The effect of an acute bout of exercise on endothelial function following ischemic-reperfusion injury

Jennifer L. Lawrence
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A Thesis

entitled

The Effect of an Acute Bout of Exercise on Endothelial Function following Ischemic-Reperfusion Injury

by

Jennifer L. Lawrence

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science degree in Exercise Science

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December 2011
Cardiovascular diseases are a major cause of death throughout the world. Furthermore, the primary pathological cause of coronary artery disease is myocardial damage due to ischemic-reperfusion (IR) injury, the vascular dysfunction and tissue damage due to reperfusion following a long duration of ischemia. According to the literature, IR injury leads to endothelial dysfunction. It has been well established that endothelial dysfunction is one of the first identifiable markers of atherogenesis and vascular disease. Furthermore, it has also been demonstrated that participation in regular, physical activity can lead to both ischemic preconditioning as well as improved endothelial function. However, it is not currently known if an acute bout of exercise performed prior to experiencing an IR injury can lead to a protective condition whereby endothelial dysfunction is either prevented or reduced. Therefore, the purpose of the present study is to determine whether an acute bout of moderate intensity exercise, such as jogging on a treadmill, can provide an alternative method of preconditioning to either prevent or reduce the effects of IR injury. The study consisted of 6 male and 3 female subjects (n=9, age = 21.7 ± 3.2 years). All subjects were all
healthy, sedentary individuals (height = 174.43 ± 8.12 cm, body mass = 77.87 ± 21.03 kg, body mass index = 25.46 ± 6.26 kg/m²) and were not currently engaged in any type of resistance or endurance exercise training. Each subject performed all three protocols; ischemic-reperfusion injury (IRI), exercise (EXER), and preconditioning (EXER/IRI) which included performing exercise prior to ischemic-reperfusion injury. Endothelial function was assessed using flow-mediated dilation (FMD) of the brachial artery, a surrogate model to the coronary arteries. The ANOVA analysis indicated that there was a significant main effect for time, however no main effect for protocol (p=0.08) or interaction was observed. Compared to the initial FMD trial at time 0, both IRI and EXER/IRI resulted in a significant decrease in brachial artery %FMD at 120 min corresponding to a 60 ± 19% and 53 ± 16% decrease in vascular reactivity, respectively. In comparison to the corresponding %FMD response at time 0, the brachial artery vascular response remained attenuated at 140 min (p<0.05) for the IRI condition but returned to pre-injury values by 160 min. The %FMD response for EXER/IRI returned to initial values by 140 min with no further change from time 0 observed at 160 min. The EXER intervention did not result in a significant change in %FMD at any of the time points examined in comparison to the initial corresponding FMD trial at time 0. In conclusion, FMD showed significant differences during the IRI and EXER/IRI protocols, but returned to normal conditions at 160 minutes and 140 minutes, respectively. Therefore, the findings of the present study indicate that the performance of a single bout of moderate intensity exercise prior to IRI does not impart any immediate protective benefits to the endothelium. However, since the exercise preconditioning protocol resulted in a faster recovery than IRI alone, this suggests there may some protection/benefits provided by the prior bout of acute exercise.
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Chapter 1

Introduction

Cardiovascular diseases are a major cause of death throughout the world. Furthermore, the primary pathological cause of coronary artery disease is myocardial damage due to ischemic-reperfusion injury (Powers, Quindry, & Kavazis, 2008). Ischemia is the occlusion of blood flow to organ tissue, in this case specifically the myocardium. Reperfusion is the restoration or return of blood flow. Ischemic-reperfusion (IR) injury is the vascular dysfunction and tissue damage due to reperfusion following a long duration of ischemia. The damage can be reversible, but following long durations of ischemia the damage may become irreversible.

Throughout the literature, there has been much speculation as to the probable events leading to IR injury, which can include decreases in cellular ATP levels, production of reactive oxygen species (ROS), accumulation of hydrogen ions, generation of reactive nitrogen species (RNS), calcium overload, calpain activation, and leukocyte activation. None of the possibilities have been proven to date. After injury has transpired, there are three potential levels of IR injury that occur depending upon the length of the ischemia. Level 1 results in the generation of reperfusion-induced cardiac arrhythmias following a 1 to 5 minute period of ischemia. Level 2 results in myocardial stunning (decreased contractility of
the heart) following a 5 to 20 minute period of ischemia. Level 3 results in irreversible damage to the tissue (i.e. necrosis, apoptosis) following a period of 20 minutes or more of ischemia (Powers, et al., 2008).

Ischemic preconditioning (IPC) is a method that has been developed to provide protection to the vasculature and tissues from IR injury through induced periods of brief, non-lethal ischemia and reperfusion cycles (Loukogeorgakis et al., 2005) prior to ischemic-induced injury. Ischemic preconditioning consists of two phases, early and late. The early phase occurs immediately and can last for approximately 2 to 3 hours, while the late phase does not develop for 12 to 24 hours but can last for days (Kavazis, 2009; Loukogeorgakis, et al., 2005). Remote ischemic preconditioning (RIPC) is an alternate method of protection through the application of multiple pre-ischemic bouts of ischemia and reperfusion in an area other than the organ affected. Ischemic preconditioning and remote ischemic preconditioning have also been referred to as cardioprotection.

More recently, the idea of exercise-induced preconditioning, or exercise-induced cardioprotection, has been the topic of research. Exercise-induced cardioprotection is the use of exercise as a form of preconditioning to IR injury (Powers, et al., 2008). Throughout literature, endurance exercise training has been shown to enhance myocardial tolerance to IR by protecting cardiac myocytes against IR-induced oxidative stresses and protect mitochondria against IR-induced damage (Powers, et al., 2008). Endurance exercise-mediated cardioprotection is observed in both short-to moderate-duration ischemia (i.e. 5-20 min) and moderate to severe (i.e. 20-60 min) ischemic insults (Powers, et al., 2008). Additionally, protection has been detected following short-term exercise in as little as 3-5 days (Powers, et al., 2008). Furthermore, investigators have examined short-term (3-5 days)
and long-term (10 weeks) exercise at moderate (55-60% VO\textsubscript{2} Max) and high intensities (75% VO\textsubscript{2} Max).

The potential mechanisms underlying the endurance exercise-induced cardioprotection have been investigated throughout the literature. The proposed mechanisms thought to have an impact against IR injury are, 1) anatomic and physiological changes in the coronary arteries (Babsky et al., 2002; Das, 2005; Kavazis, 2009; Powers, et al., 2008), 2) induction of myocardial heat shock proteins (Babsky, et al., 2002; Das, 2005; Kavazis, 2009; Lennon et al., 2004; Powers, et al., 2008), 3) increased myocardial cyclooxygenase-2 activity (Babsky, et al., 2002; Das, 2005; Kavazis, 2009; Powers, et al., 2008), 4) elevated endoplasmic reticulum stress proteins (Babsky, et al., 2002; Das, 2005; Kavazis, 2009; Powers, et al., 2008), 5) enhanced function of sarcolemmal ATP-sensitive potassium channels (Babsky, et al., 2002; Das, 2005; Kavazis, 2009; Powers, et al., 2008), 6) elevated levels of mitochondrial ATP-sensitive potassium channels (Babsky, et al., 2002; Das, 2005; Powers, et al., 2008), 7) increased myocardial antioxidants capacity (Kavazis, 2009; Powers, et al., 2008), and 8) nitric oxide production (Kavazis, 2009). However, in spite of the considerable amount of research performed, the specific mechanism leading to the protective effects remain unclear.

According to the literature, IR injury leads to endothelial dysfunction. It has been well established that endothelial dysfunction is one of the first identifiable markers of atherogenesis and vascular disease (Carden & Granger, 2000). Furthermore, it has also been demonstrated that participation in regular physical activity can lead to both ischemic preconditioning as well as improved endothelial function. However, it is not currently known if an acute bout of exercise performed prior to experiencing an IR injury can lead to
a protective condition whereby endothelial dysfunction is either prevented or reduced. This certainly has practical implications since the time required to achieve any exercise training-induced cardioprotection is typically much longer than available for either a scheduled or unscheduled event resulting in a prolonged period of ischemia. Additionally, in regards to the application of physical activity as an approach to induce preconditioning against IR injury, exercise is readily available, relatively inexpensive and for those who can perform exercise without complications or significant risk, it may be the most accessible form of preconditioning.

1.1 Purpose of Study

To our knowledge, the potential benefit of performing an acute bout of moderate intensity exercise on the vascular damage (i.e. endothelial dysfunction) caused by IR injury has not been previously examined. Therefore, the purpose of the present study is to determine whether an acute bout of moderate intensity exercise, such as jogging on a treadmill, can provide an alternative method of remote ischemic preconditioning to either prevent or attenuate the negative effects of IR injury on vascular endothelial function.

