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The Effect of Eccentric Exercise-Induced Muscle Injury on Vascular Function and Muscle Blood Flow

by

Mitchel R. Stacy

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Exercise Science

Dr. Barry W. Scheuermann, Committee Chair

Dr. Suzanne Wambold, Committee Member

Dr. David Weldy, Committee Member

Dr. John Thistlethwaite, Committee Member

Dr. Patricia R. Komuniecki, Dean
College of Graduate Studies

The University of Toledo

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An Abstract of

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The purpose of this dissertation was to examine the effect of eccentric exercise-induced muscle injury on local vascular function and muscle blood flow in humans. Healthy, male subjects performed maximal eccentric contractions to induce muscle injury to the forearm flexor muscles, with subsequent changes in maximal isometric strength and vascular responses assessed at one, 24, 48, and 96 hours post-injury. Endothelial-dependent and-independent vasodilation was measured using brachial artery flow-mediated dilation (FMD) and sublingual nitroglycerin administration, respectively. Mean blood velocities were measured by Doppler ultrasound and later used for the calculation of blood flow and the shear stress area under the curve ($SS_{AUC}$). Subjects performed submaximal handgrip exercise 48 hours following injury and muscle blood flow was assessed via Doppler ultrasound.

Eccentric exercise resulted in significant decreases in maximal isometric strength for up to 96 hours. Endothelial-dependent and –independent vasodilation was also significantly impaired for up to 96 hours following eccentric-induced injury. The shear stress stimulus ($SS_{AUC}$) responsible for the FMD response was significantly reduced from...
one to 48 hours post-injury. However, resting blood pressure and blood flow remained the same throughout the duration of the study despite an increase in brachial artery diameter at one and 48 hours following eccentric exercise. Additionally, the muscle blood flow response to dynamic sub-maximal handgrip exercise was not significantly different 48 hours post-injury.

In summary, these results suggest that skeletal muscle injury results in prolonged impairment of local vascular function without influencing subsequent muscle blood flow at rest or during sub-maximal exercise.
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Chapter 1

Introduction

Dysfunction of the vascular endothelium has been shown to play a role in both the initiation and pathogenesis of cardiovascular disease, with endothelial dysfunction representing one of the earliest events (Ross 1993). Endothelial dysfunction may manifest within the coronary vasculature, peripheral vasculature, or both prior to the diagnosis of coronary artery disease (Drexler 1997). Dysfunction of the endothelium that occurs in the diseased state can be attributed to pro-inflammatory conditions associated with the aging process (Di Francescomarino et al. 2009), poor dietary habits or obesity (Rocha and Libby 2009), and increased levels of oxidative stress (Di Francescomarino et al. 2009).

Regular exercise, which is known to promote improvements in endothelial function (Shephard and Balady 1999) and reduce the risk of cardiovascular disease (Myers et al. 2002; Sesso et al. 2000; Thompson et al. 2003), may provide an easy and effective method for the therapeutic treatment of clinical populations (Gokce et al. 2002; Kasikcioglu et al. 2005). Evidence suggests that improvements in flow-mediated dilation (FMD) are associated with long term exercise training in healthy and clinical populations.
(Gokce et al. 2002; Kasikcioglu et al. 2005), while moderate intensity (acute) exercise also improves FMD (Harvey et al. 2005) and other measures of endothelial function (Bode-Boger et al. 1994; Gill et al. 2004). Additionally, aerobic exercise has also been shown to induce increases in endothelium-dependent vasodilation in sites that are not primary working muscles (DeSouza et al. 2000; Higashi et al. 1999), indicating that a systemic adaptation of the endothelium may occur as a result of regular exercise. However, muscle injury and soreness is often associated with unaccustomed exercise, especially when eccentric (muscle lengthening) contractions are incorporated (Clarkson and Hubal 2002). Eccentric exercise can result in subsequent decreases in muscle strength, flexibility, and range of motion, while also leading to muscle soreness and inflammation (Clarkson and Hubal 2002; Ebbeling and Clarkson 1989; Newham et al. 1987; Tidball 2005). Despite the known negative effects of eccentric exercise on skeletal muscle, the potential effects on vascular function have not been clarified.

Previous research on vascular function following eccentric exercise-induced muscle injury has shown that acute injury to either a small (unilateral arm) or large muscle mass (bilateral leg press) leads to significant increases in carotid-femoral pulse wave velocity at 48 hours post-injury, indicating that eccentric resistance exercise may increase systemic arterial stiffness (Barnes et al. 2010). However, Okamoto et al. (2009) found that brachial artery FMD and brachial-ankle pulse wave velocity were unaffected following eight weeks of eccentric resistance training. This finding suggests that eccentric exercise-induced vascular dysfunction may only be an acute, temporary response.
Mediators of the post-injury inflammatory response may be associated with subsequent changes in vascular function following eccentric exercise, as cytokines have been shown to impair endothelium-dependent vasodilation (Bhagat and Vallance 1997) and C-reactive protein (CRP) can decrease the expression of endothelial-derived nitric oxide synthase (eNOS) (Schwartz et al. 2007; Venugopal et al. 2002). Further supporting the role of inflammation on vascular function, induced systemic inflammation has been found to increase arterial stiffness (Vlachopoulos et al. 2005) and impair endothelial-dependent vasodilation (Hingorani et al. 2000).

In addition to the potential effects on vascular function, eccentric-induced muscle injury has also been shown to increase muscle blood flow (Sbriccoli et al. 2001), oxygen saturation (Ahmadi et al. 2008a; Ahmadi et al. 2008b), and capillary diameter at rest (Kano et al. 2005). It has been suggested by Ahmadi et al. (2008a) that an increased oxygen demand may exist for repair processes within the damaged skeletal muscle. In agreement with this assumption, increased hemoglobin kinetics (Ahmadi et al. 2008a) and blood flow (Laaksonen et al. 2006) has been found within injured muscle during subsequent exercise. However, Ahmadi et al. (2008b) have also shown decreased hemoglobin kinetics, which may indicate an impaired ability of the injured muscle to extract circulating oxygen during exercise. In support of this argument, Kano et al. (2005) found a decrease in microvascular oxygen pressure during electrically stimulated contractions, suggestive of impaired oxygen delivery following eccentric injury.

Although it appears that vascular disruption may be a consequence of eccentric-induced muscle injury, the vascular responses have yet to be clarified. Specifically, the
effects on endothelial-dependent and endothelial-independent vasodilation following acute eccentric exercise have not been thoroughly examined in humans. Additionally, muscle blood flow responses to exercise following eccentric injury have produced conflicting results. Further investigation is warranted to provide a better understanding of how vascular function is influenced following muscle injury.

1.1 Purpose

Given the potential negative effects that eccentric exercise-induced muscle injury may have on vascular control, the objectives of this study are: i) to examine the effect of a single bout of eccentric arm exercise on local endothelial-dependent and -independent vasodilation via the flow mediated dilation technique and nitroglycerin administration, and ii) to determine the effect of eccentric exercise on muscle blood flow during subsequent dynamic handgrip exercise.

1.2 Hypotheses

We hypothesize that exercise-induced muscle injury will result in significant reductions in vascular function (as assessed by FMD and NTG), with function becoming restored as muscle strength returns to baseline levels. Furthermore, we hypothesize that eccentric muscle injury will not affect the metabolic requirements of exercise and the muscle blood flow response to subsequent submaximal handgrip exercise will remain similar between conditions.
Chapter 2

Literature Review

2.1 The Endothelium

The vascular endothelium is the largest endocrine organ in the body, consisting of a single layer of cells lining the inner layer of all blood vessels (Caramori and Zago 2000). Specific functions of the endothelium include: maintenance of the semi-permeable barrier responsible for the passage of substances from circulation to peripheral tissues, synthesis and release of a variety of cytokines and growth factors, turnover and oxidation of the lipoproteins in the arterial wall, maintenance of the collagen and proteoglycans in the structure of the basal membrane, and also as a nonthrombogenic surface for leukocytes and platelets (Caramori and Zago 2000). Additionally, the endothelium is involved in the secretion and modification of vasoactive substances which contribute to the regulation of vascular tone (Vanhoutte 2003). During instances of injury; however, endothelial cells lining the endothelium can become “activated,” and undergo a change in function as well as morphology. Some of the changes associated with endothelial cell activation are: a loss of vascular integrity, an increased expression.
of leukocyte adhesion molecules, a change in phenotype from antithrombotic to prothrombotic, an increase in cytokine production, and an upregulation of human leukocyte antigen (HLA) molecules (Hunt and Jurd 1998). Ultimately, these changes may lead to endothelial dysfunction, a condition generally characterized by an imbalance of endothelium-derived vasoactive substances, coagulation mediators, and/or cell growth and proliferation factors (Caramori and Zago 2000).

2.2 Endothelial Dysfunction and Cardiovascular Disease

Dysfunction of the vascular endothelium has been shown to be associated with both the initiation and pathogenesis of cardiovascular disease, with endothelial dysfunction representing one of the earliest events (Ross 1993). Endothelial dysfunction may manifest itself within the coronary vasculature, peripheral vasculature, or both prior to the diagnosis of coronary artery disease (Drexler 1997). Dysfunction that occurs in the diseased state has been attributed to the pro-inflammatory conditions that are associated with the aging process (Di Francescomarino et al. 2009), poor dietary habits or obesity (Rocha and Libby 2009), and increased levels of oxidative stress (Di Francescomarino et al. 2009).

