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Adequacy of muscle blood flow during handgrip exercise

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A Dissertation

Entitled

Adequacy of Muscle Blood Flow During Handgrip Exercise

By

Allison J. Harper

Submitted as partial fulfillment of the requirements for

The Doctor of Philosophy degree in

Exercise Science

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An Abstract of

Adequacy of Muscle Blood Flow During Handgrip Exercise

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The purpose of this dissertation was to determine if limb blood flow was
sufficient for the performance of handgrip exercise utilizing a variety of muscle
contraction patterns, exercise intensities, and exercise durations. Subjects first performed
heavy intensity intermittent isometric and dynamic handgrip exercise to examine the
effect of muscle contraction pattern on mean blood flow and exercise tolerance. Brachial
artery mean blood flow was measured throughout exercise using Doppler ultrasound.
Electromyography (EMG) of the forearm muscles was also measured, and the time-
tension integral was calculated to assure a similar work rate between protocols. The time
to task failure was significantly greater in intermittent isometric than dynamic exercise.
However, there were no differences in mean blood flow following the first minute of
exercise or in the normalized iEMG/time-tension integral ratio. These results indicate that
motor unit recruitment patterns, mean blood flow and presumably oxygen delivery to the

working muscles were similar between conditions, and therefore may not be a primary determinant of exercise tolerance during handgrip exercise.

Compared to the mean blood flow response during dynamic handgrip exercise, there is a transient overshoot in muscle blood flow early in recovery which has been used as evidence to suggest that blood flow is inadequate for the metabolic requirements of the exercise. If muscle blood flow is limited during handgrip exercise, one might reason that progressive increases in exercise duration would lead to greater increases in post-exercise blood flow, consistent with the results of post-occlusive studies. In a subsequent study, subjects performed four bouts each of moderate and heavy intensity dynamic handgrip exercise to examine the effect of progressively increasing exercise duration on exercise and recovery blood flow responses. Brachial artery blood flow was measured during exercise and 5 min of recovery using Doppler ultrasound. Peak blood flow was calculated for both end-exercise and early recovery periods. The area under the recovery blood flow curve (5 min) was determined and used as an index of total post-exercise hyperemia. Within each exercise intensity, peak blood flow during exercise exceeded peak recovery blood flow but was independent of exercise duration. Similarly, the area under the recovery curve was greater for heavy than moderate exercise but was not associated with exercise duration. These observations suggest that the transient overshoot in blood flow during early recovery does not reflect a blood flow limitation during exercise. Therefore, neither protocol provided evidence that brachial artery blood flow was inadequate to meet metabolic requirements.

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Chapter One

Introduction

Background and Significance

Blood flow in humans is regulated through a combination of central and peripheral mechanisms. During exercise these systems are challenged to support the significantly increased metabolic rate and oxygen demand of working muscles (Busso & Chatagnon, 2006; Schwarz *et al.*, 2006). Central hormonal and neural factors control vascular resistance at a systemic level such that mean arterial pressure can be tightly regulated even as cardiac output increases dramatically (Delp & Laughlin, 1998; Fadel, 2008; Raven, 2008). Due in part to these limitations, peripheral feedback allows relative distribution of blood flow to be altered during exercise such that a greater amount is delivered to working muscles. Local control mechanisms including metabolic, endothelium-mediated, and myogenic responses act to adjust the balance between vasoconstriction and vasodilation in each vascular bed such that blood, and thereby oxygen, is directed toward organs which need it most (Delp & Laughlin, 1998; Saltin *et al.*, 1998).

During exercise, blood flow can be occluded during muscle contractions due to increases in intramuscular pressure. Therefore, the majority of blood flow through working muscles occurs during periods of relaxation (Hoelting *et al.*, 2001; Walloe &

Wesche, 1988; Sadamoto *et al.*, 1983). With some contraction patterns these periods of relaxation may be insufficient to allow antegrade flow of all blood impeded during the contraction phase, particularly during isometric exercise in which there is no relaxation period at all. If this is the case, inadequate perfusion may play a significant role in muscle fatigue and low exercise tolerance.

In addition to direct measurement of blood flow during exercise, examination of post-exercise hyperemia occurring in the early recovery period may provide additional evidence regarding metabolic demands of the working muscle. A period of reactive hyperemia occurs following cuff-occlusion procedures which mechanically impede arterial inflow. The magnitude of this hyperemia has been shown to increase with progressively increasing occlusion duration, in effect providing a non-invasive indicator of insufficient blood flow (Leeson *et al.*, 1997; Mullen *et al.*, 2001; Toth *et al.*, 2007). Similarly, peak and total post-exercise hyperemia following heavy intensity isometric exercise reflects the degree of oxygen debt, which can be manipulated by increasing exercise intensity or duration, or by applying additional external occlusion (Osada *et al.*, 2003). Therefore, if muscle blood flow is limited during dynamic handgrip exercise, progressive increases in exercise duration should result in an increase in post-exercise hyperemia.

Inadequate blood flow and oxygen delivery could lead to decreased peak performance, low exercise tolerance, and even difficulty performing activities necessary for independent daily living in older or diseased populations. While much attention has been focused on the role of perfusion limitation during the initial transition to a higher work rate (Hughson *et al.*, 2001; Grassi, 2001), the potential implications of a cumulative

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oxygen debt throughout a bout of exercise must also be considered. Restriction of blood

flow by muscle contractions throughout a bout of exercise could be a significant predictor

of the onset of muscle fatigue and a prolonged recovery period. Therefore, further

investigation into the role of muscle blood flow in determining exercise tolerance and

post-exercise hyperemia is necessary.

Specific Aims

Muscle blood flow has been shown to vary depending on external work

performed, muscle contraction frequency and duty cycle, and metabolic rate among other

influences. Because blood flow is impeded during contraction of the surrounding

musculature, total perfusion is dependent upon the degree of impairment in the

contraction phase and the surge of flow during the relaxation phase (Kagaya & Ogita,

1992). While it is commonly believed that available blood flow is adequate to sustain

light to moderate intensity muscle contractions, evidence has been presented suggesting a

perfusion limitation during heavy intensity contractions (MacDonald *et al.*, 2001; Walloe

& Wesche, 1988; Wesche, 1986). Therefore, the purpose of this dissertation was to

determine if limb blood flow was sufficient for the performance of handgrip exercise

utilizing a variety of muscle contraction patterns, exercise intensities, and exercise

durations. To this end, this dissertation consists of two studies, each addressing a portion

of the general purpose. The first was designed to determine if differences in muscle blood

flow produced by distinct contraction patterns cause differences in exercise tolerance. In

the second study, we assessed the post-exercise hyperemic response for evidence

increasing exercise duration led to an accumulating oxygen debt, which must be repaid

following cessation of exercise. Details of the aims of each of these studies are as follows:

1. **To test the hypothesis that two different muscle contraction patterns produce different mean blood flow responses at the same work load, and that greater mean blood flow is associated with greater exercise tolerance.** This may provide information regarding one cause of muscle fatigue, and lead to further understanding of methods by which to improve exercise tolerance.

2. **To test the hypothesis that post-exercise hyperemia will incrementally increase as exercise duration increases.** As a progressive increase in post-exercise hyperemia with exercise duration would be indicative of inadequate blood flow during exercise, this will lend insight into the sufficiency of blood flow during dynamic handgrip exercise of moderate and heavy intensity.

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Chapter Two

Literature Review

Introduction

It has been recognized for some time that all human activity extending beyond a very brief period of time requires the presence of oxygen so energy can be produced through oxidative phosphorylation (Hughson *et al.*, 2001). Insufficient oxygen availability for an extended duration can lead to ischemia, fatigue, angina, and more serious cardiovascular and cerebral incidents. Since metabolic rate is rarely constant, the body's energy demand fluctuates greatly throughout many shifts in activity level each day. The body must then respond by adjusting oxygen delivery and utilization to meet these changing demands.

Control of blood flow and its adjustment between steady state levels is crucial to allow for adequate delivery of oxygen to tissues throughout the body. This is particularly important when stresses such as exercise require an increase in oxygen delivery and consumption. The pulmonary, cardiovascular, and musculoskeletal systems must work in concert to achieve maximal performance (Poole & Richardson, 1997; Ameredes *et al.*, 1998; Honig *et al.*, 1992). Recent evidence suggests blood flow and oxygen delivery to be a limiting factor during intense whole body exercise, but this may not be the case for exercise in a small muscle group (Levine, 2008; Poole & Richardson, 1997).

Doppler Ultrasound Methodology

Unlike other methods of determining blood flow which are invasive or provide only mean blood flow measurements, Doppler ultrasound makes it possible to gather blood flow values instantaneously and non-invasively (Gill, 1985; Burns, 1987). The use of this technique to measure blood flow relies on a phenomenon known as the Doppler effect, in which relative motion between the transmitter and receiver of sound waves causes the wave to be returned at a different frequency, called a Doppler shift. When operating in pulsed mode, the operator can control the depth of the tissue from which motion is being detected. When ultrasound waves are emitted by a transducer placed above a major artery, the red blood cells flowing through that artery act as receivers, and the waves are returned to the original source at a different frequency. A demodulator, using phase quadrature detection, then takes the incoming ultrasound waves and converts their frequencies into a Doppler shift signal (Burns, 1987). This signal can be broadcast through a speaker, and recorded by the system for conversion into blood velocity.

To calculate blood flow, it is also necessary to determine the cross-sectional area (CSA) of the artery in which blood velocity is being measured. This can be done using duplex Doppler scanning, with a combination of real-time imaging and pulsed Doppler. Duplex Doppler images can be collected and saved for off-line analysis, during which time the diameter of the artery is measured and used to calculate the CSA (Burns, 1987). It is not generally feasible to collect CSA and blood velocity measurements simultaneously during exercise, which would allow for the most accurate determination of blood flow. Several studies have suggested that while the cardiac cycle, body position, and hypoxia contribute to changes in arterial diameter, exercise per se does not have a

significant effect at the level of conduit arteries (Hoelting *et al.*, 2001; MacDonald *et al.*, 1998; Radegran, 1997). This allows investigators to determine CSA while the subject is at rest and vessel walls can be seen clearly.

Doppler ultrasound estimates of limb blood flow are similar to those of previously common invasive techniques such as thermodilution. The two are shown to have a linear relationship with a slope of 0.985 and intercept of 0.071, with similar coefficients of variation of approximately 6% (Radegran, 1997). Hoelting and colleagues have also used thermodilution to verify Doppler blood flow measurement during knee extension exercise (Hoelting *et al.*, 2001). Therefore, this non-invasive technique produces reliable results with minimal risk to the subject.

Adaptation of Blood Flow During Exercise

Because blood flow, even under resting conditions, oscillates in rhythm with the cardiac cycle, measurement of mean blood flow over periods of several seconds obscures the true dynamic flow patterns. Although increases in net or average blood flow with exercise can be discerned in this manner, the true response following the onset of exercise can most accurately be seen noninvasively in humans with beat-by-beat blood flow monitoring using a system such as Doppler ultrasound (Walloe & Wesche, 1988). With the use of Doppler ultrasound, it has become apparent that blood flow oscillations increase greatly with exercise, and that this increase occurs following the very first contraction in rhythmic exercise (Walloe & Wesche, 1988; Tschakovsky *et al.*, 1996; Radegran & Saltin, 1999). The rapid increase in blood flow immediately following the onset of exercise (phase 1) arises from a combination of the muscle pump and rapid

vasodilation (Shoemaker & Hughson, 1999; Saunders & Tschakovsky, 2004; Laughlin, 1987).

The muscle pump effect is thought to result from pressure changes within the intramuscular vasculature as it is alternately compressed and reopened with each muscle contraction. Early evidence suggested that rhythmic contractions could double blood flow in a maximally vasodilated human forearm (Patterson & Shepherd, 1954). More recent research shows that muscle contraction does not further enhance blood flow during conditions in which the vessels have been maximally dilated through pharmacological intervention (Hamann *et al.*, 2003; Dobson & Gladden, 2003). These studies, however, involved isolated muscle preparations and instrumented canine hindlimbs, respectively. Because a number of factors including a sufficient hydrostatic column in the venous circulation appear to be necessary for the muscle pump to be effective, data from such experiments may not be directly applicable to the response during upright exercise in humans (Laughlin, 1988; Laughlin & Joyner, 2003; Laughlin & Schrage, 1999; Shoemaker & Hughson, 1999). Most recently the relative contribution of rhythmic contractions to assist or impede muscle blood flow during single leg knee extension exercise was reported (Lutjemeier *et al.*, 2005). This data suggests a significant effect of the muscle pump at the lightest work rate, no net effect for moderate work rates, and a net impedance effect at higher work rates which required an elevation in mean arterial blood pressure in order to preserve muscle blood flow. It also highlights the importance of considering characteristics other than mean flow.