1.2 Hypothesis

We hypothesized that an acute bout of moderate intensity exercise will reduce the magnitude of endothelial dysfunction as a consequence of IR injury.
Chapter 2

Literature Review

2.1 Mechanisms of Ischemic Reperfusion Injury

Throughout the literature, there have been many proposed mechanisms leading to IR injury, such as increased and/or decreased production of bioactive agents (i.e. NO, superoxide, ROS, ATP), activation of complement system, and neutrophil activation. However, the exact pathophysiology of IR injury is still unclear. Figure 2-1 shows a cascade of the possible events leading to endothelial dysfunction due to IR injury.

![Figure 2-1. Cascade of events leading to IR injury.](image-url)
2.1.1 Reactive Oxygen Species (ROS)

During the ischemia phase, little to no oxygen is reaching muscle and organ tissue. This hypoxia alters membrane potential, the distribution of ions, increases intracellular volume, decreases membrane fluidity, impairs cytoskeletal organization of endothelial cells, is accompanied by depletion of energy stores, decreases production of certain bioactive agents (i.e. prostacyclin, NO), accelerates production of other bioactive agents (i.e. endothelin, thromboxane A2), induces genes (i.e. adhesion molecules, cytokines), and suppresses other genes (i.e. cNOS, thrombomodulin) (Carden & Granger, 2000). The ischemia is followed by the reperfusion phase, the restoration of blood flow and oxygen to the muscle and organ tissues. At the beginning of reperfusion, there is a sudden increase in oxygen (O\textsubscript{2}) that leads to a large production of ROS and superoxide. The ROS causes depletion in energy stores, decreases in nitric oxide (NO) synthesis and adenosine, calcium (Ca\textsuperscript{2+}) overload, and the release of pro-inflammatory mediators (Carden & Granger, 2000; Gourdin, Bree, & De Kock, 2009). Under normal conditions, NO scavenges low intracellular levels of superoxide (a compound that aids in vascular and tissue damage due to IR injury), prevents platelet aggregation, thrombus formation, minimizes adhesive interactions between leukocytes and endothelial cell surfaces (Beckman & Koppenol, 1996; Carden & Granger, 2000), and acts as a vasodilator within the vasculature. Adenosine works as an anti-inflammatory within tissues to prevent the effects of ischemia. Therefore a decrease in NO and adenosine following IR would lead to tissue damage and ultimately endothelial dysfunction.

Due to the large increase of ROS production, high molecular weight proteins are degraded and cause an irreversible loss of channel functioning within cells, inhibiting Ca\textsuperscript{2+} removal (Aimasheva et al., 1999). Acidosis and lactate accumulation outside the cell promote cystolic Ca\textsuperscript{2+} overload in anoxic endothelial cells and leads to decreased endothelial
functioning. The Ca²⁺ overload within the mitochondria initiates mitochondrial permeability transition pore (mPTP) activation, which leads to cellular swelling and eventually ruptures. Again, this results in a decrease in endothelial functioning (Aimasheva, et al., 1999). Furthermore, the cell rupture will cause an increase in ROS production, thus initiating a vicious cycle of cell degradation.

The large increase of ROS production from increased O₂ or cell lysis releases pro-inflammatory mediators. This guides the neutrophil interaction with the endothelium, activation of leukocytes, and enhances the expression of the adhesion molecules. The adhesion molecules promote a neutrophil-endothelium cell interaction, adhering to the endothelial basement membrane and results in endothelial cell lifting, subsequently leading to tissue damage and decreased endothelial function (Carden & Granger, 2000). This topic is discussed further in the following section.

2.1.2 Neutrophil Activation

The production and release of ROS into the blood stream triggers neutrophil activation. Neutrophil activation triggers increases in ROS, RNS, and, most importantly the enhanced expression of adhesion molecules. The adhesion molecules allow for a neutrophil-endothelial cell adhesion, which causes endothelial cell lifting (tissue damage) and ultimately endothelial dysfunction. The neutrophil-endothelial interaction leads to neutrophil adherence to the endothelium and transendothelial migration of neutrophils across the endothelium (Lefer & Lefer, 1996). Neutrophils release oxidants, proteases, and inflammatory products (Gourdin, et al., 2009; Jordan, Zhao, & Vinten-Johansen, 1999). The oxidants and proteases damage and/or kill the tissue, while the inflammatory products amplify the recruitment and activation of greater numbers of neutrophils into the affected
myocardium and extend the severity of tissue damage (Gourdin, et al., 2009; Jordan, et al., 1999). These mediators circulate, migrate, and adhere to matrix proteins and subsequently injure parenchymal cells (cardiac monocytes/hepatocytes) (Lefer & Lefer, 1996).

The interaction between neutrophil and endothelial cells is orchestrated through 3 adhesion molecules: 1) selectins, 2) β₂-integrins, and 3) immunoglobulin superfamily (Gourdin, et al., 2009; Jordan, et al., 1999; Lefer & Lefer, 1996; Vinten-Johansen et al.). Selectins (i.e., P-selectin, L-selectin, E-selectin) are glycoproteins involved in early interactions, loose tethering or “rolling” between neutrophils and coronary vascular endothelium during reperfusion (Jordan, et al., 1999; Lefer & Lefer, 1996; Menger, 2007; Vinten-Johansen, et al.). Neutralization of selectins, specifically P-selectin, reduces neutrophil adherence, neutrophil-mediated injury to endothelium, and myocardial infarction (Vinten-Johansen, et al.). β₂-integrins (i.e., CD11/CD18) are a family of glycoproteins that reside on neutrophils. β₂-integrins are triggered after the rolling phase of neutrophils and are stimulated by a number of mediators (i.e., platelet activating factor). The involvement of CD11b/CD18 with its counterligand intercellular adhesion molecule-1 (ICAM-1), an important mediator and member of the immunoglobulin superfamily, results in firm adherence of the neutrophils to the endothelium (Jordan, et al., 1999; Lefer & Lefer, 1996; Menger, 2007; Vinten-Johansen, et al.). The immunoglobulin superfamily (i.e., ICAM-1, VCAM-1, and PECAM) is expressed at a relatively low level on surface of vascular endothelial cells. The up-regulation of these adhesion molecules coincides with up-regulation of CD11/CD18 β₂-integrins (Vinten-Johansen, et al.).

2.1.3 Complement Activation

Complement activation is thought to contribute to later stages of IR injury. Endothelial function is due to increased neutrophil activation during IR through classical
and/or alternate pathways. The classical pathway develops an immune complex formation that interacts with proteins (i.e., C1q). In the end, this will lead to the cleavage of a protein (C3) that intersects with the alternate pathway (Chakraborti, Mandal, Mandal, Das, & Chakraborti, 2000; Jordan, et al., 1999).

The alternate pathway releases ROS and induces activation of proteases to accelerate cleavage of C3. This leads to the membrane attack complex (MAC), the unrestricted flow of electrolytes and water between intra- and extracellular compartments. This inability of the cell to regulate water and electrolyte concentrations causes cellular lysis (Chakraborti, et al., 2000), once again, leading to endothelial dysfunction.

2.2 Ischemic Preconditioning (IPC)

The results of previous studies (Alkhulaifi, Yellon, & Pugsley, 1994; Jia, Zhu, Wu, Xie, & Xu, 2009; Kharbanda et al., 2001; Loukogeorgakis, et al., 2005; Maulik et al., 1998; Przyklenk, Bauer, Ovize, Kloner, & Wittaker, 1993; Quindry et al., 2005; Yellon & Downey, 2003) have shown that IPC through induced periods of brief, non-lethal ischemia and reperfusion cycles provides protection to the vasculature and other tissues when applied prior to ischemic-induced injury. The benefits of IPC have been referred to as cardioprotection due to the fact that studies investigating IPC have, for the most part, targeted myocardial cells and infarct size as the outcome (J. J. Li et al., 2003; Loukogeorgakis, et al., 2005; Maulik, et al., 1998; Wu, Livainen, Pehkonen, Laurikka, & Tarkka, 2003; Wu et al., 2001). However, there is evidence that IPC may be used in other tissues and organs other than cardiac cells (Kharbanda, et al., 2001; Saito, Komiyama, Aramoto, Miyata, & Shigematsu, 2004). For example, Eberlin et al. (Eberlin et al., 2008) investigated the protective effects of IPC against IR injury on skeletal muscle in male mice. This study was designed to address three important issues including 1) development of a model of IPC in
murine skeletal muscle with a definable injury score and reproducible protective effect from IR injury, 2) determining the local protection afforded by IPC, with local injury evaluation of skeletal muscle, and 3) determining the significance of preconditioning protection on mortality rates (Eberlin, et al., 2008). The mice were placed in either the standard IR injury group or in a group that received IPC. The preconditioning protocol consisted of two 20 minute cycles of IPC followed by the standard IR injury of 20 minutes of continuous ischemia. The extent of skeletal muscle injury localized to the gastrocnemius muscle was examined following the IR injury. From these findings the authors concluded that two 20 minute cycles of IPC were successful, at least in the murine model. In fact, these results were the first to find significant reduction in mortality through IPC in a non-cardiac model of injury (Eberlin, et al., 2008).

