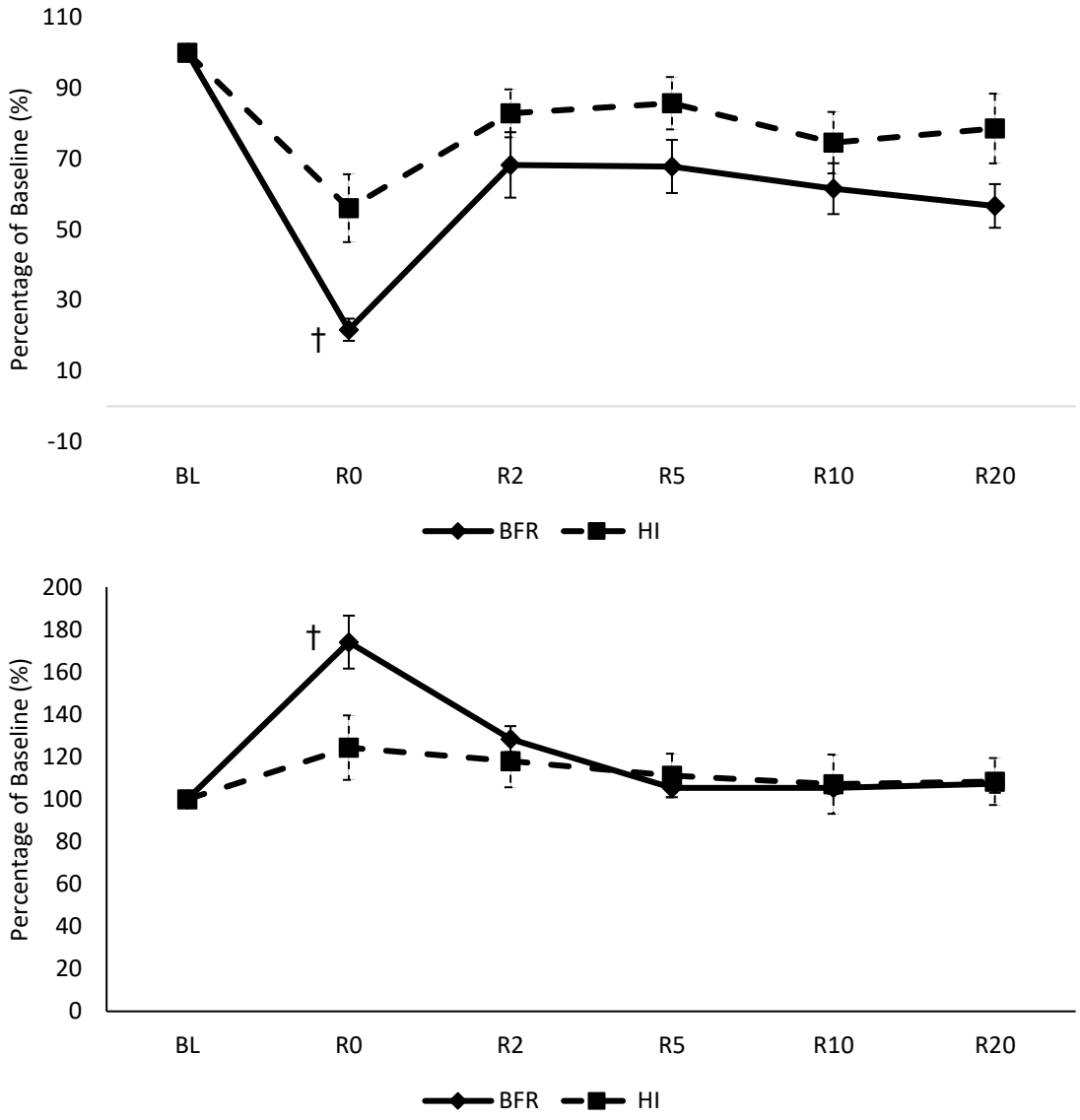

Figure 4.5 (A) Represents values of 20Hz torque percent changes during R0 – R20 minutes of recovery compared to baseline. (B) Represents values of 20Hz half-relaxation-time percent change during R0 – R20 minutes of recovery compared to baseline. † denotes significant difference between BFO and HI protocols. Values displayed are means ± SE.

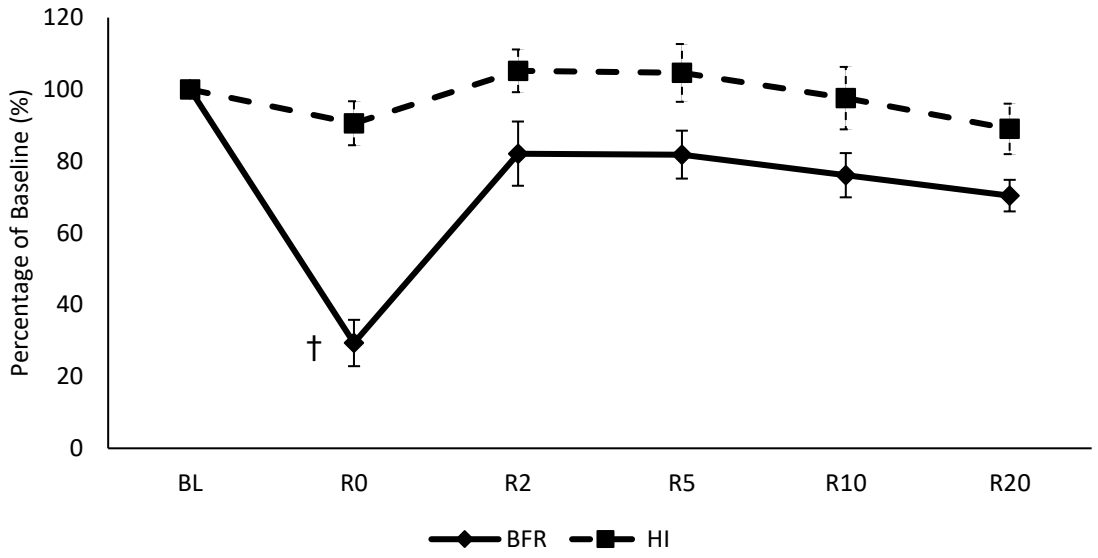

Figure 4.6 Represents values of the 20/50Hz torque ratio as percent changes during R0 – R20 minutes of recovery compared to baseline. † denotes significant difference between BFO and FF protocols. Values displayed are means ± SE.

4.3.4 EMG

Maximal EMG for BFO was reduced from baseline beginning at 25% of normalized repetitions-to-failure ($P < 0.01$), whereas during the HI protocol EMG remained unchanged from baseline values ($P > 0.05$). At the FP, the maximal EMG from the BFO was significantly less than HI at ~50% compared ~104% ($P < 0.003$), respectively (data not displayed). However, by R0 and throughout recovery maximal EMG was not statistically different from baseline values in both protocols.

4.4 Discussion

The results of the current study indicate that a bout of low-intensity dynamic concentric arm flexor contractions to failure with BFO causes a greater amount of peripheral fatigue, as well as a greater reduction of power compared with a bout of high-intensity contractions with free (unrestricted) blood flow. Although the number of repetitions required to reach failure was greater for the BFO (see Table 1.) compared with HI (21.3 ±

3.3 and 15.8 ± 2.9, respectively), the amount of total work done was less in BFO (3946.9 ± 1637.4 J) compared to the HI (8199.02 ± 2275.6 J) ($P < 0.01$). Specifically, the work completed during the BFO protocol (180.8 ± 56.5 J / repetition) was significantly lower than the HI protocol (527.5 ± 129.5 J / repetition). Although a greater amount of work was performed for the HI group, the relative decrease in peak power was greater for the BFO protocol (~90% from baseline) compared to the HI protocols (~67% from baseline), due to the reduction in velocity combined with the greater reduction in MVC torque for the BFO protocol. Despite the greater amount of work performed for the HI protocol, the BFO protocol caused more fatigability as indicated by a greater decline in power and greater low frequency fatigue. Thus, blood flow occlusion results in greater peripheral fatigue with lower submaximal intensity contractions than with higher intensity contractions when blood flow is not impeded. This may be due to greater type II fiber fatigue because BFO expedites the fatigue of type I fibers due to a non-oxygenated environment thus enhancing the recruitment of type II fibers.

