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entitled

Effect of High Intensity Interval Training (HIIT) on Vascular Function and Insulin Sensitivity

by

Shinichiro Sugiura

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in Exercise Science

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An Abstract of

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High-intensity interval training (HIIT) is characterized by short bursts of vigorous physical activity, interspersed by periods of rest or low-intensity exercise of varying durations. HIIT may be used as an effective alternative to traditional endurance training, resulting in similar or greater improvements in a number of physiological, performance, and health-related indices in both healthy and in individuals with chronic diseases. The present thesis examined the effects of a two-week HIIT program on measures of aerobic fitness, vascular function (Chapter 3) and insulin sensitivity (Chapter 4).

The first study demonstrated no effect on flow-mediated dilation or peak oxygen uptake following two weeks of HIIT with L-arginine supplement compared to HIIT alone, although both groups improved time to exhaustion and the peak work rate during progressive ramp exercise to fatigue. The results of the second study demonstrated six session of HIIT had no effect on glucose appearance and removal (i.e. oral glucose tolerance test, OGTT) or insulin sensitivity in healthy individuals with family history of type 2 diabetes mellitus (T2DM) or in healthy individuals without a family history of T2DM. However, results of the multiple regression analysis indicated that high density

lipoprotein (HDL) and low density lipoprotein (LDL) significantly predicted insulin sensitivity in healthy individuals with a family history of T2DM.

In order to prevent chronic diseases, such as T2DM, as well as other diseases that have a sedentary lifestyle as a primary risk factor, it is critical that individuals engage in regular physical activity in an effort to curb the rising trends in the prevalence of hypokinetic diseases. Although the results of the present investigation did not provide significant findings in this group of young, healthy adults, further investigations are necessary, including an examination of the physiological adaptations and compliance associated with longer term HIIT exercise programs, the inclusion of additional patient populations, and the optimal combination of exercise intensity, duration and recovery intervals in order to promote HIIT as an effective, efficient alternative training method.

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List of Abbreviations

ACE.....Angiotensin Covering Enzyme
ADA.....American Diabetes Association
ADP.....Adenosin Diphosphate
AGEs.....Glycation End Products
AMP.....Adenosin Monophosphate
AMPK.....Adenosin Monophosphate Kinase
ATP.....Adenosisn Triphosphate
A1c.....Glycated Hemoglobin

BMI.....Body Mass Index

CAD.....Coronary Artery Disease
CDC.....Center for Disease Control and Prevention
CETP.....Cholesteryl Ester Transfer Protein
CO_2.....Carbon Dioxide
CVD.....Cardiovascular Disease

EDRF.....Endothelium-Derived Relaxing Factor
END.....Endurance Exercise
eNOS.....Endothelial NOS
ET-1.....Endothilin 1

FDR.....First Degree Relatives
FFA.....Free Fat Acid
FMD.....Flow-Mediated Dilation

GLUT1.....Glucose Transporter 1
GLUT4.....Glucose Transporter 4

H^+.....Hydrogen Ion
HDL.....High Density Lipoprotein
HHb.....Deoxygenation
HIIT.....High Intensity Interval Training
HL.....Heptic Lipase

iEMG.....integrated Electromyography
IGFs.....Insulin-like Growth Factors

iNOS.....inducible NOS

K^+.....Potassium Ion

LDL.....Low Density Lipoprotein
L-NMMA.....NO Synthase Inhibitor NG-monomethyl-L-arginine
LPL.....Lipoprotein Lipase

Mfn2.....Mitofusion 2
mRNA.....messenger Ribonucleic Acid

Na^+.....Sodium Ion
NADPH.....Nicotinamide Adenine Dinucleotide Phosphate
NF-kB.....Nuclear Factor Kappa B
nNOS.....neuronal NOS
NO.....Nitric Oxide
NO_2^-.....Nitrite
NO_3^-.....Nitrate
NOS.....Nitric Oxide Synthase
NTG.....Nitroglycerin

OGTT.....Oral Glucose Tolerance Test

PGE_2.....Prostaglandin
PGI_2.....Prostacyclin
PKC.....Protein Kinase C
PO.....Peak Output

RAGE.....Receptor for Glycation End Products

SOD.....Superoxide Dismutase

TG.....Triglyceride
T2DM.....Type 2 Diabetes Mellitus

VE_{peak}.....Peak Ventilation
VLDL.....Very Low Density Lipoprotein
VO_{2max}.....Maximal Oxygen Uptake
VSMCs.....Vascular Smooth Muscle Cells

WR.....Work Rate

Chapter 1

Introduction

High-intensity interval training (HIIT) involves performing short bursts of vigorous physical activity, interspersed by periods of rest or low-intensity exercise. The specific physiological adaptations induced by this form of exercise training is determined by factors, such as intensity, duration, the number of intervals performed, as well as the duration and activity patterns during the recovery period (Gibala et al 2012). There are almost an infinite number of possibilities or combinations of exercise and recovery with interval training making it appealing to those who prefer variety and flexibility in their exercise program. It is becoming increasing evident that HIIT may be used as an effective alternative to traditional endurance training, resulting in similar or even better changes in a wide range of physiological, performance, and health-related biomarkers in both healthy and individuals with chronic diseases (Gibala et al 2012, Hwang et al 2011, Tjonna et al 2009, Wisloff et al 2007).

1.1 Thesis Outline

A Center for Disease Control and Prevention (CDC) survey (Jaslow 2013) showed that 80% of American adults do not meet the weekly recommended amount of

physical activity to maintain a healthy life-style with many of the respondents indicating that they were simply too busy to exercise on a regular basis. According to the results of previous research (Gibala et al 2012), a HIIT model consisting of 10 bouts of exercise each lasting for 60 seconds at a constant-load intensity approximating 90% of maximal heart rate, interspersed with 60 seconds of recovery is safe for most subjects to perform, is well tolerated by the subjects and is relatively appealing to those wanting to exercise but find it difficult to fit into their schedule. This approach to exercise training is also time efficient in that only 10 minutes of exercise is performed over a 20 minute training session which is considerably less than the time spent exercising during more traditional training programs (Gibala et al 2012). Therefore, HIIT may represent a viable alternative approach to exercise training, which may allow an individual to either improve or maintain their level of fitness, while at the same time spending relatively little time actually exercising.

The Review of Literature (Chapter 2) examines the physiological responses, including cardiovascular and metabolic responses, associated with HIIT in both healthy and patient populations, but with a specific focus on individuals with type 2 diabetes mellitus (T2DM).

The first study performed (Chapter 3) is an examination of the effects of HIIT with and without the consumption of a nutritional supplement containing a NO precursor (L-arginine) on vascular function (brachial artery), oxygen utilization at the pulmonary and microcirculatory level, and exercise tolerance.

In the second study carried out (Chapter 4), we determine whether a family history of T2DM is associated with improvement of insulin sensitivity over two weeks of HIIT, compared to healthy individuals without a family history of T2DM. We also investigate the improvement of absolute change in plasma glucose before and after each training session of HIIT. In addition we examine the relationship between insulin sensitivity and lipid profile, specifically triglyceride and low-density lipoprotein (LDL), over two weeks of HIIT.

Chapter 2

Literature Review

Introduction

Type 2 diabetes mellitus (T2DM) is a serious health problem, driven by disable micro-vascular complications and cardiovascular disease, and obesity is the core factor of T2DM, affecting two third of adults and reaching the warning rates in children in the modern era (Cusi 2009). The epidemic of T2DM poses a challenge to health care providers, the magnitude of the diabetes epidemic and its relationship to obesity, and the metabolic syndrome (Cusi 2009). In addition, different types of physical activities may have a positive impact on physical fitness, morbidity, and mortality among T2DM patients, based upon American Diabetes Association (ADA) position statement. We introduce high intensity interval training (HIIT), since the Centers for Disease Control and Prevention (CDC) survey (Jaslow 2013) showed that 80% of American adults do not meet the weekly-recommended exercise due to the average Americans' busy schedule. However, it does not mean they are not able to sustain or improve their wellness. We discuss HIIT, blood flow, Nitric Oxide (NO), endothelial function, flow-mediated dilation, glucose regulation, an oral glucose tolerance test (OGTT), insulin resistance, and

a disease such as T2DM. Overall, we investigated the effects of HIIT on glucose regulation in individuals with a family history of diabetes in the future.

2.1 High Intensity Interval Training (HIIT)

High-intensity interval training (HIIT) explains physical exercise by short bursts of vigorous activity, interspersed by periods of rest or low-intensity exercise. It is not finitely variable with the specific physiological adaptations induced by this form of training determined by a great number of factors of stimulus, such as intensity, duration, the number of intervals performed, as well as the duration and activity patterns during recovery (Gibala et al 2012). HIIT can be used as an effective alternative to traditional endurance training, resulting in similar or even better changes in a range of physiological, performance, and health-related biomarkers in both healthy and individuals with chronic diseases (Gibala et al 2012, Hwang et al 2011, Tjonna et al 2009, Wisloff et al 2007).

According to results of previous research (Burgomaster et al 2008, Rakobowchuk et al 2008), similar training-induced improvements in various markers of skeletal muscles and cardiovascular adaptation despite large differences in weekly training volume (~90% lower in the HIIT group) and time commitment (~67% lower in the HIIT group) were shown compared with traditional endurance training (Gibala et al 2012). In addition, other endurance-like adaptations have been reported after several weeks of low-volume HIIT, such as: an increased resting glycogen content, a reduced rate of glycogen utilization and lactate production during matched-work exercise, an increased capacity for whole-body and skeletal muscle lipid oxidation, and improved exercise performance based upon time to exhaustion tests or time trials, and increased maximal oxygen uptake (Burgomaster et al 2008, Burgomaster et al 2005, Gibala et al 2012, Gibala et al 2006,

Rakobowchuk et al 2008). Moreover, a previous study (Hood et al 2011) found that HIIT increased the protein content of citrate synthase and cytochrome c oxidase subunit IV, the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator 1α, glucose transporter content, and insulin sensitivity after HIIT.

A new practical HIIT model consists of 10 x 60 seconds work bout at a constant-load intensity that elicits ~90% of maximal heart rate, interspersed with 60 seconds of recovery based upon the potential safety, subject tolerance, and appealing for some individuals (Gibala et al 2012). This protocol is also time efficient in that only 10 minutes of exercise is performed over a 20 minutes training session (Gibala et al 2012). It is a practical and time-efficient model that is effective not only at inducing rapid skeletal muscle remodeling toward a more oxidative phenotype similar to the previous Wingate-based HIIT studies and high-volume endurance training (Little et al 2010), but also for improving functional performance (Gibala et al 2006, Little et al 2010) as shown by cycling time trials that resemble normal athletic competition (Gibala et al 2012).

2.2 HIIT with Disease Populations

2.2.1 *Metabolic Syndrome*

The metabolic syndrome is associated with increased cardiovascular morbidity and mortality, because individuals with the metabolic syndrome are three times more likely to die of coronary heart disease than healthy ones after adjustment for conventional cardiovascular risk factors (Lakka et al 2002, Tjonna et al 2008). Individuals with the metabolic syndrome are reduced by aerobic fitness and endothelial function. It has been

proposed to be independent and strong predictors of mortality related to other established risk factors (Halcox et al 2002, Myers et al 2002, Tjonna et al 2008).

One research study (Tjonna et al 2008) investigated cardiovascular function and prognosis in patients with the metabolic syndrome compared continuous moderate exercise with high intensity interval training (HIIT). Thirty-two metabolic syndrome patients participated in either group three times a week for sixteen weeks. VO_{2max} improved more after HIIT than the other group (35% versus 16%), and HIIT was superior to continuous moderate exercise in enhancing endothelial function (9% versus 5%). In addition, HIIT enhanced insulin signaling in fat and skeletal muscle, skeletal biogenesis, and excitation-contraction coupling. Finally, HIIT reduced blood glucose and lipogenesis in adipose tissue. Exercise intensity was an important factor to improve aerobic capacity and to reverse the risk factors of the metabolic syndrome (Tjonna et al 2008).

2.2.2 *Type 2 Diabetes Mellitus (T2DM)*

A previous study (Little et al 2011) examined glucose regulation and skeletal muscle metabolic capacity among T2DM patients by using HIIT, and participants performed Gibala et al's (Gibala et al 2012) protocol. Training increased muscle mitochondrial capacity as evidenced by higher citrate synthase maximal activity and protein content of Complex II 70 kDa subunit, Complex III Core 2 protein, and Complex IV subunit IV. In addition, Mitofusion 2 (Mfn2) and GLUT4 protein content were higher after training.

Since reduced mitochondrial capacity in skeletal muscle was reported in insulin resistance and T2DM (Ritov et al 2010) and muscle oxidative capacity was shown to be a significant predictor of insulin sensitivity (Bruce et al 2003), it was possible that the rapid

increase in skeletal muscle mitochondrial content after HIIT might contribute to reduced insulin resistance and improved glycemic control (Little et al 2011). The training-induced increase in GLUT4 protein content potentially played a role in improving glucose regulation (Little et al 2011). Studies in rodents indicated that the exercise-induced increase in GLUT4 protein was directly related to the increase in muscle glucose uptake at any given insulin concentration (Little et al 2011, Ren et al 1994). Finally, elevated Mfn2 might be involved in regulating the increase in mitochondrial capacity after HIIT, and a role of Mfn2 in the pathogenesis of T2DM was supported by studies reporting reduced Mfn2 expression in skeletal muscles of T2DM, suggesting alternations in mitochondrial fusion/fission might contribute to mitochondrial impairment (Hernandez-Alvarez et al 2010, Little et al 2011). However, it is unknown that a training-induced increase in muscle Mfn2 is linked to improved metabolic health.

Gillen et al. (Gillen et al 2012) examined the 24-hour blood glucose response to one session of HIIT in using continuous glucose monitoring, compared with a non-exercise control day among T2DM that is prevalence of hyperglycemia. HIIT reduced hyperglycemia measured as a part of time spent above 10 nmol/L, and postprandial hyperglycemia, measured as the sum of post-meal areas under the glucose curve, was also lower after HIIT. It showed HIIT improved glyemic control in T2DM. In contrast, Manders et al. (Manders et al 2010) found no influence of continuous high-intensity exercise (30 minutes at 70% peak output), but it might be associated with the intermittent pattern and/or the higher absolute intensity of HIIT. Mechanisms mediating the reductions in postprandial hyperglycemia after exercise cannot be ascertained, but it speculates that HIIT increased skeletal muscle insulin sensitivity (Gillen et al 2012). The

high degree of muscle fiber recruitment (Gibala & McGee 2008) and/or glycogen utilization (Larsen et al 1999) related to HIIT may increase subsequent muscle glucose uptake (Gillen et al 2012).

2.2.3 *Cardiac Diseases*

Two research studies (Guiraud et al 2010, Meyer et al 2012) investigated the optimal protocol of HIIT by using coronary heart disease or chronic heart failure patients. The first study (Meyer et al 2012) found that heart failure subjects, who performed with 100% of peak power output, 30 seconds interval duration, and passive recovery, lasted for a longer exercise time (tolerance), compared to active recovery groups, although all protocols appeared to be safe. However, its protocol was superior to others, because of time above percentages of VE_{peak} and time above percentages of O_2 $pulse_{peak}$. The second study (Guiraud et al 2010) stated that the protocol, which consisted of coronary heart disease patients asking their comfort level of exercise and time spent above 80% of VO_{2max} with 15 seconds interval and passive recovery, was optimal, when they considered perceived exertion.

A previous study (Warburton et al 2005) compared HIIT with the traditional exercise training among patients with coronary artery disease. HIIT resulted in a greater improvement in time to exhaustion and anaerobic threshold than the other exercise without any risks to patients. Another investigation (Meyer 2001) indicated that interval training should be advocated for the rehabilitation of patients with severe chronic heart failure. Preliminary research (Meyer et al 1990) also indicated that interval training is more effective in improving exercise capacity than continuous aerobic training (Warburton et al 2005). To support the importance of HIIT for functional status, research

showed that this form of training improved cardiac function, such as submaximal stroke volume, myocardial contractility, an ejection fraction at peak exercise, and exercise tolerance (Ehsani et al 1986, Hagberg et al 1983, Oberman et al 1995, Warburton et al 2005). Moreover, HIIT reduced the incidence of angina and decreased ST-segment depression at a given rate-pressure product during exercise in previously symptomatic patients (Ehsani et al 1986, Warburton et al 2005). Thus, it was obvious that HIIT could improve submaximal and maximal cardiovascular function, while decreasing ischemia at the same myocardial oxygen consumption (Warburton et al 2005).

Another research study (Munk et al 2009) evaluated HIIT on in-stent restenosis after percutaneous coronary intervention for stable or unstable angina, and they found that it was associated with a significant reduction in late luminal loss in the stented coronary segment. This effect was related to increased aerobic capacity, improved endothelium function, and attenuated inflammation. Finally, a recent research study (Normandin et al 2013) showed that HIIT provided a high-level physiological stimulus for patients with heart failure and reduced ejection fraction without any arrhythmias, inflammation, myocardial dysfunction, or myocardial necrosis, compared with moderate-intensity continuous exercise.

Traditional endurance exercise training improved endothelial function with coronary artery disease (CAD) patients (Hambrecht et al 2000), and improvements in endothelial function with exercise training were suggested to account for up to 40% of the associated risk reduction (Currie et al 2012, Green et al 2008). This training is time-consuming, and compliance is relatively low, although endurance training demonstrated to be effective for patients with CAD (Barbour & Miller 2008, Currie et al 2012).

Interval exercise training gained considerable attention as an innovative strategy for individuals with decreased aerobic functional capacity (Wisloff et al 2007). A recent review of interval exercise training studies (Cornish et al 2011) in CAD patients highlighted greater improvements in many physiological indices, such as brachial artery endothelial function, compared with moderate-intensity endurance exercise (Currie et al 2012).

A previous study (Currie et al 2012) investigated a single bout of moderate-intensity endurance exercise (END) on a cycle ergometer, resulted in similar acute changes in endothelial function with individuals with CAD, compared with HIIT. Endothelial function was assessed using brachial artery flow-mediated dilation (FMD), and brachial artery diameter and velocities were measured in using Doppler ultrasound. Endothelial-dependent function improved with no differences between exercise conditions, and time effect for FMD normalized to the shear rate area under the curve was also observed. However, endothelial-independent function did not change after END or HIIT. Thus, END and HIIT resulted in similar acute increase in brachial artery endothelial-dependent function in a population with dysfunction at rest, in spite of the different exercise intensities.