There is considerable evidence associating the excessive production of free radicals during the inflammatory process with the pathology of endothelial dysfunction and development of atherosclerosis (Droge 2002; Halliwell 2001; Vendrov et al. 2007; Zhang et al. 2006). An example of how free radicals may affect the vascular endothelium is through the oxidation of low-density lipoprotein (LDL), as elevated levels of oxidized LDL have been implicated in the pathology of atherosclerosis (Packard and Libby 2008;
Rocha and Libby 2009). Following oxidation in the arterial intima, LDL particles can be responsible for the release of inflammatory mediators and the expression of adhesion molecules via endothelial and smooth muscle cell activation (Witzum and Steinberg 1991). Leukocyte accumulation can also occur in the subendothelial space, resulting in additional recruitment of inflammatory cells and increased oxidation of LDL. Monocytes within the arterial intima can ultimately become lipid laden cells (foam cells) as they internalize local oxidized LDL particles via scavenger receptors (Libby 2002). As these intimal macrophages internalize LDL particles, they can promote local vascular damage through the release of multiple mediators, as well as contribute to cell apoptosis, resulting in the addition of antigenic and thrombogenic debris within the vascular lesion and causing subsequent atheroma progression (Rocha and Libby 2009).

The interaction of oxidized LDL with its receptor, oxidized LDL receptor-1 (LOX-1), has been suggested as a contributing factor in endothelial dysfunction in atherogenesis, as it has been shown to stimulate endothelial generation of superoxide radicals and lead to the inactivation of the vasodilator nitric oxide (NO) (Cominacini et al. 2001; Sawamura et al. 1997). Reactive oxygen species (ROS) production via oxidized LDL/LOX-1 interaction may also lead to the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (Roebuck 1999) and CD40/CD40 ligand (Li et al. 2003), resulting in increased expression of vasoconstrictive molecules, adhesion molecules, and chemokines through gene transcription in endothelial cells (Li et al. 2002). Increased expression of LOX-1 has also been shown in the aorta and veins of hypertensive rats, further supporting its potential role in endothelial dysfunction (Nagase et al. 1997).
2.3 Assessment of Endothelial Function

Modifications in endothelial function can be assessed with a variety of methods, such as: 1) measurement of morphological and mechanical characteristics of the vascular wall (intima media thickness, compliance, distensibility, and remodeling indexes); 2) determination of soluble endothelial markers (von Willebrand factor, plasminogen activator, inhibitor complex thrombomodulin adhesion molecules, and N-oxides); and 3) measurement of the endothelial-dependent regulation of vascular tone at focal sites of the circulation (Kelm 2002). The use of high resolution B-mode ultrasonography imaging, which provides a direct, noninvasive measure for endothelial function, has become a common method incorporated within many intervention studies (Thijssen et al. 2011).

2.3.1 Flow-Mediated Dilation

The technique of flow-mediated dilation (FMD), originally described by Celermajer et al. (1992), is a common and effective way to measure endothelial-dependent vasodilation (Thijssen et al. 2011). Flow-mediated dilation creates a flow stimulus following temporary limb occlusion and subsequent vasodilatory capacity of the endothelium is assessed. The FMD response has been shown to be dependent on the endothelium (Pohl et al. 1986), primarily nitric oxide dependent (Joannides et al. 1995), and reproducible at rest as well as following acute aerobic exercise (Harris et al. 2007). The clinical relevance of FMD is apparent, given that individuals who have impaired endothelial function in coronary arteries also have diminished FMD values (Anderson et al. 1995) and patients with peripheral arterial disease (PAD) who have suffered from cardiovascular events are associated with lower FMD responses (Brevetti et al. 2003).
2.3.2 *Physiology of Flow-Mediated Dilation*

Multiple studies have shown the FMD response to be primarily dependent on endothelium-derived NO (Joannides et al. 1995; Meredith et al. 1996; Mullen et al. 2001). As endothelial cells are exposed to increased levels of shear stress following arterial occlusion, calcium-activated potassium channels on endothelial cell membranes are opened, resulting in the hyper-polarization of endothelial cells and increasing the driving force for calcium entry (Cooke et al. 1991; Miura et al. 2001; Olesen et al. 1988). Calcium and Akt/protein kinase B can independently activate endothelial nitric oxide synthase (eNOS), which facilitates the conversion of NO from the amino acid L-arginine (Dimmeler et al. 1999a; Dimmeler and Zeiher 2003). Nitric oxide diffuses from endothelial cells into vascular smooth muscle cells and reacts with the haem group of the enzyme guanylate cyclase, activating the enzyme and catalyzing the production of cyclic guanosine monophosphate (cyclic GMP). Cyclic GMP activates protein kinase G (PKG) within smooth muscle cells which subsequently leads to the dephosphorylation of myosin light chains and the relaxation of vascular smooth muscle (Chen et al. 2002; Dakak et al. 1998; Joannides et al. 1995; Pohl and de Wit 1999).

Although the FMD response is thought to be primarily regulated by NO following the standard five minute ischemic period, longer durations of ischemia likely result in other mediators contributing to the FMD response (Mullen et al. 2001). Mullen et al. (2001) have shown that infusion of the nitric oxide synthase (NOS) inhibitor $N^\gamma$-monomethyl-L-arginine (1-NMMA) during 15 minute wrist occlusion produces a similar peak FMD response with a prolonged hyperemic response. This finding indicates that the
FMD response during increased periods of shear stress must be influenced by other mechanisms. Mullen et al. (2001) have suggested the response may be related to a sequential recruitment of mechanisms, with NO being the primary contributor during instances of acute shear stress, while other mechanisms potentially mediate the FMD response when the shear stress stimulus is prolonged.

In addition to NO, prostaglandins (Koller et al. 1993) and endothelium-derived hyperpolarizing factor (EDHF) (Busse et al. 2002) are other important vasoactive substances that contribute to endothelial-dependent vasodilation and may play a role in NO-independent FMD responses. The prostaglandin prostacyclin (PGI₂) is formed through the conversion of the fatty acid arachidonic acid by cyclo-oxygenase.

Prostacyclin activates the enzyme adenylate cyclase within smooth muscle cells, which catalyses the conversion of ATP to cyclic AMP (Vanhoutte 2003). Cyclic AMP then activates a phosphorylating enzyme, protein kinase A (PKA), which ultimately leads to vasodilation of vascular smooth muscle through similar actions asPKG (Ko et al. 2008).

Although the mechanisms are not completely known, endothelium derived hyperpolarizing factor (EDHF) is thought to manipulate vascular smooth muscle relaxation in a different way, through the activation of calcium-dependent potassium channels, resulting in an influx of potassium into the vascular smooth muscle cell and leading to hyperpolarization of smooth muscle cells. Hyperpolarization ultimately inhibits voltage-dependent calcium channels from opening and decreases intracellular phosphatidylinositides, resulting in reduced calcium entry in smooth muscle cells (Nelson et al. 1990).
2.3.3 Endothelial-Independent Function

In contrast to the assessment of endothelial-dependent function via the FMD technique, endothelial-independent function is commonly assessed by using an exogenous NO donor in the form of a nitroglycerin (NTG) spray or sublingual tablet to act directly on vascular smooth muscle and induce vasodilation (Thelen et al. 2008). Therefore, the response to NTG administration is considered an overall reflection of vascular smooth muscle function (Ducharme et al. 1999). Duration and amplitude of the vasodilation following NTG administration is generally more pronounced than during FMD assessment (+15% vs. +10%) (Kelm 2002), with maximal NTG-induced vasodilation occurring at approximately five minutes post-administration (Thelen et al. 2008). As the number of cardiovascular risk factors begins to increase, the response following NTG becomes less pronounced, indicating vascular smooth muscle dysfunction that is independent of endothelial dysfunction (Adams et al. 1998). For instance, hypertensive individuals have shown an attenuated response to NTG, suggesting overall vascular impairment; however, this may not automatically indicate an impairment of endothelial function (Gokce et al. 2001).

2.4 Exercise-Induced Improvements in Endothelial Function

Regular exercise, which is known to promote improvements in cardiovascular health (Shephard and Balady 1999) and reduce the risk of cardiovascular disease (Myers et al. 2002; Sesso et al. 2000), may improve endothelial function via several mechanisms. Exercise-induced increases in shear stress on the luminal surface of the endothelium are thought to be the primary stimulus for improvements, as shear stress has been found to upregulate antioxidant enzymes (Inoue et al. 1996; Takeshita et al. 2000), inhibit
apoptosis activity (Dimmeler et al. 1996; Dimmeler et al. 1999b), and increase the activation of eNOS (Dimmeler and Zeiher 2003) and production of prostacyclin (Frangos et al. 1985; Grabowski et al. 1985).