2.3 Remote Ischemic Preconditioning (RIPC)

Direct approaches of IPC require the target cells or organs to be exposed to IR injury, and therefore are more susceptible to damage during preconditioning protocols. Due to the risk of tissue damage, this technique has not lead to widespread clinical applications. Thus, the technique of RIPC has been advocated. Remote ischemic preconditioning is similar to IPC in that there is an application of multiple cycles of ischemia and reperfusion, but in an area other than the target tissues that will be exposed to IR injury. In 2005, Loukogeorgakis and colleagues (Loukogeorgakis, et al., 2005) investigated the time course for cardioprotection induced by RIPC and established that there are two separate phases of protection. This study, which examined the effect of IR injury on vascular endothelial cell function, found that if RIPC was applied directly before an ischemic-reperfusion period and at 24 and 48 hours before IR injury, RIPC could prevent endothelial cell IR injury. An early phase of protection was activated immediately but disappeared within 4 hours of the initial
insult, and a late phase of protection appeared approximately 24 hours after the application of RIPC stimulus and was sustained for about 48 hours (Loukogeorgakis, et al., 2005).

Other studies (Birnbaum, Hale, & Kloner, 1997; Cheung et al., 2006; Kharbanda et al., 2002) have found that RIPC can precondition target tissues. These findings support that this form of preconditioning may be an important and common form of myocardial protection in the clinical setting (Birnbaum, et al., 1997). Transient limb ischemia is a simple preconditioning stimulus with important potential clinical applications (Kharbanda, et al., 2002). Multiple potential clinical uses of remote ischemic preconditioning are 1) reducing cardiac damage during percutaneous coronary interventions, 2) protecting the myocardium during coronary artery bypass graft and other cardiac surgical procedures requiring cardiopulmonary bypass, 3) protecting the endothelial and vascular smooth muscle during vascular surgical procedures, 4) inducing protection before activities that reproducibly cause ischemia in patients with unstable angina, 5) protecting donor hearts before excision and transport, and 6) protecting other organs (i.e. brain, kidney) during episodes of ischemia (Kloner, 2009).

### 2.4 Exercise Induced Cardioprotection

Although the benefits of performing regular exercise on cardiorespiratory fitness are well known, the application of physical activity as a form of preconditioning has the potential to reduce or prevent IR injury, but has received relatively little attention. However, there is evidence from both animal and human studies that exercise can induce cardioprotection against IR injury (Abete et al., 2000; Abete et al., 2001; Brown, Jew, Sparagna, Musch, & Moore, 2003; Demirel et al., 2001; Freimann et al., 2005; French et al., 2008; Michaelides et al., 2003; Mussi et al., 2008; Powers et al., 1998; Quindry, et al., 2005; Starnes, Taylor, & Park, 2003; Yamashita et al., 1999). Indeed, reports in the literature
indicate that endurance exercise training in particular may enhance cellular tolerance, including myocardial cells, to IR injury (Brown, et al., 2003; French, et al., 2008; Powers, et al., 1998; Quindry, et al., 2005). Although the specific mechanism(s) leading to the protected state has not been established, there is evidence that cells are better protected against IR injury induced increases in oxidative stress and/or by protecting mitochondria against IR injury induced damage (Ascensao, Ferreira, & Magalhaes, 2007; Demirel et al., 1998; Hamilton et al., 2001; Powers, et al., 1998). Endurance exercise-mediated cardioprotection is observed in both short to moderate duration ischemia (i.e. 5-20 min) and moderate to severe (i.e. 20-60 min) ischemic insults (Demirel, et al., 2001; Powers, et al., 1998; Quindry, et al., 2005; Yamashita, et al., 1999). Additionally, protection against IR injury has been detected following short-term exercise training in as little as 3-5 days. This suggests that the underlying mechanism may be upregulated relatively quickly compared to other endurance training induced adaptations (Demirel, et al., 2001; Hamilton, et al., 2001; Powers, et al., 1998).

In addition to the potential causes of IR injury, this review by Powers and colleagues (Powers, et al., 2008), along with many other articles, discussed seven possible mechanisms behind endurance exercise-induced cardioprotection. Those mechanisms are as follows; 1) anatomic and physiological changes in the coronary arteries, 2) induction of myocardial heat shock proteins (Lennon, et al., 2004) 3) increased myocardial cyclooxygenase-2 activity, 3) elevated endoplasmic reticulum stress proteins, 4) enhanced function of sarcolemmal ATP-sensitive potassium channels, 5) elevated levels of mitochondrial ATP-sensitive potassium channels, and 6) increased myocardial antioxidants capacity (Babsky, et al., 2002; Das, 2005; Powers, et al., 2008). However, the mechanism(s) underlying the improved protective condition remains unclear in spite of being a focus of several investigations. Kavazis
(Kavazis, 2009) discussed similar mechanisms to those previously reviewed by Powers et al, including additional proposed molecular mechanisms. During exercise, it has been suggested there is an increase in nitric oxide production and adaptations to the endothelium. Exercise increases the ability of the endothelium to release vasoactive factors, including nitric oxide.

The beneficial effects of regular exercise against myocardial ischemia seem to be strongly related to increased antioxidants, specifically superoxide dismutase (SOD), a major antioxidant. Antioxidants minimize IR injury through decreasing the production of ROS (Powers, 2002), which is thought to be an important factor in endothelial dysfunction (as previously discussed). Extracellular SOD is thought to induce cardioprotection after an IR insult (Q. Li et al., 1998; Michaelides, et al., 2003). Increased NO production, through exercise, increases the ability of the endothelium to release vasoactive factors and decreases the effects of superoxide. Although more research is needed, antioxidants and NO seem to be the key defenders in the mechanisms leading to exercise-induced cardioprotection.

2.5 Exercise and Flow-Mediated Dilation (FMD)

Flow-mediated dilation (FMD) is an index of NO-mediated vasodilator function (Black, Cable, Thijssen, & Green, 2009) within the endothelium. Vascular endothelial function is essential for maintenance of health of the vessel wall and for vasomotor control (Green, Maiorana, O'Driscoll, & Taylor, 2004). Furthermore, endothelial cell function, or decline in function, is associated with a number of diseases (Faulx, Wright, & Hoit, 2003), such as atherosclerosis and hypertension, and can be used to assess age-related decreases in functioning. However, the decrease in endothelial function may be either reversed or attenuated by making changes to modifiable risk factors, such as lack of physical activity and smoking, associated with cardiovascular and related diseases (Faulx, et al., 2003).
It is well known that exercise is an important key to sustained or improved health, while decreasing the risk of certain diseases, in this case specifically cardiovascular diseases. Therefore, an exercise bout may be thought of as providing a direct dose of vascular medicine (Green, 2009). Exercise training has been shown, in many animal and human studies, to augment endothelial, NO-dependent vasodilatation (Green, Maiorana, et al., 2004).

Exercise training typically improves arterial function in subjects with cardiovascular risk factors or disease (Black, et al., 2009). Studies have shown that exercise, even short-term, can decrease the age-related decline of the endothelium function (Black, et al., 2009) through increased NO bioactivity (Green, Maiorana, et al., 2004). Improved endothelial functioning occurs with exercise training, regardless of the type of exercise training. Vona et al. (Vona et al., 2009) examined the difference between three types of exercise training (aerobic, resistance, and aerobic plus resistance) in subjects who had recently suffered an acute myocardial infarction. The results of that study demonstrated improved functioning independent of the type of training (Vona, et al., 2009).

In addition to the well documented reports of improvements in endothelial function following long term exercise training programs, studies have also shown that an acute bout of exercise performed following a high fat meal or high glucose drink improves endothelial function. For example, Padilla et al. (Padilla, Harris, Fly, Rink, & Wallace, 2006) investigated the effects of performing a single bout of aerobic exercise session on the endothelial function following a high fat meal. They found that performing a single bout of exercise counteracted the impaired endothelial function that typically occurs following consumption of a meal high in fat content as shown by a significant improvement in the FMD response with exercise. The extent that performing a single bout of moderate intensity exercise may
provide some protection from IR induced injury has not been previously investigated but given the relative ease that exercise can be performed by most individuals, it is important that potential benefits of exercise on either preventing or attenuating endothelial dysfunction due to IR injury be established.
Chapter 3

Methods

3.1 Subjects

The study consisted of 9 healthy inactive subjects (6 males, 3 females) between the ages of 18-27 years. Subjects were free of any cardiovascular, pulmonary, and/or metabolic diseases. Subjects were not currently engaged in any type of resistance or endurance training. Each subject was informed of all potential risks, benefits, and protocols prior to providing written informed consent. 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.