Another research study (Currie et al 2013) examined 12 weeks of HIIT and END on FMD and cardiopulmonary fitness (VO_{2peak}) in patients with CAD. Both groups attended two supervised sessions per week for 12 weeks, including the END group performing 30-50 minutes of continuous cycling at 58% peak power output (PO), in contrast, the HIIT group performing 10 x 60 seconds with 60 seconds intervals at 89% of PO separated by 60 seconds intervals at 10% of PO. In results, both groups improved

FMD and there was no group difference. Thus, HIIT provided an alternative to the current and more time-intensive prescription for cardiac rehabilitation, because HIIT elicited similar improvements in FMD and fitness, despite different exercise intensity and duration.

2.3 Local Regulation of Blood Flow

Klabunde et al (2005) describes local blood flow regulation. Tissues and organs have the ability to regulate their own blood flow to some extent. This intrinsic ability to regulate blood flow is called local regulation, and it can occur in the complete absence of any extrinsic neurohumoral effects. For instance, if a muscle is removed from the body, perfused under constant pressure with an oxygenated salt solution, and electrically stimulated to induce muscle contractions, the blood flow increases.

The mechanisms responsible for local regulation originate within the blood vessels, such as endothelial factors, myogenic mechanisms, and from the surrounding tissues (tissue factors). Many of which are related to ways, for example, arachidonic acid metabolites and bradykinin. Mechanical factors, such as compressive force during muscle contraction, can influence resistance and blood flow.

2.3.1 *Tissue Factors*

The tissue substances are produced by the tissue surrounding blood vessels. These substances react in the blood vessel to produce either relaxation or contraction of the smooth muscle, so they alter resistance and blood flow. In some cases, these substances indirectly act on the vascular smooth muscle by affecting endothelial function or by altering the release of norepinephrine by sympathetic nerves. In addition, some

vasoactive substances are tissue metabolites that are products of cellular metabolism or activity, such as adenosine, CO_2, H^+, K^+, and lactate. In addition, different cell types surrounding blood vessels can release vasoactive substances as local, paracrine hormones, such as histamine, bradykinin, and prostaglandins.

Increases or decreases in metabolism alter the release of some of these vasoactive substances. Metabolic activity is closely coupled to blood flow in most organs of the body. The actively metabolizing cells surrounding arterioles release vasoactive substances that cause the vasodilation. Several substances have been implicated in metabolic regulation of blood flow as follows:

1. Adenosine is a potent vasodilator in most organs, and the adenosine monophosphate (AMP) is derived from hydrolysis of intracellular adenosin triphosphate (ATP) and adenosine diphosphate (ADP).
2. Inorganic phosphate is released by the hydrolysis of adenine nucleotides (ATP, ADP, and AMP). Inorganic phosphate may have some vasodilatory activity in contracting skeletal muscle, but its importance is far less than that of adenosine, potassium, and nitric oxide in regulating skeletal muscle blood flow.
3. Carbon dioxide (CO_2) readily diffuses from parenchymal cells to the vascular smooth muscle of blood vessels as a gas, where it causes vasodilation.
4. Hydrogen ion (H^+) increases CO_2 during states of high anaerobic metabolism, when acid metabolites such as lactic acid are produced. Increased H^+ causes local vasodilation, specifically, in the cerebral circulation.
5. Potassium ion is released by contracting cardiac and skeletal muscles. Normally, the Na^+/K^+-ATPase pump is able to restore the ionic gradients, but the pump does

not keep up with a rapid depolarization during a muscle contraction and a small amount of K^+ accumulates in the extracellular space. Small increases in extra-cellular K^+ around blood vessels cause the hyperpolarization of the vascular smooth muscle cells, possibly by stimulating the electrogenic Na^+/K^+-ATPase pump and increasing K^+ conductance through potassium channels. The hyperpoloarization leads smooth muscle relaxation. Potassium ion plays a significant role in the increase in blood flow in contracting skeletal muscles.

6. Oxygen levels in the blood vessel and surrounding tissue are important in the local regulation of blood flow. The hypoxia-induced vasodilation may be direct inadequate O_2 to sustain smooth muscle contraction or indirect via the production of vasodilator metabolites, such as adenosine, lactic acid, and H^+. Although hypoxia causes vasodilation in almost all vascular beds, there is a notable exception that it causes vasoconstriction in the pulmonary circulation.
7. Osmolarity changes in the blood and in the tissue interstitium have been implicated in local blood flow regulation. It is well known that intra-arterial infusions of hyperosmolar solutions can produce vasodilation. Tissue ischemia and increased metabolic activity raise the osmolarity of the tissue interstitial fluid and venous blood.

Several tissue factors involved in regulating blood flow are not directly associated with tissue metabolism. These factors include paracrine hormones, such as histamine, bradykinin, and products of arachidonic acid (eicosanoids). Histamine, released by mast cells in response to injury, inflammation, and allergic responses, causes arteriolar

vasodilation, venous constriction in some vascular beds, and increased capillary permeability.

Bradykinin is formed from the action of kallikrein (a proteolytic enzyme) acting on alpha$_2$-globulin (kininogen), which is found in blood and tissues, and bradykinin is a powerful arterioles dilator. It acts on vascular bradykinin receptors, which stimulate nitric oxide formation by the vascular endothelium, such as vasodilation. Also, bradykinin stimulates prostacyclin formation, which produces vasodilation. One of the enzymes responsible for breaking down bradykinin is angiotensin-covering enzyme (ACE). Thus, ACE inhibition decreases angiotensin II, and it increases bradykinin, that is responsible for the vasodilation accompanying ACE inhibition.

Arachidonic acid metabolites, such as prostacyclin (PGI_2) and prostaglandin E_2 ($PG\ E_2$) are vasodilators, and other eicosanoids, such as $PGF_{2\alpha}$, thrombozanes, and leukotrienes are generally vasoconstrictors.

2.3.2 *Endothelial Factors*

The vascular endothelium plays an important role in the regulation of smooth muscle tone and organ blood flow. Two of substances are nitric oxide and prostacyclin, that are powerful vasodilators. In contrast, endothelin-1 is a powerful vasoconstrictor. Although all three of these endothelial-derived substances have important actions on the vascular smooth muscle, nitric oxide appears to be the most important in regulating blood flow under normal physiologic conditions. An increase in shearing forces acting on the vascular endothelium stimulates endothelial nitric oxide production, which causes vasodilation.

2.3.3 *Smooth Muscle (Myogenic) Mechanisms*

Myogenic mechanisms originate in the smooth muscle of blood vessels, specifically, in small arteries and arterioles. When the lumen of a blood vessel is expanded, which occurs when the intravascular pressure is suddenly increased, the smooth muscle responds by contracting in order to restore the vessel diameter and resistance. In contrast, a reduction in the intravascular pressure results in smooth muscle relaxation and vasodilation. The reduction in the blood flow associated with the increase in venous pressure activates tissue metabolic mechanisms that cause vasodilation. In most organs vasodilation occurs because the metabolic vasodilator response overrides the myogenic vasoconstrictor response.

2.3.4 *Extra-vascular Compression*

Mechanical compression forces can affect vascular resistance and blood flow within organs, and this sometimes occurs during normal physiologic conditions. At other times, compression forces can be the result of pathologic mechanisms. The pressure that distends the wall of a blood vessel is the transmural pressure (inside minus outside pressure). The veins, that have a relatively low intravascular pressure, are more likely to collapse when the extra-vascular pressure is elevated, but arteries can also become significantly compressed, when the extra-vascular pressure is elevated to very high level.

2.3.5 *Autoregulation of Blood Flow*

Autoregulation is the intrinsic ability of an organ to maintain a constant blood flow despite changes in perfusion pressure, and it occurs in isolated and perfused organs, that are not subject to neural or humoral influences. When the perfusion pressure

(arterial – venous pressure: $P_A - P_V$) initially decreases, blood flow (F) falls due to the following formula (Ohm's law) such as $F = (P_A - P_V)/R$. The reductions in flow and perfusion pressure are thought to activate metabolic or myogenic mechanisms that cause anteriolar vasodilation and a fall in resistance (R).

Autoregulation may involve both metabolic and myogenic mechanisms. If the perfusion pressure to an organ is reduced, the initial fall in the blood flow leads to a fall in tissue pO_2 and the accumulation of vasodilator metabolites. These changes cause the resistance vessels to dilate in an attempt to restore normal flow. A reduction in perfusion pressure may also be sensed by the smooth muscle in resistance vessels, which responds by relaxing myogenic response, which leads to an increase in flow.

2.3.6 *Reactive and Active Hyperemia*

Reactive hyperemia is the transient increase in organ blood flow that occurs after a brief period of ischemia, usually produced by the temporary arterial occlusion. When the occlusion is released and perfusion pressure is restored, the flow becomes elevated due to the reduced vascular resistance. During the hyperemia, the oxygen becomes replenished, and vasodilator metabolites are washed out of the tissue. Hyperemia causes the resistance vessels to regain their normal vascular tone and return the flow to normal levels. The longer the period of the occlusion, the greater the metabolic stimulus for vasodilation leads to increase the peak flow and duration of hyperemia. In the process, myogenic mechanisms may contribute to reactive hyperemia, because arterial occlusion decreases the pressure in arteiroles, that can lead to myogenic mediated-vasodilation.

Active hyperemia is the increase in organ blood flow, that is associated with increased metabolic activity of an organ or tissue. With increased metabolic activity,

vascular resistance decreases owing to vasodilation and vascular recruitment (specifically skeletal muscle). Active hyperemia occurs during muscle contractions, called exercise or functional hyperemia, and it increases cardiac activity, mental activity, and gastrointestinal activity during the food absorption. The increase in the blood flow by active hyperemia is maintained throughout the period of increased metabolic activity, and it subsides when normal metabolism is restored. The amplitude of active hyperemia is associated with an increase in metabolic activity, such as oxygen consumption. This amplitude is important, because it increases oxygen delivery to tissues at a time of increased oxygen demand. Thus, the increased blood flow enhances the removal of metabolic waste products from the tissue.

2.4 Anatomy and Physiology of Vascular Smooth Muscle Cells

2.4.1 *Cellular Structure*

The diameter in vascular smooth muscle cells is about 5 to10 μm, and their length varies from 50 to 200 μm. The sarcoplasmic reticulum is developed more poorly than those in cardiac muscles. The actin and myosin are present, but that is not organized as repeating and distinct as bands in cardiac and skeletal muscles. In other words, bands of actin filaments are joined together and anchored by dense bodies within the cell or dense bands on the inner surface of the sarcolemma. These function as Z-lines in cardiac muscles. Also, vascular smooth muscle cells are electrically connected by gap junctions, and these low-resistance intercellular connections make propagated responses along the length of the blood vessels.

2.4.2 *Vascular Smooth Muscle Contraction*

The contractile characteristics between vascular smooth and cardiac muscles are considerably different, because the former contractions are slow and sustained but the latter contractions are rapid and relatively short. These tonic contractions are determined by a number of stimulatory and inhibitory functions on the vessel, such as sympathetic adrenergic nerves, circulating hormones (epinephrine, angiotensin II, etc), substances released by the endothelial tissues along the vessel, and vasoactive substances surrounding the blood vessel.

Vascular smooth muscle contraction can start with electrical, chemical, and mechanical stimuli. The electrical depolarization of the vascular smooth muscle cell membrane makes contraction by opening voltage dependent calcium channels, that cause an increase in the intracellular concentration of calcium. Also, the electrical depolarization occurs through changes in ion concentrations or by the receptor-coupled opening of iron channels, such as calcium channels. In terms of chemical stimuli, norepinephrine, epinephrine, angiotensin II, vasopressin, endothelin-1, and thromboxane A_2 elicit contractions. The mechanical stimuli can cause contractions that originate from the smooth muscle itself and are termed a myogenic response.

2.4.3 *Vascular Endothelial Cells*

The vascular endothelial cell is a thin layer that lines all blood vessels and is a flat, single-nucleated, and elongated cell, that is 0.2-2.0 μm thick and 1-20 μm in length dependent upon a vessel type. Based upon the type of vessel, endothelial cells are joined together by different types of intercellular junctions, and some of these junctions are very tight. However, others have gaps between the cells that enable blood cells to move in and

out of the capillary easily. Endothelial cells have a few important functions that are as follows:

1. Serving as a barrier for the exchange of fluid, electrolytes, macromolecules, and cells between the intravascular and extravascular space
2. Regulating smooth muscle function through several important and different vasoactive substances, such as nitric oxide (NO), prostacyclin (PGI_2), and endothelin-1
3. Modulating platelet aggregation primarily through biosynthesis of NO and PGI_2
4. Modulating leukocyte adhesion and transendothelial migration through the biosynthesis of NO and the expression of surface adhesion molecules

Vascular endothelial cells produce NO by the enzyme NO synthase, that converts L-arginine to NO. NO production can be enhanced by (1) specific agonist such as acetylcholine, bradykinin binding to endothelial receptors, (2) increased sharing forces acting on the endothelial surface, (3) cytokines such as tumor necrosis factor and interleukins, that are released by leukocytes during inflammation and infection. The major role in NO is to relax smooth muscle, inhibit platelet function, and inhibit inflammatory responses. Also, endothelial cells synthesize endothelin-1 (ET-1), a powerful vasoconstrictor. The synthesis is stimulated by angiotensin II, vasopressin, thrombin, cytokines, and sharing forces, but NO and PGI_2 inhibit the process.

It is clear that the importance of normal endothelial function contributes to disease states, such as atherosclerosis, hypertension, diabetes, and hypercholesterolemia. The endothelial dysfunction results in less NO and PGI_2 production, causing vasoconstriction, loss of vasodilatory capacity, thrombosis, and vascular inflammation. In addition, the

damage to the endothelium at the capillary level increases capillary permeability, which leads to the tissue edema.

2.5 Discovery and Roles of Nitric Oxide

Previous research (Furchgott & Zawadzki 1980) first explained the endothelium-dependent relaxation as a phenomenon whereby acetylcholine relaxes isolated preparations of blood vessels, only if the vascular endothelium lining the vessels is present and intact (Moncada & Higgs 2006). Acetylcholine and other agents, such as brandykinin, histamine, and 5-hydroxytryptamine, release a transferable factor. It is an unstable endothelium-derived relaxing factor called EDRF that acts with stimulation of the soluble guanylate cyclase and is inhibited by hemoglobin and methylene blue (Furchgott et al 1984, Moncada & Higgs 2006).

The identification of thromboxane synthase and discovery of prostacyclin helped use bioassay experiments in cascades in order to carry out more detailed quantitative pharmacology and try to elucidate the structure of EDRF (Moncada 2005, Moncada & Higgs 2006). In addition, Moncada and his colleagues proceeded to culture porcine aortic endothelial cells on micro-carrier beads, perfused them inside a modified chromatography column and used the effluent to superfuse vascular tissues denuded of endothelium as a detection system for EDRF (Moncada & Higgs 2006).

Furchgott proposed that EDRF might be nitric oxide (NO) in 1987 (Furchgott 1988, Moncada & Higgs 2006). The proposal was based upon the observations that superoxide dismutase (SOD), which removes O_2^-, protected EDRF from rapid inactivation and that hemoglobin selectively inhibited EDRF(Furchgott et al 1984), as well as on a study of the transient relaxation of endothelium-denuded rings of rabbit aorta

to acidified inorganic nitrite (NO_2^-) (Moncada & Higgs 2006). The comparative pharmacology of EDRF and NO on vascular strips convinced the identity of EDRF as NO, but Moncada and his colleagues were interested in measuring NO using methods other than bioassay (Moncada & Higgs 2006). There are several chemical methods for the breakdown product of NO such as NO_2^- and nitrate NO_3^- (Moncada & Higgs 2006).

Moncada and his colleagues continued to investigate that NO_2^- or NO_3^- was reduced enzymically to NO or that ammonia or an amino acid was the biological precursor (Moncada & Higgs 2006). There was an interesting possibility that NO originated from the conversion of L-arginine since activated macrophages had recently been shown to generate NO_2^- or NO_3^- from this amino acid (Hibbs et al 1987, Iyengar et al 1987, Moncada & Higgs 2006). After all experiments, they identified the enzyme responsible for the production of NO as NO synthase, that is able to generate NO and L-citrulline from L-arginine. In addition, they have begun to call these biochemical reactions, "The L-arginine: NO pathway" (Moncada & Higgs 2006, Moncada et al 1989).

2.6 Regulation of Nitric Oxide Production

Endogenous nitric oxide (NO) is derived largely from enzymatic pathways, but a non-enzymatic pathway also exists (Luiking et al 2010). Enzymatic NO formation is catalyzed by NO synthase (NOS) through a series of redox reactions with degradation of L-arginine to L-citrulline and NO, and in the presence of oxygen and NADPH (nicotinamide adenine dinucleotide phosphate) (Luiking et al 2010, Moncada & Higgs 1993, Wu & Morris 1998). Three isoforms of NOS are recognized, such as endothelial NOS (eNOS or NOS3), neuronal NOS (nNOS or NOS1), and inducible NOS (iNOS or NOS2) (Luiking et al 2010). NOS1 and NOS3 are constitutive enzymes that are

controlled by intracellular Ca^{2+}/calmodulin. NOS2 is inducible at the level of gene transcription, Ca^{2+} independent, and expressed by macrophages and other tissues in response to (pro)inflammatory mediators (Luiking et al 2010). In contrast, the non-enzymatic production of NO involves production of NO from nitrite via multiple pathways, particularly under acidic conditions, such as following ischemia (Zweier et al 1995), and occurs mainly in tissue (Li et al 2008, Luiking et al 2010). The main pathway is via nitrite reduction, $e^- + 2H^+ + NO^{2-} \rightarrow NO + H_2O$ (Luiking et al 2010). The half-life of NO in blood is very short (< 1 s) due to rapid oxidation by oxyhemoglobin to nitrate and nitrite (cumulatively indicated as NOx), binding of NO to several cell structures or NO scavenging (Luiking et al 2010). Under ischemic conditions with acidosis, nitrite-mediated NO production approaches maximum constitutive NOS production, making this route an alternative under ischemic conditions in which NO production from NOS is impaired (Li et al 2008, Luiking et al 2010).

2.7 Pathophysiology of Diabetic Vascular Disease

Nitric oxide (NO) is constitutively produced by endothelial NO synthase (eNOS) through a 5-electron oxidation of the guanidine-nitrogen terminal of L-arginine, and NO causes vasodilation by activating guanylyl cyclase on subjacent vascular smooth muscle cells (Creager et al 2003, Moncada & Higgs 1993). In addition, NO protects the blood vessel from endogenous injury, such as atherosclerosis, by mediating molecular signals that prevent platelet and leukocyte interaction with vascular wall and inhibit vascular smooth muscle cell proliferation and migration (Creager et al 2003, Kubes et al 1991, Radomski et al 1987, Sarkar et al 1996).