The benefits of exercise are apparent, given that aerobically trained individuals demonstrate greater brachial artery flows at rest while also exhibiting greater peak hyperemic flows and FMD responses (Libonati 2007). Exercise-induced improvements in endothelial function are also visible in a variety of subject populations, such as overweight men (Harris et al. 2008), postmenopausal women (Harvey et al. 2005), individuals suffering from PAD (Brendle et al. 2001), and patients with chronic heart failure (Hambrecht et al. 1998). Additionally, elderly men possessing the highest functional ability are associated with the highest FMD responses (when compared to less functional peers), suggesting that continued exercise and functional capacity are important determinants of endothelial function (Welsch et al. 2008).

2.5 Eccentric Exercise-Induced Muscle Injury

Although exercise has proven to be a therapeutic intervention for patients suffering from cardiovascular disease (Myers et al. 2002) and can result in improved endothelial function (Hambrecht et al. 1998), exercise-induced muscle injury is often a consequence associated with performing unaccustomed exercise, particularly if the exercise involves a large amount of eccentric (muscle lengthening) contractions (Clarkson and Hubal 2002). During eccentric contractions the muscle is lengthened and strain is placed on sarcomeres, extending them beyond their normal length and resulting in non-uniform lengthening. In normal circumstances, sarcomeres should lengthen and
stretch uniformly as tension is increased in the muscle; however, during high intensity eccentric contractions some proposed “weaker” sarcomeres are stretched too far and can no longer maintain the tension required as the muscle lengthens (Proske and Allen 2005). It is thought that this lengthening ultimately results in disruption of weaker sarcomeres and passive structures (i.e., connective tissue) taking over as support (Proske and Morgan 2001). In addition to disruption of the sarcolemma, damage can also occur to the transverse tubules (T-tubules), myofibrils, and the cytoskeleton (Armstrong et al. 1983; Friden et al. 1984; Friden et al. 1983). These alterations to the functional units of the muscle are thought to at least partially contribute to the prolonged reductions in muscle strength commonly observed following injurious eccentric exercise (Clarkson and Hubal 2002; Newham et al. 1987; Nosaka et al. 1991).

Even though structural damage to the muscle is obvious following eccentric exercise, decrements in muscle strength may also be related to changes in excitation-contraction coupling (E-C coupling) (Proske and Allen 2005; Yeung and Allen 2004). It has been shown that stretch-induced injury results in the opening of stretch-activated ion channels and leads to increased levels of sodium ($Na^+$) (McBride et al. 2000; Yeung and Allen 2004) and calcium ($Ca^{2+}$) (Balnave and Allen 1995) in the sarcoplasm. These ions may influence E-C coupling, as increased intracellular $Ca^{2+}$ can potentially trigger calcium-activated proteases associated with muscle breakdown and repair (Proske and Allen 2005), and increased $Na^+$ permeability can affect membrane depolarization (McBride et al. 2000). In support of these claims, Yeung et al. (2004) have shown that the blocking of stretch-activated ion channels following muscle injury reduces the impairments in muscle force production normally associated with muscle damage.
2.5.1 Inflammation Following Injury

Along with damage to the muscle, inflammation is another consequence of muscle injury. Neutrophils, along with monocytes and macrophages, serve an important role in the early stages of acute inflammation through the removal of necrotic tissue or cellular debris by phagocytosis. Additionally, invading phagocytic cells can introduce proteolytic systems, resulting in the degradation of muscle proteins within the damaged tissue. As neutrophils and macrophages rapidly invade the site of injury they also promote the inflammatory process by releasing cytokines, which are responsible for attracting and activating additional inflammatory cells (Tidball 1995). Additional cytokines may then be released by other inflammatory cells, as well as local muscle, endothelial, and satellite cells to aid in the regulation of the inflammatory process (Miles et al. 2008). A further consequence of neutrophil activation is the respiratory burst and degranulation of these cells, resulting in the release of ROS and possibly leading to additional damage to cell membranes during the early stages of inflammation (Toumi et al. 2006). At the molecular level, ROS influence fundamental biological processes, such as gene expression (Datta et al. 1992), signal transduction (Abe and Berk 1999), and enzyme activity (Kotsonis et al. 1999). However, exercise-induced muscle injury can significantly increase levels of these free radicals that may augment the oxidation of circulating lipids, proteins, and deoxyribonucleic acid (DNA) (Nikolaidis et al. 2008).

2.5.2 Vascular Function Following Eccentric Exercise

Eccentric exercise is specifically known to result in elevated levels of circulating inflammatory cells (Bruunsgaard et al. 1997; MacIntyre et al. 1996) and protein degradation (Lowe et al. 1995) while also leading to localized edema (Mair et al. 1992).
In addition to the regulation of muscle injury and repair, inflammation following eccentric exercise may also have a significant impact on local and systemic vascular function. In support of this argument, acute induced systemic inflammation has been shown to increase arterial stiffness (Vlachopoulos et al. 2005) and impair endothelial-dependent vasodilation (Hingorani et al. 2000). Furthermore, cytokines released by infiltrating inflammatory cells have been shown to impair endothelial-dependent vasodilation when locally infused (Bhagat and Vallance 1997), while the acute-phase reactant, C-reactive protein (CRP), can decrease the expression of endothelial NO synthase (eNOS) and conceivably affect the subsequent production of NO (Schwartz et al. 2007; Venugopal et al. 2002).

Prior studies examining the effects of eccentric exercise on vascular function have produced conflicting results (Barnes et al. 2010; Okamoto et al. 2009). Acute eccentric-induced muscle injury to either a small (unilateral arm) or large muscle mass (bilateral leg press) has been shown to significantly increase carotid-femoral pulse wave velocity at 48 hours post-injury, indicating an increase systemic arterial stiffness (Barnes et al. 2010). However, Okamoto et al. (2009) found that an eight week eccentric resistance training program did not lead to significant differences in brachial artery FMD response or brachial-ankle pulse wave velocity, suggesting that eccentric exercise-induced changes to vascular function may only be acute.

2.5.3 Muscle Blood Flow Following Eccentric Exercise

Given the effect that eccentric exercise may have on vascular function, it is also conceivable that muscle blood flow may be influenced following exercise. Increases in muscle blood flow following eccentric exercise have been found at rest (Sbriccoli et al. 2010).
levels of oxygen saturation (Ahmadi et al. 2008a; Ahmadi et al. 2008b) and increases in capillary diameter (Kano et al. 2005) have been observed at rest. These results are suggestive of an increased metabolic demand within the injured muscle which may be related to repair processes. In support of this hypothesis, Ahmadi et al. (2008a) have shown increased hemoglobin kinetics during subsequent exercise, indicating greater unloading of oxygen to the contracting muscle. Other studies examining microvascular function following eccentric exercise have produced conflicting results; however, and found decreased hemoglobin kinetics (Ahmadi et al. 2008b) and microvascular oxygen pressure (Kano et al. 2005) during subsequent muscle contractions. These findings are not only indicative of microvascular dysfunction, but also suggest a possible impairment of oxygen delivery to the muscle during subsequent exercise.

Although eccentric exercise appears to result in alterations to microvascular function and blood flow, Walsh et al. (2001) identified no change in resting oxygen utilization following eccentric injury and Laaksonen et al. (2006) demonstrated that exhaustive stretch-shortening cycling exercise did not alter resting blood flow or oxygen uptake during subsequent exercise. These conflicting results may be due to differences in exercise-induced injury protocols, the duration of the exercise performed, and variation between measurement techniques. Future studies are warranted to elucidate the effects of eccentric exercise on subsequent vascular function and muscle blood flow.
Chapter 3

The Effect of Eccentric Exercise-Induced Muscle Injury on Vascular Function

3.1 Introduction

Dysfunction of the vascular endothelium is one of the earliest events in the pathogenesis of cardiovascular disease (Ross 1993). Endothelial dysfunction may manifest itself within the coronary vasculature, peripheral vasculature, or both prior to the diagnosis of coronary artery disease (Drexler 1997). Endothelial dysfunction that occurs in the diseased state can be attributed to pro-inflammatory conditions associated with the aging process (Di Francescomarino et al. 2009), poor dietary habits or obesity (Rocha and Libby 2009), and increased levels of oxidative stress (Di Francescomarino et al. 2009). Regular exercise, which is known to promote improvements in cardiovascular health (Shephard and Balady 1999) and reduce the risk of cardiovascular disease (Myers et al. 2002; Sesso et al. 2000), may be of significant importance for improvements in endothelial function. Aerobically trained individuals are associated with greater brachial artery flows and diameters at rest when compared to healthy controls, while also exhibiting greater peak hyperemic flows and flow-mediated dilation (FMD) responses.
(Libonati 2007). The expression of factors influencing production of the vasodilator nitric oxide (NO) are shown to be stimulated with exercise (Dimmeler and Zeiher 2003; Fukai et al. 2000; Gokce et al. 2002; Green et al. 2004). Regular exercise is also correlated with the up-regulation of superoxide dismutase expression, which may prevent superoxide-mediated inhibition of NO production (Fukai et al. 2000).