3.2 Experimental Design

All subjects reported to the Cardiopulmonary and Metabolism Research Laboratory at the University of Toledo on four separate days. On the first visit, subjects completed a medical history questionnaire, completed the informed consent form, had anthropometric measurements (height and weight), resting heart rate (HR) and resting blood pressure taken, familiarized with the testing protocols, and a resting FMD measurement was taken. The subject was asked to fast overnight for 12 hours prior to each testing day. During the second visit, subjects had a FMD baseline measurement taken followed by induction of
ischemic-reperfusion injury and three additional FMD measurements, each 20 minutes apart. The second visit lasted approximately 3 hours and 30 minutes. During the third visit, subjects had a FMD baseline measurement taken then performed an exercise preconditioning protocol. Following exercise, there was a resting period and three additional FMD measurements taken, each 20 minutes apart. The third visit lasted approximately 3 hours and 30 minutes. During the fourth visit, subjects had a FMD baseline measurement taken then performed an exercise preconditioning protocol. Following exercise, there was a resting period then ischemic-reperfusion injury was induced and three additional FMD measurements taken, each 20 minutes apart. The fourth visit lasted approximately 3 hours and 30 minutes. The second, third and fourth visits were at least 7 days apart and randomized. The testing was performed at approximately the same time of day for each subject.

3.2.1 Ischemic-Reperfusion Injury Protocol – Control (IRI)

Subjects were asked to lie down quietly in the supine position for 20 minutes. A baseline FMD measurement was taken. Subjects were given an hour and 20 minutes of rest, in which they could be in a seated position and relax. Ischemia was induced via a sphygmomanometer (blood pressure cuff) inflated to 200 mm Hg around the upper part of the non-dominant arm for 20 minutes. Immediately following the ischemic period, the cuff was deflated and subjects were then be asked to lie down in the supine position again for a 20 minute period of reperfusion. A FMD measurement was taken immediately following the 20 minutes of reperfusion, and again every 20 minutes for 1 hour (3 post-injury FMD measurements).
Figure 3-1. Ischemic-reperfusion injury protocol - Control (IRI). The time during rest prior to ischemia and reperfusion is 1 hour and 20 minutes to keep the time from the end of the initial FMD to the second FMD (2 hours) the same between the 3 protocols. Ischemia and reperfusion each lasts 20 minutes.

3.2.2 Exercise Protocol – Control (EXER)

Subjects were asked to lie down quietly in the supine position for 20 minutes. A baseline FMD measurement was taken. Subjects performed a 45 minute exercise on a commercially available treadmill at a level grade, with a 5 minute warm up at a self-selected pace prior to and 5 minute cool down at a self-selected pace following exercise. Exercise intensity was at approximately 60% of age-predicted maximal HR (predicted maximal HR = 220 – age). Heart rate was continuously monitored to ensure subjects maintain their target HR. Following exercise, there was a 1 hour period of rest. During the last 20 minutes of rest, the subject was asked to lie down in the supine position. A FMD measurement was taken immediately following the 20 minutes, and again every 20 minutes for 1 hour (3 post-injury FMD measurements).

Figure 3-2. Exercise Protocol – Control (EXER). The exercise is 1 hour and includes a warm-up and cool-down. The rest period is 1 hour to keep the time from the end of the initial FMD to the second FMD (2 hours) the same among all 3 protocols.
3.2.3 Exercise Preconditioning Protocol (EXER/IRI)

Subjects were asked to lie down quietly in the supine position for 20 minutes. A baseline FMD measurement was taken. Subjects performed a 45 minute exercise on a commercially available treadmill at a level grade, with a 5 minute warm up at a self-selected place prior to and 5 minute cool down at a self-selected pace following exercise. Exercise intensity was at approximately 60% of age-predicted maximal HR (predicted maximal HR = 220 – age). Heart rate was continuously monitored to ensure subjects maintain their target HR. Following exercise, ischemia was induced via a sphygmomanometer (blood pressure cuff) inflated to 200 mm Hg around the upper part of the non-dominant arm for 20 minutes. Immediately following the ischemic period, the cuff was deflated and subjects were asked to lie down in the supine position again for a 20 minute period of reperfusion. A FMD measurement was taken immediately following the 20 minutes of reperfusion, and again every 20 minutes for 1 hour (3 post-injury FMD measurements).

**Figure 3-3.** Exercise Preconditioning Protocol (EXER/IRI). The exercise is 1 hour and includes a warm-up and cool-down. A rest time of 20 minutes occurs before the ischemia and reperfusion to keep the time from the end of the initial FMD to the second FMD (2 hours) the same across all three protocols. Ischemia and reperfusion each lasts 20 minutes.
3.2.4 Flow-Mediated Dilation (FMD)

Flow-mediated dilation was used to assess the endothelial function of the brachial artery in the non-dominant arm. Subjects lie in a supine position. The non-dominant arm was abducted and above heart level. A forearm occlusion cuff was placed around the forearm and the ultrasound probe placed on the upper arm approximately 10 cm above the antecubital fossa (elbow joint). An initial baseline image was recorded digitally using commercially available image acquisition software. The cuff was inflated to approximately 250 mm Hg for 5 minutes. Following occlusion, the cuff was rapidly deflated and measurements continued for 2 minutes. Images were acquired using a linear array transducer with an operating frequency of 7 MHz and an echo-Doppler ultrasound imaging system (z.one ultra, ZONARE Medical Systems Inc., Mountain View, CA). A longitudinal image of the artery was recorded and examined for changes in diameter. All images were recorded digitally to a computer using imaging acquisition software (Vascular Imager, Medical Imaging Applications, LCC, Coralville, IA) and later analyzed with similar software (Brachial Analyzer, Medical Imaging Applications, LCC, Coralville, IA).

3.3 Data Analysis

The measurements of brachial artery diameter and blood velocities were used from rest and following an induced ischemic-reperfusion injury to calculate muscle blood flow. The calculation for muscle blood flow (MBF) was calculated as $\text{MBF} = \text{Mean}_{\text{vel}}(\text{CSA})(60)$; where $\text{Mean}_{\text{vel}}$ is the mean velocity (cm/s) of red blood cells through the brachial artery, while CSA is the cross-sectional area of the brachial artery ($\text{CSA} = \pi r^2$, where $r$ is the radius of the brachial artery). The calculation of the shear rate (SR) was calculated as $\text{SR} = 4(\text{Mean}_{\text{vel}}/D)$; where $\text{Mean}_{\text{vel}}$ is the mean velocity (cm/s) of red blood cells through the brachial artery, while $D$ is the brachial artery diameter (cm). Shear stress (SS) was calculated
as $SS = (SR)(5.0 \times 10^{-2} \text{ dyn}\cdot\text{s}\cdot\text{cm}^{-2})$; where SR represents shear rate and $5.0 \times 10^{-2} \text{ dyn}\cdot\text{s}\cdot\text{cm}^{-2}$ is equal to blood velocity through the brachial artery.

3.4 Statistical Analysis

A two-way analysis of variance (ANOVA) with repeated measures was used to identify differences in FMD responses between the IRI, EXER, and EXER/IRI protocols, and the time effect. A post-hoc analysis was used to find a significant main effect and/or interaction for further analyzing by using a Student-Newman-Keuls post-hoc test. Statistical significance was set at a priori of $P \leq 0.05$. All values are expressed as mean $\pm$ SD unless stated otherwise. Brachial artery diameter was measured in millimeters (mm) and expressed as a percent (%) change from baseline measurements. Data was analyzed with Sigma Stat software (Sigma Stat 3.0, Systat Software Inc., Ashburn VA).
Chapter 4

Results

Subject characteristics (6 males, 3 females) are presented in Table 4.1. All subjects reported that they were not currently participating in any regular physical activity or exercise training programs. According to the responses provided on the medical history questionnaire, all subjects were free from any known cardiovascular, pulmonary or metabolic conditions that would prevent them from participating in the study. The group mean target HR during the bout of moderate intensity exercise used to induce the remote ischemic preconditioning (RIPC) was 146 ± 5 bpm. The HR response of each subject was closely monitored resulting in a mean HR during exercise that was similar between protocols (EXER 148 ± 5 bpm; EXER/IRI 147 ± 6 bpm) and not significantly different from the target HR.