In contrast, the loss of endothelium-derived NO permits increased activity of the proinflammatory transcription factor, nuclear factor kappa B (NF-kB), resulting in expression of leukocyte adhesion molecules and production of chemokines and cytokines (Creager et al 2003, Zeiher et al 1995). This process promotes monocyte and vascular smooth muscle cell migration into the intima and formation of macrophage foam cells, characterizing the initial morphological change of atherosclerosis (Collins & Cybulsky 2001, Creager et al 2003, Libby 2000, Mohamed et al 1999, Nomura et al 2000, Zeiher et al 1995). Endothelial dysfunction is represented by impaired endothelium-dependent, NO-mediated relaxation, and it occurs in cellular and experimental models of diabetes (Bohlen & Lash 1993, Creager et al 2003, Meraji et al 1987, Pieper et al 1995, Tesfamariam et al 1990). The decreased levels of NO in diabetes may underlie its atherogenic predisposition (Creager et al 2003). Finally, many of metabolic derangements occur in diabetes, including hyperglycemia, excess free fatty acid liberation, insulin resistance, and mediate abnormalities in endothelial cell function by affecting the synthesis or degradation of NO (Creager et al 2003, King 1996).

2.8 Diabetes and Vascular Smooth Muscle Function

The impact of diabetes mellitus on vascular function is not limited to the endothelium, such as diminished vasodilator response to endogenous NO donors (Williams et al 1996) and reduced vasoconstrictor responsiveness to exogenous vasoconstrictors, for example, endothelin-1 (Creager et al 2003, Nugent et al 1996). Dysregulation of vascular smooth muscle function is exacerbated by impairments in sympathetic nervous system function (Creager et al 2003, McDaid et al 1994). Diabetes increases the protein kinase C (PKC) activity, NF-kB production, generation of oxygen-

derived free radicals in vascular smooth muscle, and diabetes is akin to these effects in endothelial cells (Creager et al 2003, Hattori et al 2000, Inoguchi et al 2000). In addition, diabetes heightens migration of vascular smooth muscle cells into nascent atherosclerotic lesions, in which they replicate and produce extracellular matrix, such as important steps in mature lesion formation (Creager et al 2003, Suzuki et al 2001). Moreover, vascular smooth muscle cell apoptosis in atherosclerotic lesions is also increased, so patients with diabetes tend to have fewer smooth muscle cells in the lesions, that increase the propensity for plaque rupture (Creager et al 2003, Fukumoto et al 1998).

2.9 Diabetes, Thrombosis, and Coagulation

Platelet function is abnormal in diabetes (Creager et al 2003). The intracellular platelet glucose concentration mirrors the extracellular environment, and it is related to increased superoxide anion formation, protein kinase activity, and decreased platelet-derived NO (Assert et al 2001, Creager et al 2003, Vinik et al 2001). Hyperglycemia changes platelet function by impairing calcium homeostasis. It also alters aspects of platelet activation and aggression, including platelet conformation and release of mediators (Creager et al 2003, Li et al 2001).

Plasma coagulation factors (eg, factor VII and thrombin) and lesion-based coagulations (eg, tissue factor) are increased, and endogenous anticoagulants (eg, thrombomodulin and protein C) are decreased (Ceriello et al 1995, Ceriello et al 1990, Creager et al 2003, Hafer-Macko et al 2002). In addition, the production of plasminogen activaor inhibitor-1 and a fibrinolysis inhibitor is increased (Creager et al 2003, Fukumoto et al 1998, Hafer-Macko et al 2002, Hattori et al 2000, Kario et al 1995,

McDaid et al 1994, Pandolfi et al 2001, Ren et al 2002, Suzuki et al 2001, Vinik et al 2001).

2.10 Hyperglycemia and NO

The intracellular glucose concentration of endothelial cells mirrors the extracellular environment (Creager et al 2003, Kaiser et al 1993). Endothelium-dependent vasodilation is reduced in healthy individuals during hyperglycemic clamping (Creager et al 2003, Williams et al 1998). Hyperglycemia induces a series of cellular events that increase the production of reactive oxygen species, such as superoxide anion, that inactivate NO to form peroxynitrite (Beckman et al 2001, Creager et al 2003, Nishikawa et al 2000). A superoxide anion promotes a cascade of endothelial processes that engage increasing numbers of cellular elements to produce oxygen derived free radicals, such as protein kinase C (PKC) (Creager et al 2003, Nishikawa et al 2000). Activation of PKC by glucose has been implicated in the regulation and activation of membrane-associated NADPH (nicotinamide adenine dinucleotide phosphate)-dependent oxidase and subsequent production of superoxide anion (Creager et al 2003, Hink et al 2001). Peroxynitrite, resulting from the interaction of NO and superoxide anion, oxidizes the NOS co-factor tetrahydrobiopterin (Creager et al 2003, Koppenol et al 1992, Laursen et al 2001).

Mitochondrial production of superoxide anion increases intracellular production of advanced glycation end products (AGEs) (Creager et al 2003, Nishikawa et al 2000). AGEs increase production of oxygen-derived free radicals, and a receptor for AGE (RAGE) activation increases intracellular enzymatic superoxide anion production (Creager et al 2003, Schmidt & Stern 2000, Tan et al 2002, Wautier et al 2001).

Increased supraoxide anion production also activates the hexosamine pathway that diminishes NOS activation by protein kinase Akt (Creager et al 2003, Du et al 2001). These processes may recruit extracellular xanthine oxidase that further augments the oxidative stress (Creager et al 2003, Desco et al 2002).

2.11 Free Fatty Acid Liberation and Endothelial Function

Circulating levels of free fatty acids are elevated in diabetes due to their excess liberation from adipose tissue and diminished uptake by skeletal muscle (Boden 1999, Creager et al 2003, Fujimoto 2000, Kelley & Simoneau 1994). Free fatty acids may impair endothelial function through several mechanisms, such as increased production of oxygen-derived free radicals, activation of PKC, and exacerbation of dyslipidemia (Creager et al 2003, Dichtl et al 1999, Dresner et al 1999, Inoguchi et al 2000). Elevation of free fatty acid concentrations activate PKC and decrease insulin receptor substrate-1-associated phosphatidylinosital-3 kinase activity (Creager et al 2003, Dresner et al 1999, Griffin et al 1999).

The liver responds to free fatty acid flux by increasing very-low-density lipoprotein production and cholesteryl ester synthesis (Creager et al 2003, Sniderman et al 2001). This increased production of triglyceride-rich proteins, and the diminished clearance by lipoprotein lipase results in hypertriglyceridemia, which is typically observed in diabetes (Creager et al 2003, Cummings et al 1995). Elevated triglyceride concentrations lower HDL by promoting cholesterol transport from HDL to very-low-density lipoprotein (Creager et al 2003, Dresner et al 1999, Inoguchi et al 2000, Montagnani et al 2002, Sniderman et al 2001). Finally, insulin resistance is associated with elevations in free fatty acid levels. Abdominal adipose tissue, found prominently in

T2DM, is more insulin resistant. This tissue releases more free fatty acids than other types of adipose located elsewhere (Creager et al 2003).

2.12 Insulin Resistance and NO

T2DM is characterized by insulin resistance (Creager et al 2003). Insulin stimulates NO production from endothelial cells by increasing the activity of NOS via activation of phosphatidylinositol-3 kinase and Akt kinase (Creager et al 2003, Kuboki et al 2000, Zeng et al 2000, Zeng & Quon 1996). In addition, insulin-mediated glucose disposal correlates inversely with the severity of the impairment in endothelium-dependent vasodilation (Creager et al 2003, Mather et al 2000). Furthermore, insulin signal transduction via the phosphatidylinositol-3 kinase pathway is impaired, and insulin is less able to activate NOS and produce NO (Creager et al 2003).

2.13 Endothelial Production of Vasoconstrictors

Endothelial dysfunction is characterized not only by decreased NO, but also by increased synthesis of vasoconstrictor prostanoids and endothelin in diabetes (Creager et al 2003, De Vriese et al 2000, Golovchenko et al 2000, Luft 2002, O'Driscoll et al 1997). Hyperglycemia increases the expression of cyclooxygenase-2 mRNA and protein levels but not the expression of cyclooxygenase-1 mRNA in cultured human aortic endothelial cells (Cosentino et al 2003, Creager et al 2003). In addition, endothelin may be particularly relevant to the pathophysiology of vascular disease in diabetes, since endothelin promotes inflammation and causes vascular smooth muscle cell contraction and growth (Creager et al 2003, Hopfner & Gopalakrishnan 1999). In fact, blockage of endothelin A and B receptors increases forearm blood flow after intra-arterial

administration of insulin, which indicates the insulin may affect vascular tone via stimulation of endothelin in healthy individuals (Cardillo et al 1999, Creager et al 2003). Blockage of endothelin A receptors also increases forearm blood flow in patients with T2DM, implicating an increase of endogenous endothelin-1 in resistance vessels (Cardillo et al 2002, Creager et al 2003).

2.14 Flow-Mediated Dilation (FMD)

The distensibility of an artery plays an important role in regulating the cardiac performance, perfusion, and homeostasis based upon previous research (Kingwell 2002), and another study (Boutouyrie et al 2002) showed that a stiff arterial tree is related to adverse cardiovascular problems (Rakobowchuk et al 2008). How about the peripheral artery distensibility in the upper and lower extremities? The brachial endothelial function indicates the coronary endothelial function and the independent measurement of atherosclerotic disease risk (Rakobowchuk et al 2008, Schachinger et al 2000, Suwaidi et al 2000, Vita 2005). In addition, coronary artery disease patients decreased popliteal artery flow-mediated dilation (FMD) (Angerer et al 2001, Rakobowchuk et al 2008). The popliteal artery, unlike the brachial artery, is a common site of peripheral vascular disease and exhibits unique elastic-like properties (Debasso et al 2004, Rakobowchuk et al 2008).

Intravascular ultrasound assessments are useful, because they allow access to arteries inaccessible by conventional ultrasound. The technique does not have limited use in clinical research environment, compared with the invasive nature of the intravascular ultrasound (Currie 2012). Flow-mediated dilation is the most widely used technique as the non-invasive assessment of endothelial-dependent function (Currie 2012).

The FMD technique is based upon the principle that an increase in blood flow through an artery increases the shear stress exerted on the endothelium, that activates the mechanoreceptors and elicits the production of NO, that causes the subsequent vasodilation (Currie 2012). Celermajer et al. (Celermajer et al 1992) described the FMD technique involved inflation of a pneumatic tourniquet on the thigh above systolic blood pressure for a duration of four-five minutes. Reactive hyperemia shows the increase in blood flow and shear stress in the artery, when the cuff is released (Currie 2012). They used longitudinal ultrasound images of the femoral artery collected at baseline and following cuff deflation to find the change in diameter in responding to reactive hyperemia. It was assumed that the FMD technique was assessing endothelial-dependent and NO-mediated vasodilation (Currie 2012). This was confirmed by Joannides et al. (Joannides et al 1995), who demonstrated the presence of the NO synthase inhibitor NG-monomethyl-L-arginine (L-NMMA) later (Currie 2012). While this study (Joannides et al 1995) investigated the radial artery, the same responses occurred in the brachial (Lieberman et al 1996) and femoral (Kooijman et al 2008) arteries (Currie 2012). However, there is suggested evidence (Tschakovsky & Pyke 2005) that may not be fully attributed to NO pathways, so results should be interpreted with caution (Currie 2012).

2.15 Flow Mediated Dilation (FMD) Technique

Numerous efforts (Corretti et al 2002, Harris et al 2010, Pyke & Tschakovsky 2005, Thijssen et al 2011a) have been made to standardize the FMD techniques, and the most recent guideline (Thijssen et al 2011a) were published in 2011 (Currie 2012). It highlighted historical progression of the FMD technique to its current application, and issues requiring consolidation included the occlusion cuff placement, the duration of

post-occlusion measurements, and data analysis (Currie 2012, Thijssen et al 2011a). The current recommendations for NO-mediated endothelial-dependent assessments are to place the occlusion cuff distally (Doshi et al 2001) above systolic blood pressure for five minutes (Currie 2012, Mullen et al 2001). Celermajer et al. (Celermajer et al 1992) described that post-occlusion measurements were collected for 60 s after cuff release, but the time to peak dilation can vary depending upon the sample population (Currie 2012). Thus, current guidelines recommended post-occlusion measurements for a minimum of three minutes (Black et al 2008, Currie 2012, Thijssen et al 2011a).

FMD can be assessed in any conduit or resistance artery, but the brachial artery is an accepted surrogate for the coronary artery, that is relevant for coronary artery disease populations (Currie 2012). In fact, relationships between coronary and brachial artery endothelial function have been shown that the positive predictive value of abnormal brachial dilation (< 3%) is relevant to 95% of predicting coronary endothelial dysfunction (Anderson et al 1995, Currie 2012).

2.16 Flow Mediated Dilation (FMD) Analysis

Artery diameter dimensions for the FMD technique can be obtained by using B-mode ultrasound or duplex ultrasound, when permitted (Currie 2012). Duplex ultrasound enables the simultaneous collection of arterial diameters using B-mode ultrasound, and blood velocity is measured by using pulsed-wave Doppler ultrasound (Currie 2012). Arterial diameters can be analyzed at end-diastole, or throughout the entire cardiac cycle (Kizhakekuttu et al 2010) to determine the pre-occlusion and peak post-occlusion diameters using ultrasonic calipers or edge-tracking software, when applicable (Currie

2012). The FMD response should be reported in absolute and relative terms (Corretti et al 2002), such as (1) and (2) equations (Currie 2012).

(1) Absolute FMD (mm) = (Peak Post-Occlusion Diameter) – (Pre-Occlusion Diameter)

(2) Relative FMD (%) = (Absolute FMD/Pre-Occlusion Diameter) x 100

Blood velocity measurements are an important element of the FMD response, since they can quantify the shear stress stimulus obtained from the application and subsequent release of the occlusion cuff (Currie 2012). Mean blood velocity (MBV) can be calculated by blood flow and shear rate, such as (3) and (4) equations (Currie 2012).

(3) Blood Flow ($ml*min^{-1}$) = πr^2 x MBV

(4) Shear Rate = (8 x MBV/Lumen Diameter)

Pyke and his colleagues (Pyke & Tschakovsky 2005) observed that the increased shear stress on the endothelium rather than increased flow is the stimulus for vasodilation despite the name of flow-mediated dilation (Currie 2012). Previous research (Pyke et al 2004) reported that a smaller arterial diameter experienced a larger shear stress stimulus and a greater FMD response compared to an artery with a larger baseline lumen diameter. However, the FMD response was no longer related to baseline diameter, when shear rate was controlled to create a uniform stimulus between different sized arteries (Currie 2012). Thus, FMD should be normalized to the shear rate to account for the magnitude of stimulus received by distinct vessels (Currie 2012, Pyke & Tschakovsky 2005). Previous research (Black et al 2008) stated that the shear rate should be calculated as the shear rate area under the curve (AUC) until the peak post-occlusion diameter, so normalized FMD is calculated as the ratio of the relative FMD to the AUC (Currie 2012). The process of normalization violates established statistical assumptions for

normalization and may not be an appropriate calculation (Atkinson et al 2009, Currie 2012).

There is no consensus on the use of normalized FMD, and caution should be used in calculating and interpreting normalized FMD values, especially for arteries of different caliber (Currie 2012). Normalization may be suitable for within-subject comparison that demonstrate strong relationships between FMD and shear rate (Currie 2012, Thijssen et al 2011a).

2.17 Endothelial-Independent Assessments

Nitric oxide (NO) diffuses to the vascular smooth muscle cells (VSMCs), where it elicits vasodilation, and consequently the VSMCs play an important role in endothelial-dependent dilation (Currie 2012). Endothelial-independent assessments are important, because they measure the functioning of VSMCs using an exogenous NO donor, such as nitroglycerin (NTG) or sodium nitroprusside (Currie 2012). Measurements are typically performed after the FMD test, at least 10 minutes following the cuff release to allow the artery to return to baseline diameter (Corretti et al 2002, Currie 2012). A 0.4 mg dose of NTG is used to elicit the maximal obtainable vasodilation. Previous guidelines recommended ultrasound assessments for 3-4 min after administration (Corretti et al 2002, Currie 2012). However, recent evidence demonstrated that peak diameter could occur past that time point (Currie 2012, Thelen et al 2008). Thus, measurements should be collected for 10 minutes following administration, and endothelial-independent responses can be presented in absolute and relative terms, as described above (Currie 2012).

Previous research (Thijssen et al 2011b) conducted a retrospective analysis of data to evaluate relationships between the brachial and superficial femoral artery endothelium-dependent FMD and endothelium-independent FMD in young and healthy individuals. They also examined the relationship between FMD between both brachial arteries. There was no correlation between brachial and superficial femoral artery FMD or between brachial and popliteal artery FMD. However, a correlation was observed in FMD between both brachial arteries. Endothelium-independent FMD between brachial and superficial femoral artery was modestly correlated, but that of brachial and popliteal artery was not correlated. Thus, these data indicated that conduit artery vasodilator function in the upper limbs was not predictive of that in the lower limbs, but measurement of FMD in one arm is predictive of FMD in the other.

2.18 Relationship between Flow-Mediated Dilation (FMD) Changes and NO with T2DM

Researchers (Green et al 2006) examined the stimulated endothelial NO function reflected the basal endothelial NO function with flow-mediated dilation (FMD) in seventeen T2DM patients. Nine subjects undertook an exercise training or control period, whereas the remaining eight subjects were administered an angiotensin II receptor blocker (losartan) or placebo. Resistance vessel basal endothelium-dependent NO function was assessed by using intrabrachial administration of N^G-monomethyl-L-arginine (L-NMMA) and plethysmographic assessment of forearm blood flow. FMD was higher after intervention compared with control/placebo, but there were no significant changes in forearm blood flow responses to L-NMMA. In addition, FMD and L-NMMA

responses did not reveal a significant correlation, and improvements in FMD with the interventions were not associated with changes in the L-NMMA responses.

2.19 Near-Infrared Spectroscopy (NIRS) and Measurement

Near Infrared Spectroscopy (NIRS) has been used extensively to evaluate changes in muscle oxygenation and blood volume during a variety of exercise modes (Bhambhani 2004). The NIRS provides multi-distance frequency-domain spectroscopy, since a single channel of eight laser diode light sources operating at two different wavelengths, and light from the diodes is coupled to fibers in a photomultiplier tube and pulsed in rapid succession (110 MHz). The diodes are set at source-detector distances of 2.0, 2.5, 3.0, and 3.5 cm, and the light is received by another detector fiber, is carried back, and is detected by a photodiode in the spectrometer (Williams et al 2013).