Another factor contributing to the increase in blood flow following the onset of exercise may be rapid vasodilation. Several studies have shown evidence of vasodilation

occurring within the first two to three seconds of exercise (Naik *et al.*, 1999; Saunders &

Tschakovsky, 2004; Tschakovsky *et al.*, 2004; Tschakovsky & Sheriff, 2004). Despite

the mounting evidence supporting the contribution of rapid vasodilation following the

onset of exercise, researchers have been unable to isolate the mechanism responsible for

this vasodilation. Studies of isolated muscle arterioles conducted by Wunsch and

colleagues (Wunsch *et al.*, 2000) hypothesized that vasodilators would have to act

directly on vascular smooth muscle to cause such a rapid relaxation. However, exposure

of isolated skeletal muscle arterioles to potassium chloride, adenosine, acetylcholine, and

sodium nitroprusside all failed to produce vasodilation within four seconds. Numerous

studies in humans have also failed to show a significant contribution of acetylcholine,

adenosine, or nitric oxide to rapid vasodilation (Shoemaker *et al.*, 1997; Radegran &

Saltin, 1999; Hamann *et al.*, 2004; Brock *et al.*, 1998). This reveals the need for further

research into the mechanisms potentially responsible for vasodilation within the first

seconds of rhythmic exercise, and suggests that a number of factors, rather than a single

mechanism, may work in conjunction to cause this dilation.

The second phase of increase of blood flow following exercise onset (phase 2),

begins 10-20 seconds into exercise and raises blood flow to a level which attempts to

match oxygen delivery to the metabolic demand of the working muscles (Shoemaker &

Hughson, 1999). Phase 2 of the blood flow increase is tightly coupled to metabolic

activity (Ferreira *et al.*, 2005), and may be controlled in part by pH, adenosine, ATP,

nitric oxide, potassium, prostaglandins, and/or a number of other metabolites (Delp &

Laughlin, 1998; Clifford & Hellsten, 2004). It appears that, like phase 1, vasodilation

during phase 2 is controlled by multiple mechanisms in combination (Clifford &

Hellsten, 2004; Tschakovsky & Hughson, 2003). In addition to vasodilation, sympathetic vasoconstriction and functional sympatholysis also contribute to the redistribution of blood flow to the capillaries of the contracting motor units following the onset of exercise (Delorey *et al.*, 2002; Delp & Laughlin, 1998; Tschakovsky & Hughson, 2003; Wray *et al.*, 2004). The net result of these multiple control mechanisms is that blood flow generally reaches a steady state within 2-3 min of the onset of moderate exercise in healthy subjects (Walloe & Wesche, 1988; Shoemaker *et al.*, 1996; Shoemaker *et al.*, 1994; Murrant & Sarelius, 2000; Koga *et al.*, 2005; Clark *et al.*, 1998; Clark *et al.*, 2001).

Muscle Contraction Cycle and Blood Flow

Even in moderate intensity exercise, intramuscular pressure can be great enough to impede muscle blood flow during contraction, forcing the majority of blood to flow through working muscle during the relaxation phase of the contraction cycle (Sadamoto *et al.*, 1983; Walloe & Wesche, 1988). Therefore, characteristics of the muscle contraction cycle including duty cycle and contraction frequency, affect the mean blood flow response and the ability to adequately perfuse the muscle (Hoelting *et al.*, 2001). A number of studies involving electric stimulation of dog diaphragms have examined the effect of duty cycle on diaphragmatic blood flow. A blood flow limitation was found at duty cycles above 10-30%, depending on mean arterial and transdiaphragmatic pressures (Hussain *et al.*, 1989; Bark *et al.*, 1987). Beyond this point, diaphragm blood flow was found to be a function of both transdiaphragmatic pressure and duty cycle (Bark *et al.*, 1987; Bellemare *et al.*, 1983). Similarly, blood flow in the dog hindlimb appears to depend upon both muscle tension development and duty cycle (Dodd *et al.*, 1994). This evidence suggests a critical duty cycle beyond which the relative time spent in relaxation

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is insufficient to compensate for limited flow during contraction. However, it is unclear if this concepts transfers to voluntary muscle contraction in human subjects.

Muscle contraction frequency is a variable which can be manipulated independently or in conjunction with changes in duty cycle and work load. Because mean blood flow is dependent upon the duration of both contraction and relaxation phases of the contraction cycle (Bellemare *et al.*, 1983; Walloe & Wesche, 1988; Gaskell, 1877), it is important to consider the influence of contraction frequency on muscle blood flow. When contraction frequency was increased by increasing walking and running speed Kagaya saw an initial increase in calf blood flow as speed increased. This is to be expected as increased speed is associated with an increased work rate for this form of exercise. However, as speed continued to increase, blood flow leveled off (walking) and eventually decreased at the highest speeds (running) (Kagaya, 1990). Since oxygen demand continued to increase with speed, this demonstrates an oxygen delivery limitation at higher contraction frequencies. Similar results have been seen when work rate is held constant. During supine knee extension exercise, Hoelting and colleagues found femoral artery blood flow to be significantly higher at 40 contractions per minute than at 80 contractions per minute at work rates above 5 watts (Hoelting *et al.*, 2001). To maintain similar workloads at different contraction frequencies muscle tension was greater at 40 than 80 contractions per minute, so it is not possible to separate the influences of muscle tension and contraction frequency on blood flow. It appears, however, that increases in contraction frequency and duty cycle may create a situation in which blood flow during brief periods of relaxation is not able to meet oxygen demand.

Post-Occlusive Reactive Hyperemia

During conditions such as cuff-occlusion where blood flow to the forearm is limited by an external mechanical force, a post-occlusive reactive hyperemic response (PORH) occurs immediately following cuff release. Blood velocity increases significantly above resting levels, putting a great shear stress on arterial walls. This shear stress then prompts a flow-mediated arterial dilation, thereby allowing easier passage for a large volume of blood (Toth *et al.*, 2007; Pyke *et al.*, 2008; Padilla *et al.*, 2008). This post-occlusive hyperemia has been shown to increase with progressively increasing occlusion duration, suggesting a relationship between oxygen debt and total hyperemia (Leeson *et al.*, 1997; Mullen *et al.*, 2001; Toth *et al.*, 2007). As such, PORH provides a non-invasive assessment of the cumulative perfusion deficit.

Post-Exercise Hyperemia

Immediately following the final muscle contraction in a bout of exercise a transient overshoot in mean muscle blood flow, known as post-exercise hyperemia, may be seen. This hyperemia is qualitatively similar to post-occlusive reactive hyperemia (PORH), and many researchers have suggested it provides evidence of insufficient blood flow during the preceding exercise (Van Beekvelt *et al.*, 2001; Wesche, 1986; MacDonald *et al.*, 2001). Like PORH, post-exercise hyperemia is influenced by a number of factors including inter-subject variability, exercise type, intensity, and duration.

Magnitude and duration of the post-exercise hyperemia associated with dynamic handgrip exercise appear to be related to exercise intensity. Moderate intensity handgrip exercise at an average of 2.4 kg a non-significant hyperemia was observed only in the first 10 s of recovery (Van Beekvelt *et al.*, 2001). However, heavy intensity exercise at

averages of 7.1 and 12.8 kg resulted in significant hyperemia of approximately 150-200% end-exercise blood flow, and hyperemia was maintained for 60-90 seconds (Van Beekvelt *et al.*, 2001; MacDonald *et al.*, 2001). Similar results are seen with isometric contractions of the quadriceps muscles, where the greatest increase in post-exercise blood velocity occurred between 20% and 50% MVC (Wesche, 1986). This increase in magnitude and duration of hyperemia with increasing exercise intensity is likely related to the higher intramuscular pressures associated with higher exercise intensity, which may occlude flow during muscle contractions.

When measured following isometric exercise, peak and total reactive hyperemia have been shown to increase with progressively increasing exercise duration. Relative blood velocity increase during early recovery was twice as great following a 30 s isometric contraction as for a 5 s contraction (Wesche, 1986). Similarly following isometric exercise under artificially ischemic conditions created with arterial occlusion, peak blood flow increased approximately two-fold as duration increased from a mean of 17.5 to 175.3 s. Total hyperemia increased more than six-fold over the same interval (Osada *et al.*, 2003). These data suggest a cumulative effect in which longer periods of insufficient blood flow during isometric contractions result in increases in peak and total post-exercise hyperemia. It is currently unclear if this pattern continues as duration increases beyond 3 to 5 min and extends to dynamic handgrip exercise, in which it could serve as an indicator of limited blood flow.

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Chapter Three

Muscle Blood Flow and Exercise Tolerance

Introduction

Adequate oxygen supply is necessary for skeletal muscle to perform repeated contractions during rhythmic or intermittent isometric exercise. Therefore muscle blood flow and oxygen delivery increase immediately upon the onset of exercise, and reach a steady state proportional to metabolic demand of the contracting muscle within 2-3 min. Insufficient blood flow, along with oxidative capacity, neuromuscular activation, muscle size, fiber type composition, and presence of reactive oxygen species, have been identified as potential contributors to skeletal muscle fatigue (Hicks *et al.*, 2001; Ferreira & Reid, 2008).

Results of a recent study suggest muscle contractions of short duration result in higher blood flow and oxygen consumption than contractions of longer duration at the same time-tension integral, despite the fact that total amount of contractile work is held constant (Hamann *et al.*, 2005). At submaximal workloads short duration intermittent isometric contractions were also associated with greater myoglobin desaturation, demonstrating a difference in intracellular oxygenation as compared to longer contractions (Wigmore *et al.,* 2008). Recent evidence also indicates that muscle blood flow and motor unit recruitment, as indicated by integrated EMG, are higher during

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dynamic than intermittent isometric knee extension exercise (Laaksonen *et al.*, 2003). These findings suggest that factors other than total work performed, including metabolic rate associated with a particular muscle contraction pattern, tension development, and muscle fiber recruitment, are tightly coupled with muscle blood flow (Hamann *et al.*, 2005; Hamann *et al.*, 2004). The effect of these relationships on muscle fatigue and exercise tolerance, however, remains unclear.

A significant gender difference in muscle fatigue in healthy young subjects has been demonstrated in a number of muscles and muscle groups including the tibialis anterior (Russ & Kent-Braun, 2003), elbow flexors (Hunter & Enoka, 2001), and inspiratory muscles (Gonzales & Scheuermann, 2006). However, under ischemic conditions there is no significant difference in indicators of fatigue between men and women (Russ & Kent-Braun, 2003). Since the gender difference in muscle fatigue appears to be blood flow dependent (Russ & Kent-Braun, 2003; Saito *et al.*, 2008), oxygen delivery to working muscles is likely a significant factor in exercise tolerance.

Although the effect of contraction type, frequency, and intensity on muscle blood flow has been examined in humans (Laaksonen *et al.*, 2003; Stebbins *et al.*, 2002; Daniels *et al.*, 2000; Hoelting *et al.*, 2001), the extent to which the interaction between contraction pattern and muscle blood flow influences exercise tolerance has received little attention. Therefore, the purpose of this study was to examine the relationship between muscle blood flow and exercise tolerance during dynamic and intermittent isometric handgrip exercise. We hypothesized that forearm muscle blood flow would be greater for intermittent isometric than for dynamic handgrip exercise, and that this would result in greater exercise tolerance during intermittent isometric exercise.

Methods

Subjects. Eleven healthy subjects (6 female, 5 male) participated in this study. The mean age of the subjects was 24 ± 6 (mean $\pm$ SD) yr, height was 169 ± 7 cm, and weight was 68 ± 14 kg. Subjects were informed of all testing procedures and any risks and discomforts associated with the testing protocol before giving their informed consent. This study was approved by the Human Subjects Committee of the Institutional Review Board at the University of Toledo, where all the tests were conducted.

Experimental Design. Subjects were studied while lying supine, with the right arm supported in an outstretched position perpendicular to the body. Subjects performed one bout each of two exercise protocols. The first exercise protocol was dynamic (DYN) handgrip exercise, which consisted of 2 s concentric forearm contraction followed by 2 s eccentric forearm contraction. The second exercise protocol was intermittent isometric (INT) handgrip exercise, comprised of 2 s isometric contractions separated by 2 s of rest. The two protocols were performed on separate days at 10% maximal voluntary contraction (MVC) in random order. Each protocol was continued until task failure, which was defined as the time at which the subject could no longer maintain contraction timing with the metronome despite visual feedback of the contraction pattern and verbal encouragement. Data acquisition began 2 minutes prior to the onset of exercise and ended 2 minutes after task failure was reached for each subject.