4.1 Brachial Artery Diameter Responses to Ischemic-Reperfusion Injury and Exercise

The pre- and post-occlusion brachial artery diameters during IRI, EXER and EXER/IRI conditions are presented in Table 4.2. Although there was a significant main effect for time, no main effect for protocol or interaction was observed for pre-occlusion brachial artery diameters between IRI, EXER and EXER/IRI conditions. At time corresponding to 0 (i.e. initial FMD trial), brachial artery diameter was similar between IRI
(3.86 ± 0.68 mm), EXER (3.90 ± 0.69 mm) and EXER/IR (3.81 ± 0.64 mm) conditions prior to occlusion. Following IRI and EXER/IRI, brachial artery diameter was increased prior to occlusion at 120 min (IRI, 4.12 ± 0.86 mm; EXER, 4.24 ± 0.86 mm) but returned to pre-occlusion values by 140 and 160 min after the FMD trial at time 0. No difference in pre-occlusion brachial artery diameter was observed at any of the times examined during the EXER protocol. The peak change in brachial artery diameter post-occlusion was similar across protocols and at all time points examined (Table 4.2).

4.2 FMD Responses of the Brachial Artery to Ischemic-Reperfusion Injury and Exercise

The mean %FMD responses to IRI, EXER and EXER/IRI protocols are presented in Figure 4-1 and summarized in Table 4.3. The ANOVA analysis indicated that there was a significant main effect for time however, no main effect for protocol (p=0.08) or interaction was observed. Compared to the initial FMD trial at time 0, both IRI and EXER/IRI resulted in a significant decrease in brachial artery %FMD at 120 min corresponding to a 60 ± 19% and 53 ± 16% decrease in vascular reactivity, respectively. In comparison to the corresponding %FMD response at time 0, the brachial artery vascular response remained attenuated at 140 min (p<0.05) for the IRI condition but returned to pre-injury values by 160 min. The %FMD response for EXER/IRI returned to initial values by 140 min with no further change from time 0 observed at 160 min. The EXER intervention did not result in a significant change in %FMD at any of the time points examined in comparison to the initial corresponding FMD trial at time 0. Although the EXER protocol resulted in an attenuated vascular response (%FMD at time 0 was 7.20 ± 2.44 vs. 4.74 ± 2.25 at 120 min), this decline in %FMD did not reach statistical significance.
4.3 Brachial Artery Blood Flow and Shear Stress Responses to Ischemic-Reperfusion Injury and Exercise

Pre-occlusion responses for brachial artery blood flow and shear stress for IRI, EXER and EXER/IRI protocols are shown in Figure 4-2 and Figure 4-3, and summarized in Table 4.4 and Table 4.5, respectively. Both IRI and EXER/IRI conditions resulted in a significant decrease in pre-occlusion brachial artery blood flow and shear stress that remained lower than values obtained at time 0 throughout the remainder of the protocol. Although the EXER protocol followed a similar pattern of change compared to IRI and EXER/IRI, there was no significant difference in either pre-occlusion brachial artery blood flow or shear stress at of the time points examined. No difference in pre-occlusion blood flow or shear stress was observed between the IRI, EXER or EXER/IRI conditions.

The peak brachial artery blood flow measured following cuff release was similar between protocols and no difference was observed for any of the time points examined (Figure 4-4; Table 4.4). Compared to the initial FMD trial at time 0, shear stress was significantly lower at 120 min in all protocols, however, there was no difference in shear stress between protocols across time. Shear stress returned to values corresponding to time 0 by 140 min for all protocols (Figure 4-5; Table 4.5).

4.4 FMD/Shear Stress Responses to Ischemic-Reperfusion Injury and Exercise

As shown in Figure 4-6, when the %FMD is expressed relative to peak post-occlusive brachial artery shear stress, the IRI protocol resulted in significant reduction in %FMD/shear stress at 120 min while the EXER/IRI protocol trended towards a decrease (p=0.09) indicating that the decrease in %FMD was greater than expected from the change in shear stress. The %FMD/shear stress remained depressed in IRI at 140 min and was significantly lower than EXER and EXER/IRI protocols at 140 min but returned to pre-
intervention values by 160 min; no difference between protocols were observed at 160 min.

EXER only was not associated with any change in %FMD/shear stress across the time.

<table>
<thead>
<tr>
<th>Table 4.1. Subject Demographics</th>
</tr>
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<tbody>
<tr>
<td>n = 9 Subjects.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Height, cm</td>
</tr>
<tr>
<td>Body mass, kg</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
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</tbody>
</table>


Table 4.2. *Arterial Diameters*.

*n* = 9 Subjects. Values are expressed as mean ± SEM. All arterial diameters are expressed in millimeters (mm). Pre-occlusion values are 30 second averages. Post-occlusion values are 5 second averages.

Pre-measurements are taken at the beginning of each protocol. The second measurement is taken 120 minutes after the pre-measurement. The third measurement is taken 140 minutes after the pre-measurement. The fourth measurement is taken 160 minutes after the pre-measurement.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>120 Min Post</th>
<th>140 Min Post</th>
<th>160 Min Post</th>
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</thead>
<tbody>
<tr>
<td><strong>Pre-Occlusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR Injury Protocol</td>
<td>3.86 ± 0.23</td>
<td>4.12 ± 0.29</td>
<td>3.96 ± 0.29</td>
<td>3.89 ± 0.25</td>
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<tr>
<td>Exercise Protocol</td>
<td>3.91 ± 0.23</td>
<td>4.00 ± 0.22</td>
<td>3.99 ± 0.23</td>
<td>3.92 ± 0.21</td>
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<tr>
<td>IR Injury/Exercise Protocol</td>
<td>3.81 ± 0.21</td>
<td>4.24 ± 0.29</td>
<td>3.93 ± 0.33</td>
<td>3.91 ± 0.28</td>
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<tr>
<td><strong>Post-Occlusion</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IR Injury Protocol</td>
<td>4.15 ± 0.23</td>
<td>4.21 ± 0.27</td>
<td>4.08 ± 0.25</td>
<td>4.13 ± 0.24</td>
</tr>
<tr>
<td>Exercise Protocol</td>
<td>4.18 ± 0.23</td>
<td>4.18 ± 0.23</td>
<td>4.01 ± 0.22</td>
<td>4.18 ± 0.21</td>
</tr>
<tr>
<td>IR Injury/Exercise Protocol</td>
<td>4.09 ± 0.22</td>
<td>4.31 ± 0.26</td>
<td>4.18 ± 0.32</td>
<td>4.13 ± 0.27</td>
</tr>
</tbody>
</table>
Table 4.3. Percent change from Pre- to Post- Diameters of the Brachial Artery.

\( n = 9 \) Subjects. Values are expressed as mean ± SEM. All changes between pre- and post-diameters are expressed as a percentage (%). Pre-measurements are taken at the beginning of each protocol. The second measurement is taken 120 minutes after the pre-measurement. The third measurement is taken 140 minutes after the pre-measurement. The fourth measurement is taken 160 minutes after the pre-measurement.

<table>
<thead>
<tr>
<th>Protocol</th>
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<tbody>
<tr>
<td>IR Injury Protocol</td>
<td>7.56 ± 1.04</td>
<td>2.57 ± 1.07</td>
<td>3.72 ± 1.53</td>
<td>6.34 ± 0.93</td>
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<tr>
<td>Exercise Protocol</td>
<td>7.20 ± 0.81</td>
<td>4.74 ± 0.75</td>
<td>6.39 ± 1.17</td>
<td>6.83 ± 1.32</td>
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<td>IR Injury/Exercise Protocol</td>
<td>7.23 ± 0.94</td>
<td>3.16 ± 1.05</td>
<td>6.96 ± 1.11</td>
<td>5.76 ± 1.08</td>
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Table 44. *Blood Flow.*

*n* = 9 Subjects. Values are expressed as mean ± SEM. All blood flow measurements are expressed in milliliters per minute (mL/min). Pre-occlusion values are 30 second averages. Post-occlusion values are 5 second averages. Pre-measurements are taken at the beginning of each protocol. The second measurement is taken 120 minutes after the pre-measurement. The third measurement is taken 140 minutes after the pre-measurement. The fourth measurement is taken 160 minutes after the pre-measurement.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>120 Min Post</th>
<th>140 Min Post</th>
<th>160 Min Post</th>
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<tr>
<td><strong>Pre-Occlusion</strong></td>
<td></td>
<td></td>
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<tr>
<td>IR Injury Protocol</td>
<td>36.0 ± 4.4</td>
<td>23.5 ± 3.9</td>
<td>25.6 ± 4.8</td>
<td>29.8 ± 4.2</td>
</tr>
<tr>
<td>Exercise Protocol</td>
<td>36.7 ± 6.5</td>
<td>32.1 ± 3.9</td>
<td>28.8 ± 3.6</td>
<td>27.8 ± 3.8</td>
</tr>
<tr>
<td>IR Injury/Exercise Protocol</td>
<td>37.2 ± 5.5</td>
<td>32.0 ± 6.1</td>
<td>25.4 ± 4.9</td>
<td>26.0 ± 5.2</td>
</tr>
<tr>
<td><strong>Post-Occlusion</strong></td>
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<td></td>
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</tr>
<tr>
<td>IR Injury Protocol</td>
<td>153.3 ± 13.8</td>
<td>133.0 ± 12.9</td>
<td>142.1 ± 16.0</td>
<td>145.7 ± 20.1</td>
</tr>
<tr>
<td>Exercise Protocol</td>
<td>154.1 ± 20.1</td>
<td>133.3 ± 13.9</td>
<td>155.7 ± 17.4</td>
<td>131.1 ± 17.5</td>
</tr>
<tr>
<td>IR Injury/Exercise Protocol</td>
<td>148.5 ± 14.2</td>
<td>159.2 ± 13.9</td>
<td>149.2 ± 18.9</td>
<td>142.0 ± 18.7</td>
</tr>
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</table>
Table 4.5. *Shear Stress.*