The NIRS system (OxiplexTS, ISS, Champaign, IL) is calibrated prior to each exercise test according to the specifications provided by the manufacturer. NIRS is used to continuously monitor the oxyhemoglobin saturation (oxy-[Hb]), deoxyhemoglobin (deoxy-[Hb]), and total hemoglobin (Hb_{tot}) throughout the exercise protocol. The NIRS probe consists of eight light-emitting diodes operating at wavelengths of 690 and 830 nm, and one detector fiber bundle. After shaving the skin, the probe is positioned longitudinally over a body part that you measure and is secured with straps around the body part. A dark, heavy cloth is secured over the probe to prevent stray visible light from affecting the operation of the optical diodes. The detector gain is adjusted for an optimal signal as a subject rested for a specific protocol.

2.20 NIRS Research

2.20.1 *Cycling-Ramp Exercise*

The study (Chin et al 2011) investigated the relationship between muscle deoxygenation (HHb) and activation was examined in three different muscles of the quadriceps during cycling ramp exercise. Pulmonary oxygen uptake was measured breath-by-breath, while muscle HHb and activity were measured by time-resolved near-infrared spectroscopy (NIRS) and EMG, respectively, at the vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM) (Chin et al 2011). Muscle deoxygenation was corrected for adipose tissue thickness and normalized to the amplitude of the HHb response, while EMG signals were integrated (iEMG) and normalized to the maximum iEMG determined from maximal voluntary contractions (Chin et al 2011). Muscle deoxygenation and activation were then plotted as a percentage of maximal work rate (%WR(max)) (Chin et al 2011). The conditioned parameter for the sigmoid fit (representing the %WR(max) at 50% of the total amplitude of the HHb response) was similar between VL and VM, but greater for RF, demonstrating a "right shift" of the HHb response compared with VL and VM (Chin et al 2011). The iEMG also showed that muscle activation of the RF muscle was lower compared with VL and VM throughout the majority of the ramp exercise, which may explain the different HHb response in RF (Chin et al 2011). These results suggest that the sigmoid function can be used to model the HHb response in different muscles of the quadriceps (Chin et al 2011). However, simultaneous measures of muscle activation are also needed for the HHb response to be properly interpreted during cycle ramp exercise (Chin et al 2011).

2.20.2 *Continuous Endurance Training versus HIIT*

A study (McKay et al 2009) investigated the O_2 kinetics, muscle deoxygenation, and exercise performance, and compared short-term HIIT with lower-intensity continuous endurance training (END). Subjects underwent eight sessions of either HIIT (8 to 12 x 1 min interval at 120% maximal O_2 uptake separated by 1 minute of rest) or END (90 to 120 minutes at 65% maximal O_2 uptake). In addition, participants completed step transitions to a moderate-intensity work rate (~90% estimated lactate threshold) on five occasions throughout training, and ramp incremental and constant-load performance tests were conducted at pre-, mid-, and post-testing periods. They found that the constant VO_2 was reduced by ~20% after only two training sessions and by ~40% after eight training sessions with no differences between HIIT and END. They also stated that the deoxygenation (change in deoxygenated hemoglobin concentration) in the vastus lateralis muscle did not change over the course of eight training sessions by monitored NIRS.

2.20.3 *Pulmonary VO_2 and HIIT*

A previous study (Williams et al 2013) tested the effects of HIIT on pulmonary O_2 uptake during transitions from low and elevated metabolic rates within the moderate-intensity domain. Eight young untrained males completed 12 sessions of HIIT (spanning 4 weeks), and it consisted of 8-12 1-minute intervals in cycling at a work rate corresponding to 110% of per-training maximal work rate. Pre-, mid-, and post-training, subjects completed a ramp-incremental test to determine maximum O_2 uptake, work rate max, and estimated lactate threshold. In addition, participants completed double-step constant-load tests, such as step transition from 20 W to Δ45% of lactate threshold (low step) and Δ45% to 90% of lactate threshold (upper step). HIIT led to increases in VO_{2max}

and maximum work rate, and time-constant pulmonary O_2 uptake of both lower and upper moderate-intensity domain step transitions were reduced by ~40%. However, adjustment of local muscle deoxygenation over time was unchanged in both lower and upper steps that potentially resulted in an improved matching of muscle O_2 utilization to microvascular O_2 delivery within the working muscle after 12 sessions of HIIT, although muscle metabolic adaptation should not be ignored.

2.20.4 *NIRS and Doppler Ultrasound*

The precise role of the sympathetic nervous system in the regulation of skeletal muscle blood flow during exercise has been difficult to define in humans, partly due to limited techniques available for blood flow measurement in active muscles (Fadel et al 2004). A visible alternative to the more traditional hemodynamic approaches, such as perivascular Doppler ultrasound, microsphere deposition, intravital microscopy, plethysmography, and tracer dilution, for evaluating vascular responses in human skeletal muscle may be the use of NIRS to measure changes in muscle oxygenation (Chavoshan et al 2002, Fadel et al 2004, Hansen et al 1996, Sander et al 2000). However, simultaneous measurements of muscle oxygenation and blood flow were never performed to fully validate the response.

Fadel et al. (2004) conducted the simultaneous tissue oxygenation measurement with NIRS and blood flow with Doppler ultrasound in skeletal muscle of conscious humans and anesthetized rats. In resting forearm of humans, reflex activation of sympathetic nerves with use of lower body negative pressure produced graded decreases in tissue oxygenation and blood flow velocity that were highly correlated. During rhythmic muscle contraction, the decreases in tissue oxygenation and blood flow evoked

by sympathetic activation were significantly attenuated but remained highly correlated in both humans and rats. Thus, this data indicated that changes in tissue oxygenation can be used to reliably assess sympathetic vasoconstriction in both resting and exercising skeletal muscle during steady-state metabolic conditions. Tissue oxygenation and muscle blood flow were dissociated at the onset of dynamic exercise, when both oxygen utilization and delivery were increasing, just as venous oxygen saturation and muscle blood flow were dissociated (Fadel et al 2004, MacDonald et al 1999).

2.21 Glucose Regulation

Insulin regulates glucose entry into all tissues, primarily muscle and adipose, except for the brain, and insulin action mediates facilitated diffusion (McArdle et al 2020). During this process, glucose combines with a carrier protein on the cell's plasma membrane for transport into cells. In this way, insulin regulates glucose metabolism. Following a meal, increased insulin-mediated glucose uptake by cells and correspondingly reduced hepatic glucose output decrease blood glucose levels. In essence, insulin reduces meal-induced elevated blood glucose concentration to a normal level. Conversely, with insufficient insulin secretion or decreased insulin sensitivity, blood glucose concentration increases above a normal level of about 90 mg/dl to a high level such as 200 to 350 mg/dl depending upon a level of insulin resistance. When blood glucose levels remain high, glucose ultimately spills into the urine, and fatty acids metabolizes as a primary energy substrate without insulin.

Insulin exerts a pronounced effect on fat synthesis. A rise in blood glucose levels after a meal stimulates insulin release, and this causes some glucose uptake by fat cells for synthesis to triacylglycerol. Insulin action also triggers intracellular enzyme activity

that facilitates protein synthesis. This occurs by one or all of the following actions:

1. Increasing amino acid transport through the plasma membrane
2. Increasing cellular levels of RNA
3. Increasing protein formation by ribosomes

2.21.1 *Insulin Transport of Glucose into Cells*

Muscle fibers contain GLUT-1 and GLUT-4 with most glucose entering by the GLUT-1 carriers during rest. With high blood glucose and insulin concentrations following a meal, muscles cells transport glucose via the GLUT-4 transporter. GLUT-4 action is mediated through a second messenger that permits migration of the intracellular GLUT-4 protein to the surface of the plasma membrane to promote glucose uptake. The fact that GLUT-4 moves to the cell surface through a separate insulin-dependent mechanism coincides with observations that active muscles transport glucose during physical activity without insulin.

2.21.2 *Glucose-Insulin Secretion Interaction*

Blood glucose levels within the pancreas directly control insulin secretion. Elevated blood glucose levels cause insulin release, which in turn, induces glucose entry into cells, removing the stimulus for insulin release. In contrast, a decrease in blood glucose concentration dramatically lowers blood insulin levels to prevent the development of hypoglycemia. The interaction secretion between glucose and insulin serves as a feedback mechanism to maintain blood glucose concentration within narrow limits. Rising levels of plasma amino acids also increase insulin secretion.

The insulin concentration decreases as exercise duration extends or intensity

increases, since it results from inhibitory effects of an exercise-induced catecholamine release on insulin release from pancreatic β-cell. Suppression effect of Catecholamine on insulin secretion relates directly to physical activity intensity, and its inhibition of insulin secretion explains why no excessive insulin releases and possible rebound hypoglycemia occurs with a concentrated glucose feeding during physical activity. Prolonged physical activity derives progressively more energy from free fatty acids mobilized from the adipocytes from reduced insulin secretion and decreased carbohydrate reserves. Blood glucose lowering with prolonged physical activity directly enhances hepatic glucose output and sensitizes the liver to the glucose-releasing effects of glucagon and epinephrine, whose actions help to stabilize blood glucose levels.

2.22 Glucose Transport System in Skeletal Muscle

Insulin stimulation and physical exercise are the most physiologically relevant stimulators of glucose transport in skeletal muscle (Fujii et al 2000, Goodyear & Kahn 1998). Interestingly in patients with T2DM, insulin but not contraction-stimulated glucose transport is impaired (Jessen & Goodyear 2005, Kennedy et al 1999, Zierath et al 1998). Contractile activity can stimulate GLUT4 translocation in absence of insulin (Goodyear & Kahn 1998, Hayashi et al 1997), and a few studies (Coderre et al 1995, Douen et al 1990, Roy & Marette 1996) suggest there are different intracellular "pools" of GLUT4, such as one stimulated by insulin and one stimulated by exercise (Jessen & Goodyear 2005).

2.22.1 *AMP-Activated Protein Kinase*

The hypothesis has been indicating that AMP-activated protein kinase (AMPK) is a critical signaling molecule for the regulation of multiple metabolic, protein synthetic, and transcriptional processes in contracting skeletal muscle (Jessen & Goodyear 2005). AMPK is a member of protein kinase family with 12 molecules that extend from plants to mammals, and it is the mammalian homolog of the SNF-1 protein kinase in Saccharomyces cerevisiae, that is critical for the adaptation of yeast to nutrient stress (Hardie et al 1998, Jessen & Goodyear 2005, Mitchelhill et al 1994, Stapleton et al 1994). When the cell senses low fuel such as decreased ATP, AMPK acts to switch off ATP-consuming pathways and switch on alternative pathways for ATP regeneration (Jessen & Goodyear 2005). Contractile activity changes the fuel status of skeletal muscle, and there can be significant decreases in both phosphocreatine and ATP concentrations depending upon the intensity of the contractions (Jessen & Goodyear 2005).

The serine/threonine kinase LKB1 in complex with the two accessory subunits, such as STRAD and MO25 was identified as an upstream kinase for AMPK (Hawley et al 2003, Hong et al 2003, Jessen & Goodyear 2005, Shaw et al 2004). LKB1 is a kinase for 11 of 12 proteins in the AMPK family (Jessen & Goodyear 2005, Lizcano et al 2004, Spicer et al 2003). Moderate intensity aerobic cycle exercise (Fujii et al 2000, Stephens et al 2002, Wojtaszewski et al 2000) and high intensity sprint exercise (Chen et al 2000) increased skeletal muscle AMPK activity (Jessen & Goodyear 2005). LKB1 appeared to be constitutively active in skeletal muscle, and neither muscle contraction nor 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) treatment increased LKB1 activity (Jessen & Goodyear 2005, Sakamoto et al 2002). Interestingly, the increased

LKB1 expression seen following long-term endurance training also had no effect on activity (Jessen & Goodyear 2005, Taylor et al 2004). Then, experiments, using AICAR as an AMPK activator, generated important information for the function of AMPK. Specific activation and/or inhibition of AMPK by pharmacological agents would be a valuable approach to more clearly define the role of AMPK in glucose transport and other metabolic effects (Jessen & Goodyear 2005). Unfortunately, such compounds are not currently available (Jessen & Goodyear 2005).

2.22.2 *Calcium-Regulated Signaling to Glucose Transport*

The increase in myocellular calcium concentrations has been proposed to be a signal in the initiation of contraction-stimulated glucose transport and GLUT4 translocation (Holloszy et al 1986, Holloszy & Hansen 1996, Jessen & Goodyear 2005). The mechanism by which calcium might regulate exercise-stimulated glucose transport is unknown, but it is unlikely that calcium ions directly activate the glucose transport system, since cytoplasmic calcium concentrations are elevated for only a fraction of a second after each muscle contraction (Jessen & Goodyear 2005). In contrast, the increase in muscle glucose transport can remain elevated for a considerable period of time after the contractile activity ends (Jessen & Goodyear 2005). Thus, one or more of the calcium-regulated intracellular proteins might lead to GLUT4 translocation, and potential candidates include calmodulin, the family of calmodulin-dependent protein kinases (CaMK), the protein kinase C (PKC) family, and all of which are important intermediaries in cellular signal transduction (Jessen & Goodyear 2005).

Some observations questioned whether changes in calcium concentrations could be the sole mediator of contraction-induced transport with their occurrence depending

upon the stimulation frequency (high calcium concentration) rather than the force production (high metabolic stress) (Jessen & Goodyear 2005). However, the role for calcium as the only mediator of contraction-stimulated glucose uptake seems less likely, but further studies needs to be conducted using other approaches than the inhibitors (Jessen & Goodyear 2005).

2.22.3 *Nitric Oxide (NO) Synthase and Glucose Transport*

Nitric oxide (NO) is produced in various tissues through the activation of different isoforms of NO synthase (NOS) (Moncada & Higgs 1993), and skeletal muscles express both neuronal NOS and endothelial NOS (Jessen & Goodyear 2005, Kobzik et al 1994). Treadmill running exercise activated NOS in gastrocnemius muscles (Roberts et al 1999), providing additional evidence that NO production in skeletal muscle increased during exercise (Jessen & Goodyear 2005). Exogenous NO, which is generated from the NO donor sodium nitroprusside, stimulated glucose transport in isolated skeletal muscles (Balon & Nadler 1997, Etgen et al 1997, Higaki et al 2001, Young et al 1997) by increasing GLUT4 concentrations at the cell surface (Etgen et al 1997, Jessen & Goodyear 2005).

2.23 Blood Glucose Homeostasis during Exercise

The plasma glucose concentration is maintained through four processes such as 1) Mobilize glucose from liver glucogen stores, 2) Mobilize plasma free fat acid (FFA) from adipose tissue to spare plasma glucose, 3) Synthesize new glucose in the liver (gluconeogenesis) from amino acids, lactic acid, and glycerol, 4) Block glucose entry into cells to force the substitution of FFA as a fuel (Powers & Howley 2009).

2.23.1 *Permissive and Slow-Acting Hormones*

Thyroxine, cortisol, and growth hormone regulate carbohydrate, fat, and protein metabolism, but they either facilitate the actions of other hormones or respond to stimuli in a slower manner (Powers & Howley 2009).

1. Thyroid Hormones

The thyroid hormones T_3 and T_4, whose free concentration do not change dramatically from resting to the exercising state (Powers & Howley 2009). However, T_3 and T_4 are removed from the plasma by tissues during exercise at a greater rate than at rest. In turn, thyroid stimulating hormone secretion from the anterior pituitary enhances to stimulate the secretion of T_3 and T_4 from the thyroid gland to maintain the plasma level (Galbo et al 1977).

2. Cortisol

Cortisol stimulates FFA mobilization from adipose tissue, mobilizes tissue protein to yield amino acids for glucose synthesis in the liver, and decreases the rate of glucose utilization by cells. The plasma contisol concentration measured at 60 minutes into each of the exercise tests plotted against % VO_{2max}. When people exercise with low intensity and long duration, the concentration of cortisol does not change very much (Powers & Howley 2009). Perhaps, high intensity exercise with short duration might increase the concentration of cortisol.

3. Growth Hormone (GH)

Growth hormone (GH) plays a major role in protein synthesis, acting either directly or through the enhanced secretion of insulin-like growth factors (IGFs) from the liver, and it influences fat and carbohydrate metabolism in slow-acting effect. GH supports the action of cotisol, decreases glucose uptake by tissues, increases FFA mobilization, and enhances gluconeogenesis in the liver (Powers & Howley 2009).

2.23.2 *Fast-Acting Hormones*

1. Epinephrine and Norepinephrine

Epinephrine (E) and norepinephrine (NE) are not only relative to glucogen utilization, but also the mobilization of glucose from the liver, FFA from adipose tissue, and may interfere with the uptake of glucose by tissues (Coker & Kjaer 2005, Rizza et al 1979). Plasma E and NE increase linearly with duration of exercise (Howley et al 1983, Powers et al 1982). These changes are related to cardiovascular adjustments to exercise-increased heart rate, blood pressure, and the mobilization of fuel. It is sometimes difficult to separate the effect of E from NE, but E seems to be more responsive to changes in the plasma glucose concentration, while NE responses to changes of blood pressure during the increased heat load (Powers et al 1982). E binds to β-adrenergic receptors on the liver and stimulates the breakdown of liver glycogen to form glucose for release into plasma.

Over seven weeks of endurance training causes a very rapid decrease in the plasma E and NE responses to a fixed exercise bout. Within three weeks, the concentration of both catecholamines is greatly reduced (Winder et al 1978). Paralleling

this rapid decrease in E and NE with endurance exercise training is a reduction in glucose mobilization (Mendenhall et al 1994). In spite of this, the plasma glucose concentration is maintained, since there is a reduction in glucose uptake in muscles at the same fixed workload after endurance training (Rizza et al 1979, Wojtaszewski & Richter 1998).

2. Insulin and Glucagon

These two hormones are discussed together, since they respond to the same stimuli but exert opposite actions relative to the mobilization of liver glucose and adipose tissue FFA. Insulin, the primary hormone, is involved in the uptake and storage of glucose and FFA. Glucagon causes the mobilization of those fuels from storage, as well as increases gluconeogenesis. Epinephrine (E) and norepinephrine (NE) stimulate α-adrenergic receptors on the beta cells of the pancreas to decrease insulin secretion during exercise, when the plasma glucose concentration is normal. In addition, E and NE stimulate β-adrenergic receptors on the alpha cells of the pancreas to increase glucagon secretion, when the plasma glucose is normal (Coker & Kjaer 2005). The effect of sympathetic nervous system increases the mobilization of fuel for muscle contractions. Endurance training decreases the sympathetic nervous system response to a fixed exercise bout, resulting in less stimulation of adrenergic receptors on the pancreas and less change in insulin and glucagon.