Numerous studies have explored the chronic effects of blood flow restriction during isometric contractions, and observed increases in strength following a training program[22]. However, the acute effects of BFO have not been explored comprehensively to understand the underlying factors. One study by Copithorne and Rice (2019) comparing low-intensity contractions with and without BFO, observed that a sustained low-intensity (20% of MVC) isometric contraction of the arm flexors with blood flow occlusion produced greater peripheral fatigue, compared with a low-intensity sustained contraction with no obstruction to blood flow. Only a few studies have explored the acute effects of BFO or BFR during dynamic contractions[22]. The current study contrasted the acute effects of BFO at low-intensity in comparison to a high-intensity protocol with no blood flow restriction. We observed a greater decrease in peak power following BFO (90% reduction from baseline) than the HI protocol (67% reduction from baseline). This larger reduction in peak power with BFO could indicate that fast type II fibers that generate greater velocity and torque[8,23] are affected more than the slow type I fibers. Conversely, type II fibers during the BFO protocol could be activated for a longer amount of time, as recruitment of type II fibers is enhanced with greater force demand[24]. MVC during the BFO protocol was also reduced to a greater extent than during HI (~77% and ~23%,

respectively), however, this result is expected due to the failure criteria. That is, the HI protocol terminated when participants were unable to produce full range of motion at 80% of MVC, and the BFO protocol terminated when participants were unable to produce full range of motion at 25% of MVC. Type I fibers that contract more slowly and generate less mechanical power therefore require less ATP, whereas type II fibers require more ATP to generate force quickly and to reach the "optimal velocity" for the required movement[24]. Furthermore, fibers are not only recruited in order for force development but also in relation to the speed of contraction[8,23]. Therefore during the BFO condition the reduction of peak power is likely due to expedited fatigue of type I fibers in an oxygen depleted environment leading to a greater activation of type II fibers that consume a greater amount of ATP, as compared to the HI protocol which may be able to use type I fibers to produce force for a longer duration. This increased demand placed on type II fibers during BFO causes greater reductions in torque and velocity compared with HI.

For both protocols VA was reduced from baseline at FP, but was not different between protocols (see Figure 4.2) and returned to baseline in both protocols by the end of the recovery period. Karabulut et al. (2010) found that low-intensity dynamic leg extensions with vascular restriction resulted in greater voluntary inactivation compared to non-vascular restricted control participants. It was proposed that the greater decrease in VA with vascular restriction may indicate a greater inhibition of central drive to motor units. However, that study was not performed to failure, but rather to a pre-determined termination criteria of 20 repetitions. Despite the differences in repetitions to FP between the two protocols in the current study, the criterion for task termination was failure and thus VA was similarly affected. The loss of VA of ~10% indicates a greater amount of peripheral fatigue than central (supraspinal) when protocols are terminated at voluntary failure. Maximal normalized EMG during the BFO protocol was reduced to a greater level at FP compared to the HI group. Greater reductions in normalized maximal EMG with BFR compared to free flow protocols at the same low-intensity have been previously reported[17]. This result can likely be attributed to either reductions in motor unit firing rates or reductions in the number of active motor units, or both[25]. The findings of the current study indicate the greater reduction in maximal EMG following BFO is likely due to reductions in the number of active motor units caused by a greater metabolic

stress of the working muscle, activation and fatigue of the fibers, as a result of the non-oxygenated environment. Following the termination of both protocols M_{max} area was not significantly reduced (data not displayed), indicating that despite that greater amount of peripheral fatigue experienced following the BFO protocol, muscle fibers can still be activated fully with imposed stimulation on the system as previously noted[26,27]. Thus, the greater impairments in MVC, velocity and power with BFO compared with HI are due mainly to peripheral factors as indicated by the greater decrease in maximal EMG.

After a sustained muscular contraction of several minutes, recovery of voluntary force is largely completed in a few minutes; however, the long lasting fatigue component observed at low-frequencies (20Hz) of excitation compared with those at high frequencies (50 Hz) can remain reduced for hours or days[28]. This low frequency fatigue indicates some damage to the structure of the muscle fiber and EC coupling mechanisms[29]. In the current study, this was evident by finding that MVC and 50Hz recovery following both protocols as previously observed[28], whereas the 20Hz and twitch responses were more depressed at R0 after BFO than HI (~78% and ~48%, respectively). By R2 there was no difference in low frequency fatigue between protocols but each remained depressed from baseline for the 20 minute recovery period. Furthermore, there was greater slowing in the 20Hz HRT measures in the BFO protocol compared with HI indicating the BFO protocol likely caused a greater slowing of type II fibers which resulted in a reduction in velocity and impacting power[30].

The results of the current study have shown that although less work is performed during a low-intensity single bout of dynamic biceps contraction with BFO to failure, compared to high-intensity with no external impediment to blood flow, power as a function of strength and velocity is depressed ~23% more. Measures of VA, maximal EMG, and peripheral contractile properties measured using tetanic involuntary contractions, have been combined to provide a comprehensive evaluation of the determining factors during the different fatiguing protocols. We have shown that central (supraspinal) factors are not likely a major determining cause for the level of fatigability experienced during these protocol as reflected by the VA measure, but rather peripheral fatigue is the major contributing factor. During fatigue the greater loss of velocity, torque and therefore

power during the BFO protocol may be due to structural damage and EC coupling failure of type II fibers, which are recruited for a longer duration, This is further indicated by the greater reductions in low-frequency involuntary tetanic contractions (20Hz), and the slowing of the 20 Hz HRT following the BFO compared with HI. These results may indicate a greater challenge to the system when training with blood flow restriction compared with an alternative high-intensity bout without blood flow restriction. This indicates that positive compensatory adaptations of strength and hypertrophy may be realized during chronic dynamic training using low intensity exercise with blood flow restriction that may be beneficial in compromised situations such as rehabilitation and aging.

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Chapter 5

5 The effect of blood flow occlusion on the tibialis anterior motor unit firing rates during sustained low-intensity isometric contractions

5.1 Introduction

Over the past two decades research on low-intensity exercise augmented by blood flow restriction has shown similar or greater hypertrophy and strength adaptations[1,2,3,4] than traditional high-intensity exercise training. However, a limitation of blood flow restriction or occlusion is that it can only be applied practically and safely to limb muscles and cannot directly benefit trunk or core muscles. Despite this, some studies have observed that when blood flow restriction was applied to the most proximal region of the arm, using elastic cuffs, following either an acute (single bout), or after 2-6 weeks of training that cross sectional area (CSA) of the pectoralis major was increased by 8-16%[5,6], strength increased 6-9%[5,6]. Other research has shown greater pectoralis major electromyography (EMG)[7]. Because blood flow restriction in addition to exercise results in an increase in venous pooling and cell swelling[8], the resulting increase in CSA of the pectoralis major was attributed to an anabolic response activation triggered by cell swelling[9]. This interaction between the pectoralis musculature and arm musculature (biceps, triceps, brachialis) may have been caused by blood pooling and cell swelling as both the arm and pectoralis muscle receive blood flow from a common arterial supply arising from the axillary artery. Alternatively, increases in CSA and EMG to the pectoralis major muscle may be explained by activation of the muscle during the exercise task (bench press). Thus, it is unclear whether there are upstream (proximal) effects possible from occlusion or restriction of muscle blood flow. Exploration of changes in neural drive proximal and distal to the site of occlusion in an acute paradigm may provide some important insight into this concept.

Muscle activation assessed by EMG, provides insight into the level of motor unit activation during voluntary contractions[10]. However, to overcome some of the limitations of surface EMG, a more direct measure of changes in neural drive during

voluntary contractions can be achieved by recording motor unit firings rate (MUFRs) from indwelling electrodes. A study by Moritani and colleagues[11] used intramuscular fine wire electrodes to record the effect of blood flow occlusion (BFO) on motor unit (MU) spike amplitude and spike frequency during low-intensity contractions (20% of MVC). The results of that study noted that prior to blood flow occlusion, MU spike amplitudes and spike frequency were lower compared to after the application of BFO. Moritani and colleagues[11] concluded that the resulting increase in spike amplitude and frequency was explained by an increase in the activation of higher threshold MUs, due to the lower availability of oxygen. However this study was unable to track the firing rates of individual MUs.

Several studies exploring low-intensity fatiguing tasks have reported that the mean firing rates of early recruited low-threshold motor units (<25% MVC) during sustained isometric contractions progressively decreased during the fatiguing task whereas the rates of higher threshold MUs (>50% MVC) initially increased before decreasing[12,13,14]. Presumably, the increase in surface EMG with declines in firing rates during a submaximal fatiguing task indicates that recruitment of likely larger type II MUs is required[14,15]. In high-intensity fatiguing contractions decreased MU firing rates remain depressed when blood flow is occluded immediately following task termination[16]. Those authors concluded that occlusion trapped some metabolic products in the muscle that inhibited MUFRs through a reflex pathway. Together, these studies indicate that there is a relationship between blood flow and neural drive to the fatigued muscle, however, it is unclear the impact that the obstruction of blood flow *during* sustained low-intensity contractions will have on neural activation of muscle as assessed by MU firings rates.

Thus, to assess the effects of BFO on MUFRs proximal and distal to the site of occlusion we recorded changes in single motor unit firing rates of the TA, strength reduction, and surface EMG characteristics prior to and following a sustained fatiguing submaximal dorsiflexion contraction to failure. Arterial supply to the TA arises from a branch of the popliteal artery just inferior to the knee joint. Occlusion on the distal thigh would impede blood flow into the TA and occlusion at the ankle would impede blood flow leaving the TA. Therefore, when blood flow occlusion occurs either proximal (upstream) or distal

(downstream) to the TA (the main dorsiflexor) during a sustained low-intensity isometric dorsiflexion contraction, we hypothesize that: H1: BFO proximal to the TA will cause the greatest reduction in MUFR recorded from the TA, whereas H2: BFO distal to the TA will cause greater MUFR decreases recorded from the TA than control (without any occlusion), but less than proximal BFO.