Measurements. Anthropometric measurements including forearm volume, anterior forearm skinfold thickness, and circumference of the right forearm were taken for each subject. Forearm volume was obtained by water displacement and MVC

handgrip force was determined with the use of a grip strength dynamometer (Creative Health Products, Inc., Ann Arbor, MI) for each subject.

Surface EMGs of the wrist and finger flexor muscle group were also recorded throughout each exercise protocol. Skin was shaved if necessary and cleaned with isopropyl alcohol before two sets of disposable Ag/AgCl electrodes (1.4 cm diameter, 2.0 cm interelectrode distance; Noraxon Dual Electrodes, Noraxon, AZ) were placed over the belly of the muscle group on the right anterior forearm. A reference electrode was placed over the left olecranon process. Raw EMG was sampled at 2000 Hz by a differential biopotential amplifier (PowerLab, ADInstruments) and saved to a computer hard drive for offline analysis. EMG signals were bandpass filtered (20-500 Hz), rectified, and integrated.

Brachial artery blood velocity was measured continuously during exercise with Doppler ultrasound velocimetry (Model 500-V, Multigon Industries, NY) operating in pulse mode with an operating frequency of 4MHz. The fixed crystal transducer produced an ultrasound beam angle of 45° relative to the skin. The Doppler transducer was placed flat on the distal third of the upper arm above and parallel to the brachial artery. Mean arterial pressure (MAP) was measured every 15 seconds by a noninvasive wrist-cuff (Vasotrac APMZO5A) on the wrist of the non-exercising hand which was maintained at heart level. Heart rate was monitored and recorded using an electrocardiogram.

The diameter of the brachial artery was measured, internal border to internal border, at rest in the supine position using 2-D sonography with a multi-frequency linear array probe (5-9 MHz; center frequency of 7.5 MHz) operating in two-dimensional echo mode (Logiq 400, GE Medical System, Milwaukee, WI). Imaged data was stored on a

DVD for offline analysis to determine mean brachial artery diameter over 10-15 cardiac cycles at peak systole and end diastole. This cross-sectional view of the artery allowed the computation of the cross sectional area ($CSA = \pi r^2$) of the brachial artery and muscle blood flow [MBF (ml/min) = MBV (cm/sec) x CSA (cm^2)]. All brachial artery diameter measurements were taken by the same operator during cardiac cycles which provided optimal resolution of the arterial borders.

Data Processing and Analysis. Time-tension integral (TTI) of each exercise bout was determined by integrating the handgrip displacement curve, and was used as an index of contractile work performed. Due to differences in exercise duration between subjects and protocols, mean blood flow (MBF) was calculated at rest, in 15 s intervals during the first 2 min of exercise, and during the last 15 s of exercise. Integrate EMG (iEMG) was normalized to resting values and divided by TTI in the same 15 s intervals to assess motor unit recruitment patterns.

Time to task failure, mean blood flow, and iEMG responses were compared using paired t-tests and two-way repeated measures ANOVA with the Tukey-Kramer post hoc test for pairwise comparisons when appropriate. Significance was set a priori at $P \leq 0.05$, and values are reported as mean ± standard error.

Results

Subject Characteristics. The descriptive characteristics of the subjects are presented in Table 3.1. The group mean MVC handgrip strength was 387 ± 34 N with a mean forearm volume of 974 ± 106 ml. A significant positive relationship was observed between maximal handgrip strength and forearm volume ($r = 0.82$, $p < 0.05$). However, there was no correlation between MVC and time to task failure for either INT or DYN

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with r = 0.21 for INT and r = 0.37 for DYN (Figure 3.6). One subject's time to task

failure for DYN of 383 s appears to be an outlier, and when considered without this data

point the correlation between MVC and time to task failure for DYN is significant (r =

0.89, p<0.05).

Central Cardiovascular Response. Heart rate increased similarly with exercise

duration during INT and DYN exercise with no significant difference in heart rate

observed between conditions at task failure (INT, 87 ± 4 bpm; DYN, 99 ± 6 bpm).

Compared to resting conditions, mean arterial pressure increased with exercise duration.

However, this increase was similar between INT (96 ± 13 mm Hg) and DYN (105 ± 11

mm Hg) exercise trials. Vascular conductance (i.e. the product and blood flow and mean

arterial pressure), although significantly higher during exercise than resting conditions,

was not significantly different between conditions (INT, 1.46 ± 0.38 ml/min/mm Hg;

DYN, 1.38 ± 0.39 ml/min/mm Hg).

Determinants of Exercise Tolerance. Although the work performed per unit time

during the INT and DYN trials was similar, the time to task failure (TTF) was

significantly longer during the INT (847 ± 137 s) compared to DYN (247 ± 22 s) exercise

(Figure 3.1). Thus total work, defined as time-tension integral (TTI), was also

significantly greater during INT (3947 ± 609 W) than DYN exercise (979 ± 88 W).

Brachial artery blood flow during INT and DYN for a representative subject is

presented in Figure 3.2, illustrating the similarity of the two over the first five min. of

exercise. Since TTF differed significantly between subjects and protocols, a comparison

of blood flow responses could only be performed during the initial 2 min of exercise and

at TTF. As indicated in Figure 3.3 and Table 3.2, brachial artery blood flow increased

significantly from resting conditions with exercise duration during both INT and DYN

exercise trials and mean blood flow was similar at task failure in INT and DYN. Mean

blood flow as also similar in INT (82.9 ± 11.3 ml/min) and DYN (92.8 ± 22.5 ml/min) at

the same time-tension integral (Figure 3.4).

The ratio of normalized iEMG to TTI was similar for INT and DYN at rest,

during each 15 s interval in the first 2 min of exercise, and at end exercise (Figure 3.5 and

Table 3.2). Therefore, although the time to task failure was considerably different

between exercise modes, differences in brachial artery blood flow and motor unit

recruitment do not appear to be significant contributors to the difference in exercise

tolerance.

	Mean ± SD	Range
Age (yr)	24 ± 6	21-39
Height (cm)	169 ± 7	160-183
Weight (kg)	68 ± 14	50-97
Body Mass (kg/m^2)	20 ± 3	15-26
Forearm Volume (ml)	1019 ± 364	599-1831
Brachial Artery Diameter (cm)	0.37 ± 0.08	0.28-0.56
MVC (N)	387 ± 113	263-572
Workload (kg) - 10% MVC	7 ± 1	5-10

Table 3.1. Subject characteristics.

	Dynamic	Intermittent Isometric
TTI per minute	237.8 ± 5.2	282.6 ± 7.3
Resting Blood Flow	22.4 ± 5.1	26.3 ± 8.1
End Exercise Blood Flow	86.8 ± 18.7	87.6 ± 18.1
End Exercise Normalized iEMG/TTI	31.7 ± 5.4	26.5 ± 3.8