Although exercise has proven to be an effective therapeutic intervention for improving endothelial function (Shephard and Balady 1999), exercise-induced muscle injury is often a consequence associated with performing unaccustomed exercise, particularly if eccentric (muscle lengthening) contractions are incorporated (Clarkson and Hubal 2002). Eccentric injury can result in 50-60 percent decrements in muscle force production immediately after exercise and losses in muscle strength may be apparent for one to two weeks post-injury (Newham et al. 1987; Nosaka et al. 1991; Saxton et al. 1995). Structural damage to the sarcolemma, T-tubules, myofibrils, and the cytoskeleton may contribute to these losses in muscle strength (Armstrong et al. 1983; Friden et al. 1984; Friden et al. 1983); however, inflammation is also a consequence of muscle injury (Tidball 2005).

Post-injury inflammation may contribute to vascular dysfunction, as induced systemic inflammation has been shown to increase arterial stiffness (Vlachopoulos et al. 2005) and impair endothelial-dependent vasodilation (Hingorani et al. 2000). Cytokines produced during the inflammatory response have also been shown to impair endothelial-dependent vasodilation (Bhagat and Vallance 1997), while acute-phase reactants such as
C-reactive protein (CRP) have been implicated in the decreased expression of endothelial nitric oxide synthase (eNOS) (Schwartz et al. 2007; Venugopal et al. 2002).

Despite the negative implications inflammation may have on vascular function, the effects of muscle injury and the subsequent inflammatory responses on vascular function are relatively unknown. Acute eccentric-induced muscle injury to either a small (unilateral arm) or large muscle mass (bilateral leg press) has lead to significant increases in carotid-femoral pulse wave velocity at 48 hours post-injury, indicating that resistance exercise may increase systemic arterial stiffness (Barnes et al. 2010). However, no significant differences in brachial artery FMD response or brachial-ankle pulse wave velocity were apparent following eight weeks of eccentric resistance training (Okamoto et al. 2009), suggesting that exercise-induced vascular dysfunction may only be an acute, temporary response. Therefore, the primary aim of this study was to examine the effect of acute, local eccentric exercise-induced muscle injury on local endothelial-dependent and endothelial-independent vasodilation.

3.2 Methods

3.2.1 Subjects. Ten healthy male subjects (22-34 years) participated in the research protocol. All individuals were void of cardiovascular, pulmonary, and metabolic disease as determined through a standard medical history questionnaire. The study was approved by the Institutional Review Board for Human Subjects Research and Review Committee at the University of Toledo and was in accordance with the guidelines set forth by the Declaration of Helsinki. Subjects reported to the Cardiopulmonary and Metabolism Research Laboratory located at The University of Toledo for testing following an
overnight fast and having abstained from any endurance or weight training for a week prior to their arrival. Additionally, subjects also abstained from any exercise regimen throughout the duration of their study participation. All individuals provided written informed consent after explanation of experimental procedures and potential risks associated with the study.

3.2.2 Experimental Protocol. Subjects were asked to visit the laboratory on four separate occasions. During the first visit, anthropometric measurements including height and weight were measured. Subjects were asked to rest quietly in the supine position for approximately 20 minutes prior to any measurements. A blood pressure measurement was taken while the subject rested comfortably, and following the rest period, endothelial-dependent function was assessed via the flow-mediated dilation technique. Following another 10 minute period of rest, subjects were administered nitroglycerin sublingually and endothelial-independent function was assessed. Maximal isometric strength of the forearm flexor muscles was determined to examine subsequent force decrements resulting from eccentric exercise. Subjects performed the eccentric exercise protocol (2 x 25 maximal eccentric contractions) following strength determination and then returned one hour later to undergo the same processes of flow-mediated dilation, nitroglycerin administration, and strength assessment.

On visits two through four, subjects returned 24 (visit two), 48 (visit three), and 96 (visit four) hours following the first visit (eccentric exercise). Each subsequent visit consisted of the same process of blood pressure measurement and the assessment of flow-mediated dilation, nitroglycerin administration, and strength assessment.
3.2.3 *Determination of Maximal Strength.* Subjects were asked to perform an isometric, maximal voluntary contraction (MVC) of the forearm flexor muscles at a fixed angle of 90° elbow flexion for approximately three seconds while seated on a standard arm curl bench. Forces were measured by using a force transducer (OMEGA LCCA-1K, Stamford, CT) on three separate trials, with no less than two minutes of rest allowed between each trial. The highest force attained out of the three trials was representative of each subject’s MVC.

3.2.4 *Eccentric Exercise.* Subjects performed two sets of 25 maximal eccentric contractions of the forearm flexor muscles on a standard arm curl exercise bench, with each contraction lasting approximately four seconds. An investigator provided manual resistance with each contraction as the subject maximally contracted and resisted throughout a range of motion consisting of approximately 45° to 180° of elbow flexion. Subjects were allowed five minutes of rest between each set of exercise.

3.2.5 *Measurement of Endothelial-Dependent Vasodilation.* The noninvasive technique of flow-mediated dilation (FMD) was used to measure endothelial-dependent vasodilation. Subjects rested quietly in the supine position for 20 minutes prior to any measurements. The left arm was slightly abducted at heart level as an echo-Doppler ultrasonography system (z.one ultra, ZONARE Medical Systems Inc., Mountain View, CA) with a 7 MHz ultrasound probe was used to obtain baseline images of the brachial artery. Once baseline images were acquired, a forearm occlusion cuff was inflated to a suprasystolic pressure of approximately 250 mmHg for a period of five minutes. Following the occlusion period, the cuff was rapidly deflated as ultrasound images were
continuously measured for two minutes to examine changes in brachial artery diameter. All images and blood velocities were recorded digitally to a computer and later analyzed using commercially available software (Medical Imaging Applications, LCC, Coralville, IA). The flow-mediated dilation response was expressed as the peak percent change from resting, baseline diameter following the five minute occlusion period.

3.2.6 Calculation of Blood Flow and Shear Stress. Brachial artery diameter and mean blood velocities were used from rest and following the FMD occlusion period to calculate blood flow and shear stress. Blood flow was calculated using the equation \( MBV \times CSA \times 60 \); where \( MBV \) is the mean blood velocity and \( CSA \) is the cross-sectional area of the brachial artery \( (CSA = \pi r^2, \text{ where } r \text{ is arterial radius}) \). Shear stress \( (SS) \) was calculated using the equation \( 4 \times \mu \times MBV / D \); where \( \mu \) is blood viscosity (assumed to be 0.05 dyne s cm\(^{-2}\)), \( MBV \) is the mean blood velocity, and \( D \) is brachial artery diameter (cm). The post occlusion shear stress area under the curve \( (SS_{AUC}) \) was calculated above baseline for 60 seconds following occlusion by using the trapezoidal rule.

3.2.7 Measurement of Endothelial-Independent Vasodilation. Endothelial-independent vasodilation was assessed via sublingual nitroglycerin (GTN) administration. Subjects rested comfortably in the supine position for approximately 10 minutes following the flow-mediated dilation test. After the rest period, sublingual nitroglycerin was administered and subsequent changes in vasodilation were continuously measured for six minutes and 30 seconds. The peak vasodilatory response from baseline, resting brachial diameter was expressed as a percent change and indicative of endothelial-independent vasodilation.
3.2.8 **Statistical Analysis.** A one-way analysis of variance (ANOVA) with repeated measures was used to identify differences across time within FMD, GTN, hemodynamic and maximal strength responses following eccentric exercise. Significant main effects and interactions were further analyzed using a Student-Newman-Keuls post-hoc test, with statistical significance set at $P \leq 0.05$. Associations were examined by linear regression modeling. All values are expressed as mean ± SEM unless stated otherwise.

3.3 **Results**

Subject demographics are presented in table 3.1. All subjects were void of cardiovascular, pulmonary, or metabolic disease and arrived for testing following an overnight fast. Mean resting blood pressure was not different following eccentric exercise (Post) and resting blood flow remained similar throughout the protocol duration (Table 3.2). Resting brachial artery diameter was significantly higher from pre-injury (Pre) baseline value at one and 48 hours post-exercise (Table 3.2; $P < 0.05$).

The eccentric exercise protocol was effective in producing muscle injury, as indicated by significant decreases ($P < 0.05$ vs. Pre) in maximal isometric strength at one, 24, 48, and 96 hours post-injury (Fig. 3-1; Table 3.2). On average, maximal strength was approximately 32 percent below Pre baseline level at one hour Post and a 12 percent decrement was still apparent at 96 hours Post (Fig. 3-1).

Endothelial-dependent vasodilation (FMD) was significantly reduced at one, 24, 48, and 96 hours Post ($P < 0.05$) with the most pronounced decreases in vasodilatory capacity occurring at one hour (60.9 ± 8.3 percent of Pre) and 48 hours Post (63.7 ± 7.6 percent of Pre; Fig. 3-2A). The shear stress stimulus ($SS_{AUC}$) responsible for the FMD
response was significantly reduced from one to 48 hours Post ($P < 0.05$; Fig. 3-2B). Endothelial-independent vasodilation following nitroglycerin administration significantly decreased at one, 24, 48, and 96 hours Post ($P < 0.05$; Fig. 3-3). No significant associations were found between the loss in muscle strength and the loss in FMD and GTN-mediated dilatory capacity following exercise ($P < 0.05$).

Table 3.1. Subject Demographics
$n = 10$ Subjects.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
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</tr>
<tr>
<td>Height, cm</td>
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<td>Body mass, kg</td>
<td>81.0 ± 6.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.6 ± 1.3</td>
</tr>
</tbody>
</table>
Table 3.2. *Hemodynamic and Muscle Injury Variables*.