5.2 Methods

5.2.1 Participants

Five healthy male subjects (for participant characteristics see Table 5-1) participated in three testing protocols, with each protocol repeated twice, administered in a pseudo-randomized order and each was separated by at least 48 hours. All procedures were approved by the local institutional ethics committee and conformed to the declaration of Helsinki. Written and verbal consent were obtained from each participant. The left leg was tested for all participants to prevent any limb dominance effects.

Parameters		**Participants (n = 5 males)**
Age (years)		25 ± 4
Height (cm)		175 ± 7
Mass (kg)		88.5 ± 20.4
Protocol	BFO_{prox}	Control/BFO_{dis}
Time to Failure (s)	340 ± 98 †	638 ± 182

Table 5-1 Participant characteristics. Data are displayed as means ± SD. † denotes differences between BFO_{prox} and BFO_{dis}/control protocols.

5.2.2 Experimental set-up

Participants were seated placing their hip and knee joints at ~90 degrees, with the ankle joint secured in a dynamometer. To record dorsiflexion, the left leg was in the dependent position and the ankle joint was plantar flexed to 30 degrees with inelastic straps to affix the foot to the dynamometer. A brace was adjusted to press down firmly on the top of the

leg to minimize any extraneous leg movement. EMG of the TA was recorded, via adhesive surface electrodes (GE Healthcare, resting ECG electrodes) arranged in a monopolar fashion. Recording electrodes were placed on the skin over the mid belly of the TA and a reference electrode secured over the distal tendon portion at the ankle.

In all protocols, torque and EMG data were recorded using an A/D converter (CED micro 1401; Cambridge Electronic Design Ltd, Cambridge, UK) in conjunction with Spike2 software (v. 7.02; Cambridge Electronic Design). The torque and EMG data were sampled at 500 and 5000Hz, respectively. EMG data indwelling needle data were amplified (x1000) and bandpass filtered (10Hz – 20KHz) using Neurolog; NL844, Digitimer, Welwyn Garden City, UK.

5.2.3 Experimental protocol

Participants were randomly assigned to complete either the low-intensity (~15% of MVC) to failure protocol with blood flow occlusion (300mmHg) around the distal thigh (BFO_{prox}) or the low-intensity to failure protocol with blood flow occlusion at the ankle (BFO_{dis}) (see Appendix H for details). A third control protocol with unrestricted blood flow was time-matched to the BFO_{dis} protocol (Control). Each of the five participants repeated each protocol twice to improve numbers of recorded MUs. Except for the addition of the blood pressure cuff to restrict blood flow all procedures were identical in all protocols. Pressure was maintained at 300mmHg using an adult sized sphygmomanometer during the BFO protocols to abolish blood flow during the resting state as tested during pilot experiments by absence of a pulse at the ankle. Two pairs of custom made bipolar indwelling wires with hooked tips (0.004mm diameter, CFW Company, Grover Beach, CA) were inserted with a hypodermic needle (BD Eclipse 25G applicator needle) to a depth of ~1cm. The needles were inserted and immediately removed, leaving the two pairs of fine wire electrodes embedded in the TA muscle. After the insertion of the fine wire indwelling electrodes, participants were instructed to perform two brief (~3-5 seconds) dorsiflexion MVCs. If variability in maximal torque was 5% or greater between MVCs then a third MVC was performed. Two to three minutes of rest was given between each MVC. Strong verbal encouragement and visual feedback were provided during all voluntary contractions. The greatest dorsiflexion MVC was selected as the baseline value and used to estimate 15% of

MVC used in the protocols. After baseline MVC measures were acquired (at the beginning of each testing day), participants completed one of the three protocols. Participants were instructed to maintain a sustained isometric contraction (~15% MVC), that allowed for the recording of single motor units, until failure. Failure was defined as the point at which participants were unable to maintain the testing contraction after being verbally warned, twice consecutively. TA EMG was recorded using a monopolar electrode setup, the recording electrode was placed over the muscle belly, with the references electrode over the tendon area over the ankle. Following the completion of each protocol a post-MVC was recorded in order to establish fatigue induced strength loss.

5.2.4 Data analyses and statistics

Offline, Spike2 software (v. 7.02; Cambridge Electronic Design) was used to analyze all data. Peak force was recorded as the greatest value generated during the brief MVCs prior to and following each protocol. Maximal voluntary EMG (root mean square, RMS) was measured over a 500ms epoch centered about the peak force for the greatest pre-fatigue MVC. During the protocol contractions, mean RMS EMG was calculated over a 500ms epoch 2s after testing contraction was stabilized, and at 25 - 100% of normalized time-to-failure and expressed as a percentage of the value obtained during the pre-fatigue MVC. For the MU train analysis a template shape algorithm was utilized (Spike2 v. 7.02, Cambridge Electronic Design) that overlays sequential action potentials to identify action potentials of the same MU. Visual inspection by an experienced operator was used to confirm a specific MU train. Trains of MUs were included in the analysis with interspike intervals coefficient of variance of <30%[17]. Averages of 10 continuous interspike intervals at each of the time-to-failure percentages were then analyzed for changes in MUFRs.

A one-way repeated measures ANOVA with a Bonferroni correction was performed in order to test for differences among protocols for baseline values of MVC force, RMS EMG, as well as MUFR. A three-way repeated-measures ANOVA was used to assess the effects of protocol, protocol x time, and time on mean RMS EMG and MUFR. Paired sample ttests were used to assess differences in reductions of MVC pre and post protocol, and for the time-to-failure measures. When only a main effect of time was observed, data were pooled across protocols and paired sample ttests were used in conjunction with a Dunnett's table

to determine time points different from baseline. All statistical analyses were performed using SPSS version 25. All data are reported in the text as the mean ± SD and displayed as the mean ± SE. The significance level was $P < 0.05$.

5.3 Results

5.3.1 MVC, time-to-failure and surface EMG

Time-to-failure (TTF) for the BFO_{dis} and control protocols (638 ± 182s) were equal, and significantly longer than the BFO_{prox} protocol (340 ± 98s) ($P < 0.01$). MVC force prior to each protocol was not different ($P > 0.05$), and all protocols had a significant reduction in MVC (see Figure 5.1) from pre to post ($P < 0.01$) ranging from control at ~15%, BFO_{dis} at ~18% and BFO_{prox} at ~40%. However, the only difference between protocols was between control and BFO_{prox} ($P < 0.01$). RMS EMG between protocols had a main effect of time ($P < 0.01$), but not a protocol, or a protocol x time interaction. EMG during the control protocol increased from baseline to ~28% of normalized EMG at 100% of TTF, whereas EMG during the BFOdis and BFOprox protocols increased from baseline ~30% and ~48% of normalized EMG, respectively at 100% .

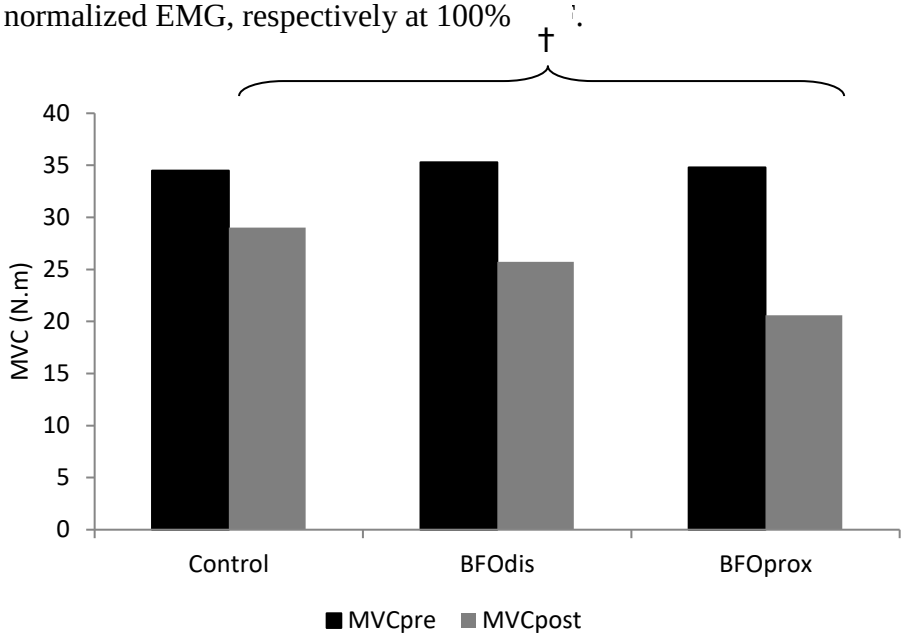

Figure 5.1 MVC data for protocols. Fatigue induced reductions in MVC force following termination of protocols compared to baseline pre-fatigue values. Data are displayed as means ± SE. † denotes differences between BFO_{prox} and control protocols.