Table 3.2. Comparison of dynamic and intermittent isometric exercise. Summary of work performed per unit time, blood flow, and EMG during DYN and INT. All values are mean ± standard error.
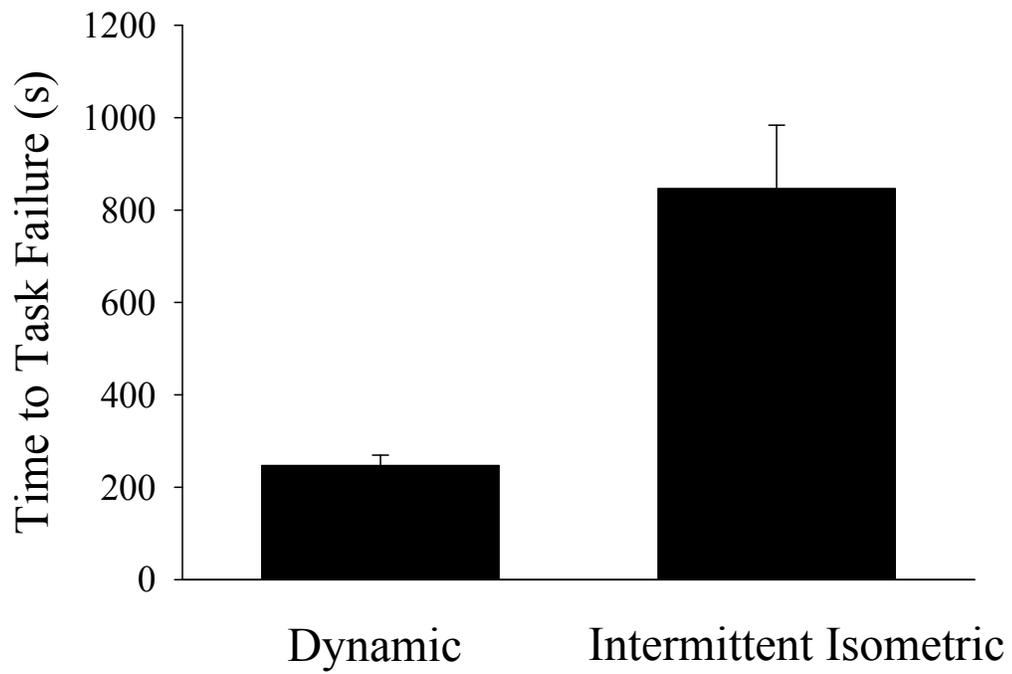
Figure 3.1. Exercise tolerance was 265% greater for intermittent isometric contractions as compared to dynamic contractions during handgrip exercise.
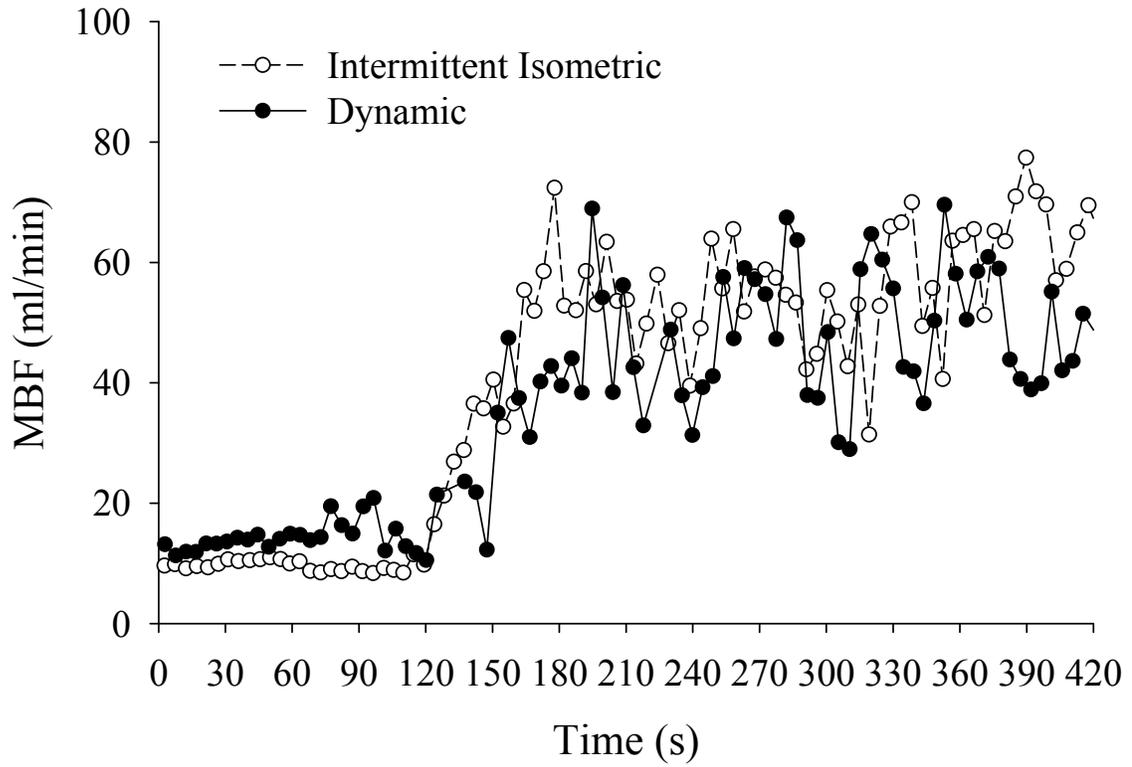
Figure 3.2. Mean forearm blood flow (average over a cardiac cycle) response of a single subject during intermittent isometric and dynamic handgrip exercise. For comparison purposes, intermittent isometric exercise is truncated to the time to fatigue for dynamic exercise.
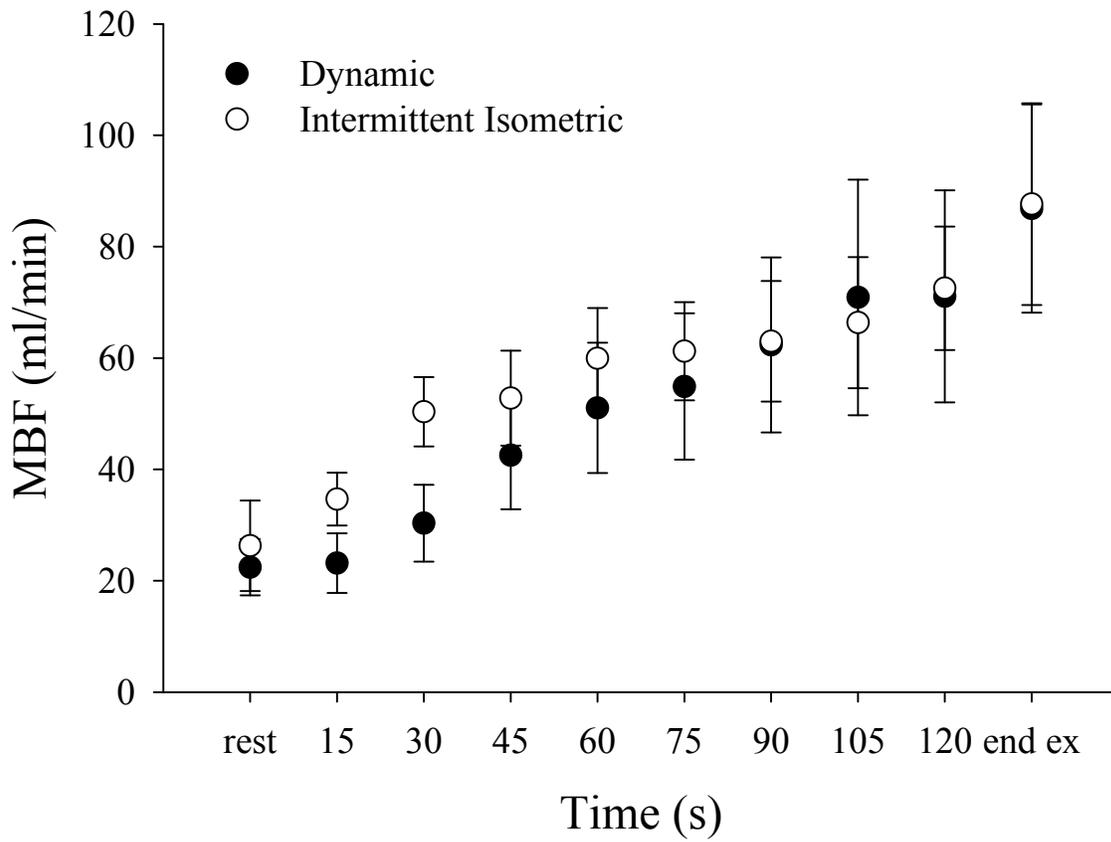
Figure 3.3. Following the initial rise (~60 s), mean forearm blood flow was similar between dynamic and intermittent isometric handgrip exercise.
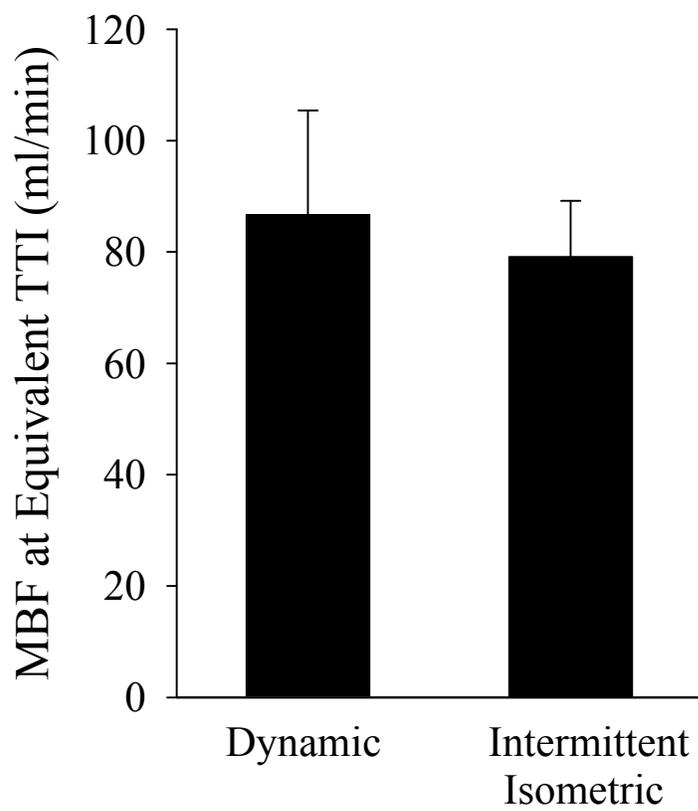
Figure 3.4. The group mean forearm blood flow response was similar between dynamic and intermittent isometric exercise at an equal tension time integral.
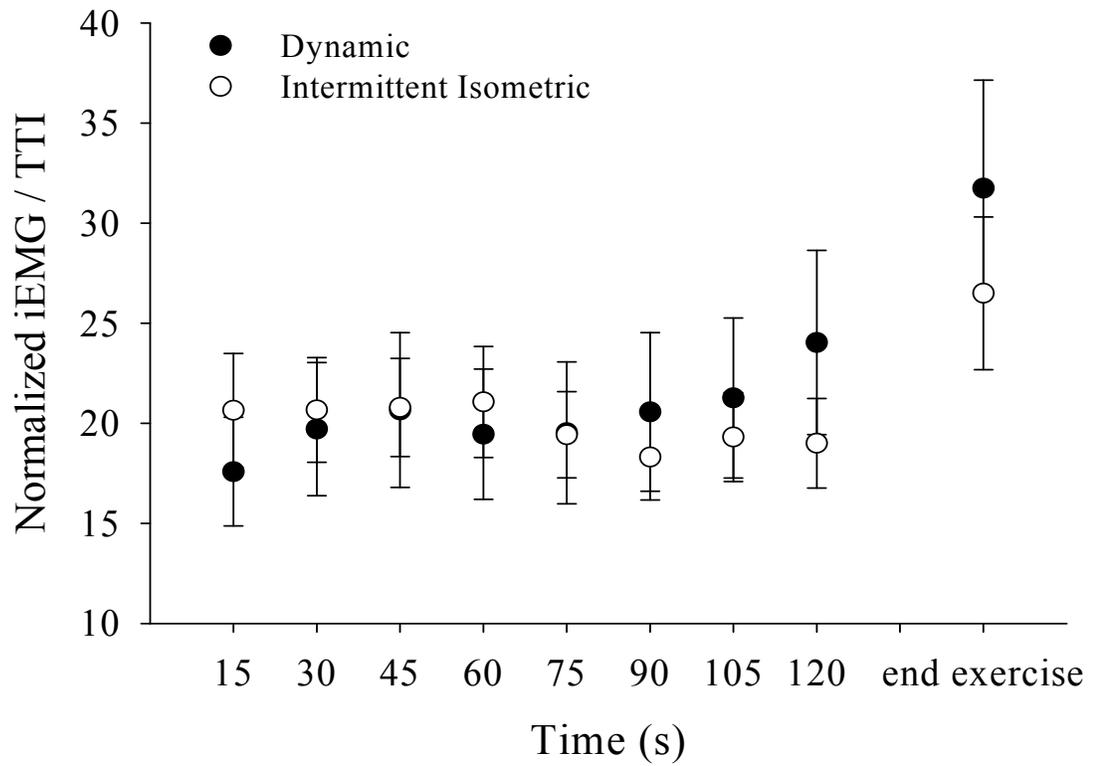
Figure 3.5. The ratio of forearm muscle activity and time tension integral showed no difference in motor unit recruitment patters between dynamic and intermittent isometric handgrip exercise.
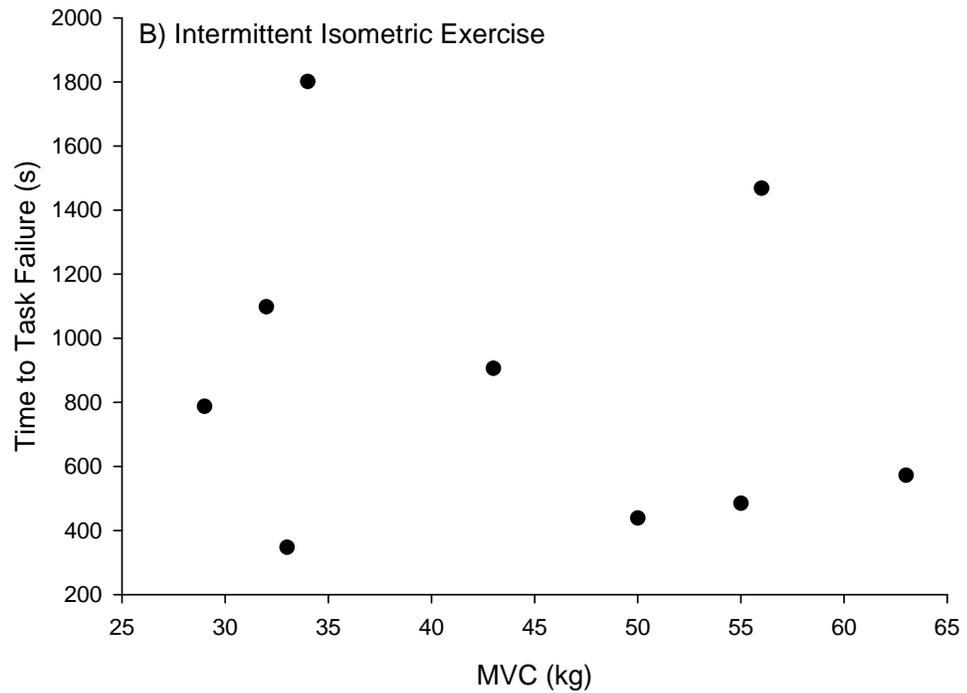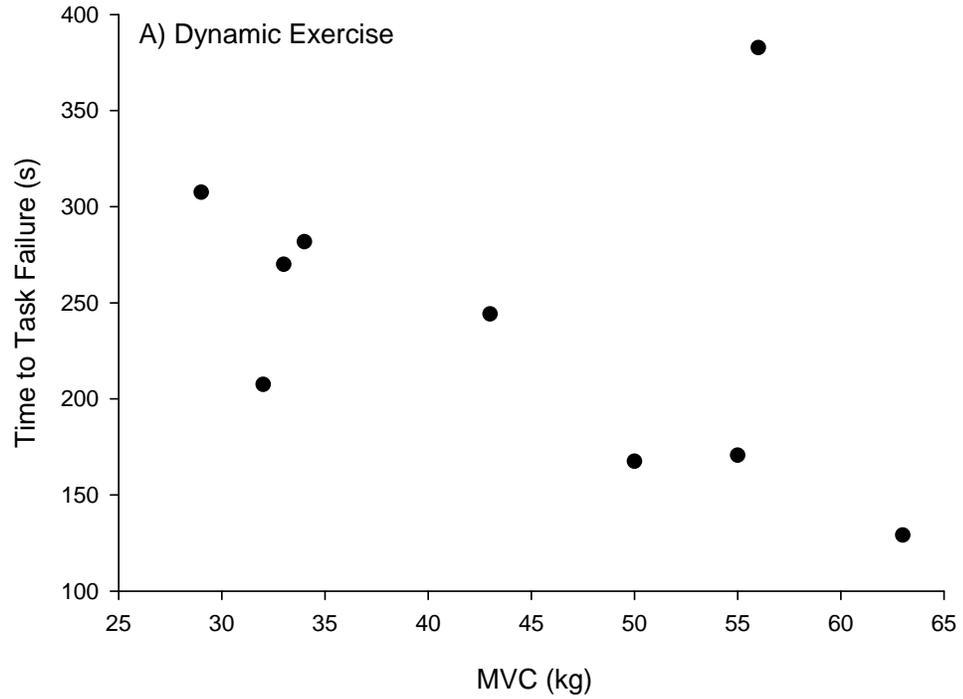
Figure 3.6. Relationship between MVC and time to task failure for A) dynamic and B) intermittent isometric exercise. Neither condition resulted in a significant correlation with r = 0.21 for dynamic and r = 0.37 for intermittent isometric exercise.