$n=10$ Subjects. Values are expressed as mean ± SEM. *, significantly different from pre-injury baseline value ($P \leq 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Pre-Injury</th>
<th>1 Hr Post</th>
<th>24 Hr Post</th>
<th>48 Hr Post</th>
<th>96 Hr Post</th>
</tr>
</thead>
<tbody>
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<td>Systolic blood pressure, mmHg</td>
<td>121.2 ± 2.57</td>
<td>120.8 ± 2.13</td>
<td>120.0 ± 2.07</td>
<td>119.8 ± 2.20</td>
<td>120.4 ± 2.06</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>81.2 ± 2.44</td>
<td>79.8 ± 2.03</td>
<td>79.8 ± 2.24</td>
<td>79.6 ± 2.15</td>
<td>79.6 ± 2.00</td>
</tr>
<tr>
<td>Resting brachial artery diameter, mm</td>
<td>3.92 ± 0.11</td>
<td>4.19 ± 0.09*</td>
<td>4.02 ± 0.08</td>
<td>4.09 ± 0.09*</td>
<td>4.05 ± 0.07</td>
</tr>
<tr>
<td>Resting blood flow, ml/min</td>
<td>51.14 ± 7.90</td>
<td>51.2 ± 9.32</td>
<td>48.24 ± 6.62</td>
<td>44.50 ± 6.88</td>
<td>58.62 ± 7.90</td>
</tr>
<tr>
<td>Hyperemic shear stress AUC, dynes/cm²</td>
<td>2545.9 ± 62.0</td>
<td>2053.9 ± 147.3*</td>
<td>2086.2 ± 135.2*</td>
<td>2193.8 ± 142.3*</td>
<td>2363.5 ± 169.6</td>
</tr>
<tr>
<td>Flow-mediated dilation, %</td>
<td>8.4 ± 0.99</td>
<td>5.12 ± 0.69*</td>
<td>6.34 ± 0.69*</td>
<td>5.33 ± 0.63*</td>
<td>6.02 ± 0.96*</td>
</tr>
<tr>
<td>Nitroglycerin administration, %</td>
<td>26.3 ± 2.05</td>
<td>20.7 ± 1.50*</td>
<td>21.9 ± 1.58*</td>
<td>19.3 ± 1.91*</td>
<td>22.4 ± 1.47*</td>
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<tr>
<td>Maximal isometric strength, kg</td>
<td>17.1 ± 1.14</td>
<td>11.6 ± 1.32*</td>
<td>12.8 ± 1.39*</td>
<td>13.4 ± 1.34*</td>
<td>15.0 ± 1.49*</td>
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</tbody>
</table>
Figure 3-1. Maximal isometric strength following exercise-induced injury. Bars are means ± SEM. *, significantly different from pre-injury ($P < 0.05$).
Figure 3-2. Percent change in peak FMD responses following eccentric muscle injury (panel A) and the associated magnitude of the shear stress stimulus (panel B). Values are means ± SEM. *, significantly different from pre-injury baseline value ($P < 0.05$).
**Figure 3-3.** Percent change in peak nitroglycerin responses following eccentric muscle injury. Bars are means ± SEM. *, significantly different from pre-injury baseline value ($P < 0.05$).
3.4 Discussion

It is well known that eccentric exercise can result in significant reductions in muscle force generation (Newham et al. 1987; Nosaka et al. 1991) and that inflammation is a common response associated with muscle injury (Tidball 2005). However, the effects of exercise-induced muscle injury on vascular function are poorly understood. The most significant finding of the present study was that local, exercise-induced muscle injury resulted in significant reductions in local endothelial-dependent and –independent vasodilatory capacity for up to 96 hours post-injury. Additionally, the associated magnitude of the shear stress stimulus during flow-mediated assessment was significantly impaired for 48 hours following muscle injury. To our knowledge, this is the first study that has examined the acute effect of local exercise-induced muscle injury on local endothelial-dependent and –independent function.

The effects of inflammation have significant clinical relevance given that excessive production of free radicals during the inflammatory process can induce endothelial dysfunction and lead to the development of atherosclerosis (Droge 2002; Halliwell 2001; Vendrov et al. 2007; Zhang et al. 2006). Previous research investigating the effects of induced inflammation on vascular function have shown that acute inflammation increases arterial stiffness (Vlachopoulos et al. 2005) and impairs endothelial-dependent vasodilation in vivo (Hingorani et al. 2000; Nosaka et al. 1991). However, the effects of exercise-induced inflammation are less clear. Barnes et al. (2010) found increased central arterial stiffness 48 hours following acute eccentric exercise in small or large muscle masses. The findings of Okamoto et al. (2009),
however, indicate that an eccentric resistance training program does not affect vascular function, as shown by no significant difference in baseline brachial-ankle pulse wave velocity or brachial artery FMD. Cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) produced during inflammation may contribute to the acute impairments in vascular function following muscle injury, as each has been implicated in endothelial dysfunction (Bhagat and Vallance 1997; Vlachopoulos et al. 2005). Tumor necrosis factor-alpha has been shown to shorten the half-life of mRNA coding for nitric oxide synthase (Yoshizumi et al. 1993), conceivably reducing the subsequent production of NO. Additionally, CRP has also been shown to downregulate endothelial derived nitric oxide synthase (Schwartz et al. 2007).

Interestingly, a study by Nakajima et al. (2010) indicated significant increases in serum levels of CRP immediately following peak cycling exercise and after completion of leg resistance exercise to exhaustion, however, subsequent effects on vascular function were not assessed.

Although speculative, the changes in endothelial-dependent and –independent vasodilation in the present study may also be indicative of mechanical disruption to endothelial cells or the vascular smooth muscle supporting local blood flow to the injured muscle. Indeed, it is known that eccentric exercise can result in mechanical disruption of skeletal muscle (Clarkson and Hubal 2002) while also impairing local microvascular function (Kano et al. 2005) and vasodilator responses in the microcirculation of skeletal muscle in rats (Heap et al. 2006). However, it is not known if mechanical disruption of the skeletal muscle also translates into mechanical disruption of the endothelial cells and/or vascular smooth muscle of the conduit artery. Bhagat et al. (1997) found that the
administration of cytokines to a local venous site was only capable of impairing endothelial function for up to 24 hours. This suggests that additional circulating mediators of inflammation or local mechanical disruption of the vasculature may be contributing to the prolonged vascular dysfunction observed in the present study.

Increased blood flow following eccentric-induced muscle injury has been estimated in previous studies utilizing Doppler ultrasound (Sbriccoli et al. 2001) and near-infrared spectroscopy (NIRS) (Ahmadi et al. 2008b), possibly indicating an increased metabolic demand by the surrounding tissue for post-injury repair processes. However, a significant difference in resting blood flow was not found in the present study despite significant decreases in maximal strength. Significant increases in brachial artery diameter were observed at one and 48 hours following injury, which closely corresponds with Kano et al. (2005), who found an increase in capillary diameter following eccentric exercise. Additionally, the shear stress stimulus during FMD assessment was significantly decreased for up to 48 hours following injury. These changes may be suggestive of a decrease in the blood flow pressure gradient within the conduit artery, however, no significant differences were found in the resting blood pressure of individuals. This finding is also in agreement with a previous study by Barnes et al. (2010) that performed a similar muscle injury protocol.

Some limitations exist within the present study. Although significant decreases in muscle strength were observed, additional biomarkers and mediators associated with muscle injury and the subsequent inflammatory process were not measured. As previously discussed, these markers may have an important regulatory role in the
decreased vasodilatory responses shown following muscle injury. Additionally, endothelial function in other sites was not determined; however, the findings of Barnes et al. (2010) indicated that muscle injury to the same muscle mass as the present study was capable of significantly influencing macrovascular function. This suggests that endothelial dysfunction resulting from muscle injury may be systemic in nature. Future research examining the precise mechanisms associated with muscle injury and the resultant inflammatory and vascular responses are warranted to elucidate the findings of the present study.

Collectively, the results of the present study indicate that acute, local muscle injury leads to subsequent impairment of local vascular function in healthy male individuals, as indicated by decreased flow- and nitroglycerin-mediated vasodilation. These findings may provide a non-invasive, alternative method for inducing vascular dysfunction in humans for future research studies aimed at determining the relationship between muscle injury and vascular function. Additionally, the results of the present study might provide insight into the structuring of therapeutic exercise interventions in patient populations suffering from various forms of cardiovascular disease.
Chapter 4

Muscle Blood Flow Following Eccentric Exercise

4.1 Introduction

Exercise is a well known therapeutic intervention for patients suffering from cardiovascular disease (Shephard and Balady 1999) and can result in improved endothelial function (Libonati 2007). However, exercise-induced muscle injury is often a consequence associated with performing unaccustomed exercise, particularly if the exercise involves eccentric (muscle lengthening) contractions (Clarkson and Hubal 2002). Eccentric exercise protocols typically result in losses of 50-60 percent in muscle force production immediately after exercise and force decrements may be apparent for one to two weeks post-injury (Newham et al. 1987; Nosaka et al. 1991; Saxton et al. 1995). Structural damage to the sarcolemma, T-tubules, myofibrils, and the cytoskeleton may contribute to losses in muscle strength (Armstrong et al. 1983; Friden et al. 1984; Friden et al. 1983). However, inflammation is also a consequence of muscle injury (Tidball 2005).
Post-injury inflammation may contribute to vascular dysfunction, as induced systemic inflammation has been shown to increase arterial stiffness (Vlachopoulos et al. 2005) and impair endothelial-dependent vasodilation (Hingorani et al. 2000). Cytokines produced during the inflammatory response have also been shown to impair endothelial-dependent vasodilation (Bhagat and Vallance 1997), while acute-phase reactants such as C-reactive protein (CRP) decrease the expression of endothelial nitric oxide synthase (eNOS) (Schwartz et al. 2007; Venugopal et al. 2002). Additionally, eccentric exercise-induced muscle injury can significantly increase arterial stiffness (Barnes et al. 2010) while also significantly decreasing endothelial-dependent and –independent vasodilation (unpublished data).