$n = 9$ Subjects. Values are expressed as mean ± SEM. All shear stress measurements are expressed in dynes per centimeter squared (dyn/cm²). Pre-occlusion values are 30 second averages. Post-occlusion values are 5 second averages. Pre-measurements are taken at the beginning of each protocol. The second measurement is taken 120 minutes after the pre-measurement. The third measurement is taken 140 minutes after the pre-measurement. The fourth measurement is taken 160 minutes after the pre-measurement.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>120 Min Post</th>
<th>140 Min Post</th>
<th>160 Min Post</th>
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<tr>
<td><strong>Pre-occlusion</strong></td>
<td></td>
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<tr>
<td>IR Injury Protocol</td>
<td>3.40 ± 0.34</td>
<td>1.90 ± 0.23</td>
<td>2.35 ± 0.31</td>
<td>2.19 ± 0.30</td>
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<td>Exercise Protocol</td>
<td>3.32 ± 0.47</td>
<td>2.84 ± 0.36</td>
<td>2.60 ± 0.31</td>
<td>2.57 ± 0.25</td>
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<tr>
<td>IR Injury/Exercise Protocol</td>
<td>3.25 ± 0.38</td>
<td>2.26 ± 0.24</td>
<td>2.26 ± 0.26</td>
<td>2.37 ± 0.30</td>
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<tr>
<td><strong>Post-occlusion</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IR Injury Protocol</td>
<td>16.0 ± 1.4</td>
<td>12.4 ± 1.0</td>
<td>15.1 ± 1.7</td>
<td>14.3 ± 0.9</td>
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<tr>
<td>Exercise Protocol</td>
<td>15.0 ± 0.9</td>
<td>12.6 ± 1.1</td>
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<td>IR Injury/Exercise Protocol</td>
<td>15.8 ± 1.3</td>
<td>13.7 ± 1.5</td>
<td>13.6 ± 1.3</td>
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Figure 4-1. The mean %FMD responses to IRI, EXER and EXER/IRI protocols. Bars are means ± SEM. *, significantly different from pre-occlusion (rest) measurements ($P < 0.05$).
Figure 4-2. The resting blood flow (mL/min) during pre-occlusion of IRI, EXER and EXER/IRI protocols. Bars are means ± SEM. *, significantly different from pre-occlusion (rest) measurements ($P < 0.05$).
Figure 4-3. The resting shear stress (dyn/cm²) prior to occlusion during IRI, EXER and EXER/IRI protocols. Bars are means ± SEM. *, significantly different from pre-occlusion (rest) measurements ($P < 0.05$).
Figure 4-4. The peak blood flow (mL/min) responses following cuff release during IRI, EXER and EXER/IRI protocols. Bars are means ± SEM. *, significantly different from pre-occlusion (rest) measurements ($P < 0.05$).
Figure 4-5. The peak shear stress (dyn/cm²) responses following cuff release of IRI, EXER and EXER/IRI protocols. Bars are means ± SEM. *, significantly different from pre-occlusion (rest) measurements (P < 0.05).
Figure 4-6. %FMD expressed relative to peak brachial artery shear stress responses during IRI, EXER and EXER/IR protocols. Bars are means ± SEM. *, significantly different from pre-occlusion (rest) measurements ($P < 0.05$).
Chapter 5

Discussion

The results of previous studies have shown that the performance of habitual physical activity or exercise training improves endothelial function (Allen, Geaghan, Greenway, & Welsch, 2003; Clarkson et al., 1999; Green, Maiorana, et al., 2004; Green et al., 2003; Green et al., 2004; Linke et al., 2001). Furthermore, the results of a recently published study have shown that performing regular physical activity may also provide some protection against IR injury (DeVan et al., 2011). Although the performance of a prior acute bout of moderate intensity exercise has shown to prevent the fall in endothelial function following consumption of a meal that is either high in fat (Silvestre et al., 2008; Tylldum et al., 2009) or glucose (Weiss, Arif, Villareal, Marzetti, & Holloszy, 2008; Zhu, Zhong, Yu, & Li, 2007) content, the potential benefits of performing a prior bout of moderate intensity exercise on preventing endothelial dysfunction due to IR injury has not been previously investigated. Thus, the purpose of this study was to determine if a prior bout of moderate intensity exercise on a treadmill would either prevent or attenuate the IR injury induced decrease in brachial artery endothelial dysfunction as determined using the flow-mediated dilation (FMD) approach. In agreement with previous studies (DeVan, et al., 2011; Loukogeorgakis, et al., 2005; Loukogeorgakis, Panagiotidou, Yellon, Deanfield, & MacAllister, 2006; Pernow, Bohm, Beltran, & Gonon, 2003), IR injury resulted in a significant decrease in endothelial
function that returned to pre-injury values within 1 hour. Similarly, when exercise was performed alone, endothelial function tended to decline which has also been demonstrated in previous studies (Gonzales, Thompson, Thistlethwaite, & Scheuermann; Harris, Padilla, Hanlon, Rink, & Wallace, 2008). However, in contrast to our hypothesis, performing a prior bout of moderate intensity exercise did lead to attenuation in endothelial function following IR injury suggesting that an acute bout of exercise may not afford any significant vascular protection such as that provided by remote ischemic preconditioning. It should be noted that endothelial dysfunction persisted longer into recovery following IR injury alone compared to when exercise was performed prior to IR injury indicating that exercise may not have been entirely inconsequential in aiding endothelial cell recovery from the ischemic insult. Interestingly, the ratio of %FMD to peak shear stress decreased significantly immediately following IR injury in the IRI condition and tended to decrease during EXER/IRI condition (p=0.09). This finding suggests that the decrease in %FMD following IR injury was greater than expected relative to any changes in shear stress following IR injury and thus, factors other shear stress may have contributed to the decrease in endothelial-dependent vasodilatation following reperfusion.

In a recent study by DeVan et al. (DeVan, et al., 2011), the consequences of long-term habitual physical activity and the potential protective benefits on IR injury were examined on healthy young and older adults who were considered to be sedentary or active. These investigators found that brachial artery FMD decreased significantly after 20 minutes of forearm ischemia and reperfusion, similar to the findings of the present study. However, the magnitude of endothelial dysfunction as a result of IR injury appeared to be greatest in the older sedentary group, while the older endurance trained group showed less of a decline in function compared to their older counterparts. In addition, these authors also reported
that young subjects, irrespective of whether they were sedentary or endurance trained, recovered more quickly from IR injury than the older subjects. These findings lead these investigators to conclude that habitual endurance exercise may impart partial protection against endothelial IR injury with advancing age. Thus, in humans, habitual exercise may attenuate the age-related impairment of endothelial function and alleviate decreases in endothelial function induced by cardiovascular diseases and associated bouts of ischemic insults.