Discussion

      The present study was performed to examine the relationship between muscle blood flow and exercise tolerance during intermittent isometric (INT) and dynamic (DYN) handgrip exercise. The major finding of this study is that although the TTI, or amount of work performed, was similar per contraction cycle, DYN exercise resulted in considerably lower exercise tolerance compared to INT exercise. Muscle blood flow and presumably oxygen delivery to the working skeletal muscle was similar between conditions and therefore is not a primary determinant of exercise tolerance during handgrip exercise. Therefore, other unknown factors contributed to the reduced exercise tolerance observed during DYN handgrip exercise.

      Muscle Blood Flow. It is well established that blood flow is impeded at 10-30% MVC during both rhythmic (Walloe & Wesche, 1988; Shoemaker *et al.*, 1994) and continuous isometric (Bonde-Petersen *et al.*, 1975; Humphreys & Lind, 1963; Lind & McNicol, 1967; Sjogaard *et al.*, 1986; Gaffney *et al.*, 1990) skeletal muscle contractions. This restriction is presumably caused by an increase in intramuscular pressure during the contraction phase constricting the vasculature. Because the majority of muscle blood flow during exercise occurs in the relaxation periods between contractions, we hypothesized the 2 s rest periods in the INT protocol would result in greater blood flow than the DYN protocol which lacks a true relaxation phase. However, our results did not confirm this hypothesis. Though there were slight differences during the transitional phase, following the first minute of exercise blood flow was similar for INT and DYN until task failure.

      The 10% MVC performed here is at the low end of work rates typically associated with contraction limitation of blood flow, and may not have been a sufficient stimulus to

elicit anticipated results. Increased intensity resulting in decreased exercise duration would not be feasible as for some subjects the current protocol was sustainable for as little as 129 s. The additional slow component in the blood flow response observed during higher intensity exercise would also be problematic. Because flow would continue to increase with time, accurate comparison of mean blood flow could not be made for exercise bouts of significantly different duration.

Mechanisms of Fatigue. Similar external work was performed in INT and DYN as indicated by motor unit recruitment and TTI. Therefore, other intra-muscular factors related to continuous muscle tension held in DYN exercise may have lead to earlier task failure. These may include substrate depletion, reactive nitrogen species, acidosis, high inorganic phosphate levels, and muscle-derived reactive oxygen species whose effect can be delayed by antioxidants (Reid, 2008; Reid *et al.*, 1992; Barclay, 1991; Gollnick *et al.*, 1986; Ferreira & Reid, 2008). A single controlling mechanism will likely never be isolated as muscle fatigue depends upon the interaction between a number of central and peripheral factors, the relative contributions of which appear to change with exercise type, age, and gender (Kent-Braun, 1999; Kent-Braun *et al.*, 2002; Giannesini *et al.*, 2003; Gibson & Edwards, 1985).

Methodological Limitations. In the present study, blood flow was assumed to be representative of oxygen delivery and extraction within the exercising muscle. Because blood flow was measured at the conduit artery level, we cannot determine the distribution of this blood among the active and inactive portions of the forearm. Additionally, oxygen content was not measured. Therefore, we are uncertain if delivery of oxygenated blood and subsequent oxygen extraction were similar for INT and DYN. Potential occlusion of

blood flow at the microvascular level would not have been detected with conduit artery blood flow measurement. It could, however, impair oxygen delivery to active motor units and contribute toward muscle fatigue.

While it is difficult to non-invasively target blood flow measurements to a working muscle or muscle group, use of near infrared spectroscopy (NIRS) to assess oxy-hemoglobin and myoglobin levels of the forearm muscles could provide data regarding the relative availability of oxygen during various muscle contraction patterns. Oxygen saturation as determined by NIRS appears to closely reflect saturation in the venules of the sampled muscle (Boushel *et al.*, 2001; Boushel *et al.*, 1998; Mancini *et al.*, 1994). In addition to calculated saturation, NIRS can also detect changes in total hemoglobin/myoglobin and in oxygenated and deoxygenated hemoglobin/myoglobin levels during exercise (Kowalchuk *et al.*, 2002; Delorey *et al.*, 2004).

Recently, NIRS has been used to examine the changing deoxy-hemoglobin/myoglobin within a muscle contraction cycle. While red blood cells were present in the microcirculation throughout the contraction cycle, there was a cyclical pattern to deoxy-hemoglobin/myoglobin, suggesting oxygen extraction occurred during periods of both contraction and relaxation (Lutjemeier *et al.*, 2008). If this technique were employed in the protocol of the present study, potential differences in intra-muscular oxygenation and its fluctuations throughout the INT and DYN contraction cycles may be revealed.

Myoglobin oxygenation can also be assessed with magnetic resonance spectroscopy during some types of exercise such as isometric ankle dorsiflexion. Wigmore and colleagues have shown greater deoxy-myoglobin levels, accompanied by

greater fatigue, at submaximal workrates during short as compared to long isometric contractions (2008). This relationship was thought to be caused by the greater time total time spent generating contractions, which requires more ATP than maintaining a contraction, in bouts of exercise composed of short contractions. Therefore, the greater time spent generating contractions in DYN could result in a greater deoxy-myoglobin which was not measured in the present study, but could be related to metabolic rate and time to task failure.

   Conclusion. Although there was no difference in mean brachial artery blood flow following the first minute of exercise, the mean time to task failure was over 10 minutes longer in intermittent isometric than in dynamic exercise. This finding suggests that muscle blood flow, at least when measured at the conduit artery, may not be a significant determinant of exercise tolerance. The extent to which the contraction-relaxation duty cycle utilized in the present study affected O_2 exchange in the microvasculature and thus availability to the mitochondria during exercise remains unclear at this time.

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Chapter Four

Exercise Duration and Post-Exercise Hyperemia

Introduction

Compared to mean blood flow response during dynamic exercise, an immediate but transient overshoot in muscle blood flow occurs early in recovery (Shoemaker *et al.*, 1994). This overshoot has been used by some as evidence to suggest that blood flow during exercise is insufficient to support the metabolic requirements of the active muscles (MacDonald *et al.*, 2001; Wesche, 1986; Van Beekvelt *et al.*, 2001), while other investigators have focused on peak rather than mean flows such that no overshoot is seen (Lutjemeier *et al.*, 2005). Because of the interaction between muscle contraction-relaxation phases and intramuscular pressure and the effect of this interaction on muscle blood flow, it is rather difficult to non-invasively assess the adequacy of muscle blood flow during exercise. However, observation of post-exercise hyperemia may provide insight into the perfusion requirements of exercise.

During conditions such as cuff-occlusion where blood flow to the forearm is artificially limited, a post-occlusive hyperemic response occurs which is qualitatively similar to the post-exercise overshoot described by Shoemaker and colleagues (Shoemaker *et al.*, 1994). This post-occlusive hyperemia has been shown to increase with progressively increasing occlusion duration, suggesting a relationship between oxygen

debt and total hyperemia (Leeson *et al.*, 1997; Mullen *et al.*, 2001; Toth *et al.*, 2007). A similar phenomenon has also been seen following heavy and severe isometric exercise, which is known to impede blood flow. Performance of severe intensity exercise and exercising under ischemic conditions both resulted in siginificantly greater post-exercise hyperemia than heavy intensity exercise. Peak and total post-exercise hyperemia following severe isometric exercise were also linearly related to exercise duration and time-tension index (Osada *et al.*, 2003; Bellemare *et al.*, 1983). Based on these data, we can surmise that the relationship between oxygen debt and total hyperemia extends to conditions in which exercise is performed with inadequate perfusion.

Therefore, if muscle blood flow is limited during dynamic handgrip exercise and the transient overshoot is direct evidence of this inadequacy, one might reason that a progressive increase in exercise duration would lead to an increase in post-exercise blood flow, similar to the response observed when occlusion duration is progressively increased. The purpose of this study was to investigate the relationship between exercise duration and post-exercise hyperemia in both moderate exercise, during which blood flow is thought to be sufficient, and heavy exercise, which may impede muscle blood flow. We hypothesized a) for moderate intensity exercise there would be no significant difference in muscle blood flow with increasing exercise duration, and b) for heavy intensity exercise muscle blood flow would increase with increasing exercise duration.

Methods

Subjects. Five healthy male subjects participated in this study. The mean age of the subjects was 30.6 ± 6.2 (mean $\pm$ SD) yr, height was 179 ± 8 cm, and weight was 78.2 ± 19.1 kg. Subjects were informed of all testing procedures and any risks and discomforts

associated with the testing protocol before giving their informed consent. This study was approved by the Human Subjects Committee of the Institutional Review Board at the University of Toledo, where all the tests were conducted.

Experimental Design. Subjects were studied while sitting upright with the right arm supported in front of the body and the elbow at an angle of approximately 90˚. Subjects performed dynamic handgrip exercise with a 33% duty cycle at moderate (5% maximum voluntary contraction, MVC) and heavy (10% MVC) intensities. Each subject performed bouts of 3, 5, 7, and 9 min duration for each exercise intensity. Brachial artery blood velocity was measured continuously at rest, throughout the exercise bout, and for 5 min. of recovery using Doppler ultrasound velocimetry. No more than 3 exercise bouts were performed in each visit, and bouts were performed in random order with moderate exercise completed prior to heavy exercise on a given day.

Measurements. Anthropometric measurements including forearm volume, anterior forearm skinfold thickness, and circumference of the right forearm were taken for each subject. Forearm volume was obtained by water displacement and MVC handgrip force was determined with the use of a grip strength dynamometer (Creative Health Products, Inc., Ann Arbor, MI) for each subject for calculation of subsequent exercise intensity.

Brachial artery blood velocity was measured continuously during exercise with Doppler ultrasound velocimetry (Model 500-V, Multigon Industries, NY) operating in pulse mode with an operating frequency of 4MHz. The fixed crystal transducer produced an ultrasound beam angle of 45° relative to the skin. The Doppler transducer was placed flat on the distal third of the upper arm above and parallel to the brachial artery.

During an additional visit, each subject performed 9 min of continuous exercise at each intensity while images of the brachial artery were obtained at 3, 5, 7, and 9 min. of exercise using 2-D sonography with a multi-frequency linear array probe (5-9 MHz; center frequency of 7.5 MHz) operating in two-dimensional echo mode (Logiq 400, GE Medical System, Milwaukee, WI). Imaged data was stored on a hard drive for offline analysis by commercial edge detection software (Brachial Analyzer for Research, Medical Imaging Applications, Coralville, IA) to determine brachial artery diameter. This cross-sectional view of the artery allowed the calculation of the cross sectional area ($CSA=\pi r^2$) of the brachial artery and muscle blood flow [MBF (ml/min)= MBV (cm/sec) x CSA (cm^2)].

Data Processing and Analysis. The 5 highest peak velocities during the last 15 s of each bout of exercise were measured and averaged. This average was reported as peak exercise blood flow. Similarly, the peak velocities of the first 5 cardiac cycles following the offset of exercise were measured and averaged. This average was reported as peak recovery blood flow. Area under the recovery curve was determined by integrating the blood flow curve for the first 5 min of recovery. Area under the curve provides an index of total post-exercise hyperemia.

Exercise versus recovery peak blood flow values and area under the curve responses were compared using two-way repeated measures ANOVA with the Tukey-Kramer post hoc test for pairwise comparisons when appropriate (SigmaStat). Linear regression and correlation coefficients were determined using SigmaPlot. Significance was set a priori at $P \leq 0.05$, and values are reported as mean ± standard error.

Results

Subject Characteristics. Descriptive characteristics of the 5 male subjects are
presented in Table 4.1. The group mean MVC handgrip strength was 53.8 ± 2.4 kg,
which resulted in moderate exercise intensities ranging $2.3 - 3.0$ kg and heavy exercise
intensities ranging $4.7 - 6.0$ kg resistance. Subjects had a mean resting heart rate of 67.2
± 2.8, systolic blood pressure of 123 ± 9, and diastolic blood pressure of 85 ± 10.
Brachial artery diameter and blood flow at rest averaged 4.9 ± 0.3 mm and 22.7 ± 1.0
ml/min respectively.

Brachial Artery Diameter. For moderate exercise, there was no significant
difference in group mean brachial artery diameter at rest (4.86 ± 0.29 mm) and that after
exercise of any duration (range $4.83 - 4.90$ mm). With heavy exercise, mean diameter
increased from 4.86 ± 0.35 mm at rest to $5.03 - 5.16$ mm following exercise. This
difference was significant at each exercise duration from 3 to 9 min as demonstrated in
Figure 4.1.