Studies that have investigated muscle blood flow following eccentric-induced muscle injury have reported increased muscle blood flow at rest (Sbriccoli et al. 2001) and during exercise (Laaksonen et al. 2006), increased resting muscle oxygen saturation (Ahmadi et al. 2008a; Ahmadi et al. 2008b), and increased capillary diameter at rest (Kano et al. 2005). A decrease in microvascular oxygen \( (O_2) \) pressure during electrically stimulated contractions has also been shown (Kano et al. 2005), which may indicate impaired oxygen delivery within the microvascular circulation following injury. In line with this assumption, another study has shown decreased oxygen desaturation and re-saturation kinetics during exercise for up to six days post-injury (Ahmadi et al. 2008b), possibly indicating an impaired ability of oxygen to unload from hemoglobin and/or impairment of the injured muscle to extract circulating oxygen. However, contradicting results from Ahmadi et al. (2008a) found an increase in oxygen saturation kinetics at rest and during exercise. These findings indicate that oxygen unloading occurred to a greater
degree, and it has been suggested that an increased oxygen demand may exist within the damaged muscle for repair processes (Ahmadi et al. 2008a). In disagreement with the previously mentioned study, Walsh et al. (2001) identified no change in resting oxygen utilization following eccentric injury and Laaksonen et al. (2006) demonstrated that exhaustive stretch-shortening cycle exercise did not result in significant differences in resting blood flow or oxygen uptake during post-injury exercise.

Despite the fact that muscle injury is known to increase arterial stiffness (Barnes et al. 2010) and decrease endothelial function (unpublished data), conflicting results exist on the specific effects of acute, local muscle injury on local blood flow responses during exercise. Therefore, the primary aim of this study was to examine the effect of local eccentric exercise-induced muscle injury on muscle blood flow during sub-maximal exercise.

4.2 Methods

4.2.1 Subjects. Nine healthy male subjects (22-34 years) participated in the research protocol. All individuals were void of cardiovascular, pulmonary, and metabolic disease as determined through a standard medical history questionnaire. The study was approved by the Institutional Review Board for Human Subjects Research and Review Committee at the University of Toledo and is in accordance with the guidelines set forth by the Declaration of Helsinki. Subjects reported to the Cardiopulmonary and Metabolism Research Laboratory located at The University of Toledo for testing following an overnight fast and having abstained from vigorous exercise for a week prior to their arrival. Additionally, subjects also abstained from any exercise regimen throughout the
duration of their study participation. All individuals provided written informed consent after explanation of experimental procedures and potential risks associated with the study.

4.2.2 Experimental Protocol. Subjects were asked to visit the laboratory on two separate occasions. During the first visit, resting blood pressure and anthropometric measurements including height and weight were measured. Subjects were asked to rest quietly in the supine position for approximately 20 minutes prior to any measurements. Brachial artery diameter and blood pressure was measured while the subject rested comfortably. Following the rest period, maximal handgrip strength was determined for the calculation of handgrip exercise workload. Subjects then performed five minutes of sub-maximal handgrip exercise while brachial artery blood velocities were continuously measured. Following handgrip exercise, maximal isometric strength of the forearm flexor muscles was first determined, and then subjects performed an eccentric exercise protocol (2 x 25 maximal eccentric contractions).

Subjects returned for subsequent testing approximately 48 hours following their first visit. On the second visit, resting brachial artery diameter and blood pressure was measured. Subjects then performed the same handgrip exercise protocol as on visit one. Following the completion of exercise, maximal isometric strength was once again assessed.

4.2.3 Determination of Maximal Strength. Subjects were asked to perform an isometric, maximal voluntary contraction (MVC) at a fixed angle of 90° elbow flexion for approximately three seconds while seated on a standard arm curl bench. Forces were
measured by using a force transducer (OMEGA LCCA-1K, Stamford, CT) on three separate trials, with no less than two minutes of rest allowed between each trial. The highest force attained out of the three trials was representative of each subject’s MVC.

Maximal handgrip strength was measured using a handgrip dynamometer (Takei, Tokyo, Japan) on three separate trials, with no less than two minutes of rest allowed between each trial. The highest force attained out of the three trails was representative of maximal handgrip strength.

4.2.4 Eccentric Exercise. Subjects performed two sets of 25 maximal eccentric contractions of the forearm flexor muscles on a standard arm curl exercise bench, with each contraction lasting approximately four seconds. An investigator provided manual resistance with each contraction as the subject maximally contracted and resisted throughout a range of motion consisting of approximately 45° to 180° of elbow flexion. Subjects were allowed five minutes of rest between each set of exercise.

4.2.5 Sub-Maximal Handgrip Exercise. Subjects performed sub-maximal exercise at 10 percent of initial handgrip strength. Five minutes of exercise was performed while the duty cycle (1 sec contraction / 2 sec relaxation), resistance (10% MVC) and distance traveled (0.08 m) remained constant throughout the duration of exercise.

4.2.6 Measurement of Brachial Artery Diameter and Blood Velocity. An echo-Doppler ultrasonography system (z.one ultra, ZONARE Medical Systems Inc., Mountain View, CA) with a 7 MHz ultrasound probe was used to obtain baseline images of the brachial artery prior to each bout of sub-maximal exercise. All images were recorded digitally to
a computer and later analyzed using commercially available software (Medical Imaging Applications, LCC, Coralville, IA).

Brachial artery blood velocities were continuously measured during sub-maximal exercise using a Doppler ultrasound velocimetry system (model 500-M, Multigon Industries, Yonkers, NY) operating in pulsed mode. The Doppler transducer was placed 6-10 cm proximal to the medial humeral condyle while operating at a frequency of 4 MHz, with a fixed transducer crystal and sound beam angle of 45° relative to the skin. All blood velocity data was digitized at 100 Hz (PowerLab 16SP, ADInstruments, Grand Junction, CO) and stored for offline analysis. Blood velocities were later averaged over each cardiac cycle and analyzed in 15 second intervals at rest and during exercise.

4.2.7 Calculation of Blood Flow. Brachial artery diameter and mean blood velocities were used from rest and during exercise to calculate blood flow. Blood flow was calculated using the equation $MBV \times CSA \times 60$; where $MBV$ is the mean blood velocity and $CSA$ is the cross-sectional area of the brachial artery ($CSA = \pi r^2$, where $r$ is arterial radius). Since a previous study using the same contraction frequency and similar workload indicated no change in exercising brachial artery diameter (Shoemaker et al. 1997), baseline diameter was used for the calculation of blood flow during exercise.

4.2.8 Statistical Analysis. A two-way analysis of variance (ANOVA) with repeated measures was used to identify differences in muscle blood flow between conditions and across time. Significant main effects and interactions were further analyzed using a Student-Newman-Keuls post-hoc test, with statistical significance set at $P \leq 0.05$. All values are expressed as mean ± SEM unless stated otherwise.
4.3 Results

Subject demographics are presented in table 4.1. All subjects were void of cardiovascular, pulmonary, or metabolic disease and arrived in a fasted state. The average workload performed during sub-maximal exercise was $4.5 \pm 0.15$ kg. Mean resting blood pressure was not significantly different following eccentric exercise (Post) and resting brachial artery diameter remained similar at 48 hours Post (Table 4.2).

The eccentric exercise protocol was effective in producing muscle injury, as indicated by significant decreases ($P < 0.05$ vs. Pre) in maximal isometric strength at 48 hours Post (Table 4.2). On average, maximal strength was approximately 26 percent below pre-injury baseline level (Table 4.2).

Resting blood flow was not significantly different from pre-injury baseline value at 48 hours Post (Table 4.3; Fig. 4-1). Although significant differences ($P < 0.05$) were found in exercising blood flow with increasing exercise duration, no significant differences were found between Pre and Post exercise conditions (Table 4.3; Fig. 4-1).
Table 4.1. Subject Demographics

$n = 9$ Subjects.

<table>
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<tr>
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<td>Age, years</td>
<td>26.6 ± 1.4</td>
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<td>Height, cm</td>
<td>175.7 ± 2.2</td>
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<td>Body mass, kg</td>
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<td>Body mass index, kg/m²</td>
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<td>Handgrip Strength, kg</td>
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Table 4.2. *Hemodynamic and Muscle Injury Variables*

*n = 9 Subjects.* Values are expressed as mean ± SEM.