2.24 Glucose Metabolism during Moderate Exercise versus High Intensity Exercise

During moderate exercise (60% VO_{2max}) of short duration in individuals without diabetes, increased glucose uptake by muscles is balanced by an equal rise in hepatic

glucose production, and glucose levels remain unchanged (Adams 2013, Colberg et al 2010b, Marliss & Vranic 2002). There is a decrease in insulin level, which sensitizes the liver to glucagon, which causes increasing glucose production (Adams 2013, Marliss & Vranic 2002). Catecholamines play a role in increasing glucose production only during moderate exercise greater than two hours duration (Adams 2013). With T2DM, blood glucose uptake by muscles usually increases more than hepatic production (Adams 2013, Minuk et al 1981). This is also normally corresponding to a decline in plasma insulin levels and greatly reducing a risk of hypoglycemia in T2DM without using insulin or insulin secretagogues (Adams 2013, Colberg et al 2010b).

In high intensity exercise (> 80% VO_{2max}), unlike at lesser intensities, glucose is the exclusive muscle fuel (Adams 2013, Marliss & Vranic 2002). Catecholamine levels rise markedly and cause glucose production to rive seven to eight-fold, while glucose utilization is only increased three to four-fold (Adams 2013). In individuals without T2DM, there is a small blood glucose increase during intense exercise that increases further immediately at exhaustion and persists for up to one hour (Adams 2013). Plasma insulin levels rise, correct the glucose level, and restore muscle glycogen, but this physiological response in T1DM would be absent (Adams 2013).

2.25 Oral Glucose Tolerance Test (OGTT)

OGTT evaluates blood sugar levels 2 hours after drinking 75g of a concentrated glucose solution, and delayed removal of ingested glucose indicates diabetes (McArdle et al 2010). Based upon the American Diabetes Association criteria, diabetes is diagnosed at 2-hour blood glucose of greater than or equal to 200 mg/dl at http://www.diabetes.org/diabetes-basics/diagnosis.

The deregulation of insulin secretion and insulin sensitivity leads to hyperglycemia (Kahn 2003), so they are targeted by therapies for diabetes (Fowler 2007, Seike et al 2011). OGTT plays an important role in evaluating this problem, since it helps enhance the quality of diabetic medical care. In order to maintain reasonable assessment accuracy, a study (Seike et al 2011) investigated indices for measuring insulin secretion and insulin sensitivity using OGTT, compared with analogous indices obtained by a glucose clamp technique (DeFronzo et al 1979) among Japanese with various degrees of glucose tolerance. They found insulin secretion index was significantly correlated with an analogous index obtained from a hyperglycemic clamp test, and it was also significantly correlated with an analogous index obtained from a hyperinsulinemic-euglycemic clamp test. These results suggested that their methods could provide an accurate and convenient tool in order to improve the diabetes management in clinical practice. Thus, potentially analogous indices obtained by a glucose clamp technique seems to be better to manage diabetes in clinical practice than OGTT, but it needs more consistent results for clinicians to perform the test, since OGTT has been used for a long time.

2.26 VLDL Catabolism in Insulin Resistance

Modest reductions in post-heparin lipoprotein lipase (LPL) levels have been reported (Ginsberg 1991) in some T2DM, and it may contribute significantly to elevated TG levels, particularly in severely hyperglycemic patients (Ginsberg et al 2005). Very low density lipoprotein (VLDL) and chylomicrons can compete for the same LPL-mediated pathway for TG removal from the circulation, and hepatic uptake of VLDL remnants is a complex process involving several parallel and interactive pathways

(Ginsberg et al 2005). Insulin resistance might lead to reduced LDL receptors, limiting remnant removal (Ginsberg et al 2005). Hepatic lipase (HL) is increased in many individuals with diabetic dyslipidemia, and it is suggested that HL-mediated TG hydrolysis of VLDL remnants is unimportant, although high HL activity may be important for the low HDL levels and the predominance of small dense LDL characteristic of this lipid complex (Ginsberg et al 2005).

2.27 Role of Insulin Resistance in the Generation of Small Dense LDL

The regulation of plasma levels of low-density lipoprotein (LDL), such as its precursor of very low-density lipoprotein (VLDL), is complex in individuals with insulin resistance and T2DM (Ginsberg et al 2005). In the presence of hypertriglyceridemia, dense, cholesteryl ester-depleted, triglyceride-enriched LDL are present, and the basis for small, dense LDL in insulin resistance is derived in large part from action of cholesteryl ester transfer protein (CETP) (Ginsberg et al 2005). This protein, related to lipoproteins in the blood, a particularly high density lipoprotein (HDL), can mediate the exchange of VLDL (or chylomicron) triglyceride (TG) for LDL cholestrol ester, creating a TG-enriched, cholesteryl ester depleted LDL particles (Ginsberg et al 2005). The TG in LDL can be lipolyzed by lipoprotein lipase (LPL) or hepatic lipase (HL), generating the small, dense LDL. Small, dense LDL is present in insulin resistance and T2DM patients, even though their TG levels are normal (Ginsberg et al 2005). One of these factors is HL that is increased in insulin resistance and hydrolyzes any TG in LDL. In addition, higher levels of blood free fatty acid have been shown to stimulate an in exchange cholesteryl ester and TG between LDL or HDL and VLDL (Ginsberg et al 2005).

2.28 The Relationship between Insulin Resistance and Lipid Profile

There is evidence that insulin plays an important role in lipid metabolism (Steinberger et al 1995). Hyperinsulinemia has been documented to enhance hepatic VLDL synthesis and may directly contribute to the increased plasma triglyceride and LDL in obese adolescents (Orchard et al 1983, Stalder et al 1981, Steinberger et al 1995). Resistance to the action of insulin on lipoprotein lipase in peripheral tissues may also contribute to elevated triglyceride and LDL levels (Pykalisto et al 1975, Sadur et al 1984, Steinberger et al 1995). Golay et al. (Golay et al 1987) suggests that insulin resistance may be responsible for the reduced levels of HDL in individuals with non-insulin-dependent diabetes (Steinberger et al 1995). These investigators show that the plasma HDL concentration is significantly reduced in participants with non-insulin-dependent diabetes versus a control group despite enhanced HDL synthesis, and they indicate that the decrease in plasma HDL level is entirely accounted for by an increase in the rate of apo-A1/HDL degradation, which exceeds the enhanced rate of its synthesis (Steinberger et al 1995). Within both the control and non-insulin-dependent diabetic groups, the plasma insulin concentration and plasma HDL concentration are strongly and inversely correlated (Steinberger et al 1995).

2.29 The Epidemic of T2DM

T2DM is more problematic in the Unites States as well as worldwide, and experts agree that the sedentary life-styles combined with excessive caloric intake, including high carbohydrates and saturate fats, have led to the prevalence of obesity that potentially causes T2DM (Cusi 2009).

A previous study (Wild et al 2004) estimates that the worldwide prevalence of diabetes will increase almost twice by 2030 affecting 366 million people, and Mainous et al. (Mainous et al 2007) projects the number of diabetics in ten-year increments into the future, and they estimate that the diabetics will grow up to 32.6 million by 2021. By 2031, the number will increase to 37.7 million people, which is 14.5% of the entire adult population in the United States, with an overwhelming 20.2% of adults of Hispanic origin having diabetes (Cusi 2009).

About one in three individuals with diabetes or an estimated 6 million people are believed to be undiagnosed in the United States, and one major problem of a delayed diagnosis of diabetes has progressed the complications, such as cardiovascular disease (CVD) (Cusi 2009). The risk of CVD developing in the years before the development of hyperglycemia is 2.8 times higher in subjects with a normal fasting plasma glucose at baseline that developed T2DM during follow-up than the control group that never developed T2DM (Cusi 2009, Hu et al 2002). In addition, T2DM and excess adiposity are believed to be the development factors of early cardiovascular disease (CVD) and increased overall mortality in obese individuals in population-based studies (Abbasi et al 2002, Bray & Bellanger 2006, Cusi 2009, Flegal et al 2007, Gunnell et al 1998, Hamilton et al 2007, National Institutes of Health 1985, Peeters et al 2003, Pi-Sunyer 1993, Poirier et al 2006, van Dam et al 2006, Wyatt et al 2006). Moreover, diabetics are known to have cardiovascular death rates that are three to four times higher in the presence of similar traditional factors, such as elevated blood pressure, dyslipidemia, and smoking, compared with matched non-diabetic individuals (Cusi 2009). In the United States,

medical care for obesity-related conditions, including T2DM, were estimated to exceed $117 billion or almost 10% of the total healthcare costs (Cusi 2009).

2.30 Mechanisms related to Obesity with T2DM

The normal body mass index (BMI) is considered to be between 18.5 and 25 kg/m^2 (Cusi 2009, National Institutes of Health 1998). If the BMI is between 25 and 29.9 kg/m^2, this individual is considered to be overweight (Cusi 2009). It is obese, if > 30 kg/m^2, and obesity has been further classified into stage I (BMI from 30.0 to 34.9 kg/m^2), stage II (BMI from 35.0 to 39.9 kg/m^2), stage III (if $\geq$ 40.0 kg/m^2 or morbid obesity) (Cusi 2009).

Impairments in insulin secretion and insulin action contribute to glucose intolerance and development of T2DM (DeFronzo 1988), and glucose can promote its own disposal, such as independence of insulin (Lopez et al 2009). Glucose effectiveness or insulin-independent glucose disposal represents the ability of glucose per se under basal insulin conditions in order to increase glucose disposal and to suppress endogenous glucose production (Lopez et al 2009). Moreover, there is a possibility that some metabolic abnormalities, such as insulin resistance (Perseghin et al 1997), intramyocellular lipid accumulation (Perseghin et al 1999), and impaired suppression of lipolysis by insulin (Eriksson et al 1999), are present in lean predisposed subjects (Straczkowski et al 2003).

A key physiological mechanism in linking obesity with T2DM is that increased general and abdominal obesity is strongly correlated with insulin resistance, that represents the factor leading to T2DM (Stewart 2009). Consequently, there is a gradual rise in insulin production that eventually cannot compensate for increasing levels of

insulin resistance which can cause a complete halt in the ability to produce insulin among patients, who do not exercise or lose weight to prevent their insulin resistance (Stewart 2009). Insulin resistance is a central pathogenic factor for the metabolic syndrome and is related to both generalized obesity and the accumulation of fat in the omental and intramyocellular compartments (Esler et al 2001a, Stewart 2009). The accumulation of intramyocellular lipids may be due to reduced lipid oxidation capacity (Esler 2000, Stewart 2009).

The adipose tissue is a dynamic endocrine organ that secretes a number of factors that are increasingly recognized to contribute to systemic and vascular inflammation based upon previous research (Christensen & Galbo 1983, Esler et al 2001b, Johnson et al 2005, Stewart 2009), and many of these factors, referred to as adipokines, appear to regulate a number of the processes directly or indirectly that contribute to the development of atherosclerosis, including hypertension, endothelial dysfunction, insulin resistance, and vascular remodeling (Stewart 2009). In addition, a lower adiponectin level was associated with increased levels of insulin resistance, triglyceride, C-reactive protein, tissue plasminogen activator, and alanine aminotransferase and with lower levels of HDL-cholesterol and Factor VIII, factors associated with diabetes (Stewart 2009).

Leptin, a protein hormone secreted from adipose tissue, plays an important role in regulating energy intake and energy expenditure, and obese people appear to resist the effects of leptin, although it is a signaling protein that reduces appetite (Stewart 2009). Obesity is also associated with an increase in adipose tissue macrophages that participate in the inflammatory process through the elaboration of cytokines (Stewart 2009, Sullivan 1982). According to a previous study (Et-Taouil et al 2003), baseline leptin levels

predicted the development of obesity, glucose intolerance, insulin resistance, and metabolic syndrome after adjustment for obesity (Et-Taouil et al 2003, Stewart 2009). In addition, inflammation is related to endothelial dysfunction and is recognized as one of the cardiovascular risk factors clustering in metabolic syndrome (Christensen & Galbo 1983, Stewart 2009). Lastly, obesity is associated with oxidative stress, and the oxidation of LDL contributes to the development of atherosclerotic lesions (Stewart 2009).

2.31 Genetic and Environmental Factors of T2DM

Both genetic and environmental factors involved in T2DM, and first-degree relatives have 40% lifetime risk of developing this disease (Straczkowski et al 2003). The concordance rates of T2DM in monozygotic twins range from 55 to 90% (Barnett et al 1981, Newman et al 1987, Straczkowski et al 2003). Parental history of T2DM increases risk of developing disease more than threefold with one and six-fold with two affected parents (Lopez et al 2009, Meigs et al 2000). An acquired factor, such as obesity, is also associated with increased risk of T2DM (Chan et al 1994), and obesity and impaired glucose tolerance are more common in relatives of diabetic patients (Haffner et al 1996, Straczkowski et al 2003). Impaired glucose tolerance also predicts risk with approximately six-fold higher cumulative T2DM incidence (Gabir et al 2000, Lopez et al 2009).

Compared with individuals without a family history of diabetes, cross sectional studies of normal glucose-tolerance offspring of T2DM parents show glucose effectiveness is unchanged (Nielsen et al 2000) or even increased (Henriksen et al 1994, Henriksen et al 2000). The latter postulated as a compensatory mechanism in the setting of insulin resistance (Lopez et al 2009). A prospective study in offspring of two diabetic

parents shows that insulin resistance and decreased glucose effectiveness independently predict the development of disease (Lopez et al 2009, Martin et al 1992). In contrast, insulin resistance and glucose effectiveness predict disease less strongly among people without a family history of diabetes (Goldfine et al 2003, Lopez et al 2009).

2.32 First-degree Relatives of T2DM

2.32.1 *Glycogen Synthesis Factor*

First-degree relatives (FDR) have been frequently found to be insulin resistance compared to a family history of T2DM (Eriksson et al 1989, Laws et al 1989, Nyholm et al 1996, Ostergard et al 2007, Vaag et al 1992, Vaag et al 2001). A number of potential explanatory pathophysiologic mechanisms for insulin resistance in muscles have not been fully understood, but it is well established that defective muscle glycogen synthesis plays a major role in the insulin resistance found in T2DM (Damsbo et al 1991, Ostergard et al 2007, Rothman et al 1995, Schalin-Jantti et al 1992, Shulman et al 1990, Vaag et al 1992). Similar conditions can exist in healthy FDR (Vaag et al 1992), although it is not clear whether such abnormality is inherited or acquired (Ostergard et al 2007, Vaag et al 1996). Although a defect in glycogen synthase activation can directly explain the decrease in insulin stimulation of non-oxidative glucose disposal, it is unlikely that this is the proximal abnormality in insulin resistance (Ostergard et al 2007). Increased plasma-free fatty-acid concentrations are typically associated with insulin-resistant states, such as T2DM (Boden & Shulman 2002, Reaven et al 1988) and the severity of insulin resistance (Ostergard et al 2007, Perseghin et al 1997).

2.32.2 *Physical Fitness Factor*

Low physical fitness has emerged as a consistent trait in individuals with T2DM (Koivisto et al 1986, Ostergard et al 2007, Regensteiner et al 1995, Schneider et al 1984). Nyholm et al. (Nyholm et al 1996) demonstrated not only reduced insulin sensitivity, but also reduced maximal oxygen uptake in FDR comparing FDR with activity-matched controls without diabetic predisposition. Lower maximal oxygen uptake was identified (Berntorp & Lindgarde 1985), and it has been confirmed (Nyholm et al 2004, Ostergard et al 2007, Thamer et al 2003). Thus, reduced physical capacity could be a primary trait in the development of T2DM (Ostergard et al 2007).

2.32.3 *Muscle Fiber Type Factor*

The insulin resistance found in individuals with T2DM might be due, in part, to a more "insulin resistant" muscle fiber type mixture, since individual muscle fibers differ in insulin sensitivity (Ostergard et al 2007). There is evidence that individuals with T2DM have a low percentage of type I fibers and a higher proportion of type II fibers (Hickey et al 1995a, Kriketos et al 1996, Marin et al 1994), and these muscle characteristics have been found to correlate with insulin sensitivity (Hickey et al 1995b, Kriketos et al 1996, Lillioja et al 1987, Marin et al 1994, Ostergard et al 2007). A previous study (Nyholm et al 1997) found an increased number of type IIb fibers in insulin-resistant FDR (Ostergard et al 2007). Because the human skeletal muscle fiber type distribution is largely determined by genetic factors (Simoneau & Bouchard 1995), these findings could explain an inherited insulin-resistant muscle fiber type mixture as an important abnormality in T2DM (Ostergard et al 2007).

2.32.4 *Mitochondrial ATP Production Factor*

Evidence of reduced mitochondrial ATP production, such as impaired mitochondrial dysfunction, was demonstrated in FDR (Ostergard et al 2007, Petersen et al 2004). This mitochondrial dysfunction, such as reduced oxidative capacity, is associated with impaired insulin-stimulated glucose uptake (Simoneau & Kelley 1997), and it entails a diminished capacity for lipid oxidation (Ostergard et al 2007). Because the content of intramyocellular triglycerides predicts insulin resistance very strongly (Kelley & Goodpaster 2001, Pan et al 1997, Perseghin et al 1999), reduced oxidative capacity could partly explain the accumulation of intramyocellular triglycerides in insulin-resistant conditions (Ostergard et al 2007). It has been suggested that mitochondrial dysfunction is an inherited primary defect in individuals prone to T2DM (Lowell & Shulman 2005, Petersen et al 2004), and decreased oxidative capacity in T2DM is an evaluation of the expression of the gene responsible for oxidative processes in muscle (Ostergard et al 2007). The expression of a compilation of "oxidative genes" was fond to be suppressed in individuals with T2DM, impaired glucose tolerance, or a family history of T2DM (Mootha et al 2003, Ostergard et al 2007, Patti et al 2003).

2.33 Diagnosis and Classification of T2DM

The American Diabetes Association recommends the use of four criteria to diagnose diabetes as follows (Colberg et al 2010b):

1. Glycated hemoglobin (A1C) value of 6.5% or higher
2. Fasting plasma glucose ≥126 mg/dl (7.0 mmol/l)
3. 2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test using 75g of glucose, and/or

4. Classic symptoms of hyperglycemia, (e.g., polyuria, polydipsia, and unexplained weight loss), or hyperglycemic crisis with a random plasma glucose of 200 mg/dl (11.1 mmol/l) or higher

The major forms of diabetes are type 1 diabetes (5 to 10%) and T2DM (90 to 95%) (Colberg et al 2010b). Less common forms include gestational diabetes mellitus, that is associated with a 40 to 60% chance of developing T2DM in 5 to 10 years (Colberg et al 2010b, U.S. 2008).