Exercise Blood Flow. While mean brachial artery blood flow during the final 30 s
of exercise increased significantly above resting levels during both moderate and heavy
exercise, within each intensity blood flow was similar regardless of exercise duration.
Mean blood flow for heavy exercise approached a significant duration effect with $p =$
0.055. A similar pattern was seen with peak blood flow, as reported in Table 4.2 and
Figure 4.3, with a no significant difference between exercise durations within a given
intensity level. There was a significant positive relationship between both mean and peak
blood flow during exercise and handgrip resistance ($r = 0.86$ and 0.81; $p < 0.05$),
demonstrating the increased oxygen delivery demands of higher work loads (Figure 4.2).

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Post-Exercise Hyperemia. Immediate hyperemic response was represented by peak blood flow following the final muscle contraction cycle. This peak recovery flow was significantly higher than resting blood flow for all trials. However, as shown in Figure 4.3, peak recovery blood flow was lower than peak end-exercise blood flow for all moderate exercise durations and for heavy exercise durations 5 min and longer. There was no increase in peak hyperemia with progressively increasing exercise duration for either moderate or heavy intensity exercise. Area under the recovery blood flow curve (AUC) is reported in Figure 4.4 as an indicator of total hyperemia during the initial 5 min recovery period. As with peak hyperemia, within each exercise intensity there was no significant difference in AUC between exercise durations, though there was a significant intensity effect (see also Table 4.2). These results suggest that brachial artery blood flow is sufficient during dynamic handgrip exercise at both 5% and 10% MVC.

Age (yr)	30.6 ± 2.8
Height (m)	1.97 ± 0.04
Weight (kg)	78.2 ± 8.5
MVC (kg)	53.8 ± 2.4
Moderate Intensity (kg)	2.7 ± 0.1
Heavy Intensity (kg)	5.3 ± 0.2

Table 4.1. Subject characteristics and exercise intensities. All values are mean ± SE.

	3 min	5 min	7 min	9 min
Moderate Intensity				
Pre-Exercise	26.8 ± 2.0	20.7 ± 1.5	27.2 ± 3.9	22.3 ± 2.2
Mean Exercise [a]	93.7 ± 8.9	93.2 ± 10.8	93.9 ± 11.3	91.7 ± 14.5
Peak Exercise	401.7 + 29.8	390.2 + 27.4	409.6 + 41.4	407.1 + 38.6
Peak Recovery [b]	356.6 + 35.5	351.4 + 32.6	360.4 + 43.5	374.6 + 42.0
AUC	10155 ± 1468	8345 ± 1027	9981 ± 1375	9837 ± 1306
Heavy Intensity				
Pre-Exercise	20.9 + 3.2	20.7 + 2.9	18.7 + 2.9	24.4 + 1.2
Mean Exercise [a]	157.6 ± 21.2	196.9 ± 22.7 [d]	186.1 ± 21.6	220.3 ± 29.7 [d]
Peak Exercise	536.2 ± 51.1	635.5 ± 48.0	536.8 ± 69.9	657.7 ± 61.5
Peak Recovery [b]	506.1 ± 47.5	562.3 ± 28.4	565.5 ± 59.2	584.3 ± 64.7
AUC [c]	15321 ± 1278	17388 ± 3249	19176 ± 3627	18794 ± 1701

Table 4.2. Summary of exercise and recovery blood flow data. Blood flow values are given in ml/min. AUC is area under the recovery blood flow curve. All values are mean ± standard error. [a] Significantly different from pre-exercise blood flow within the same intensity. [b] Significantly different from peak exercise blood flow within the same intensity. [c] Significantly different from AUC of moderate exercise. [d] Significantly different from mean exercise blood flow for 3 min heavy intensity ($p < 0.05$)
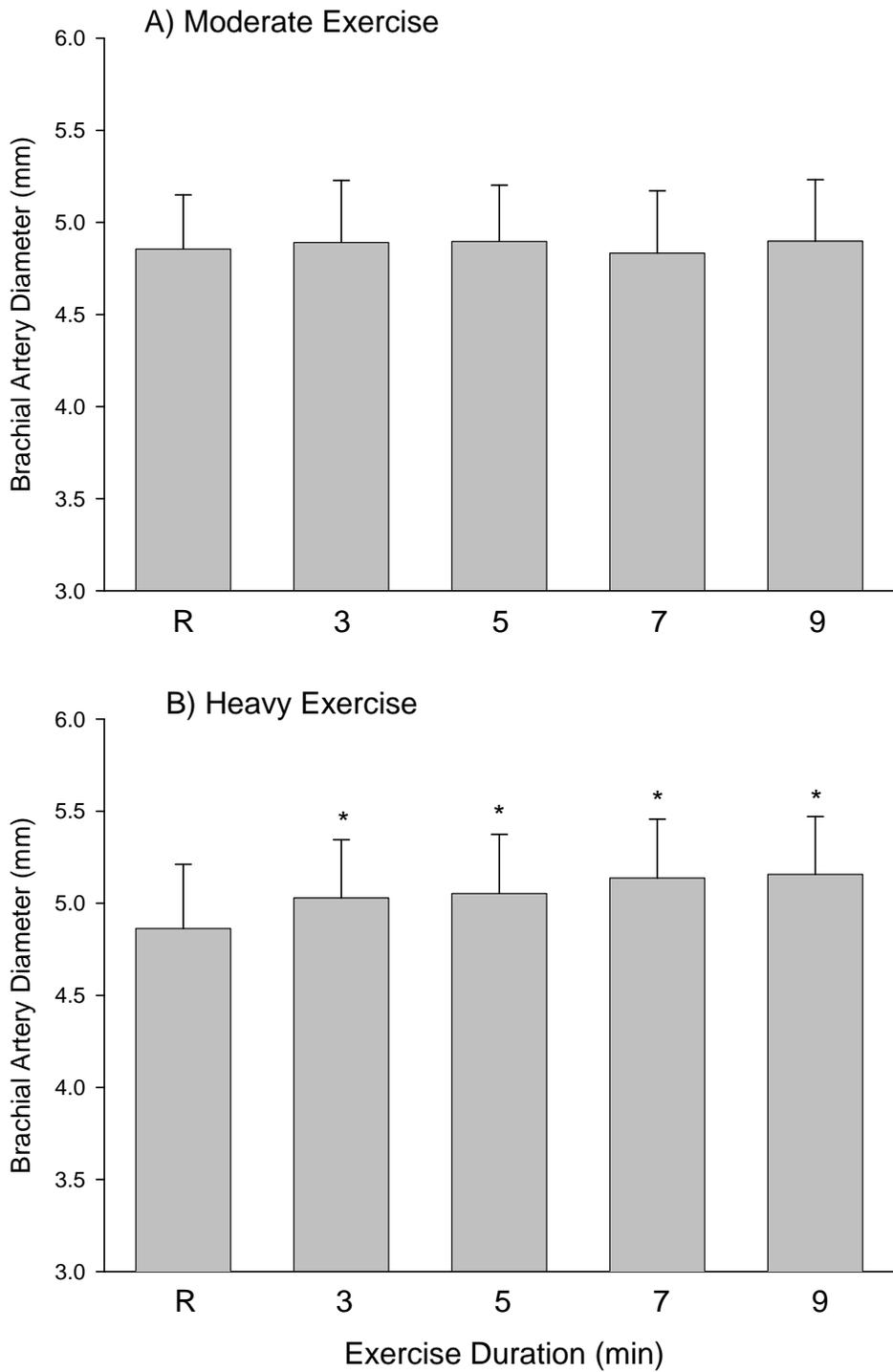
Figure 4.1. Group mean (± SE) brachial artery diameter measurements determined during a continuous bout (9 min) of A) moderate and B) heavy exercise. * Significantly different from the resting arterial diameter for the corresponding exercise intensity.
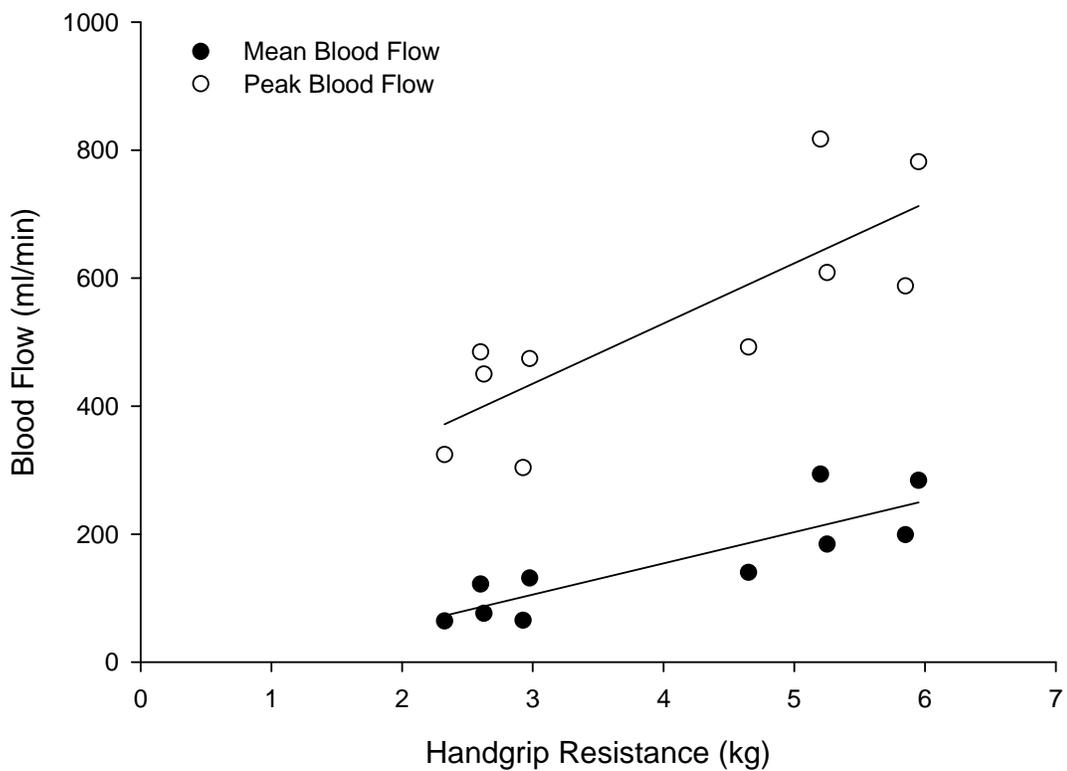
Figure 4.2. Mean (closed circles) and peak (open circles) brachial artery blood flow during the final 30 s of 9 min handgrip exercise. Both mean and peak flows are significantly correlated with handgrip resistance (r = 0.86 and 0.81 respectively; $p < 0.05$).
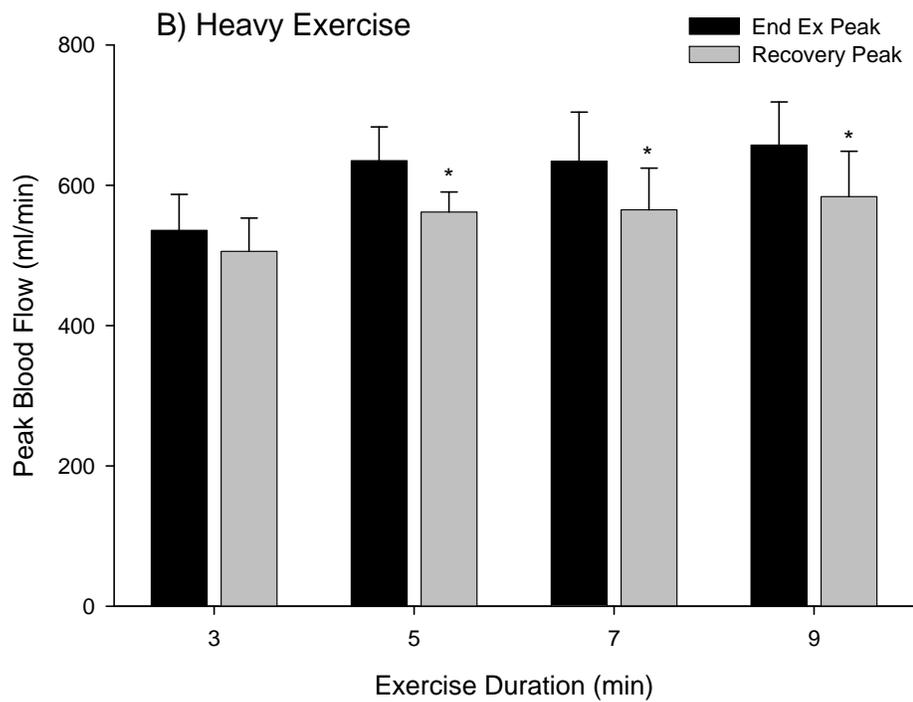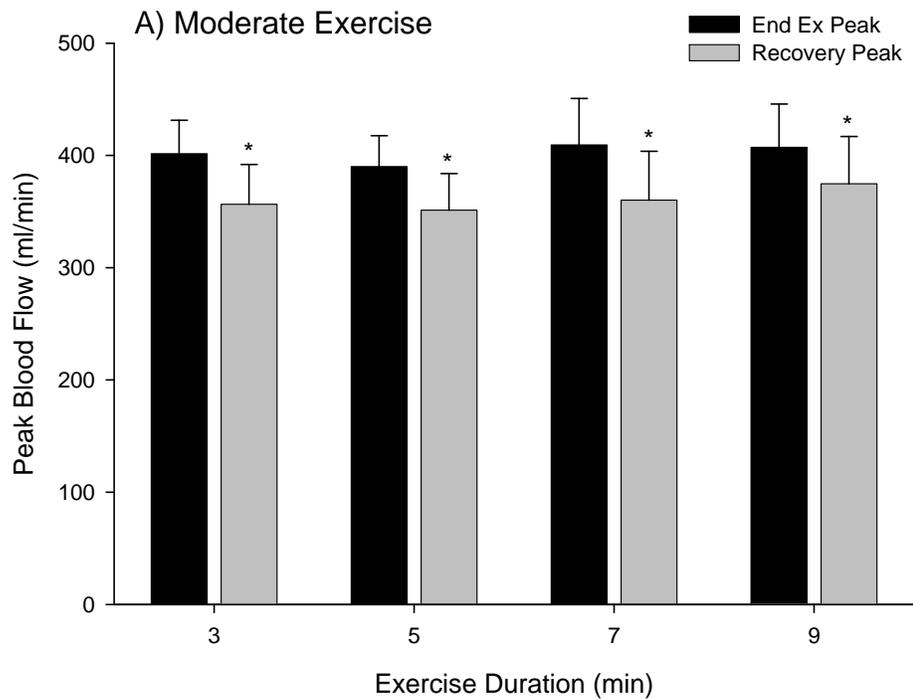
Figure 4.3. Group mean (±SE) peak blood flow responses at end exercise compared to peak blood flow responses during early recovery from A) moderate and B) heavy exercise. * Significantly different from corresponding end exercise value.