*, significantly different from pre-injury baseline value (P ≤ 0.05).

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<tr>
<th></th>
<th>Pre-Injury</th>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>120.4 ± 2.9</td>
<td>120.2 ± 2.6</td>
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<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>80.2 ± 2.5</td>
<td>79.8 ± 1.8</td>
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<tr>
<td>Brachial artery diameter, mm</td>
<td>4.0 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>Maximal isometric strength, kg</td>
<td>16.5 ± 3.4</td>
<td>12.2 ± 1.4*</td>
</tr>
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Table 4.3. *Brachial Artery Blood Flow During Exercise*

*n = 9 Subjects.* All values are reported as mean ± SEM.

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Pre-Injury (ml/min)</th>
<th>48 Hr Post (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34.2 ± 2.9</td>
<td>37.5 ± 3.3</td>
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<tr>
<td>120</td>
<td>84.6 ± 9.5</td>
<td>91.5 ± 8.6</td>
</tr>
<tr>
<td>135</td>
<td>108.9 ± 8.5</td>
<td>110.7 ± 10.3</td>
</tr>
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<td>150</td>
<td>127.5 ± 11.5</td>
<td>136.7 ± 10.8</td>
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<td>165</td>
<td>152.3 ± 14.9</td>
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<td>390</td>
<td>291.8 ± 21.0</td>
<td>308.1 ± 16.1</td>
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Figure 4-1. Exercise blood flow responses before and 48 hours following exercise-induced injury. Values are means ± SEM.
4.4 Discussion

Unaccustomed eccentric exercise can result in mechanical disruption of skeletal muscle (Clarkson and Hubal 2002) and lead to significant reductions in muscle force generation (Newham et al. 1987; Nosaka et al. 1991) while also impairing microvascular function (Heap et al. 2006; Kano et al. 2005). However, the effect of muscle injury on arterial blood flow at rest and during exercise is not clearly defined. Therefore, the most significant finding of the present study was that local, exercise-induced muscle injury did not result in significant changes to local resting arterial blood flow or the blood flow response during sub-maximal exercise at 48 hours post-injury.

The significant decrease in isometric strength is consistent with other studies examining the effects of eccentric exercise (Clarkson et al. 1992; Newham et al. 1987; Nosaka et al. 1991) and suggests that the exercise protocol incorporated within the present study was effective in producing muscle injury.

Previous studies utilizing Doppler ultrasonography (Sbriccoli et al. 2001) and near-infrared spectroscopy (NIRS) (Ahmadi et al. 2008b) have found an increase in resting blood flow values following eccentric injury, suggesting an increase in metabolic demand within the injured tissue during the recovery process. However, the present study revealed no change in resting muscle blood flow from pre-injury baseline values. This finding is in agreement with previous research from our lab (unpublished data) which indicated that resting blood flow was unaffected for up to 96 hours following an identical eccentric injury protocol. Additionally, brachial artery diameter was not
significantly different from pre-injury values 48 hours following injury. Given that resting blood flow did not change in the present study, it is not surprising that arterial diameter and blood pressure also remained similar.

In addition to similar pre- and post-injury resting blood flow values, the blood flow response to exercise was also closely paralleled at 48 hours Post. In contrast to the findings of the present study, Laaksonen et al. (2006) found significant increases in muscle blood flow during exercise; however, a different exercise modality (stretch-shortening cycle exercise) and instrumentation (positron emission tomography) was incorporated. Studies that have utilized NIRS following eccentric-induced injury have shown decreased hemoglobin kinetics at the onset of severe intensity cycling exercise (Davies et al. 2008) and isometric contractions (Ahmadi et al. 2008b), but have also found increased kinetics during isometric exercise (Ahmadi et al. 2008a). These contradictory findings create further questions as to what metabolic alterations the injured muscle undergoes during subsequent exercise. An increase in blood flow or hemoglobin kinetics during exercise would suggest a higher metabolic requirement following muscle injury, but decreased hemoglobin kinetics may indicate an impaired ability of muscle to extract and utilize oxygen. The findings of the present study indicate that despite muscle injury, arterial blood flow appears to remain similar at rest and during sub-maximal exercise, suggesting that the exercise workload determines the blood flow requirements for exercise, not the extent of muscle injury.

Some limitations exist within the present study. Although significant decreases in muscle strength were observed, additional biomarkers and mediators associated with
muscle injury and the subsequent inflammatory process were not measured. As previously discussed, these markers may play an important role in the vascular responses following muscle injury (Bhagat and Vallance 1997; Schwartz et al. 2007). Additionally, we did not measure blood pressure or arterial diameter during exercise; however, a previous study by Shoemaker et al. (1997) reported no change in blood pressure or diameter when handgrip exercise of a similar relative intensity was performed.

In conclusion, the results of the present study indicate that acute, local muscle injury does not affect resting or exercising blood flow responses in healthy male individuals during submaximal handgrip exercise. Additional research is warranted to elucidate the effects of eccentric exercise-induced muscle injury and the associated inflammatory mediators on skeletal muscle blood flow during exercise.
Chapter 5

Concluding Remarks

5.1 Summary

The specific aims of this dissertation were 1) to test the hypothesis that eccentric exercise-induced muscle injury would lead to impairments in local endothelial-dependent and -independent vasodilation, and 2) to test the hypothesis that muscle injury would not affect the muscle blood flow response to submaximal handgrip exercise. In agreement with our first hypothesis, we found that muscle injury resulted in significant reductions to both endothelial-dependent and –independent vasodilation for up to 96 hours. Additionally, we found significant decreases in the shear stress stimulus associated with FMD assessment for up to 48 hours post-injury. These findings suggest that factors associated with muscle injury are capable of impairing vascular function and the hyperemic response. No change in resting blood pressure or blood flow was observed throughout the duration of the study despite an increase in brachial artery diameter at one and 48 hours following eccentric exercise. These findings suggest that although muscle
injury leads to a temporary vasodilatory response, other hemodynamic variables may not be affected at rest.

In support of our second hypothesis, we found that the muscle blood flow response to submaximal handgrip exercise was not different following eccentric-induced muscle injury. This finding suggests that the mechanisms contributing to the flow stimulus during exercise are not influenced by muscle injury. However, since submaximal exercise was performed, it is unknown whether heavy intensity exercise would elicit a similar response. The findings of the present study also indicate that despite muscle injury, the metabolic cost of exercise appears to remain similar when submaximal exercise is performed.

5.2 **Future Direction**

Given our finding of decreased endothelial-dependent and -independent vasodilation following exercise-induce muscle injury; it would be beneficial to study the underlying mechanisms responsible for these impairments. Mediators of the post-injury inflammatory response could be measured in the plasma to see if a systemic increase in one or multiple biomarkers is closely correlated with the decrements in vasodilatory capacity. Additionally, circulating markers of muscle injury could be assessed and muscle biopsies sampled so the extent of injury associated with our eccentric exercise protocol could be further evaluated. Our findings suggest that the eccentric injury protocol incorporated within the present study offers a potential method for examination of vascular dysfunction in humans. This protocol may provide a viable option for future
studies interested in examining the mediators associated with exercise-induced changes in vascular function.

The blood flow response to exercise following muscle injury may be further elucidated by performing a higher exercise intensity that would elicit a significant increase in arterial diameter. Given that we found impaired dilatory capacity during endothelial-dependent and -independent assessment, it would also be of interest to see if dilatory capacity and subsequent blood supply to exercising muscle is affected during high intensity exercise. Future studies examining the effects of exercise-induced muscle injury on blood flow are warranted to elucidate the potential impact of muscle injury on vascular control during exercise.
References


Appendix A

Subject Information and Consent Form

The Effect of Eccentric Exercise on Endothelial Function and Muscle Blood Flow

Principal Investigator: Barry W. Scheuermann, Ph.D.
Other Staff (identified by role): Mitchel R. Stacy, MS (Co-investigator)
                                  David L. Weldy, MD, Ph.D. (Co-investigator)
                                  Jennifer L. Lawrence (Graduate Student Assistant)
                                  Kallie J. Bladon (Undergraduate Student Assistant)

Contact Phone number(s): (419) 530-2692 Office
                         (419) 530-2058 Lab

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 that will measure how your blood vessels respond (endothelial function) following a strenuous bout of arm exercise that causes your muscles to become sore and stiff, similar to the feelings you experience following exercising for the first time after a long period of inactivity.

You were selected as someone who may want to take part in this study because you expressed interest in this study by contacting either Dr. Barry Scheuermann or Mitchel Stacy and you meet the criteria outlined below. This study will include approximately 20 subjects recruited from the University of Toledo community.

To participate in this study, you must be between 18-45 years of age and be free of any known cardiovascular, pulmonary, or metabolic disease as determined by the medical history questionnaire. Individuals not meeting these criteria will be excluded from the study.

If you are pregnant, you will be excluded from participating in this study. If you are a woman and would like to participate in the study, you must undergo a pregnancy test prior to participating in the study. You will not be asked to pay any expenses associated with the pregnancy test.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT

If you decide to take part in this study, you will be asked to visit the Cardiopulmonary and Metabolism Research Laboratory within the Department of Kinesiology located in the Health and Human Services building, room 1407, where all testing will take place. You will be asked to visit the laboratory on 5 separate occasions. The first visit will last approximately 2 hours, with the following 4 visits lasting approximately 1-3 hours.