2.34 Treatment Goals in T2DM

The goal of treatment in T2DM is to achieve and maintain optimal blood glucose, lipid, and blood pressure levels to prevent or delay chronic complications of diabetes (Colberg et al 2010b, Standards 2010). Diet and physical activity are critical to manage and control T2DM because they help treat the related glucose, lipid, blood pressure control abnormalities, and aid in weight loss and maintenance (Colberg et al 2010b). Genetic and environmental factors are strongly correlated to developing T2DM, and the risk increases with age, obesity, and physical inactivity (Colberg et al 2010b). According to researchers (Colberg et al 2010b), "When medications are used to control T2DM, they should augment lifestyle improvement not replace them".

2.35 Type 1 Diabetes Mellitus (T1DM)

Clinical definitions of diabetes often obscure different mechanistic subtypes, such as type 1 and type 2, and both type are characterized by progressive β-cell failure (Cnop et al 2005). In type 1 diabetes mellitus (T1DM), it is typically caused by an autoimmune assault against β-cells, including progressive β-cell death. The genetic factor is HLA-

related in T1DM versus non HLA-related in T2DM, and putative environmental factor triggers T1DM by viral infection versus obesity in T2DM (Cnop et al 2005). β-cell of T1DM is reduced by 70-80% at the time of diagnosis, and it is suggested that β-cell loss occurs slowly over years due to the variable degrees of insulitis and absence of detectable β-cell necrosis (Cnop et al 2005, Kloppel et al 1985). These pathology findings are relevant to the progressive decline in first-phase insulin secretion in antibody positive individuals, long before the development of overt diabetes (Cnop et al 2005, Srikanta et al 1983).

2.36 Pre-diabetes

Pre-diabetes is defined as impaired glucose tolerance (IGT) such as a plasma glucose >140 mg/dL and <200 mg/dL 2 hours after a 75g oral glucose tolerance or impaired fasting glucose (fasting glucose between 100 and 126 mg/dL) (Rynders et al 2014). Impaired glucose tolerance has been an independent predictor of future adverse cardiovascular events (Cavalot et al 2006, Woerle et al 2007), and approximately 50% of adults with IGT progress to T2DM over their lives (Cowie et al 2009, Rynders et al 2014). Regular aerobic exercise is the treatment for the delay and/or prevention of T2DM, since its transient effects on postprandial metabolism and skeletal muscle insulin sensitivity can increase for approximately 48 hours after the last exercise bout (Colberg et al 2010a, Rynders et al 2014). Current American Diabetes Association (ADA) guidelines recommend that all pre-diabetic individuals engage in at least 150 minutes of moderate intensity physical activity or vigorous intensity physical activity for at least 90 minutes each week with no more than 48 hours separating bouts (American Diabetes Association 2012, Colberg et al 2010a, Rynders et al 2014).

Rynders et al. (Rynders et al 2014) investigated the effects of acute moderate intensity exercise and high intensity exercise on glucose disposal and insulin sensitivity in pre-diabetes adults. Although there were no differences in the insulinogenic index between the exercise-conditions, the index improved by 51% and 85% on the moderate intensity and high intensity exercises compared with that of control. Its improvement correlated to significant decrease of the glucose, insulin, and C-peptide area under the curve values during the last phase of the glucose tolerance test after high intensity exercise, with only a trend for reductions after moderate intensity exercise.

2.37 Conclusions

Exercise is a cornerstone for effective diabetes prevention and management, and it promotes weight loss and maintenance, hepatic and peripheral insulin sensitivity, glucose uptake and utilization, and cardiovascular health (Younk et al 2011). However, most of Americans are very busy every day, so they do not have time to exercise for an hour. This does not mean they are not able to improve their health and wellness, since high intensity interval training (HIIT) has been shown to improve cardiovascular and pulmonary function as well as improve metabolism with less time than traditional moderate intensity exercise training. Indeed, an effective HIIT program requires a participant to perform as little as 10 bouts of near-maximal effort exercise each lasting only 60 seconds interspersed with bouts of 60 seconds of rest or recovery between each bout. The reduced time commitment of HIIT protocols has led to the widespread use of HIIT for both healthy and patient populations.

The overall purposes of the first study is to determine the effects of high intensity interval training with and without the consumption of a nutritional supplement containing

a NO precursor on vascular function (brachial artery), oxygen utilization at the pulmonary and microcirculatory level, and exercise tolerance. First, we examine the endothelial response in the brachial artery over two weeks of HIIT with and without the nutritional supplement, L-arginine. Second, we compare the effects over two weeks of HIIT with and without L-arginine supplementation on O_2 uptake, microvascular changes, and the time to exhaustion during progressive ramp exercise.

The chronic disease, T2DM, is very difficult to recover from, although exercise and proper diet manage the symptoms. We will assess individuals with a family history of T2DM that can prevent pre-diabetes and T2DM, and it will make a big difference in their lives. The overall purpose of the second study is to determine whether a family history of T2DM influences high intensity interval training (HIIT) in insulin sensitivity, compared to healthy individuals without a family history of T2DM. This has not been previously investigated, but the results of such a study have the potential to help a large segment of our population because this exercise can be applied in many settings.

We assess insulin sensitivity over two weeks of HIIT, by comparing a family history of T2DM with healthy individuals using an oral glucose tolerance test (OGTT), which includes: a) fasting plasma glucose and b) two hours plasma glucose. Second, we investigate the improvement of absolute change in plasma glucose before and after each training session of HIIT. We examine the relationship between insulin sensitivity and lipid profile, specifically triglyceride and low-density lipoprotein (LDL), over two weeks of HIIT.

2.38 References

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Chapter 3

The Effects of High Intensity Interval Training (HIIT) with L-arginine versus HIIT on Cardiovascular Function

3.1 Introduction

High-intensity interval training (HIIT) is characterized by short bursts of high intensity, vigorous physical activity that is interspersed by periods of rest or low-intensity exercise. The specific physiological adaptations induced by this form of training are determined by several of factors including, the exercise intensity, the exercise duration, the number of intervals performed, as well as the intensity, duration and type of activity performed during each of the recovery periods (Gibala et al 2012). However, it appears as though HIIT can be used as an effective alternative to traditional endurance training in both healthy and diseased individuals, since the reslts of several investigations demonstrate similar or even greater improvements in a range of physiological (i.e. central and peripheral adaptations), performance, and health-related variables (Gibala et al 2012, Hwang et al 2011, Tjonna et al 2009, Wisloff et al 2007). For example, the results of previous studies (Burgomaster et al 2008, Rakobowchuk et al 2008), demonstrated similar training-induced improvements in mitochodrial biogenesis and an upregulation of the enzymes involved in metabolism as well as cardiovascular adaptations including

improvements in endothelial function, despite large differences in weekly training volume (~90% lower in the HIIT group) and time commitment (~67% lower in the HIIT group) compared with traditional endurance training (Gibala et al 2012). Perhaps what makes HIIT so attractive to many individuals is that current research suggests that significant improvements can be achieved with as few as 10 exercise bouts of 60 seconds at a constant-load intensity that elicits ~90% of maximal heart rate separated by 60 seconds of recovery. This protocol appears to be well tolerated and has now been applied to many patient populations based upon safety, subject tolerance, and appeal (Gibala et al 2012, Currie et al 2012).

According to previous investigations (Cameron & Dart 1994, Hayashi et al 2005, Sugawara et al 2006, Tanaka et al 2000), long-term moderate-intensity exercise has been shown to increase central artery stiffness in populations with reduced baseline central elasticity. However, other research (Rakobowchuk et al 2008, Tanaka et al 2000) indicates that younger populations tended to have the higher baseline central artery stiffness and showed less propensity for training-related increases (Rakobowchuk et al 2009). Compared with the moderate intensity exercise training, a sprint interval training that utilized repeated 30 seconds bouts of all-out exercise improved the popliteal artery function and structure (Rakobowchuk et al 2008), skeletal muscle metabolic efficiency (Burgomaster et al 2007, Burgomaster et al 2008), and aerobic fitness and performance (Burgomaster et al 2007, Burgomaster et al 2006, Burgomaster et al 2008, Burgomaster et al 2005, Rakobowchuk et al 2009). Recently, brachial artery endothelial function was investigated in patients with coronary artery disease in response to high intensity interval exercise compared to a more traditional moderate-intensity endurance exercise training

program (Currie et al 2012). The results of this study demonstrated similar improvements in brachial artery endothelial-dependent function, based on the flow-mediated dilation (FMD) technique, despite considerable differences in the training volume. While the comparison between high intensity interval training and endurance training is of interest, perhaps more significant was the improvement in endothelial function following short-term (i.e. two weeks) high intensity interval training.

Arterial stiffness plays an important role in regulating the cardiac performance, perfusion, and homeostasis based upon the results of previous research (Kingwell 2002). Other research (Boutouyrie et al 2002) shows that a "stiff" arterial tree is related to adverse cardiovascular problems including hypertension (Rakobowchuk et al 2008). The response of the endothelial cells to shear stress stimuli in the brachial artery reflect the function of the endothelial cells in the coronary arteries and thus, is considered to be a very good independent measurement of atherosclerotic disease risk (Rakobowchuk et al 2008, Schachinger et al 2000, Suwaidi et al 2000, Vita 2005). L-arginine is a precursor of nitric oxide (NO) that plays an important role in maintaining vascular tone since it functions as a potent vasodilator. The signaling molecule, NO, is produced by the NO synthase (NOS) family of enzymes, that catalyze the oxidation of L-arginine, yielding NO and L-citrulline (Bailey et al 2010b, Bredt et al 1991, Moncada & Higgs 2006, Moncada et al 1988, Moncada et al 1989). A complementary NOS-independent pathway for NO production, such as the reduction of inorganic nitrite (NO_2^-) to NO, especially in acid/hypoxic conditions, has been described (Bailey et al 2010b, Cosby et al 2003, Gladwin et al 2006) but the benefits of this pathway to vascular control during exercise are not well described. Thus, several studies (Heitzer et al 2000, Lucotti et al 2009,

Lucotti et al 2006, Martina et al 2008, Maxwell 2002, Natarajan Sulochana et al 2002, Settergren et al 2009, Wascher et al 1997) have argued that L-arginine supplementation in humans may be effective and improve vascular function by reducing oxidative stress, and increasing NO availability where there appears to be endothelial dysfunction such as cardiovascular complications and diabetes (Fayh et al 2012).

To date, a number of studies have examined the benefits of performing HIIT exercise on brachial artery function and there is some evidence that L-arginine supplementation also improves endothelial function. However, the potential benefits of combining HIIT while also ingesting a L-arginine supplement have not been previously investigated in individuals performing whole body exercise. The results of a previous study (Hambrecht et al 2000) that investigated the effects of daily handgrip exercise with L-arginine supplementation did show a significant improvement in endothelium dependent vasodilation, compared with L-arginine alone or training group alone. Thus, the results from this study suggest that both interventions seem to produce additive effects with respect to endothelium-dependent vasodilation (Hambrecht et al 2000). The purpose of this study was primarily to investigate endothelial-dependent function in the brachial artery alone with measures of performance before after six sessions of HIIT only or HIIT with L-arginine supplementation among healthy individuals. We hypothesized that HIIT with L-arginine supplementation would result in greater improvements in endothelial-dependent function in the brachial artery, and improved performance (aerobic capacity and time to exhaustion) compared to performing HIIT only.

3.2 Methods

3.2.1 *Participants*

Fifteen healthy and young adult men (22 ± 1 yr (Mean ± SEM), 178.6 ± 2.2cm, 84.0 ± 4.2 kg) volunteered to participant in this study. Participants with any previous medical history of cardiovascular or pulmonary disease, musculoskeletal injuries, diabetes mellitus, or individuals who are currently taking any prescribed medications and/or vasodilation supplements were excluded from this study. All procedures, experimental protocol and risks associated with participating in the investigation were explained to each subject. Written informed consent was obtained before participants performed any exercise and were provided any nutritional supplement. This study was approved by the Human Subjects Research Review Committee at the University of Toledo and is in accordance with the Declaration of Helsinki.

3.2.2 *Experimental Procedures*

Subjects reported to the Cardiopulmonary and Metabolism Research Laboratory (CMRL) at the University of Toledo on ten separate occasions over a four week period. Subjects were asked to refrain from any strenuous exercise while participating in this study. Each subject was instructed to consume only a light meal and to abstain from vigorous exercise and caffeinated beverages for ≥ 12 hours prior to arriving for testing.

During the first session, anthropometric data (body height, body mass, and body composition) were measured and recorded using standard clinical practice. In addition, a measurement of the left brachial artery endothelium-dependent function was determined by using the flow-mediated dilation (FMD) technique. During the second session,

subjects performed a maximal exercise test using an electromagnetically braked stationary cycle ergometer for the determination of peak oxygen uptake (VO_{2peak}). This exercise test involves four minutes of warm-up pedaling at 20 W followed by a progressive ramp increase in work rate using a linear forcing function of 20 - 25 W/min to volitional fatigue. In addition, the muscle microvacular oxygenation-deoxygenation status was also measured using near-infrared spectroscopy (NIRS) in the left vastus lateralis during maximal ramp exercise test.

Following the maximal exercise test, subjects were randomly assigned to either the high intensity interval training (HIIT) or the high intensity interval training with L-arginine supplementation (HIIT-L-arginine) (Table 3.1). Both the HIIT and HIIT-L-arginine groups performed the same exercise training program consisting of 10 bouts of exercise at an intensity equivalent to 80% of the peak work rate achieved during their maximal exercise test for 60 seconds, followed by 60 of recovery at 30 W between each bout. All exercise was performed on cycle ergomenter three times a week for a period of two weeks, similar to the training program used in a previous study (Gibala et al 2012). Subjects who were assigned to the HIIT were given a placebo while the HIIT-L-arginine group was given 5.4 g per day of L-arginine supplement. For both the HIIT and the HIIT-L-arginine groups, they consumed either the placebo or the L-arginine supplement for 2 days prior to the first day of training and continued to receive daily dosages until the subjects completed all post-training assessments.

Within two days of completing the training program, subjects reported to the laboratory for the post-training assessment including anthropometric data (height, weight and body composition). In addition, the post-training assessment of endothelial function

was performed using the same FMD technique on the same limb. During the final visit to the laboratory, each subject completed a progressive ramp exercise test on an electronically braked cycle ergometer to volitional fatigue for the post-training assessment of aerobic capacity (i.e. VO_{2peak}).

3.2.3 *Stationary Cycling Exercise Protocol*

All exercise testing and training was performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Prior to each exercise maximal exercise test, the participants were prepared by adjusting the cycle ergometer for seat height and handlebar position, the NIRS probe was placed over the vastus lateralis muscle and the subject was given a mouthpiece to breathe through for the measurement of pulmonary gas exchange. Heart rate was continuously monitored using a 4-lead configuration. The participants were instructed to pedal at a cadence of 80 rpm during each trial. Each ramp exercise test was preceded by four minutes of a constant load exercise at 20 W to obtain baseline measures. Following the four minutes lead-in, the work rate was progressively increased in a linear forcing function a rate of 20 or 25 W/min depending upon their previously recorded activity level. The exercise trial was terminated either upon request by the participant, when volitional fatigue was reached, or when the participant could no longer maintain pedal cadence above 40 revolutions per minute despite strong verbal encouragement by the investigator.

3.2.4 *Pulmonary Gas Exchange (VO_2, VCO_2 and VE)*

Pulmonary gas exchange (including O_2 uptake, CO_2 output and minute ventilation VE) was measured breath-by-breath using a commercially available metabolic

measurement system (Vmax, Sensormedics, Yorba Linda, CA). The metabolic system was calibrated according to the specifications provided by the manufacturer prior to each exercise test using a large pump with a known value (3.0 L) to calibrate the flow meter and known gas concentrations to calibrate the O_2 and CO_2 analyzers. The subject was required to breathe through a rubber mouthpiece while the nose was occluded with a nose clip.

3.2.5 *Near-Infrared Spectroscopy*

Muscle oxygenation was evaluated by NIRS system (OxiplexTS, ISS, Champaign, IL). Based upon a similar approach to previous research (Ferreira et al 2005), NIRS was used to continuously monitor the oxyhemoglobin saturation (oxy-[Hb]), deoxyhemoglobin (deoxy-[Hb]), and total hemoglobin (Hb_{tot}) throughout the exercise protocol. The NIRS probe consists of eight light-emitting diodes operating at wavelengths of 690 and 830 nm, and a photomultiplier tube. The laser diodes and photomultiplier tube are connected to a lightweight plastic probe by optical fibers consisting of two parallel rows of emitter fibers and one detector fiber bundle comprising source-detector separations of 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. After shaving the skin, the NIRS probe was positioned longitudinally over the muscle belly of left vastus lateralis muscle (~15 cm above the patella) and secured with a Velcro strap around the thigh. NIRS was calibrated on each test after a warm-up period of at least 30 minutes. A dark, heavy cloth was secured over the probe to prevent stray visible light from affecting the operation of the optical diodes. The calibration was performed with the optical probe placed on a calibration block with known absorption and reduced scattering coefficients previously determined (i.e. a phantom calibration block).

Correction factors were determined and automatically implemented by he equipment's software for the calculation of the absorption coefficient (μ_A) and reduced scattering coefficient (μ'_S) for each wavelength during the data collection (Hueber et al 2001). The NIRS probe placement was visually inspected after each exercise session; no appreciable movement was observed in any exercise test.

3.2.6 *Flow-Mediated Dilation (FMD)*

Similar to the approach used in a previous study (Stacy et al 2013), FMD in the brachial artery was conducted in the supine position using echo-Doppler ultrasonography (Zonare Medical System, Inc., Mountain View, CA). Prior to obtaining any measurements, subjects were instructed to lie in the supine position for approximately 20 minutes. The left arm was slightly abducted at heart level and positioned so that the ultrasound probe could be easily moved and held in place with the assistance of a custom-designed device to maintain consistent probe position and tissue compression while imaging. The echo-Doppler ultrasonography system was used to obtain baseline measurements of the brachial artery and the associated blood velocities. A 7 MHz ultrasound probe operating in B-mode was placed in a longitudinal orientation on the skin over the brachial artery positioned between 2 and 10 cm proximal to the antecubital fossa. All measurements were acquired and analyzed by the same investigator to limit inter-observer variability both between and within subjects. Once baseline measurements were obtained, an occlusion cuff that had been placed around the forearm was inflated to a suprasystolic pressure of 250 mmHg (D.E. Hokanson, Inc., Bellevue, WA) for 5 minutes. After the occlusion period, the cuff was immediately deflated and ultrasound images were continuously measured for 3 minutes in order to capture the maximal

change in brachial artery diameter and. blood velocities. All images and blood velocities were recorded at a sampling rate of 2 Hz using digitizing frame grabber software on a computer, and were later analyzed using commercially available software (Medical Imaging Application, LLC, Coralville, IA). The FMD response was quantified for each subject by calculating the percent change from resting baseline diameter to the peak change in brachial artery diameter following the release of the cuff after the 5 minute occlusion period (i.e. %FMD).