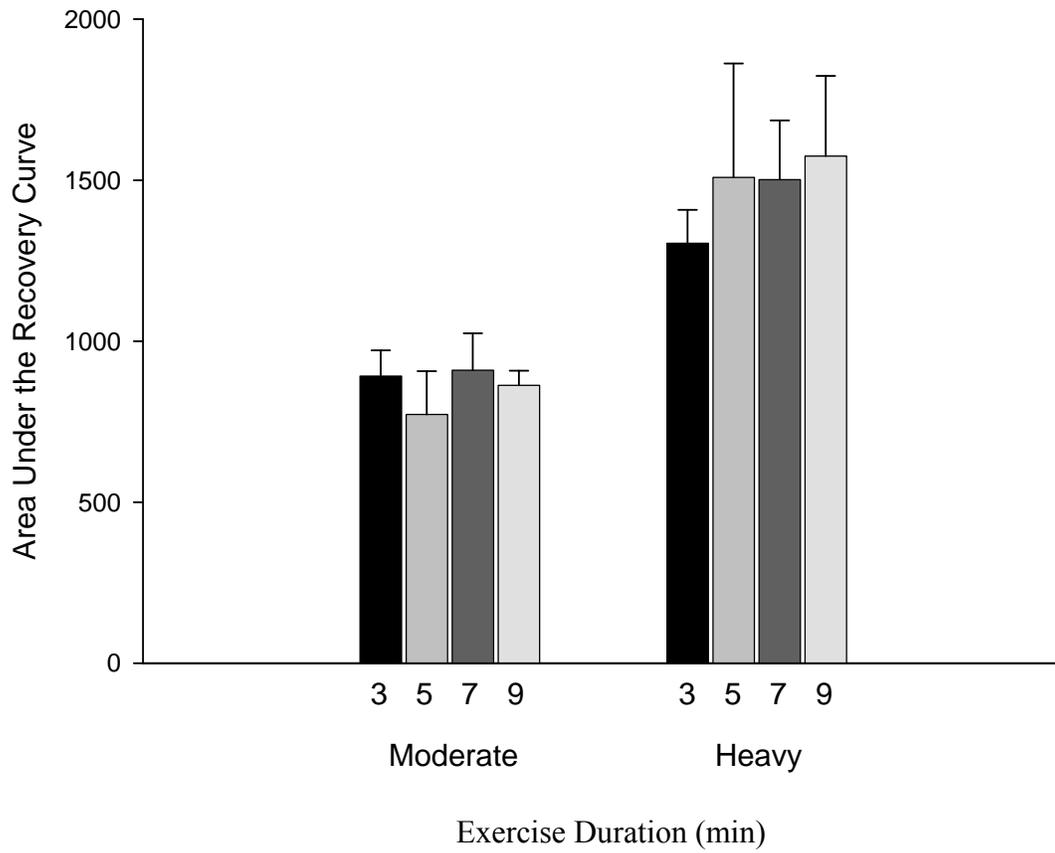**Figure 4.4.** Group mean (±SE) area under the blood flow curve during the first 5 min of recovery from moderate and heavy exercise. Values within each exercise intensity are not significantly different.

Discussion

The present study sought to determine if there was a relationship between exercise duration and post-exercise hyperemia in moderate and heavy intensity dynamic handgrip exercise. Consistent with our first hypothesis, we found hyperemia following moderate exercise to remain constant despite progressively increasing exercise duration from 3 to 9 min. However, in contrast with our second hypothesis, we found hyperemia following heavy exercise also remained constant for exercise durations ranging from 3 to 9 min. Therefore, there does not appear to be evidence of insufficient blood flow during handgrip exercise at 5% or 10% MVC.

Moderate Exercise. As anticipated, no evidence of inadequate perfusion was found for moderate exercise. Consistent with previous studies showing a minimal post-exercise hyperemia at light to moderate intensities (Van Beekvelt *et al.*, 2001; Wigmore *et al.*, 2006), peak and total recovery blood flow following moderate exercise were significantly lower than following heavy exercise. While several researchers have suggested post-exercise hyperemia to be indicative of inadequate flow during exercise (MacDonald *et al.*, 2001; Walloe & Wesche, 1988), this interpretation may depend on data analysis methodology.

Due to the highly oscillatory nature of blood flow during exercise, there can be a large difference between mean and peak flow. While mean data provides valuable information regarding average perfusion requirements, it does not address the manner in which those requirements are fulfilled. Peak flow, seen during relaxation phases of the contraction cycle, may be a more accurate reflection of the intermittent cycle of oxygen delivery seen during dynamic exercise. If the first seconds of recovery are interpreted by

the body as a prolonged relaxation phase, comparison of peak exercise to peak recovery blood flow may be a more representative figure. Others who have addressed the issue from this vantage point found, as seen here, that there was no increase in peak flow during recovery (Lutjemeier *et al.*, 2005). Rather, the rapidly decreasing blood flow seen during recovery may simply reflect a dynamic return to the resting state.

Heavy Exercise. Mean and peak exercise blood flow as well as peak recovery blood flow following heavy exercise were consistent with those previously reported for this and higher workloads (MacDonald *et al.*, 2001; Van Beekvelt *et al.*, 2001). Recovery blood flow and area under the curve did not, however, increase with progressively increasing exercise duration as would be anticipated based on data from occlusive and isometric exercise studies (Osada *et al.*, 2003; Toth *et al.*, 2007; Mullen *et al.*, 2001; Leeson *et al.*, 1997) if perfusion were in fact insufficient. As peak recovery blood flow was significantly less than peak exercise blood flow for all but the shortest exercise duration, flow between muscle contractions was likely sufficient to support metabolic requirements.

Due to the disparity between present results and previous evidence of blood flow impairment during heavy exercise (Walloe & Wesche, 1988; Bonde-Petersen *et al.*, 1975; MacDonald *et al.*, 2001; Osada *et al.*, 2003), further consideration of the exercise protocol is warranted. With a duty cycle of 33%, two thirds of the exercise time was spent with forearm musculature in a relaxed state. A threshold duty cycle in skeletal muscle may exist below which blood flow during relaxation periods is sufficient to offset limitations during contractions as is the case in the diaphragm (Bellemare *et al.*, 1983; Bark *et al.*, 1987; Hussain *et al.*, 1989). Additionally, increasing exercise intensity

beyond 10% MVC would further increase intramuscular pressure during contractions and amplify resistance to blood flow, although this would create other complications as discussed in Chapter 3.

Methodological Limitations. There are a number of methodological limitations which must be considered when interpreting the results of the present study. First, recovery blood flow responses were tracked for five minutes following the offset of exercise for calculation of area under the curve, or total post-exercise hyperemia. For some bouts of exercise, particularly longer bouts of heavy intensity, the hyperemic response may extend much longer than 5 minutes. If this were the case our AUC results may underestimate the hyperemic response and obscure any differences present later in the recovery period. Secondly, we must consider the power of our statistical analysis given the number of subjects studied. The power of our results ranged from 0.05 for the duration effects of blood flow at moderate exercise and area under the curve to 0.446 for duration effect of mean blood flow during heavy exercise and 0.976 for the intensity effect of area under the curve. Clearly a larger sample size would increase the power of these calculations, though the results we saw were quite consistent qualitatively and we would expect a similar trend to continue if more subjects were tested.

Conclusion. Although duration of dynamic handgrip exercise was incrementally increased from 3 to 9 minutes, a time range in which increases in PORH and post-isometric exercise hyperemia have been seen, there were no corresponding increases in peak or total post-exercise hyperemia following either moderate or heavy intensity exercise. This suggests sufficient blood flow was present during exercise such that there was no accumulation of oxygen debt in the longer exercise durations. While our heavy

intensity, 10% MVC, has been shown to impede blood flow in the past, the duty cycle of

the present study may have allowed adequate blood flow between muscle contractions to

sustain oxygen requirements.

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Chapter Five

Concluding Remarks

Summary

This dissertation was comprised of two studies that were designed to address the following specific aims; 1) to test the hypothesis that two different muscle contraction patterns produce different mean blood flow responses at the same work load, and 2) to test the hypothesis that post-exercise hyperemia will incrementally increase as exercise duration increases. In contrast to our first hypothesis, we found dynamic and intermittent isometric handgrip exercise to result in similar steady state blood flow responses. This is consistent with previous research showing a tight coupling between blood flow and metabolic rate or work performed. However, these similar perfusion rates were associated with markedly different times to task failure. This finding suggests blood flow, and presumably oxygen delivery, is not a key factor in determining exercise tolerance and muscle fatigue. In addition, there was no relationship between iEMG and exercise tolerance, with similar motor unit recruitment in both dynamic and intermittent isometric trials. As the study was limited to healthy young subjects exercising a small, isolated muscle mass, it remains unclear if these findings translate to populations with impaired circulation or to whole body exercise.

Our second hypothesis was tested with both moderate and heavy intensity dynamic handgrip exercise. In contrast to the hypothesis, we found no relationship between exercise duration and measures of post-exercise hyperemia at either exercise intensity. At the moderate intensity, this result adds support to the view that muscle contracting at light to moderate work rates is adequately perfused. The similar post-exercise hyperemia following heavy exercise ranging from 3 to 9 min conflicts with previous findings which suggested that muscle blood flow was impeded during heavy to severe intensity exercise. It is plausible increased flow during relaxation phases of the dynamic exercise allowed for sufficient perfusion despite any limitations caused by high intramuscular pressure during contraction phases. Further examination of oscillations in blood flow throughout the contraction cycle is necessary to address this issue.

Direction for Future Research

As our first study has found no relationship between limb blood flow and exercise tolerance, a further investigation into physiological causes of task failure is warranted. It is likely that a number of mechanisms contribute toward muscle fatigue and task failure, and the proportional influence of each may vary depending on exercise demands and subject characteristics. In this case, the 50% duty cycle of intermittent isometric exercise allowed for some recovery between contractions whereas the constant repetition of eccentric and concentric contractions of the dynamic condition did not. A comparison of sustained isometric exercise to the two conditions observed here would lend further insight. One limitation of the methods employed here is the ability to measure blood flow only at the conduit artery level. Distribution of blood flow among the active and inactive portions of the forearm and oxygen content of that blood were not measured. While it is

difficult to non-invasively target blood flow measurements to a working muscle or muscle group, use of near infrared spectroscopy to assess oxy-hemoglobin and myoglobin levels of the superficial forearm muscles could provide data regarding the relative availability of oxygen during various muscle contraction patterns.