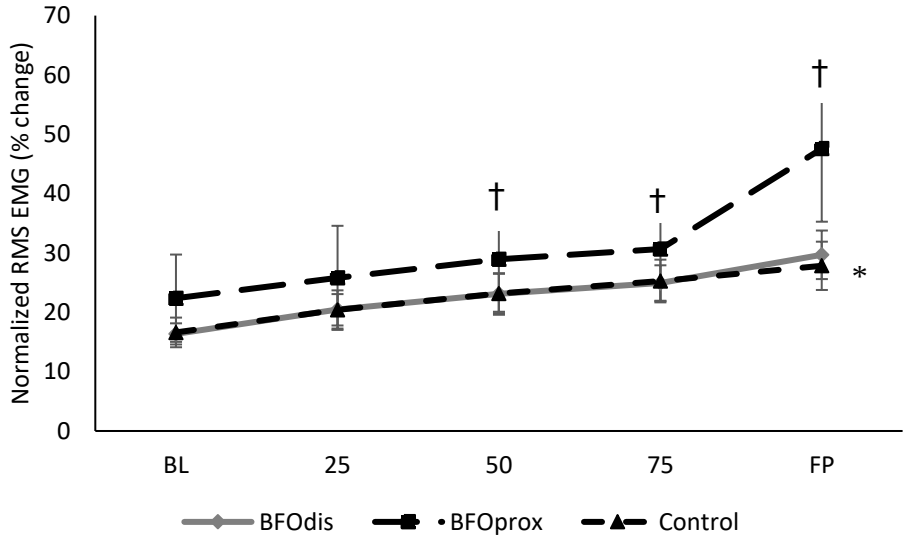

Figure 5.2 Submaximal RMS EMG normalized to pre-fatigue MVC RMS EMG. Data are displayed as means ± SE. † denotes differences for BFO_{prox} and BFO_{dis} from baseline. * denotes difference in control for baseline.

5.3.2 Motor unit firing rates

Motor unit firing rates at baseline were not different between protocols ($P > 0.05$). MUFRs for the control protocol remained unchanged throughout the protocol, whereas the BFO_{dis} and BFO_{prox} protocols showed reductions in MUFRs throughout each normalized time point of the protocols (see Table 5-2 and Figure 5.3). Between protocol analysis identified a main effect of time ($P < 0.01$), protocol ($P < 0.01$), and protocol x time ($P < 0.01$). There were no protocol differences at 25% of normalized TTF, however both BFO_{dis} and BFO_{prox} were different than control at 50% ($P = 0.025$; $P < 0.01$, respectively) and 75% ($P < 0.01$; $P < 0.01$, respectively) of normalized TTF, but not different from each other. At 100% of TTF MUFRs for the BFO_{prox} protocol were reduced to a greater amount than the BFO_{dis} protocol ($P < 0.01$).

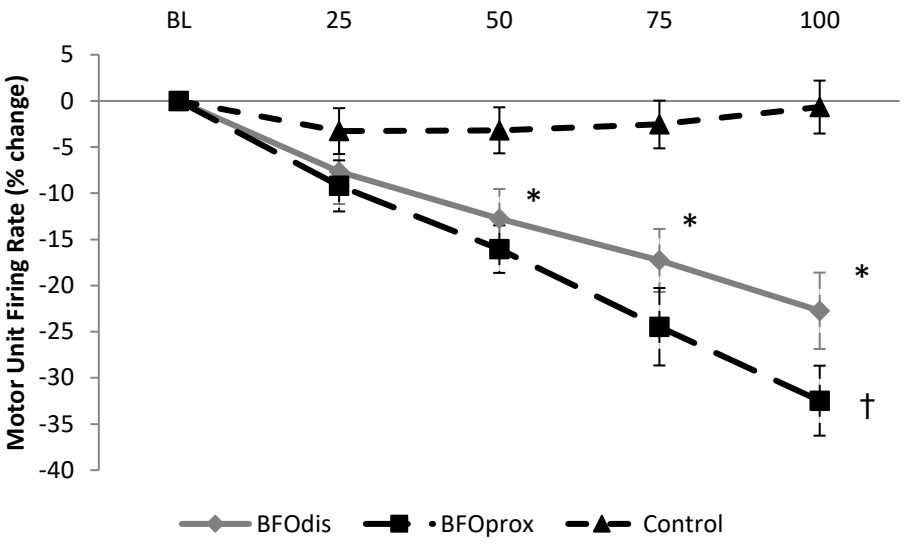

Figure 5.3 MUFR reductions displayed as % changes from baseline values. Data are displayed as means ± SE. * denotes differences between BFO protocols and control. † denotes differences between BFO_{prox} and BFO_{dis} protocols.

Protocol	*Control*		*BFO_{dis}*		*BFO_{prox}*	
MVC	MVC Pre	MVC Post	MVC Pre	MVC Post	MVC Pre	MVC Post
	34.5 ± 4.9	29.0 ± 4.6*	35.3 ± 4.3	25.7 ± 4.9*	34.8 ± 4.5	20.6 ± 5.6*†
Mean testing force (N.m)	15.2 ± 1.6		15.2 ± 0.9		14.7 ± 1.6	
Total # of Motor Units	20		20		20	
# of Motor Units / Participant	4 ± 1.2		4 ± 1.0		4 ± 1.6	
Baseline MUFR (Hz)	10.3 ± 0.9		10.3 ± 1.3		10.2 ± 1.7	
MUFR at 25% of TTF	9.9 ± 0.9		9.5 ± 1.3		9.2 ± 1.6	
MUFR at 50% of TTF	9.9 ± 0.8		8.9 ± 1.1*		8.5 ± 1.5*	
MUFR at 75% of TTF	10.0 ± 0.8		8.5 ± 1.2*		7.7 ± 1.4*	
MUFR at 100% of TTF	10.2 ± 0.7		7.9 ± 1.2*		6.8 ± 1.1*†	

Table 5-2 Raw MVC and MUFR data, representing absolute values. Data are displayed as means ± SD. * denotes differences between BFO protocols and control. † denotes differences between BFO_{prox} and BFO_{dis} and control protocols.

5.4 Discussion

The current study sought to observe modulation of MUFRs, submaximal EMG, and strength output, following either occlusion of blood flow through external pressure of the femoral artery, or occlusion inferior to the TA at the ankle, compared with an unrestricted control protocol. Results indicated that low-intensity contractions in combination with BFO (proximal and distal) may alter neural strategies of rate coding and recruitment, with respect to decreased MUFRs (see Figure 5.3) and increased submaximal EMG (see Figure 5.2), compared to unrestricted blood flow contractions. As hypothesized, the resulting strength impairments following the control protocol caused the smallest decrease in isometric strength, while both the BFO_{dis} and BFO_{prox} protocols showed greater decreases, respectively. Despite the significantly shorter amount of TTF (340 ±

98s) compared to the time matched BFO_{dis} and control protocols (638 ± 182s), the BFO_{prox} protocol caused a significantly greater reduction in isometric force compared to control, with no other differences between the BFO protocols. For MUFRs, the BFO_{prox} caused greater reductions than BFO_{dis} at 100% TTF even though it was almost 48% shorter in duration, indicating that although both these protocols caused greater neural impairment compared with control, proximal occlusion has a greater effect than the distal. Nevertheless, these results provide support that occlusion can affect muscle function located upstream of the occlusion site.

The TA receives its arterial supply from the femoral artery, which becomes the popliteal artery in the popliteal fossa at the knee, and branches into the anterior tibial artery, posterior tibial artery, and the peroneal artery. Distally, the anterior tibial artery leaves the dorsiflexor compartment to become the dorsalis pedis artery of the foot. Occlusion at the distal thigh should impede flow from entering the muscle and therefore depriving the working muscle of oxygen, whereas impeding flow distal to the muscle should reduce arterial flow leaving the muscle as well as venous return from cutaneous veins of the lower part of the leg and foot. Together this distal occlusion would cause blood pooling and cell swelling of the dorsiflexor compartment. Previously observed increases in CSA in trunk musculature (pectoralis major) following bench press exercise with external restriction of blood flow at the upper arm[5] proposed that cell and muscle swelling and blood pooling[8] in a common arterial vasculature (axillary artery) may cause an anabolic response by activation of signaling pathways[9]. This proposed mechanism for an anabolic response in the muscle is likely a similar mechanism that would be observed in the current study.

During low-intensity (15% of MVC) isometric contractions the dorsiflexors without occlusion should not cause any impediment to blood flow over the ~11 minutes of contraction, verified by the presence of a pulse at the ankle during pilot testing throughout the protocol. Pulse pressure however was not present in the proximal and distal occluded protocols. As indicated in the introduction, several studies have shown the importance of blood flow on neuromuscular factors related to fatigability[5,6]. It has been previously observed that MUFRs at low-intensities without obstructed blood flow

will at first decrease for the first 10-20% of TTF before increasing[18]. A similar trend, although not statistically significant, was observed in the control protocol of the current study and this small initial decline may offset the short lasting muscle potentiation that might occur, while the subsequent increase in MUFR may compensate for fatigue[14,19,18]. However, when BFO is applied to a sustained low-intensity contraction, reductions in MUFRs are observed immediately (by at least 25% of TTF), indicating that any initial potentiation is not offsetting the immediate reduction in MUFRs. The greater reduction in BFO_{prox} of MUFRs indicates that impeded blood flow likely causes a greater amount of fatigue, confirmed by the resulting decrease in isometric strength which are greater for BFO_{prox} compared with control. Reductions in MUFRs of BFO_{dis} are also likely related to impediments in blood circulation. These results are consistent with other studies that have explored effects of fatigue and occlusion on changes in MUFRs[14,16].