During the ischemia phase, hypoxia disrupts membrane potential, the distribution of ions, and impairs cytoskeletal organization of endothelial cells (Carden & Granger, 2000). As the reperfusion phase occurs, the sudden increase in O₂ leads to a large production of ROS and superoxide, resulting in energy store depletion, decreases in NO and adenosine, Ca²⁺ overload, and the release of pro-inflammatory mediators (Carden & Granger, 2000; Gourdin, et al., 2009), leading to tissue damage and ultimately endothelial dysfunction. In our study, 20 minutes of forearm occlusion at a suprasyntolic pressure followed by 20 minutes of reperfusion lead to a significant decrease in endothelial function, as assessed by FMD of the brachial artery. After IR injury, the decline and recovery of the brachial artery FMD within 60 minutes is in agreement with previous studies young healthy populations (DeVan, et al., 2011; Kharbanda, et al., 2001; Loukogeorgakis, et al., 2006; Loukogeorgakis et al., 2007). Interestingly, previous studies have shown that endothelial-independent vasodilatation does not appear to be affected by IR injury. The administration of either sodium nitroprusside or nitroglycerin which act directly on vascular smooth muscle, resulted in similar vasodilatory responses compared to placebo trials providing evidence that the endothelial-independent vasodilatory mechanisms are not susceptible to IR injury
Studies have shown that FMD responses to reactive hyperemia are NO-dependent following the release of a 5 minutes distal occlusion, and the decrease in FMD is believed to be due to a decrease of NO bioavailability (Pyke et al., 2010). In the present study, peak shear stress was significantly reduced following IR injury in all three protocols which could lead one to speculate that NO release was also reduced due to IR injury and consequently, FMD response should also be reduced compared to pre-injury conditions. While the FMD responses were reduced considerably following IR injury, the decrease in the ratio of FMD to peak shear stress was greater than expected if the decline in endothelial-dependent vasodilatation was simply proportional to decrease in peak shear stress. Our findings suggest that factors other than peak shear stress may have contributed significantly to the endothelial dysfunction observed soon after the IR injury. Indeed, it has been shown that NO is not the only vasodilator that can be released in response to shear stress stimuli. Other vasodilators include prostaglandins and endothelial-derived hyperpolarizing factor (EDHF). There is evidence the EDHF may be able to compensate when NO production is either compromised or NO bioavailability is reduced (Bellien et al., 2007; Pyke, et al., 2010). For example, in a recent study by Pyke and colleagues (Pyke, et al., 2010), they examined radial artery FMD response to three different reactive hyperemia shear stress stimuli and found that NO may not be obligatory for radial artery FMD in response to either 5 or 10 minutes of occlusion in healthy volunteers. We did not examine the effect of IR injury on NO release or other potential vasodilators and therefore, elucidation of the mechanism(s) underlying the increase in endothelial dysfunction observed in the present study awaits further investigation.
In the present study, FMD measurements were repeatedly obtained during recovery from IR injury which may lead to the question whether consecutively repeated FMD measurements can alter the FMD response due to sensitivity of the vasodilator response of the endothelium. In a study by Harris et al. (Harris, Padilla, Rink, & Wallace, 2006), they examined the effect of repetitive reactive hyperemia on brachial artery FMD. The subjects were tested on 3 separate days. The protocols tested repetitive FMD measurements every 30 minutes, 60 minutes, and 120 minutes. They found that repetitive reactive hyperemia, similar to the approach we used in the present study had no effect on sequential FMD measurements. Although we did not systematically examine the FMD results for this effect, the fact that FMD measurements were performed repeatedly over a relatively short time period, it does not appear that our approach led to any prolonged endothelial dysfunction since the FMD responses returned to pre-injury values within 60 minutes.

5.1 Limitations

One limitation to our study is the involvement of female subjects, due to the potential effects that hormonal changes during the menstrual cycle may have on endothelial-dependent vasodilatation. We did not account for which phase in the menstrual cycle the women were currently in at the time of testing nor did we include a sufficient number of women in the study to compare results between sexes. Women exhibit changes in endothelial-dependent vasodilation during the menstrual cycle, which could affect the results (Chan, McAllister, Colhoun, Vallance, & Hingorani, 2001). Estrogen is believed to increase the release of NO through increased activation of endothelial NO synthase (eNOS), leading to increased vasodilation. A study by Chan et al. (Chan, et al., 2001) found that vascular responses in resistance vessels change during the menstrual cycle in women, and that the menstrual phase of women should be taken into account during data interpretation.
However in a recent study by Black et al. (Black, et al., 2009), they compared the FMD response of young men and young women and did not find a significant difference between them. These findings suggest that changes in female hormones during the menstrual cycle may or may not potentially affect endothelial function, however we did not observe any differences between the sexes that would suggest that the results of the present study would be any different if the trials had been better timed with specific phases of the menstrual cycle.

5.2 Conclusion(s)

In conclusion, brachial artery FMD showed no significant difference between protocols, but did show a main effect for time. Flow-mediated dilation showed significant differences during the IRI and EXER/IRI protocols, but returned to baseline values within 60 minutes of IR injury. Although peak shear stress was reduced following IR injury, the decrease in FMD was proportionally greater than the decrease in peak shear stress. Thus, the decrease in FMD in our study was greater than predicted by the decrease in peak shear stress suggesting that factors other than shear stress contribute to the fall in FMD following IR injury. Finally, the findings of the present study indicate that the performance of a single bout of moderate intensity exercise prior to IR injury does not impart any immediate protective benefits to the endothelium. Although other factors such as exercise duration, intensity and timing of application need to be considered, moderate intensity exercise immediately before IR injury is not appear to be a viable approach for remote ischemic preconditioning. However, since the exercise preconditioning protocol resulted in a faster recovery than IRI alone, this suggests there may some protection/benefits provided by the prior bout of acute exercise.
5.3 Future Directions

There are three different levels to the tissue damage and endothelial dysfunction seen in IR injuries. Level 3 poses the largest threat with irreversible damage to the tissue (i.e. necrosis, apoptosis) following a period of 20 minutes of ischemia or more (Powers, et al., 2008). Further investigation into the mechanisms behind the cause(s) of IR injury and the mechanism that imparts cardioprotection following exercise training could allow researchers and physicians to develop preventative measures that limit the amount of irreversible damage that occurs in many of the patients undergoing procedures that inevitably lead to ischemic-reperfusion injury.

Our study focused on young, healthy adults however, it is well known that the risk of cardiovascular diseases due to IR injury increases with advancing age. Thus, it would be beneficial to examine the potential benefits that exercise may have on middle-aged and/or elderly subjects, particularly in those individuals that typically lead a more sedentary lifestyle. DeVan and colleagues (DeVan, et al., 2011) stated that middle aged sedentary subjects demonstrated greater injury and delayed recovery from endothelial IR injury compared with younger subjects, and that habitual exercise training may have provided partial protection against the magnitude of endothelial IR injury with advancing age. Since many of the vascular complications associated with IR injury are observed in older adults, it is important that the potential benefits of acute and long-term exercise programs be systematically investigated in older adults where the effects may be more readily apparent because of the aging process.

Another key component to investigate would be the timing of the bout of exercise as strategy to induce remote ischemic preconditioning. The results of our study indicated that when exercise was used alone (i.e., EXER protocol), there was no significant effect on the
FMD response. This is contrast to other studies that have shown a decline in endothelial function following an acute bout of exercise. The different results between our study and those of others may be explained, at least in part, by the longer recovery phase between FMD measurements in the present study. Furthermore, whether the bout of exercise is performed before or after the IR injury may also be an important consideration. For example, the timing of exercise relative to the IR injury may have a significant effect on a number of variables that are known to affect endothelial function and the FMD response (Silvestre, et al., 2008; Weiss, et al., 2008) and is an issue that requires further investigation. Assessing endothelial function over a longer period of time from 2 to 24 hours could also provide additional information that could not be revealed with the approach used in the present study. Other studies (Eberlin, et al., 2008; Kharbanda, et al., 2002; Loukogeorgakis, et al., 2005) that have assessed FMD for a longer period following IR injury have not used exercise as an ischemic preconditioning method, but the effects of the more widely used approach of multiple cycles of ischemia and reperfusion have been examined.

Most studies using exercise to induce remote ischemic preconditioning have typically employed an exercise training program over a much longer period of time rather than a single bout of exercise. Although the results of the present study indicate that a single bout of exercise may not provide any significant benefits by preventing IR injury from inducing endothelial dysfunction, it would be interesting to determine the time-line that is required for exercise to induce benefits. To our knowledge, no studies have been undertaken that specifically investigates the interaction between IR injury and the time course of the benefits provided by exercise training induced remote ischemic preconditioning. Furthermore, determining the intensity “threshold” required for eliciting exercise training induced protection could lead to better strategies in providing recommendations for the use of
physical activity as a way to prepare for scheduled events (i.e. voluntary or planned surgeries) involving IR injury.

Our study used an acute bout of moderate intensity exercise as a way to induce remote ischemic preconditioning of the brachial artery. If this study had been performed using some form of forearm or handgrip as the exercise mode rather than the lower limb exercise on the treadmill exercise, this would have allowed us to investigate the effects of local ischemic preconditioning on IR induced injury. The protocol in the present study could be adapted so that endothelial function (or dysfunction) of brachial artery in response to remote ischemic preconditioning could be assessed simultaneously with the popliteal artery in response to local ischemic preconditioning. In this way, a direct comparison of these conduit arteries could be made and perhaps, additional information regarding the underlying protective mechanism(s) elicited by remote versus local ischemic preconditioning may be determined.
References


Appendix A

IRB Approval

TO: Barry Scheuermann, Ph.D.
    UT Department of Kinesiology

FROM: Roland Skorel, M.D., Chair
       Deepak Malhotra, M.D. Vice Chair
       Gregory Siegel, R.Ph., J.D., Chair Designee
       UT Biomedical Institutional Review Board

SIGNED: [Signature]

DATE: 12/22/11

SUBJECT: IRB # 107159
            TITLE: The Effect of an Acute Bout of Exercise on Ischemic-Reperfusion Induced Endothelial Injury

On December 10, 2010 the above named project was reviewed and approved by the Chair and Chair Designee of the University of Toledo Institutional Review Board as an expedited review (categories #4 and #6). A signed and dated Adult Research Subject Consent form is required from each participant prior to that individual taking part in this research. This research is approved for a period of up to one year from the date of this review and approval. This action will be reported to the full committee at its meeting on 01/20/2011.