Results from study two pertaining to moderate intensity exercise confirmed the long-held belief that blood flow is sufficient in this exercise domain. However, the unexpected finding that hyperemia following heavy exercise was not dependent on exercise duration invites further study. It is unknown if this result is due to the technique used to identify blood flow limitation, the subject pool, or exercise protocol utilized in this study. Follow-up research altering the exercise protocol is warranted due to overwhelming evidence showing increases in hyperemia following both cuff occlusion and sustained isometric exercise. With a duty cycle of 33%, two thirds of the exercise time was spent with forearm musculature in a relaxed state. A threshold duty cycle may exist below which blood flow during relaxation periods is sufficient to offset limitations during contractions. Additionally, increasing exercise intensity beyond 10% MVC would further increase intramuscular pressure during contractions and amplify resistance to blood flow.

In both studies presented here a healthy young adult population was tested. As a group these subjects are unlikely to experience circulatory limitations during exercise, particularly during small muscle mass exercise as performed here. Future investigations of blood flow adequacy and its role in exercise tolerance and recovery should include subjects with impaired vascular function. Older persons and those with peripheral or central vascular disease are more likely to show signs of perfusion limitation during

exercise. Therefore, the primary factors leading to task failure and delayed recovery may depend on both the exercise challenge itself and the physical condition of the specific subject group studied.

Appendix I: Medical History Questionnaire

Medical History Questionnaire

Name:_____ Age:_____ Sex:_____

DOB:_____ Weight (lbs):_____ (kg):_____ Height (in): _____ (cm):_____

Do you have any of the following health conditions?*	Yes	No
Family history of heart disease? i.e. Heart attack, bypass, stroke, or sudden death before age 55 in 1st degree male relative (father, brother, son) or before age 65 in 1st degree female relative (mother, sister, daughter)		
Smoking habit? i.e. Current cigarette smoker or one who has quit within the previous 6 months		
High blood pressure? i.e ≥140/90 on two separate occasions or currently on antihypertensive medication		
Abnormal cholesterol levels? i.e. Total Cholesterol >200mg/dL, or LDL >130 mg/dL, or HDL <35 mg/dL, or currently on lipid lowering medication		
High fasting glucose? i.e Fasting blood glucose ≥110 on two separate occasions		
Are you inactive? i.e. Accumulate <30 minutes of moderate physical activity on most days of the week		
BMI _____ *For Office Use Only*		

If you can answer yes to *2 or more* above please obtain *medical clearance* for exercise from your *personal physician*.

Contact Information	
Street:	
City:	
Zip:	
Home Phone:	
Work:	
Cell:	
E-mail:	
Preferred contact method:	

Emergency Contact Information	
Name:	
Relationship:	
Home:	
Work:	
Cell:	

Do you currently have any of the following?*	Yes	No
Pain in the chest, neck, jaw, or arms?		
Shortness of breath at rest or with mild exertion?		
Dizziness or fainting?		
Difficulty breathing while lying down, relieved by sitting up?		
Awakened by shortness of breath?		
Swelling in your ankles?		
Rapid heart rate while at rest?		
Leg pain or cramping while walking, relieved with rest?		
Heart murmur?		
Unusual fatigue or shortness of breath with usual activities?		

If you can answer yes to *any* of the above please obtain *medical clearance* for exercise from your *personal physician*.

Prescribed Medications:

_____

_____

Allergies: _____

Supplements (nutritional/athletic): _____

_____

Do you have an orthopedic condition/arthritis that may limit your activity?

_____

Are you pregnant? If so, how many weeks?

_____

Do you have any other problems or medical conditions not addressed on this form?

_____

Do you have a history of the following?*	Yes	No
Heart attack os stroke?		
Heart surgery (CABG, angioplasty)?		
Metabolic disorder (diabetes, kidney, thyroid)?		
Respiratory problems (asthma, COPD)?		
Hospitalization or surgery within the last 6 months?		

If you can answer yes to *any* of the above please obtain *medical clearance* for exercise from your *personal physician*.

How long have you had your medical condition(s)?

_____

Signature:_____

Date: _____

* Adapted from ACSM's Guidelines for Exercise Testing and Prescription Sixth Edition

Appendix II: Informed Consent

EFFECT OF EXERCISE DURATION ON POST-EXERCISE HYPEREMIA

Principal Investigator: Barry W. Scheuermann, Ph.D.

Other Staff (identified by role):   Allison Harper, MS

Contact Phone number(s):   (419) 530-2058

What you should know about this research study:

- We give you this consent/authorization form so that you may read about the purpose, risks, and benefits of this research study. All information in this form will be communicated to you verbally by the research staff as well.

- Routine clinical care is based upon the best-known treatment and is provided with the main goal of helping the individual patient. The main goal of research studies is to gain knowledge that may help future patients.

- We cannot promise that this research will benefit you. Just like routine care, this research can have side effects that can be serious or minor.

- You have the right to refuse to take part in this research, or agree to take part now and change your mind later.

- If you decide to take part in this research or not, or if you decide to take part now but change your mind later, your decision will not affect your routine care.

- Please review this form carefully. Ask any questions before you make a decision about whether or not you want to take part in this research. If you decide to take part in this research, you may ask any additional questions at any time.

- Your participation in this research is voluntary.

PURPOSE (WHY THIS RESEARCH IS BEING DONE)

You are being asked to take part in a research study of handgrip exercise and post-exercise blood flow. The purpose of the study is to determine if there is a relationship between handgrip exercise duration and the peak or total amount of blood flow in the first five minutes following exercise.

You were selected as someone who may want to take part in this study because you have expressed an interest in exercise physiology research. A maximum of 30 volunteers will take part in this study.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT

If you decide to take part in this study, you will be asked to make a total of 4-6 visits to the Cardiopulmonary and Metabolism Research Laboratory.

During the first visit, all of the procedures and exercise protocol will be explained to you and you will be asked to complete an informed consent form and medical history questionnaire. Standard measurements of height, weight, forearm volume, and forearm muscle strength will also be made during the first visit. Forearm volume will be measured by placing your hand and forearm in a container of water and maximal forearm muscle strength will be determined using a standard handgrip strength device. This visit should last approximately 30 minutes.

During each subsequent visit, you will be asked to perform several bouts of handgrip exercise ranging in duration from 3-9 minutes at both 5% and 10% of your maximal handgrip strength according to the values measured during your first visit. A rest period will be provided between each bout of exercise. While you are performing the handgrip exercise, and for 5 minutes following completion of exercise, we will continue to measure the diameter of your brachial artery and your brachial artery blood velocity by placing an ultrasound probe on top of your skin just above your elbow. Each of these visits will last approximately 45-60 minutes.

RISKS AND DISCOMFORTS YOU MAY EXPERIENCE IF YOU TAKE PART IN THIS RESEARCH

As with any exercise regimen, there is a very small risk of heart attack. This risk is greatly minimized since you will be exercising the muscles of your forearm only. You may stop the test at any time if you feel need to do so. There is also the possibility of muscle soreness 24-48 hours after you exercise. Potential soreness should be limited to the exercising forearm, and be minimal due to the short duration and moderate intensity of the exercise.

POSSIBLE BENEFIT TO YOU IF YOU DECIDE TO TAKE PART IN THIS RESEARCH

There is no direct benefit from participating in this study to the participants. Students from the Department of Kinesiology who participate in this study will be exposed to current research topics and techniques. We cannot and do not guarantee or promise that you will receive any benefits from this research.

COST TO YOU FOR TAKING PART IN THIS STUDY

There are no associated costs for participating in this study.

PAYMENT OR OTHER COMPENSATION TO YOU FOR TAKING PART IN THIS RESEARCH
If you decide to take part in this research you will not receive any payment or compensation for participation in this research.

ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH
No alternative procedures or treatments will be made available since this research does not incorporate any procedures or treatments that affect the participant.

CONFIDENTIALITY - (USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION)
By agreeing to take part in this research study, you give to The University of Toledo (UT), the Principal Investigator and all personnel associated with this research study your permission to use or disclose health information that can be identified with you that we obtain in connection with this study. We will use this information for the purpose of conducting the research as described in the research consent/authorization form.

The information that we will use or disclose includes the data collected as described in the procedures section. We will only use this information for ourselves as part of the research study. Under some circumstances, the Institutional Review Board and Research and Sponsored Programs of the University of Toledo may review your information for compliance audits. We may also disclose your protected health information when required by law, such as in response to judicial orders.

The University of Toledo is required by law to protect the privacy of your health information, and to use or disclose the information we obtain about you in connection with this research study only as authorized by you in this form. Subjects are assigned a subject number, and only that number will appear in data files to protect subject confidentiality. There is a possibility that the information we disclose may be re-disclosed by the persons we give it to, and no longer protected. However, we will encourage any person who receives your information from us to continue to protect and not re-disclose the information.

Your permission for us to use or disclose your protected health information as described in this section is voluntary. However, you will <u>not</u> be allowed to participate in the research study unless you give us your permission to use or disclose your protected health information by signing this document.

You have the right to revoke (cancel) the permission you have given to us to use or disclose your protected health information at any time by giving written notice to Dr. Barry Scheuermann or Allison Harper at the Cardiopulmonary and Metabolism Research Lab. However, a cancellation will not apply if we have acted with your permission, for example, information that already has been used or disclosed prior to the cancellation. Also, a cancellation will not prevent us from continuing to use and disclose information that was obtained prior to the cancellation as necessary to maintain the integrity of the research study.

Except as noted in the above paragraph, your permission for us to use and disclose your protected health information has no expiration date.

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A more complete statement of University of Toledo's Privacy Practices is set forth in its Joint Notice of Privacy Practices. If you have not already received this Notice, a member of the research team will provide this to you. If you have any further questions concerning privacy, you may contact the University of Toledo's Privacy Officer at 419-383-3413.

IN THE EVENT OF A RESEARCH-RELATED INJURY
In the event of injury resulting from your taking part in this study, treatment can be obtained at a health care facility of your choice. You should understand that the costs of such treatment will be your responsibility. Financial compensation is not available through The University of Toledo or The University of Toledo Medical Center. By signing this form you are not giving up any of your legal rights as a research subject.

In the event of an injury, contact: Dr. Barry Scheuermann (419.530.2692) or
Allison Harper (419.530.2058)

VOLUNTARY PARTICIPATION
Taking part in this study is <u>voluntary</u>. You may refuse to participate or discontinue participation at any time without penalty or a loss of benefits to which you are otherwise entitled. If you decide not to participate or to discontinue participation, your decision will not affect your future relations with the University of Toledo or The University of Toledo Medical Center.

NEW FINDINGS
<u>You will be notified of new information that might change your decision to be in this study if any becomes available.</u>

OFFER TO ANSWER QUESTIONS
Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over. If you have questions regarding the research at any time before, during or after the study, you may contact Dr. Barry Scheuermann (419.530.2692) or Allison Harper (419.530.2058).

If you have questions beyond those answered by the research team or your rights as a research subject or research-related injuries, please feel free to contact the Chairperson of the University of Toledo Biomedical Institutional Review Board at 419-383-6796.

SIGNATURE SECTION (Please read carefully)

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ THE INFORMATION PROVIDED ABOVE, YOU HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND YOU HAVE DECIDED TO TAKE PART IN THIS RESEARCH.

BY SIGNING THIS DOCUMENT YOU AUTHORIZE US TO USE OR DISCLOSE YOUR PROTECTED HEALTH INFORMATION AS DESCRIBED IN THIS FORM.

The date you sign this document to enroll in this study, that is, today's date, MUST fall between the dates indicated on the approval stamp affixed to the bottom of each page. These dates indicate that this form is valid when you enroll in the study but do not reflect how long you may participate in the study. Each page of this Consent/Authorization Form is stamped to indicate the form's validity as approved by the UT Biomedical Institutional Review Board (IRB).

Name of Subject (please print)	Signature of Subject or Person Authorized to Consent	Date

			a.m.
Relationship to the Subject	(Healthcare Power of Attorney authority or Legal Guardian)	Time	p.m.

Name of Person Obtaining Consent (please print)	Signature of Person Obtaining Consent	Date

Name of Witness to Consent Process (when required by ICH Guidelines) (please print)	Signature of Witness to Consent Process (when required by ICH Guidelines)	Date