Increases in submaximal surface EMG with low-intensity contractions have been previously observed with[7,4] and without blood flow impediments[20,12,21]. It has been proposed that the increase in EMG is due to facilitated activation of type II muscle fibers and their high-threshold motor units[7]. However, it has been previously observed that during low-intensity submaximal contractions to an endurance limit (failure) that submaximal EMG fails to reach maximal values[17]. This observation was also shown in the current study with both protocols that reached volitional failure (BFO_{dis} and BFO_{prox}) showing submaximal normalized EMG reaching values of only ~30% and ~48%, respectively. A study by Moritani and colleagues[11] observed that during arterial occlusion (200mmHg) at low-intensities (20% of MVC), RMS EMG amplitudes progressively increased, along with larger MU spike amplitudes. The authors suggested that this was due to an increase in activation of larger MUs due to a lack of oxygenated blood flow reaching the muscle, as well due to an increase in firing rates of MUs to compensate for a loss of overall force generating capacity of the muscle. However the results of the current study found that despite an increase in submaximal surface EMG there was a concomitant decline in firing rates indirectly indicating the surface EMG increase was due mainly to enhanced recruitment and not rate coding. Presumably due to BFO there was an increase in type II fiber recruitment, while motor unit firing rates of smaller and early recruited MUs showed a decrease due to metabolic stress placed upon type I fibers

that fatigue faster in the hypoxic environment than type II fibres[22]. A study by Vollestad and colleagues[23] found that during prolonged contraction of the vastus lateralis during exhaustive cycling and based on glycogen depletion, that type I and type IIa muscle fibers were activated from the beginning of exercise, while larger type IIb fibers were only recruited at near exhaustion. The current study may indicate that by depriving the working muscle of oxygen, these larger MUs are activated early in order to compensate for the loss of muscle force. The decline in firing rates with distal BFO is likely also related to similar fatigue of type I fibers observed with proximal BFO. The difference perhaps being due to continuation of inflow to the working muscle during BFO_{dis} that does not have the same immediate affect as BFO_{prox} and thus changes are muted or delayed in BFO_{dis} when compared with BFO_{prox} (see figure 5.3 and table 5-2). However the end result and mechanisms for changes in these factors compared with control responses are likely similar.

The novelty of the current study and the main finding was that blood flow occlusion distal to the TA causes substantial fatigue, assessed by decreases in motor unit firing rates and force that is greater than an intensity matched unrestricted control protocol, yet less effective than BFO proximal to the TA. This indicates the importance in fatigue of constant oxygenated blood flow in reaching the working muscle. Observing changes in motor unit firing rate behaviour, and overall muscle fatigue, greatly increases the application basis of blood restriction and occlusion training. Blood-flow restriction training when used chronically promotes a more aggressive anabolic response from the working muscle in order to overcome impairments the current study has observed in an acute setting. The greater activation of type II fibers promotes hypertrophy and strength adaptations that may be more beneficial for individuals or groups that are unable to perform traditional high-intensity exercise. Furthermore, these results provide support that occlusion distal to the working muscle may induce similar effects on muscles located proximal that share the same circulatory anatomy, but whose blood supply per se is not impeded.

5.5 References

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6 General discussion and summary

6.1 General Discussion

This thesis provides a novel approach to the impact of acute blood flow occlusion on the neuromuscular system, building upon previous literature that reports how restriction or occlusion of blood flow results in positive neuromuscular adaptations (strength and hypertrophy) with chronic training. Prior studies on the effects of blood flow restriction have provided some insights into neuromuscular activation through the examination of surface EMG[1,2,3,4], increases in CSA[2,5,6], and strength[1,2,4]. Studies in this thesis have expanded on those factors with models of acute fatiguing tasks.

Chapters 2, 3, and 5, all examine the effects of blood flow occlusion during low-intensity contractions in an acute setting compared to low-intensity contractions without blood flow obstructions. In order to explore the assumptions of some of the more foundational studies on chronic blood flow restriction in more detail, the experiments presented in this thesis approached the impact of blood flow occlusion in acute fatiguing tasks allowing for a variety of measures to be made. For example, the studies completed by Yasuda and colleagues[1,2] suggested increased recruitment in type II fibres due to chronic restriction training based on increases in submaximal RMS EMG presumably leading to the resulting increases in strength and hypertrophy due to type II fiber impairment and adaptation. The results of the experiments presented in this thesis are generally in agreement but factors have been more comprehensively explored using acute muscle fatigue with measures of both voluntary and tetanic stimulated contractions, isometric and dynamic contractions, corticospinal excitability, strength and power, and both surface and indwelling (MUFRs) EMG.

Low-intensity exercise that manipulates both training volume and intensity regimes have concluded that low-intensity exercise training at high volumes (number of sets x repetitions) provides sufficient stimulus to increase muscle volume that is equivalent to high-intensity exercise of similar volume[7]. These studies indicate that a low-intensity exercise with sufficient volume can be a relatively low-impact alternative (with regard to joint health and rehabilitation) to traditional high-intensity exercise for muscle growth.

The significant impact from the studies in this thesis relates to the idea that low-intensity exercise to failure with blood flow occlusion can be completed in less time or with fewer repetitions than an equivalent high-intensity exercise without occlusion. When extrapolated to longer term training studies using blood flow restriction or occlusion these results from acute interventions highlight the value of this training modality in enhancing factors that stimulate muscle growth and strength.

In relation to these measures, results presented in this thesis include greater low-frequency fatigue with blood flow occlusion during low-intensity sustained isometric contractions in less total time to failure compared to a time-matched low-intensity sustained contraction as reported in study 1. Study 2 provided further support that BFO impacts corticospinal excitability, specifically at the spinal motoneuron level with low-intensity blood flow occlusion contractions compared to an intensity matched control producing greater excitation of the motor pool at voluntary failure, and in ~80% less time. Study 3 showed that muscle power can be positively affected at low-intensities with blood flow occlusion and still provide sufficient impact to reduce velocity and strength, and therefore power. Chronic low-intensity exercise with blood flow obstruction has been previously observed to increase strength measures[1,2,4]. However, muscle power is a product of both strength (force production) and velocity. Traditional high-intensity exercise for power adaptations focuses on the increase of only one "pillar" of power which is strength. However, high-intensity exercise directly effects the speed or velocity in which movement can be performed. Therefore this mode of training may not be as beneficial as a training technique to enhance both "pillars" of power. The benefit of a low-intensity contraction is that it can be performed at a greater velocity than a high-intensity contractions. By comparing the effect of a low-intensity dynamic elbow flexion (biceps curl) exercise set vs. a high-intensity blood free flow set, study 3 supports the findings of Mitchell and colleagues[7], indicating that a low-intensity alternative is a viable method to provide greater fatigue, with less work performed (~50% less work total, and ~65% less work/contraction). Study 4 provided evidence to support and better define neural activation changes found with surface EMG in previous studies. The decline in MUFRs with circulatory impediments whether proximal or distal were similar and support that increases in surface EMG likely reflect greater recruitment of presumably

larger type II motor units. The result that distal occlusion produced findings similar to proximal occlusion supported prior speculations that muscles upstream of the occlusion site can be affected to cause positive muscle adaptations when used for chronic training.

The greater benefit to these results, is that it may provide a viable low-impact alternative to high-intensity power training in aging adults or rehabilitation settings. With adult aging muscle size is reduced and results in the functional loss of muscle contractile capacity, referred to as sarcopenia[8]. However loss of muscle strength with aging is only one "pillar" of the more functional measure of power, which is a better determinant of quality of life[9]. Due to morphological changes with muscle during aging in addition to loss of strength there is loss of contractile speed or velocity. Results here support that the application of blood flow obstructed exercise when used chronically may provide sufficient stimulus to adequately improve or minimize loss of muscle power with aging, thus increasing or prolonging positive quality of life in old age. When acute injury occurs, strength deficits and muscle atrophy can prolong the rehabilitation process. By using low-intensity exercise in combination with blood flow obstruction, therapists using this modality may be able to prevent some of the muscle atrophy and help improve recovery of strength and power.

The physiological factors that were examined throughout these experiments provide greater insights into the acute effects of blood flow obstruction on several neuromuscular factors related to exercise and that may help direct or inform further studies related to chronic use of blood flow restriction exercise The more extreme occlusion model used here in acute situations presumably confers maximum stress on the system to highlight the relative importance of various factors worthwhile to explore further.

6.2 Limitations

The main limitation of the studies presented in this thesis is that there was no quantitative measure of blood flow obstruction. The majority of literature presented to date used a level of blood flow restriction that is realistic for chronic use. For chronic use, an external

pressure provided by either elastic bands or a blood pressure cuff is restricted to varying low levels 0 – 100mmHg[4] of restriction of systolic blood pressure[3], or moderate blood flow restriction of 160 - 230mmHg[1,5,6,10]. Whereas in this thesis complete occlusion at 250mmHg or more was used in an acute task[1,11]. These varying levels of restriction provide differing insights into the effects of blood flow obstruction. For the studies outlined in this thesis a quantitative value of obstruction could have been obtained using a Doppler ultrasound system. However, the limited surface area of the muscles investigated (arm and leg) prevented the use of such a system. Therefore, in order to ensure that a constant level of restriction was applied throughout the protocols and between participants, a high level of external pressure (250mmHg or 300mmHg) was selected. Although this level of external restriction was considered extreme, it allowed the study of acute single bouts of exercise to be examined to assess relative contribution of some key factors.