Items Available for Review:
- IRB Application Requesting Initial Review of Expedited Research
- Protocol (assigned version date 12/10/2010)
- Adult Research Subject Consent Form (assigned version date 12/10/2010)
- Data Collection Tool (assigned version date 10/25/2010)
- Medical History Questionnaire (assigned version date 10/25/2010)
- Verbal Research Announcement (assigned version date 10/25/2010)

This research is approved until the expiration date listed below, unless the IRB notifies you otherwise.

Only the most recent IRB approved Consent form listed above may be used when enrolling participants into this research.

APPROVAL DATE: 12/10/2010               EXPIRATION DATE: 12/09/2011

Please read the following attachment detailing Principal Investigator responsibilities.
Subject Information and Consent Form

The Effect of an Acute Bout of Exercise on Endothelial Function following Ischemic-Reperfusion Injury

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

Other Staff (identified by role): Jennifer Lawrence (Co-investigator)  
Mitch Stacy (Graduate Student Research Assistant)  
Sarah McGlinchy (Graduate Student Research Assistant)

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

What you should know about this research study:

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

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

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

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

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

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

• Your participation in this research is voluntary.
PURPOSE (WHY THIS RESEARCH IS BEING DONE)
You are being asked to take part in a research study of an acute bout of exercise preconditioning and ischemic-reperfusion injury. The purpose of the study is to determine whether an acute bout of exercise can provide an alternative method of preconditioning to prevent ischemic-reperfusion injury.
You were selected as someone who may want to take part in this study because you have expressed an interest in exercise physiology research. A maximum of 20 volunteers will take part in this study.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT
If you decide to take part in this study, you will be asked to make a total of 4 visits to the Cardiopulmonary and Metabolism Research Laboratory.

During the first visit, all of the procedures and exercise protocol will be explained to you and you will be asked to complete an informed consent form and medical history questionnaire. Standard measurements of height, weight, resting heart rate and resting blood pressure will also be made during the first visit. A flow mediated dilation measurement of your brachial artery will be taken. This is done non-invasively using an echo-doppler ultrasonography system. The visit should last approximately 1 hour.

During the second visit, you will be asked to lie down quietly in the supine position for 20 minutes. A baseline FMD measurement will be taken. You will be given an hour and 20 minutes of rest, in which you can be in a seated position and relax. Ischemia will be induced via a blood pressure cuff inflated to 200 mm Hg around the upper part of your non-dominant arm for 20 minutes. Immediately following the ischemic period, the cuff will be deflated and you will then be asked to lie down in the supine position again for a 20 minute period of reperfusion. A FMD measurement will be taken immediately following the 20 minutes of reperfusion, and again every 20 minutes for 1 hour (3 post-injury FMD measurements). This visit will last approximately 3 hours and 30 minutes.

During the third visit, you will be asked to lie down quietly in the supine position for 20 minutes. A baseline FMD measurement will be taken. You will be asked to perform a 45 minute bout of exercise on a commercially available treadmill at a level grade, with a 5 minute warm up at a self-selected place prior to and 5 minute cool down at a self-selected pace following exercise. Exercise intensity will be at 60% of age-predicted maximal HR (predicted maximal HR = 220 – age). Following exercise, you will be given 1 hour of rest. During the last 20 minutes of rest, you will be asked to lie down in the supine position again for 20 minutes. A FMD measurement will be taken immediately following the 20 minutes, and again every 20 minutes for 1 hour (3 post-injury FMD measurements). This visit will last approximately 3 hours and 30 minutes.
During the fourth visit, you will be asked to lie down quietly in the supine position for 20 minutes. A baseline FMD measurement will be taken. You will be asked to perform a 45 minute bout of exercise on a commercially available treadmill at level grade, with a 5 minute warm up at a self-selected pace prior and 5 minute cool down at a self-selected pace following exercise. Exercise intensity will be at 60% of your age-predicted maximal HR (predicted maximal HR = 220 – age). Ischemia will be induced via a blood pressure cuff inflated to 200 mm Hg around the upper part of your non-dominant arm for 20 minutes. Immediately following the ischemic period, the cuff will be deflated and you will be asked to lie down in the supine position again for a 20 minute period of reperfusion. A FMD measurement will be taken immediately following the 20 minutes of reperfusion, and again every 20 minutes for 1 hour (3 post-injury FMD measurements). This visit will last approximately 3 hours and 30 minutes.

**RISKS AND DISCOMFORTS YOU MAY EXPERIENCE IF YOU TAKE PART IN THIS RESEARCH**
As with any exercise regimen, there is a very small risk of heart attack. You may stop the test at any time if you feel need to do so. There is also the possibility of muscle soreness 24-48 hours after you exercise. Potential soreness should be limited to the legs, and be minimal due to the moderate intensity of the exercise.

**POSSIBLE BENEFIT TO YOU IF YOU DECIDE TO TAKE PART IN THIS RESEARCH**
There is no direct benefit from participating in this study to the participants. Students from the Department of Kinesiology who participate in this study will be exposed to current research topics and techniques. We cannot and do not guarantee or promise that you will receive any benefits from this research.

**COST TO YOU FOR TAKING PART IN THIS STUDY**
There are no associated costs for participating in this study.

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

**ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH**
No alternative procedures or treatments will be made available since this research does not incorporate any procedures or treatments that affect the participant.
CONFIDENTIALITY - (USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION)

By agreeing to take part in this research study, you give to The University of Toledo (UT), the Principal Investigator and all personnel associated with this research study your permission to use or disclose health information that can be identified with you that we obtain in connection with this study. We will use this information for the purpose of conducting the research as described in the research consent/authorization form. The information that we will use or disclose includes the data collected as described in the procedures section. We will only use this information for ourselves as part of the research study. Under some circumstances, the Institutional Review Board and Research and Sponsored Programs of the University of Toledo may review your information for compliance audits. We may also disclose your protected health information when required by law, such as in response to judicial orders.

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

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

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

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

A more complete statement of University of Toledo’s Privacy Practices is set forth in its Joint Notice of Privacy Practices. If you have not already received this Notice, a member of the research team will provide this to you. If you have any further questions concerning privacy, you may contact the University of Toledo’s Privacy Officer at 419-383-3413.
IN THE EVENT OF A RESEARCH-RELATED INJURY
In the event of injury resulting from your taking part in this study, treatment can be obtained at a health care facility of your choice. You should understand that the costs of such treatment will be your responsibility. Financial compensation is not available through The University of Toledo or The University of Toledo Medical Center. By signing this form you are not giving up any of your legal rights as a research subject. In the event of an injury, contact: Dr. Barry Scheuermann (419.530.2692) or Jennifer Lawrence (419.530.2058).

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 any questions regarding the research at any time before, during or after the study, you may contact Dr. Barry Scheuermann (419.530.2692) or Jennifer Lawrence (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 or Person Authorized to Consent</th>
<th>Date</th>
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<tr>
<th>Name of Person Obtaining Consent (please print)</th>
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<th>Name of Witness to Consent Process (when required by ICH Guidelines) (please print)</th>
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Appendix B

Medical History Questionnaire

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td>Family history of heart disease? i.e. Heart attack, by-pass, stroke, or sudden death before age 55 in 1st degree male relative (father, brother, son) or before age 65 in 1st degree female relative (mother, sister, daughter)</td>
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<td>Smoking habit? i.e. Current cigarette smoker or one who has quit within the previous 6 months</td>
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<td>High blood pressure? i.e. systolic pressure &gt; 140/90 on two separate occasions or currently on antihypertensive medication</td>
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<td>Abnormal cholesterol levels? i.e. Total Cholesterol &gt; 200mg/dL, or LDL &gt; 130mg/dL, or HDL &lt; 35mg/dL, or currently on lipid lowering medication</td>
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<td>High fasting glucose? i.e. Fasting blood glucose &gt; 110 on two separate occasions</td>
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<td>Are you inactive? i.e. Accumulate 30 minutes of moderate physical activity on most days of the week</td>
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<td>Do you currently have any of the following?*</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Pain in the chest, neck, jaw, or arms?</td>
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<td>Shortness of breath at rest or with mild exertion?</td>
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<tr>
<td>Dizziness or fainting?</td>
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<tr>
<td>Difficulty breathing while lying down, relieved by sitting up?</td>
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<td>Awakened by shortness of breath?</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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<tr>
<td>Do you have a history of the following?**</td>
<td>Yes</td>
<td>No</td>
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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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<tr>
<td>Do you have any of the following?*</td>
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</table>

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