3.2.7 *Heart Rate*

Electrocardiography (ECG) (ADInstruments, Colorado Springs, CO) was monitored by placing four electrodes over specific sites on the surface of the chest. HR was measured by determining the R-R interval during off-line analysis. To obtain an optimal ECG signal, the chest was prepared by shaving (if necessary) any hair in the area of the electrode placement, slightly abrading with gauze, and cleaning with an alcohol wipe prior to placement. In addition, a heart rate monitor (Polar USA, Lake Success, NY) was placed around the chest before each training session which allowed the investigator and a subject to monitor HR throughout each of the training sessions.

3.2.8 *Statistical Analyses*

The pre- and post-training values for the primary variables of interest (%FMD, VO_{2peak}, peak deoxy-[Hb]) were analyzed using a two-way analysis of variance (ANOVA) with one repeated measure (group x time). A significant main effect or interaction term was further analyzed using the Student-Newman-Keuls post-hoc multiple comparison test. All data was presented as the group mean ± SEM unless

indicated otherwise. Statistical significance was set *a prior* at $P < 0.05$. All statistical analyses were performed using Sigma Stat 3.0 (Systat Software, San Jose, CA).

3.3 Results

3.3.1 *Cardiovascular Response during the Progressive Ramp Exercise Test*

The ANOVA results for the time to exhaustion indicated that there was no main effect for training program ($P > 0.05$) but there was a main effect for time ($P < 0.05$). There was no significant interaction between the training program and time ($P > 0.05$) and therefore, the time to exhaustion was improved from pre- to post-training but there were no differences between the training programs post-training (HIIT-L-arginine, pre-training, 930 ± 67 vs. post-training, 998 ± 25; HIIT, pre-training, 972 ± 63 s vs. 1,033 ± 36 s) (Figure 3-1). Similarly, the results of the ANOVA comparison for peak WR achieved indicated no significant main effect for training program ($P > 0.05$), however there was a significant main effect for time ($P < 0.05$) but the interaction term for training program and time did not reach significance ($P > 0.05$). Therefore, when the pre-training and post-training data were pooled across the training conditions, there was significant improvement in the peak WR achieved (HIIT-L-arginine, pre-training, 291 ± 21 W vs. post-training, 307 ± 20 W; HIIT, pre-training, 290 ± 11 s vs. 302 ± 13 W) (Figure 3-2)

The results of the ANOVA procedure indicated that there was no significant main effect for training program ($P > 0.05$) or time ($P > 0.05$) and no significant interaction ($P > 0.05$) indicating that there was no improvement in VO_{2peak} between the HIIT-L-arginine and the HIIT groups either before or following the short term training program. (Figure 3-3, Table 3.2).

3.3.2 *Microvascular Response*

One subject in HIIT-L-arginine group was removed from the analysis due to technical difficulties retrieving the data from the storage device. The results of the ANOVA indicated that there was not a main effect for training protocol nor was a main effect for pre- to post-training. The interaction term was not significant and change in muscle deoxy-[Hb] from baseline was not significantly different between training protocols nor was there a difference from pre- to post-training (Figure 3-4).

3.3.3 *Flow-Mediated Dilation*

The results of the ANOVA indicated that there was no main effect for training protocol ($P > 0.05$) and there was no main effect for pre- to post-training ($P > 0.05$) for %FMD. In addition, the there was no significant interaction between the training program and time and therefore, the there was no significant training or L-arginine effect on the brachial artery FMD response (HIIT-L-arginine, pre-training, 6.6 ± 1.0 % vs. post-training, 7.5 ± 1.3 % ; HIIT, pre-training, 5.8 ± 1.1 % vs. 6.7 ± 0.9 %) (Figure 3-5).

Table 3.1. Subject Demographics

Group	Pre		Post	
	HIIT + L-A (N=8)	HIIT + P (N=7)	HIIT + L-A (N=8)	HIIT + P (N=7)
Age (y/o)	21.1 ± 0.3	22.6 ± 1.4	21.1 ± 0.3	22.6 ± 1.4
Height (cm)	181.1 ± 3.4	175.8 ± 2.8	181.1 ± 3.4	175.8 ± 2.8
Weight (kg)	91.3 ± 5.8	75.6 ± 5.0	91.6 ± 5.8	75.9 ± 5.1
BMI (kg/m^2)	27.8 ± 1.5	24.5 ± 1.7	27.9 ± 1.5	24.6 ± 1.7

Note:
Age, Height, Weight, BMI: Mean ± SEM.
HIIT + L-A: High Intensity Interval Training with L-arginine.
HIIT + P: High Intensity Interval Training with placebo.

Table 3.2. Progressive Ramp Exercise

Group	Pre		Post	
	HIIT + L-A (N=8)	HIIT + P (N=7)	HIIT + L-A (N=8)	HIIT + P (N=7)
Peak VO_2 (L/min)	3.8 ± 0.3	3.8 ± 0.1	4.0 ± 0.3	3.8 ± 0.3
Peak VO_2 (ml/kg/min)	42.8 ± 4.1	51.6 ± 3.2	45.0 ± 4.1	50.4 ± 4.0
Peak VE (L/min)	142.3 ± 12.0	134.7 ± 9.4	160.1 ± 8.9	137.4 ± 15.3
Peak VE/O_2	37.8 ± 2.2	35.1 ± 1.7	40.3 ± 1.9	36.0 ± 1.6
Peak VE/CO_2	31.9 ± 1.6	29.4 ± 1.5	32.9 ± 1.4	30.1 ± 1.3
Peak Heart Rate (bpm)	192 ± 2	189 ± 4	192 ± 3	187 ± 4

Note:
Progressive Ramp Exercise: Mean ± SEM.
HIIT + L-A: High Intensity Interval Training with L-arginine.
HIIT + P: High Intensity Interval Training with placebo.

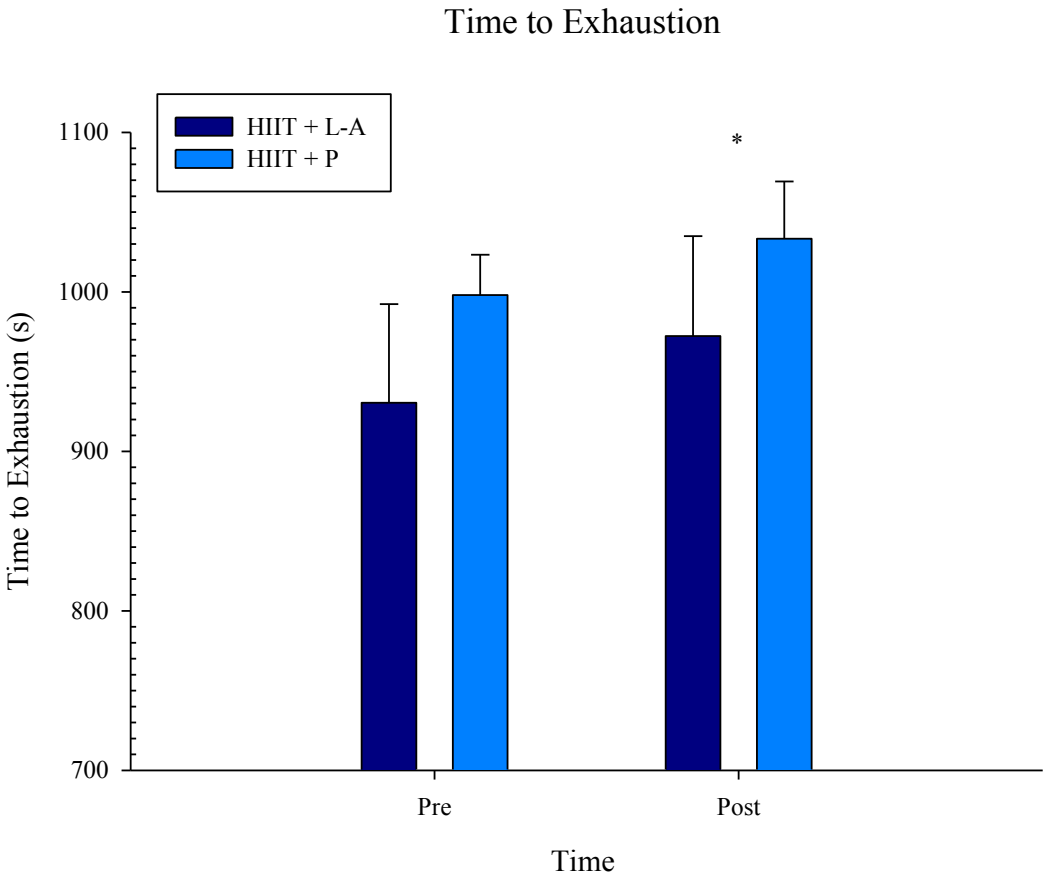

Figure 3-1. Time to exhaustion was assessed before and after two weeks of high intensity interval training (HIIT) with L-arginine (N = 8) and HIIT with placebo (N = 7).
*, Significant difference between pre- and post-training for pooled response ($P < 0.05$).
Data was represented by mean ± SEM.
HIIT + L-A: High intensity interval training with L-arginine.
HIIT + P: High intensity interval training with placebo.

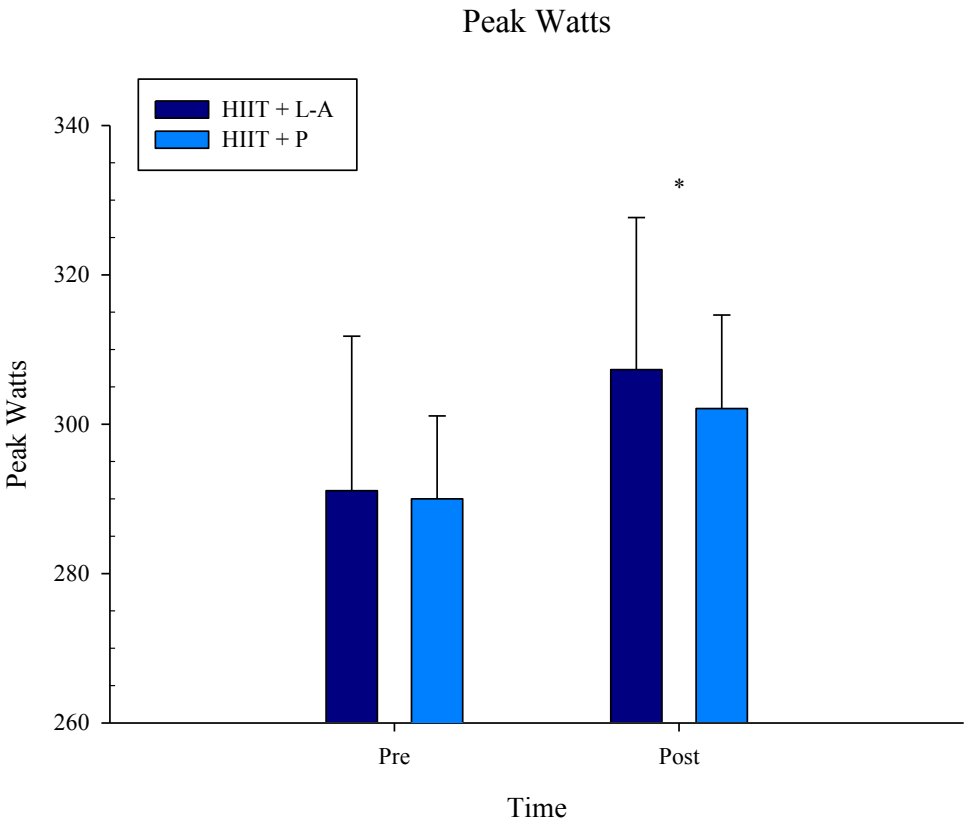

Figure 3-2. Peak work-rate was assessed before and after two weeks of high intensity interval training (HIIT) with L-arginine (N = 8) and HIIT with placebo (N = 7).
*, Significant difference between pre- and post-training for pooled response ($P < 0.05$).
Data was represented by mean ± SEM.
HIIT + L-A: High intensity interval training with L-arginine.
HIIT + P: High intensity interval training with placebo.

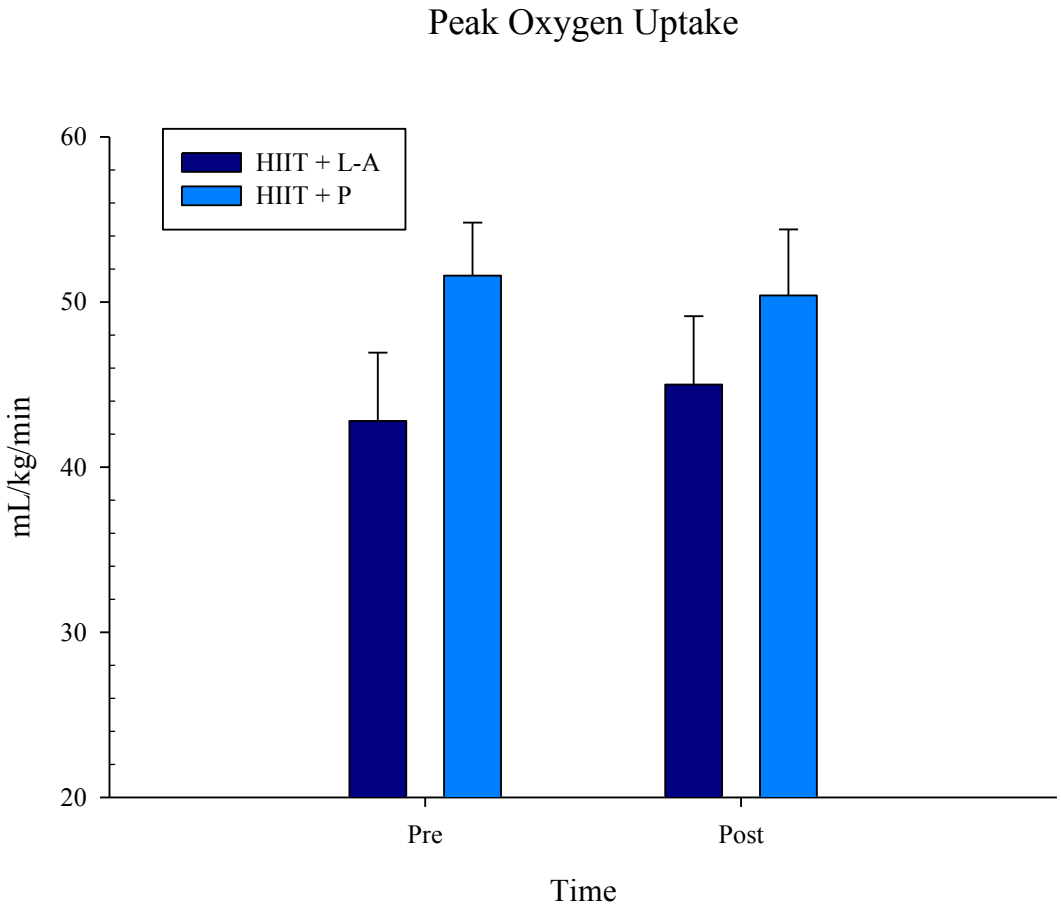

Figure 3-3. Peak oxygen uptake was assessed before and after two weeks of high intensity interval training (HIIT) with L-arginine (N = 8) and HIIT with placebo (N = 7). No significant difference between pre- and post-training in HIIT + L-A ($P > 0.05$). No Significant difference between pre- and post-training in HIIT + P ($P > 0.05$). Data was represented by mean ± SEM.
HIIT + L-A: High intensity interval training with L-arginine.
HIIT + P: High intensity interval training with placebo.

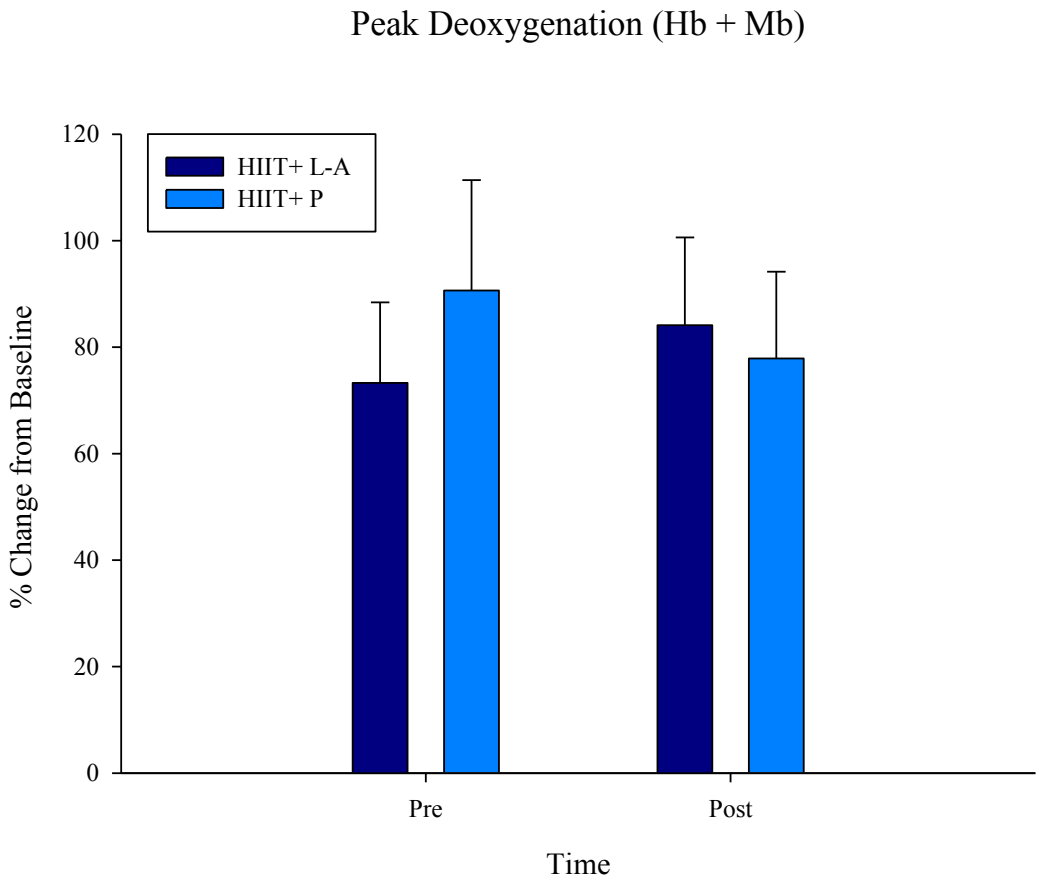

Figure 3-4. Peak deoxygentated hemoglobin was assessed before and after two weeks of high intensity interval training (HIIT) with L-arginine (N = 7) and HIIT with placebo (N = 7).
No significant difference between pre- and post-training in HIIT + L-A ($P > 0.05$).
No Significant difference between pre- and post-training in HIIT + P ($P > 0.05$).
Data was represented by mean ± SEM.
HIIT + L-A: High intensity interval training with L-arginine.
HIIT + P: High intensity interval training with placebo.