The use of the Borg scale for rating perceived exertion (RPE) may have been a useful tool to have added into these studies The application of external pressure to restrict or occlude blood flow causes an unpleasant sensation for most people that although may not be described as “painful”, certainly adds a level of discomfort. For example, a study completed by Martín-Hernández and colleagues[12] found over six consecutive training session of either a low-intensity (20% of MVC) BFR group or a high-intensity (85% of MVC) group, that RPE was reduced following each consecutive session. These results indicate that with practice and familiarization RPE and pain do not limit highly motivated individuals when BFR is applied. When muscle contractions are sustained until exhaustion (failure) regardless of the application of BFR or BFO, individuals certainly experience greater RPE and pain as they approach failure[12]. All individuals tested throughout the thesis experiments had previously participated in experimentation involving blood flow obstruction to some degree, and were therefore experienced with the perceived “pain” involved. Thus, it is likely that termination of protocols at failure was due to muscle fatigue, rather than to pain. However, it would have been helpful to have attempted to assess those factors objectively.

Ideally, experimentation involving reductions in muscle strength (fatigue), should include some assessment of muscle activation such as the interpolated twitch technique (ITT).

Voluntary activation can be variable[13], however it does provide some objective assessment of motivation and muscle activation. Although the ITT was used in studies 1 and 3, it was not used in studies 2 and 4. For study 2 the use of MVCs, in which ITT would have been applied, may have affected the results of corticospinal excitability as greater intensities[14] of contractions will cause differing results than low-intensity contractions. However, for experiment 4 it may have provided a better insight into the fatigue experienced during the sustained isometric contraction that resulted in the reduction of MUFRs, but was challenging to apply with the setup available and the indwelling wire recordings.

6.3 Future Directions

Although the main body of literature surrounding this topic has been based upon chronic adaptations observed following weeks or months of training, those experiments have provided limited insight into relevant factors involved. The mechanisms that influence these neuromuscular adaptations are still not well understood and warrant further investigation. Perhaps greater focus should be placed upon mechanisms, such as the signaling pathways that are activated within a working muscle that is deprived of readily available oxygen, and how these signaling pathways promote muscle growth in relation to varying degrees of BFR. Also, it would be important to understand the metabolic stress that is placed upon working muscle fibers from being in a low oxygen environment. To understand the interaction with blood flow restriction or occlusion, and the vasculature upon which an added stress is artificially produced, requires the examination of how each part of the muscle, nervous system, vasculature, and cardio-respiratory complex is affected by obstructed blood flow.

Exploration into the benefits that can be provided for populations that makes traditional high-intensity exercise impractical should be undertaken. For example an aged population may find it difficult or unsafe to provide enough stimuli through traditional high-intensity exercise to effect positive changes. The effects of osteoporosis or a life time of joint stress may make the impact of high-intensity training for muscle power and growth unsafe. Although high degrees of external pressure to obstruct flow may not be warranted for populations that are more likely to have vascular disease (such as arteriosclerosis), but lower levels of restriction may be safely beneficial. Perhaps the

increase in arterial pressure that accompanies blood flow obstructed exercise may help reverse the effects of vascular disease such as arteriosclerosis by increasing the elastic properties that have been lost with aging. Investigating the impacts of blood flow obstructed low-intensity exercise in an aging population would be a worthwhile study to follow up the results found in the studies of this thesis with a young active population. For example, exercise has positive influences on bone remodeling and prevention of osteoporosis[15]. However, the question to investigate would be whether low-intensity exercise in combination with blood flow obstruction, provides enough stimulus for bone remodeling to occur. Along with bone remodeling, exercise strengthens the musculotendinous properties to prevent injury.

Another population that may benefit from low-intensity blood flow obstructed exercise, would be individuals that have acute injuries and are seeking rehabilitation. For example, individuals with broken limbs or joint ligament injuries will initially have muscle atrophy, which makes recovery a prolonged process. The use of a low-intensity intervention with blood flow restriction may provide not only physiological benefits in regards to neuromuscular recovery, but also psychological benefits. Thus, more controlled longer term studies in special populations are important.

Although the experiments presented in this thesis have explored the effects of blood flow occlusion and fatigue. The muscles examined in these experiments (arm flexors and TA) have a relative 50-50% morphology of type I and type II muscle fibers. However, an investigation that explored the effects of blood flow obstruction on a predominantly type I fiber muscle may provide further insights into the importance of proper blood flow to working muscle. For example the soleus has a much larger type I fiber morphology (~80% type I). The experiments presented in this thesis propose that type I fiber fatigue is exacerbated with obstructed blood flow, therefore one could hypothesize that fatigue would be greater in a muscle that predominantly relies on readily available oxygen to produce ATP and thus sustained muscle contraction.

6.4 Summary

The results of the four studies presented in this thesis indicate that when blood flow occlusion is combined with low-intensity exercise as a training technique it may provide a stimulus that is similar, or more beneficial than traditional training techniques without blood flow obstruction. For example the time required to reach muscle fatigue is greatly reduced with BFO with less work required, however this reduction in time and work does not negatively affect muscle adaptations such as strength, power, and hypertrophy. Although the studies explored acute factors related to blood flow occlusion, the results can be extrapolated cautiously to chronic training, or will help direct future investigations based on factors identified from acute fatiguing tasks. Because high-intensity training can be for some populations impractical, or may be harmful, the exploration of an alternative low-intensity method that provides beneficial results is an important research area.

6.5 References

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vascular occlusion or hypoxia on neuromuscular function in athletes. Eur J Applied Physiol. **113**(7): 1767-1774. (2013b).

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Appendices

Appendix A

Western Research

Date: 22 December 2017

To: Charles Rice

Project ID: 107212

Study Title: The effect of chronic low-frequency electrical stimulation on neuromuscular properties in the human muscles

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

Full Board Reporting Date: January 9 2018

Date Approval Issued: 22/Dec/2017

REB Approval Expiry Date: 21/Dec/2018

Dear Charles Rice ,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Kelly Patterson, Ethics Officer, on Behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix B

Western Research

Date: 25 July 2018

To: Charles Rice

Project ID: 107212

Study Title: The effect of chronic low-frequency electrical stimulation on neuromuscular properties in the human muscles

Application Type: HSREB Amendment Form

Review Type: Delegated

Full Board Reporting Date: August 7, 2018

Date Approval Issued: 25/Jul/2018

REB Approval Expiry Date: 21/Dec/2018

Dear Charles Rice ,

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
Chronic Electrical Stimulation Study - version 4.0 - tsDCS (1) - clean version	Consent Form	06/Jul/2018	4.0
Chronic Electrical Stimulation Study - version 4.0 - tsDCS (1) - clean version	Protocol	06/Jul/2018	4.0

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

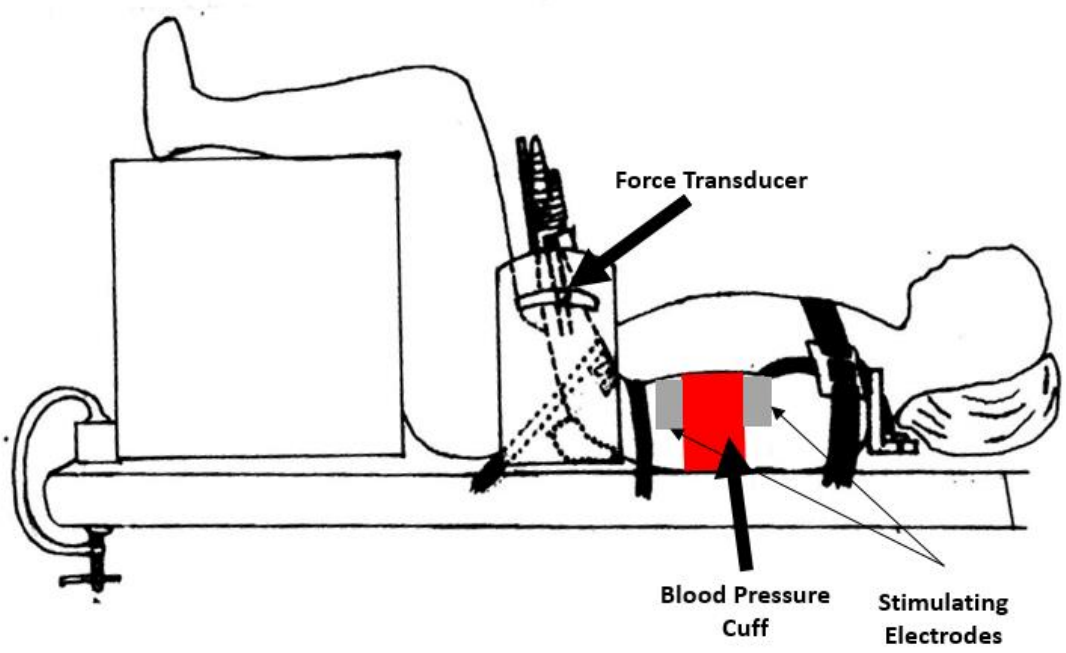
Please do not hesitate to contact us if you have any questions.