STUDY VISITS

First Visit: During your first visit, all of the experimental procedures will be explained to you and you will be asked to complete an informed consent form and a medical history questionnaire. You will be asked to arrive to the laboratory in a fasted state (no food or
caffeinated drinks for 8 hours) and have avoided any strenuous physical activity for 24 hours prior to your visit. Standard measurements of height and weight will be obtained.

The following experimental procedures will also be conducted:

- During this visit you will have your blood collected following the completion of the informed consent and medical history questionnaire. The flow-mediated dilation (FMD) and nitroglycerin tests of blood vessel function, which are described below, will then be administered. You will also be tested for your maximal voluntary strength (MVC). A description of the MVC test is also provided below. Once your maximal strength is determined, you will perform 10 minutes of submaximal arm exercise while blood flow is measured in your arm.
- The first testing session is expected to require approximately 2 hours of your time.

Second Visit:

- During the second visit, your blood will be collected in the same manner as visit 1. A flow mediated dilation (FMD) test will be performed, followed by the administration of nitroglycerin, which is described below. After the FMD test, your maximal strength will be measured by an MVC test.
- You will be asked to perform 2 trials of strenuous arm exercise, with a 5 minute break between each trial, as described below. Following a 1 hour rest period, you will return to the lab and undergo the same process of blood collection, FMD/nitroglycerin, and MVC as previously performed prior to the bout of arm exercise.
- It is expected that it will require approximately 3 hours to complete all testing on your second visit.

Visits three to five:

- During your final 3 visits, you will undergo the same procedures of blood collection, FMD/nitroglycerin, and MVC as previously described on your first 2 visits; however, your fourth visit will differ slightly as it will also include 10 minutes of submaximal exercise (as performed on visit 1 and described below) while blood flow is measured in your arm.
- It is expected that it will require approximately 2-3 hours to complete all testing during each of visits three to five.

STUDY PROCEDURES

Flow Mediated Dilation (FMD) – Endothelial-Dependent Function: This test will last approximately 40 minutes. You will be asked to lie quietly for 15-20 minutes on a standard treatment table.
• One blood pressure cuff will be placed around your forearm, just below the elbow.
• A plastic probe (Doppler ultrasound) with gel will be placed on the skin of your upper arm to acquire an image of your artery.
• The blood pressure cuff on your forearm will be inflated to a high pressure for five minutes. At the five minute mark, the cuff around your arm will be rapidly deflated and an image of your artery will be measured for 2 minutes.

Nitroglycerin Administration – Endothelial-Independent Function: This test will last approximately 15 minutes. You will continue to lie quietly for 10 minutes following the FMD test.

• Following a 10 minute rest period, you will be given a tablet that will dissolve under your tongue and an image of your artery will be measured for 5 minutes using the same probe placement as previously described.

Maximal Voluntary Contraction (MVC): This test will last approximately 5 minutes. You will be asked to sit comfortably on a standard arm curl exercise bench.

• You will hold a handle attached to a force transducer by a cable and pulley system.
• You will be asked to try and flex your upper arm as hard as possible, exerting maximal effort for approximately 5 s.
• You may be asked to repeat this task 3 to 5 times but will be provided with a rest period between each effort.

Blood Collection: This test will last approximately 5 minutes. You will be asked to sit comfortably in a chair while you rest your arm on a table.

• An experienced individual will insert a small needle into a vein located on the inside portion of your arm near your elbow so that a small amount of blood can be taken. You may feel slight discomfort which will only last for a few seconds.
• Approximately 2 ½ teaspoons of blood will be collected each day.

Arm Exercise: The study protocol requires you to perform two different types of arm exercise, with each lasting approximately 10-15 minutes. For both exercises, you will be asked to sit comfortably on a standard arm curl exercise bench. The “arm curl” exercise involves bending your arm at your elbow.

• During one of the arm exercise sessions you will be asked to hold a handle attached to a cable and pulley system. An individual will pull down on the cable and pulley system as you resist and pull in the opposite direction as forcefully as possibly. You will do this a total of 25 times, rest for 5 minutes, and then you will be asked once again to do the same effort 25 more times.
During the other arm exercise session you will be asked to hold the same handle but it will now be attached to a different pulley system that has weights attached to it. You will be asked to perform submaximal exercise for 5 minutes at an effort requiring equivalent to 5% of your maximal effort. You will have 5 minutes of rest after 5 minutes of exercise and then will be asked to perform 5 more minutes of the same exercise at the same effort as the first exercise bout.

RISKS AND DISCOMFORTS YOU MAY EXPERIENCE IF YOU TAKE PART IN THIS RESEARCH
Immediate risks may include muscle cramping, strain, or soreness during the arm exercises. In addition, subjects may also experience numbness or a tingling sensation in their hand and forearm during the occlusion phase (blood pressure cuff is pumped up) of the flow-mediated dilation test. Numbness and tingling sensations will be alleviated immediately upon release of the blood pressure cuff. Some common side effects of nitroglycerin use are dry mouth, headache, skin irritation, sweating and a sudden drop in blood pressure. The sensation of vertigo, dizziness, weakness, and/or heart palpitation may also accompany the use of nitroglycerin, but side effects should subside shortly after use. Some discomfort may occur during the collection of blood samples and slight bruising around the insertion site of the needle may appear; however, any bruising or soreness should fade within one day.

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 that participate in this study will be exposed to current research topics and techniques.

COST TO YOU FOR TAKING PART IN THIS STUDY
There are no costs associated with 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 participating in this research nor will you be given “extra credit” in any academic courses that you are enrolled.

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 subject.

CONFIDENTIALITY - (USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION)
The researchers will make every effort to prevent anyone who is not on the research team from knowing that you provided this information, or what that information is. The
consent forms with signatures will be kept separate from responses, which will not include names and which will be presented to others only when combined with other responses. Although we will make every effort to protect your confidentiality, there is a low risk that this might be breached.

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 a study-related injury, you may contact Dr. Barry Scheuermann any time of the day or night at 419-460-4901.

VOLUNTARY PARTICIPATION
Taking part in this study is voluntary. 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
You will be notified of new information that might change your decision to be in this study if any becomes available.

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 Mitch Stacy (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).

<table>
<thead>
<tr>
<th>Name of Subject (please print)</th>
<th>Signature of Subject</th>
<th>Date</th>
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<tr>
<th>Name of Witness</th>
<th>Signature of Witness</th>
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Appendix B

Medical History Questionnaire

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<tr>
<th>Family history of heart disease?</th>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td>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 (aunt, sister, daughter)</td>
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<tr>
<th>Smoking habit?</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>i.e. Current cigarette smoker or one who has quit within the previous 6 months</td>
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<tr>
<th>High blood pressure?</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>i.e. Blood pressure ≥140/90 on two separate occasions or currently on antihypertensive medication</td>
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<tr>
<th>Abnormal cholesterol levels?</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>i.e. Total Cholesterol ≥200mg/dL, or LDL ≥130mg/dL, or HDL ≤55mg/dL, or currently on lipid lowering medication</td>
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<th>High fasting glucose?</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>i.e. Fasting blood glucose ≥110 on two separate occasions</td>
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<tr>
<th>Are you inactive?</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>i.e. Accumulate ≥30 minutes of moderate physical activity on most days of the week</td>
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[Form for Office Use Only]

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

<table>
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<tr>
<th>Do you currently have any of the following?*</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>Pass out in the cold, heat, or exertion?</td>
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<td>Shortness of breath at rest or with mild exertion?</td>
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<td>Dizziness or fainting?</td>
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<td>Difficulty breathing while lying down, relieved by sitting up?</td>
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<td>Swelling in your ankles?</td>
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<td>Rapid heart rate while at rest?</td>
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<td>Leg pain or cramping while walking, relieved with rest?</td>
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<td>Heart murmur?</td>
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<td>Unusual fatigue or shortness of breath with usual activities?</td>
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If you can answer yes to any of the above please obtain medical clearance for exercise from your personal physician.

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<th>Do you have a history of the following?*</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>Heart attack or stroke?</td>
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<td>Heart surgery (CABG, angioplasty)?</td>
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<td>Metabolic disorder (diabetes, kidney, thyroid)?</td>
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<td>Respiratory problems (asthma, COPD)?</td>
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<td>Hospitalization or surgery within the last 6 months?</td>
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If you can answer yes to any of the above please obtain medical clearance for exercise from your personal physician.

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

Contact Information

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<th>Name:</th>
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<td>City:</td>
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<td>State:</td>
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<td>Home Phone:</td>
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<td>Work:</td>
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<td>Cell:</td>
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<td>E-mail:</td>
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Preferred contact method:

Emergency Contact Information

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<td>Relationship:</td>
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<td>Home:</td>
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<td>Work:</td>
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Prescribed Medications:

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<th>Allergies:</th>
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<td>Supplements (nutritional/athletic):</td>
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Do you have an orthopedic condition/arthritus 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?

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

Signature: ____________________________

Date: ____________________________

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