Sincerely,

Karen Gopaul, Ethics Officer on behalf of Dr. Joseph Gilbert, HSREB Chair

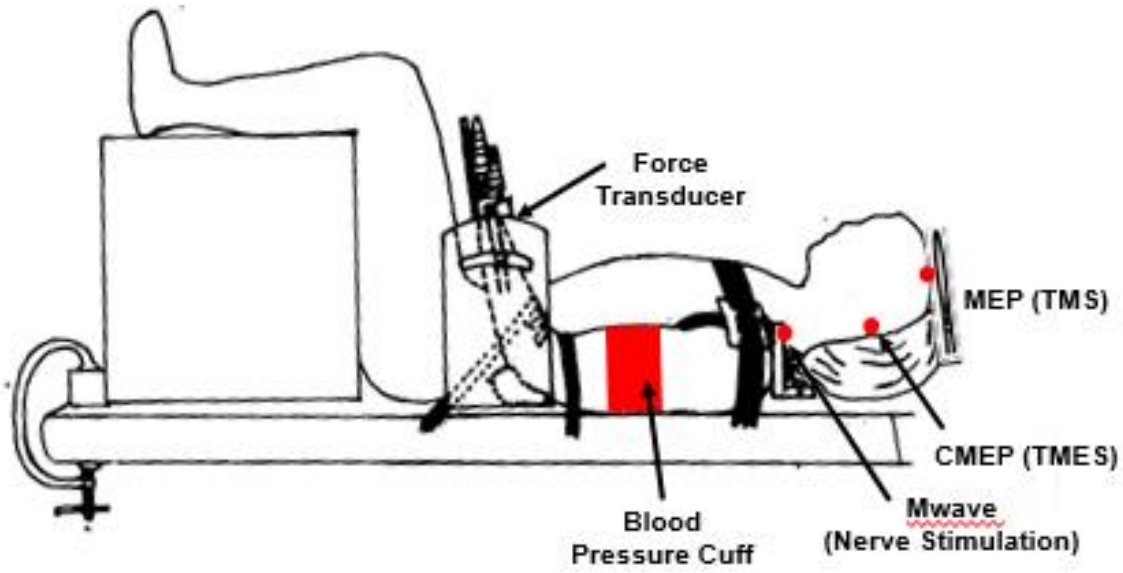
Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix C



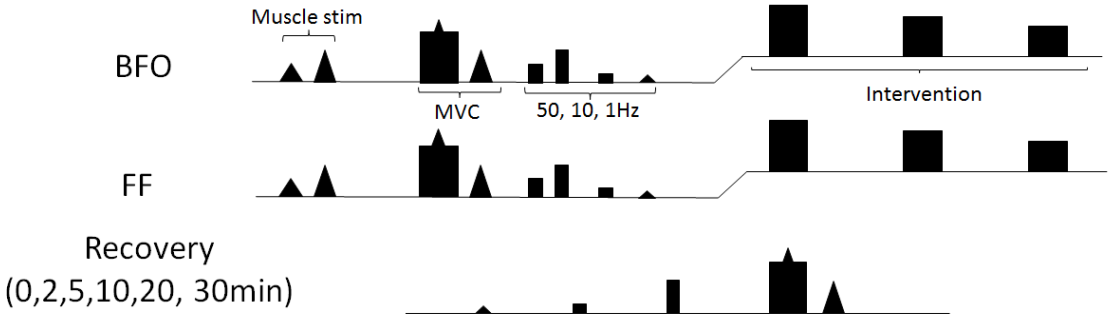
Experimental setup for experiment 1 (Chapter 2)

Appendix D

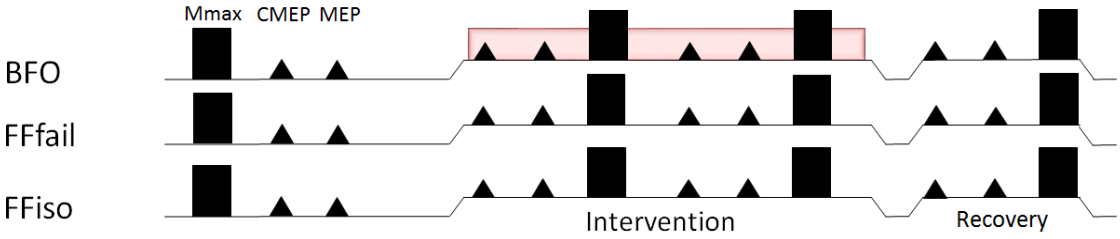


Experimental setup for experiment 2 (Chapter 3)

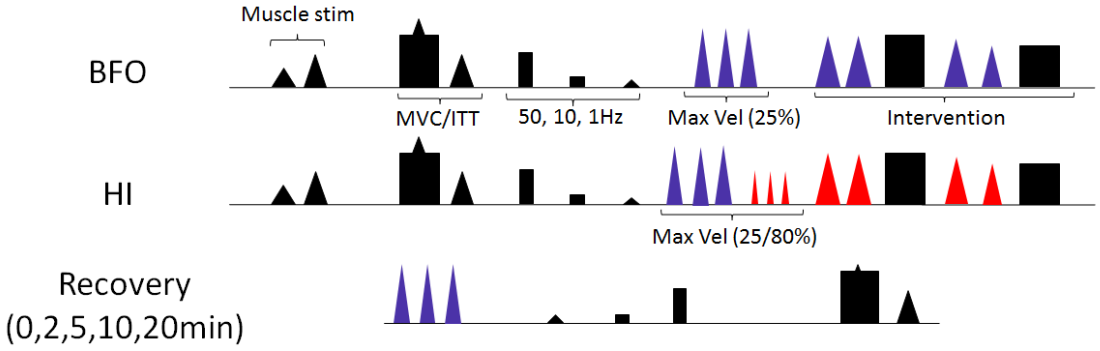
Appendix E


Muscle stim
BFO
MVC
50, 10, 1Hz
Intervention
FF
Recovery
(0,2,5,10,20, 30min)

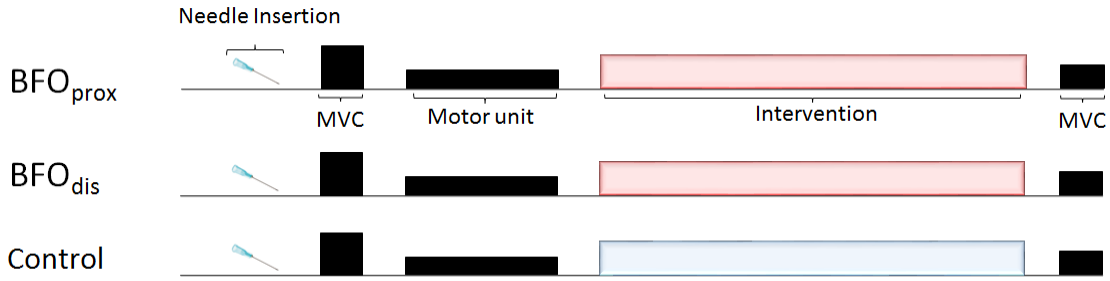
Appendix F



Appendix G


Muscle stim
BFO
MVC/ITT
50, 10, 1Hz
Max Vel (25%)
Intervention
HI
Max Vel (25/80%)
Recovery
(0,2,5,10,20min)

Appendix H


Needle Insertion
BFO prox
BFO dis
Control
MVC
Motor unit
Intervention
MVC

Curriculum Vitae

David Brian Copithorne

Education

Doctorate, Ph.D. Kinesiology, Physiology of Exercise, Neuromuscular Physiology, University of Western Ontario (present)

Research Disciplines: Kinesiology
Supervisors: Dr. Charles Rice

Master's Thesis, Masters of Science, Work and Exercise Physiology, Kinesiology, Memorial University of Newfoundland (2012/9 - 2014/4)

Thesis Title: Premovement excitability changes of the corticospinal tract are not dependent on the forthcoming task but due to a general excitation of the motor system

Areas of Research: Plasticity / Neuronal Regeneration
Research Disciplines: Kinesiology
Supervisors: Dr. Kevin Power

Bachelor's Honours, Health Science, Kinesiology, University of Ontario Institute of Technology (2008/9 - 2012/4)

Recognitions

2016/10 Graduate Student Award Poster Competition - PhD level - 300 (Canadian dollar)
CSEP
Prize / Award
PhD level poster award

Research Interests:

Motor unit changes following chronic stimulation, at the level of the motoneuron and muscle fiber. Central nervous system (CNS) modulation due to aging, exercise, and artificial stimulation. The acute effects of blood flow restricted exercise at low-intensities, on fatigue the corticospinal modulation

Affiliations

CSEP CEP/CPT IE, CSEP
CSEP certified CEP and CPT instructor/examiner (2014/4)

Research Funding History

2015/5 - 2018/4
Ontario Graduate Scholarship recipient
Funding Sources: OGS
Total Funding - 15,000/year (Canadian dollar)

Teaching Experience

2015/01/01 - 2015/04/30
Teaching Assistant, University of Western Ontario
Course Title: Introduction to Kinesiology
Course Level: Undergraduate
Number of Students: 600
Lecture Hours Per Week: 3
Tutorial Hours Per Week: 1

2014/09/01 - 2014/12/23
Teaching Assistant, University of Western Ontario
Course Title: Exercise Physiology: Muscle Function and Metabolism
Course Level: Graduate and Undergraduate
Number of Students: 100
Number of Credits: 1
Lecture Hours Per Week: 3
Guest Lecture: Yes

2014/01/01 - 2014/04/30
Course Instructor, Memorial University of Newfoundland
Course Title: Human Anatomy Online

2013/09/01 - 2013/12/23
Course Instructor, Memorial University of Newfoundland
Course Title: Human Anatomy
Course Level: Undergraduate

2013/05/01 - 2013/08/30
Teaching Assistant, Memorial University of Newfoundland
Course Title: Fitness Leadership
Number of Students: 100
Lecture Hours Per Week: 2
Lab Hours Per Week: 1

2013/01/01 - 2013/04/30
Teaching Assistant, Memorial University of Newfoundland
Course Title: Advanced exercise physiology

Course Level: Undergraduate
Number of Students: 200
Lecture Hours Per Week: 3
Guest Lecture: Yes

2012/09/01 - 2012/12/20
Teaching Assistant, Memorial University of Newfoundland
Course Title: Motor Learning
Course Level: Undergraduate
Number of Students: 200
Number of Credits: 1
Lecture Hours Per Week: 3

2012/01/01 - 2012/04/30
Teaching Assistant, University of Ontario Institute of Technology
Course Title: Exercise Physiology
Course Level: Undergraduate
Number of Students: 50
Number of Credits: 1
Lecture Hours Per Week: 3
Lab Hours Per Week: 2

2011/09/01 - 2011/12/20
Teaching Assistant, Health Sciences, University of Ontario Institute of Technology
Course Title: Principals of Fitness & Exercise
Course Level: Undergraduate
Number of Students: 50
Number of Credits: 1
Lecture Hours Per Week: 2
Lab Hours Per Week: 2

Course Development

2013/9 Course Instructor/Website Operator, Human Kinetics and Recreation, Memorial University of Newfoundland (2013/9)
Course Title: HKR 2310 Human Anatomy
Course Level: Undergraduate

Presentations

1. (2017). The Acute Effects of Blood Flow Restricted Exercise on Elbow Flexor Function. ENG at Guelph University, Guelph, Canada
Main Audience: Researcher

2. (2016). Effect of Blood Flow Restriction on Biceps Brachii Function and Corticospinal Excitability. UOIT seminar lecture, Oshawa, Canada

Main Audience: Researcher
Invited: Yes

3. (2015). Muscular Training Principles and Adaptations. Advanced Exercise Physiology Course, Oshawa, Canada
Main Audience: Knowledge User
Invited: Yes

4. (2015). The Effect of Chronic Low-Frequency Electrical Stimulation on Neuromuscular Properties in Human Muscle. ENG at Brock University, St. Catharines, Canada
Main Audience: Researcher

5. (2014). Techniques Used to Test Supraspinal and Spinal Excitability. Advanced Exercise Physiology Course, St. John's, Canada
Main Audience: Knowledge User
Invited: Yes

6. (2014). Techniques Used to Test Supraspinal and Spinal Excitability. Exercise Physiology Course, London, Canada
Main Audience: Knowledge User
Invited: Yes

7. (2013). Renal Responses to Exercise and Training. Advanced Physiology Course, St. John's, Canada
Main Audience: Knowledge User
Invited: Yes

8. (2013). Increased corticospinal excitability prior to arm cycling is due to enhanced cortical but not spinal motoneurone excitability. Memorial University Seminar Lecture, St. John's, Canada
Main Audience: Researcher
Invited: Yes

9. (2013). Gastrointestinal Responses to Exercise and Training. Advanced Physiology Course, St. John's, Canada
Main Audience: Knowledge User
Invited: Yes

10. (2013). The effect of repeated submaximal contractions on central and perihperal excitability in the biceps brachii. ENG at UOIT, Oshawa, Canada
Main Audience: Researcher

11. (2012). Corticospinal Excitability is Enhanced Prior to Rhythmic Upper-Body Movement in Humans. UOIT Research Days, CanadaMain Audience: Researcher

Publications

Journal Articles

1. Copithorne DB, Rice CL. The effect of blood flow restriction during acute low-intensity isometric elbow flexion exercise. Euro J Applied Physiol. **119**(3): 587-595. (2019).

2. Hodgson MD, Keir DA, Copithorne DB, Rice CL, Kowalchuk JM. Power reserve following ramp-incremental cycling to exhaustion: implications for muscle fatigue and function. J Appl Physiol. **125**(2): 304-312. (2018).

3. Keir DA, Copithorne DB, Hodgson MD, Pogliaghi S, Rice CL, Kowalchuk JM. The slow component of pulmonary O2 uptake accompanies peripheral muscle fatigue during high-intensity exercise. J Appl Physiol. **121**(2): 493-502. (2016).

4. Kirk EA, Copithorne DB, Dalton BH, Rice CL. Motor unit firing rates of the gastrocnemii during maximal and sub-maximal isometric contractions in young and old men. Neurosci. **330**: 376-385. (2016).

5. Cowling BL, Harwood B, Copithorne DB, Rice CL. Rate modulation of human anconeus motor units during high-intensity dynamic elbow extensions. Journal of Applied Physiology. **121**(2): 475-482. (2016).

6. Aboodarda SJ, Copithorne DB, Pearcey GE, Button DC, Power KE. Changes in supraspinal and spinal excitability of the biceps brachii following brief, non-fatiguing submaximal contractions of the elbow flexors in resistance-trained males. Neurosci Lett. **607**: 66-71. (2015).

7. Copithorne DB, Forman DA, Power KE. Premovement Changes in Corticospinal Excitability of the Biceps Brachii are not Different Between Arm Cycling and an Intensity-Matched Tonic Contraction. Motor Control. **19**(3): 223-241. (2015).

8. Aboodarda SJ, Copithorne DB, Power KE, Drinkwater E, Behm DG. Elbow flexor fatigue modulates central excitability of the knee extensors. Appl Physiol Nutr Metab. **40**(9): 924-930. (2015).

9. Halperin I, Copithorne DB, Behm DG. Unilateral isometric muscle fatigue decreases force production and activation of contralateral knee extensors but not elbow flexors. Appl Physiol Nutr Metab. **39**(12): 1338-1344. (2014).

10. Power KE, Copithorne DB. Increased corticospinal excitability prior to arm cycling is due to enhanced supraspinal but not spinal motoneurone excitability. Appl Physiol Nutr Metab. **38**(11): 1154-1161. (2013).

Translations

1. Increased corticospinal excitability prior to arm cycling is due to enhanced supraspinal but not spinal motoneurone excitability. Applied Physiology, Nutrition, and Metabolism. **38**(11): 1154-1161.

Conference Publications

1. Copithorne, D.B., & Rice, C.L. (2018). Power and velocity changes during low-intensity blood flow restricted elbow flexion contractions compared to high-intensity free flow contraction. Applied Physiology, Nutrition, and Metablosim. CSEP, ,Canada (S52). Preceedings of the CSEP annual general meeting.

Conference Date; 2018/10
Abstract First Listed Author

2. Copithorne, D.B., Stolworthy, C.S., McNeil, C.J., & Rice, C.L.Corticospinal excitability during sustained elbow flexion contractions with and without blood flow restriction. Applied Physiology, Nutrition and Metabolism. CSEP, Victoria, Canada (S349). Preceedings of the CSEP annual general meeting.

Conference Date: 2016/10
Abstract First Listed Author

3. Halperin, I., Copithorne, D.B., & Behm, D.G. (2014). Unilateral isometric muscle fatigue decreases force production andactivation of contralateral knee extensors but not elbow flexors. Applied Physiology, Nutrition and Metabolism. CSEP, St John's, Canada (S20). Preceedings of the CSEP annual general meeting.

Conference Date: 2014/10
Abstract Co-Author

4. Copithorne, D.B., Forman, D.A, & Power, K.E. (2014). Premovement changes in corticospinal excitability of the biceps brachii are not different between arm cycling and an intensity-matched tonic contraction. Applied Physiology, Nutrition and Metabolism. CSEP, St John's, Canada (S11). Preceedings of the CSEP annual general meeting.

Conference Date: 2014/10
Abstract First Listed Author

5. Monks, A.M. Andersen, O.R.A. Drodge, Manning, O.J., Forman, D., Copithorne, D.B., Button, D.C., & Power, K.E. (2014). Modulation of supraspinal and spinal excitability following an upper-body Wingate Test. Applied Physiology, Nutrition and Metabolism. CSEP, St. John's, Canada (S33). Preceedings of the CSEP annual general meeting.

Conference Date: 2014/10
Abstract Co-Author

6. Copithorne, D.B., Pearcey, G.E.P., Aboodarda, S.J., Button, D.C., & Power, K.E. (2013). The effect of repeated submaximal contractions on central and peripheral

excitability in the biceps brachii. Applied Physiology, Nutrition and Metabolism. CSEP, Toronto, Canada (1033). Preceedings of the CSEP annual general meeting.
Conference Date: 2013/10
Abstract Co-Author