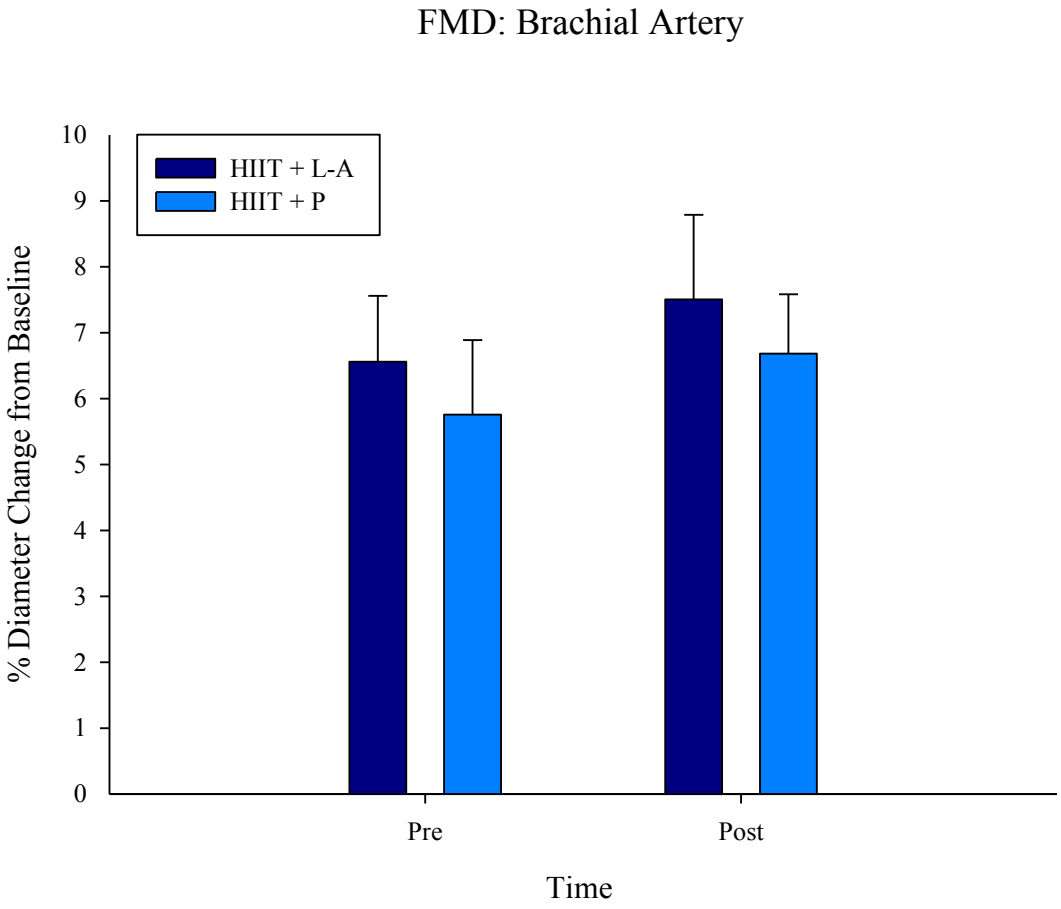

Figure 3-5. Flow mediated dilation (FMD) was assessed before and after two weeks of high intensity interval training (HIIT) with L-arginine (N = 8) and HIIT with placebo (N = 7).
No significant difference between pre- and post-training in HIIT + L-A ($P > 0.05$).
No Significant difference between pre- and post-training in HIIT + P ($P > 0.05$).
Data was represented by mean ± SEM.
FMD: Flow-mediated dilation.
HIIT + L-A: High intensity interval training with L-arginine.
HIIT + P: High intensity interval training with placebo.

3.4 Discussion

Although there are many models of HIIT currently being used, many typical approaches involve short exercise bouts at an intensity equivalent to at proximately 90% maximal HR interspersed with brief period of recovery or exercise at a considerably lower intensity, based upon the potential safety, subject tolerance, and appeal for some individuals (Gibala et al 2012). It is a practical and time-efficient model, such as effective skeletal muscle remodeling toward a more oxidative phenotype and functional performance (Gibala et al 2006, Little et al 2010) as shown by cycling time trials that resemble normal athletic competition (Gibala et al 2012).

Of the performance variables examined in the present study, both the time to exhaustion and the peak work rate achieved was significantly improved in the HIIT and the HIIT-L-arginine groups when pooled, compared to a pre-test. The results of a previous study (Bailey et al 2010b) reported that plasma lactate concentration and VO_2 measured following 6 minutes of severe-intensity exercise and at the point of exhaustion were not significantly altered by L-arginine ingestion, but exercise tolerance was extended by approximately 20% for the L-arginine condition. This improvement in performance was associated with an increase in plasma $[NO_2^-]$ prior to exercise and improved VO_2 dynamics as measured during submaximal steady-state exercise (Bailey et al 2010b). There is evidence that plasma $[NO_2^-]$ is a good indication of nitric oxide synthase (NOS) activity (Kleinbongard et al 2003, Lauer et al 2002, Lauer et al 2001, Rassaf et al 2007). This may provide an important measure for the capacity to tolerate high-intensity exercise (Bailey et al 2010a, Bailey et al 2009c, Larsen et al 2007, Larsen et al 2010, Rassaf et al 2007) if this functions as a reservoir for NO production (Bailey et

al 2010b, Gladwin et al 2006). L-arginine supplementation would be expected to spare the utilization of the anaerobic reserves thereby contributing to an improvement in performance, particularly at the higher exercise intensities (Bailey et al 2010a, Krustrup et al 2004, Rossiter et al 2002).

In the current study, improvement in performance measures were similar between the HIIT-Larginine and the HIIT suggesting that L-arginine in combination with HIIT did not provide any further benefits than the HIIT exercise alone. There may be a number of reasons for this observation including the notion of the "clinical paradox of L-arginine" (Dioguardi 2011). The much-needed L-arginine may become ineffective or actually detrimental, when it is supplemented chronically, especially if the individual already has sufficient amounts of L-arginine readily available. Since the subjects in the present study were only exposed to L-arginine for two weeks, it appears unlikely that this explains the lack of an effect on performance. However, when the body is exposed to large amounts of L-arginine, another pathway of the complex arginine metabolism which is controlled by the ubiquitous enzymes arginases such as Arginase 1 and Arginase 2 may play a significant role since these enzymes compete with NOS for L-arginine as a substrate. (Dioguardi 2011). Although ARG 2 is widely expressed, mostly in the kidneys, gut, and brain, the overexpression of ARG 2 is reported to play a critical role in the pathophysiology of cholesterol-mediated endothelial dysfunction (Dioguardi 2011, Vanhoutte 2008). Although a difference in %FMD was not observed in the present study (see below), this avenue of study is warranted given the benefits of regular physical activity on endothelial function and the ongoing use of L-arginine as a nutritional supplement.

3.4.1 *HIIT with L-arginine and VO_{2peak}*

The results of a previously conducted study (Lansley et al 2011) indicated that the ingestion of a dietary supplement containing nitrate would enhance athletic performance including power output and VO_2, during a simulated competition that included a 4 km and 16.1 km cycling time trial. Nine club-level competitive male cyclists were assigned using a randomized, crossover design, to consume 0.5 L of beetroot juice or 0.5 L of nitrate-depleted beetroot juice 2.5 hours before the completion of a 4- and 16.1-km cycling time trials. The beetroot juice group, which led to a significant increase in plasma [nitrate], also resulted in greater mean power output during both the short and longer time trials but again, the VO_2 values were not significantly different between the conditions.

Although an increase in peak power output and time to exhaustion was observed in the present study, there was no evidence of an improvement in VO_{2peak} pre- to post-training for either the HIIT-L-arginine or HIIT groups. One possibility that has been raised by others is that an increase in plasma [NO_2^-] improves efficiency by decreasing the ATP turnover rate for a given power output. For example, the results of previous studies have shown a significant blunting of the exercise induced fall in [PCr] and an attenuated increase in [ADP] and [Pi] for the same power output using ^{31}P magnetic resonance spectroscopy following beetroot juice supplementation (Bailey et al 2010a, Lansley et al 2011). The ATP cost of actin-myosin interaction or Ca^{2+} handling continue to be a matter of debate but there are suggestions that nitrite/NO can modulate these processes (Galler et al 1997, Heunks et al 2001, Viner et al 2000), as well as the efficiency of mitochondrial respiration (Clerc et al 2007, Lansley et al 2011, Larsen et al

2011). The design of the present study precludes us from speculating on this mechanism and it may well be that the lack of a significant finding in the present study may also be due to the fact that the individuals were subjected to a relatively low intensity compared to other HIIT models. In the present study, subjects trained at an intensity equivalent to 80% of the peak work rate achieved during the first maximal exercise test based on a protocol previously established that found a significant improvement in aerobic capacity (Hood et al 2011). However, the subjects were middle-aged and sedentary adults (i.e. average age 45 years old and 30 mL/kg/min) compared to the college-age group of physically active individuals in our study. In fact, the HIIT-L-arginine group had a VO_{2peak} of ≈43 mL/kg/min and the HIIT group had a VO_{2peak} ≈52 ml/kg/min (Table 3.2) and therefore, there initial level of fitness was considerably higher than the group reported by Hood et al (2011).

3.4.2 *Effect of HIIT with L-arginine and Deoxygenation*

In the current study, deoxy-[Hb] did not show any significant changes at peak exercise when comparing pre-training to post-training values in both groups which is consistent with results of previous studies. Researchers (McKay et al 2009) investigated VO_2 kinetics, muscle oxygenation-deoxygenation, and exercise performance in response to short-term HIIT and lower-intensity continuous endurance training. These subjects underwent eight sessions of either HIIT (consisting of 8 to 12 x one minute interval at 120% VO_{2peak} separated by one minute of rest) or 90 to 120 minutes at 65% maximal O_2 uptake). In addition, participants completed step transitions to a moderate-intensity work rate (~90% estimated lactate threshold) on five occasions throughout the training program. Progressive ramp exercise test to exhaustion and constant-load performance

tests were conducted at pre-, mid-, and post-testing periods. These authors reported that the time constant for VO_2 was reduced by ~20% after only two training sessions (i.e. a significant speeding of the VO_2 response) and by ~40% after eight training sessions with no differences between HIIT and the more traditional low intensity training program. While these authors observed improvements in VO_2 during submaximal exercise testing, this did not appear to be associated with an appreciable change in the deoxy-[Hb] signal measured in the vastus lateralis and this did not appear to change considerably over the course of eight training sessions.

The results of a previous study (McKay et al 2009) suggested that the faster rate of increase in muscle O_2 utilization was not accompanied by a faster and/or greater muscle O_2 extraction. It has not been well-established whether the early adaptation of microvascular blood flow is accompanied by a faster adaptation of conduit artery blood flow, but one study (Shoemaker et al 1996) provided the information on early training-induced adaptations to conduit artery blood flow (McKay et al 2009). Researchers presented that the kinetics of conduit artery blood velocity and arterial conductance became faster after ten days of endurance training (McKay et al 2009, Shoemaker et al 1996).

3.4.3 *HIIT with L-arginine and Flow-Mediated Dilation (FMD)*

In contrast to the stated hypothesis, %FMD was not improved following either HIIT-L-arginine or HIIT alone in the present study. The FMD response can be assessed in many conduit arteries, but the brachial artery has been an accepted surrogate for the coronary artery, that is relevant for coronary artery disease populations (Currie et al 2013). In fact, the relationship between coronary and brachial artery endothelial function

has been shown to positively predict abnormal brachial dilation so that an increase in less than 3% is equivalent to a 95% chance of having coronary endothelial dysfunction (Anderson et al 1995, Teragawa et al 2005). In the current study, participants were healthy and they were physically active individuals, so potentially the addition of L-arginine and/or short term HIIT was not sufficient to elicit significant improvements in %FMD. Further studies in individuals and patient populations that are known to suffer from endothelial dysfunction and limited NO availability are warranted.

Endothelial dysfunction, or a decrease in endothelial-dependent vasodilation, is considered to be one of the earliest biomarkers of atherogenesis and may aid in identifying early stages of other chronic diseases such as diabetes mellitus (Fayh et al 2012). A potent vasodilator, such as nitric oxide (NO), is reduced in diabetes patients that compromise their vascular tonus control (Fayh et al 2012, Newsholme et al 2010). L-arginine, considered a semi-essential or conditionally essential amino acid (Barbul 1986, Flynn et al 2002), has been used in order to improve the endothelial function (Maxwell 2002), insulin secretion and pancreatic β-cell protection (El-Missiry et al 2004, Krause et al 2011), in addition to adiposity control in obesity and diabetes (Fayh et al 2012, Fu et al 2005). Thus, several studies (Heitzer et al 2000, Lucotti et al 2009, Lucotti et al 2006, Martina et al 2008, Maxwell 2002, Natarajan Sulochana et al 2002, Settergren et al 2009, Wascher et al 1997) have identified L-arginine supplementation in humans as a potential treatment for cardiovascular complications and diabetes, improving the endothelial function, reducing oxidative stress, and increasing NO availability (Fayh et al 2012). However, our subjects were relatively healthy and physically active individuals, thus we did not see any differences in endothelial-dependent response.

3.5 Limitations

A limitation of the present study was that for the most part, subjects were relatively healthy and engaged in some form of regular physical activity as opposed to healthy but relatively sedentary individuals. In healthy individuals, they may already have sufficient amounts of L-arginine readily available for NOS and therefore, the additional amount provided by the nutritional supplement do not give any added benefit to these individuals. This is supported by the results of those studies that have reduced availability of substrates for NOS thereby showing improvements in endothelial function using a supplement containing L-arginine. As mentioned above, the exercise intensity for the HIIT protocol was set at 80% of the peak work rate achieved during the first maximal exercise test with no progressive overload built in to the training program. Based upon the activity-level of participants, the exercise training intensity needed to be considerably higher and progressively increased throughout the training protocol in order to see improvement after two weeks of HIIT.

3.6 Conclusions

The results of the present study demonstrated that the time to exhaustion and peak work rate achieved were significantly improved in both groups. However, the use of a nutritional supplement containing L-arginine does not appear to contribute to the improvement observed in these measures of exercise tolerance since there was no difference between training groups. In addition, VO_{2peak} peak deoxy-[Hb], an estimate of O_2 extraction in the microvasculature, was not improved by L-arginine supplementation, at least during the short-term exercise training used in the present study. In contrast to the widely held view that the use of a nutritional supplement containing L-arginine

improves vasodilation thereby improving O_2 delivery, the FMD response was not improved in the present study suggesting that L-arginine supplementation may have limited benefits, particularly in relatively healthy individuals.

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Chapter 4

The Effects of High Intensity Interval Training (HIIT) on Insulin Sensitivity in Individuals with a Family History of Type 2 Diabetes Mellitus (T2DM)

4.1 Introduction

The Centers for Disease Control and Prevention (CDC) estimated that 59.2% of Americans were either overweight or obese in late 1990s (Cusi 2009)(National Institutes of Health 1998, Wyatt et al 2006), but it increased to 68.8% in 2009-2010 (National Institutes of Health 2012). Obesity alone affects 60 million adult Americans in late 90s (Cusi 2009), but it affects at least 78.6 million of adults American now (Ogden et al 2014). In addition, health care expenditures increase significantly once an individual reaches 30 kg/m^2 (Cusi 2009, Runge 2007, Wyatt et al 2006). Obesity is associated with a reduced life span, and estimated 100,000 to 400,000 excess deaths per year, depending upon which model is used to evaluate the impact of obesity on mortality rates (Cusi 2009, Flegal et al 2007, Olshansky et al 2005, Peeters et al 2003).

When obesity progresses, individuals are prone to developing chronic diseases such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). The epidemic of T2DM is a serious healthcare problem that contributes significantly to cardiovascular and other related chronic healthcare problems. T2DM is problematic in

the United States as well as worldwide, and experts agree that the sedentary life-styles combined with excessive caloric intake, including high carbohydrates and saturated fats, have contributed significantly to the prevalence of T2DM (Cusi 2009). Some ethnic groups appear to be genetically predisposed to T2DM including Hispanics, African-Americans, Native Americans, and South Asians, which affects over 8% of the United State's population between the ages of 20 and 74 years (Boyle et al 2001, Cusi 2009). In the United States, medical care costs for obesity-related conditions, including T2DM, were estimated to exceed $117 billion or almost 10% of the total healthcare costs in the late 1990s (Cusi 2009), but the estimated annual medical cost of obesity in 2008 was $147 billion (Center for Disease Control 2014). In addition, based on the findings of epidemiological studies, T2DM and excess adiposity are believed to be significant risk factors involved in the early development of CVD and the increased overall mortality rates observed for obese individuals. (Abbasi et al 2002, Bray & Bellanger 2006, Cusi 2009, Flegal et al 2007, Gunnell et al 1998, Hamilton et al 2007, National Institutes of Health 1985, Peeters et al 2003, Pi-Sunyer 1993, Poirier et al 2006, van Dam et al 2006, Wyatt et al 2006).

Genetic and environmental risk factors, such as family history, age, obesity, and physical inactivity, play a critical role in the early development of diabetes (Bianco et al 2013, Fletcher et al 2002). Studies examining the maternal influence have helped to confirm the hereditary role in the diabetes pathogenesis. For example women with a positive family history for T2DM were more likely to develop gestational diabetes, confirming the inter-generative transmission of this disease (Bianco et al 2013, Bjornholt et al 2001, Bjornholt et al 2000, Carstens et al 2013, Crispim et al 2006, Erasmus et al

2001, Grill et al 1999, Plagemann et al 2002, Rodekamp et al 2005, Rosenbaum et al 2013). The precocious effects of a positive family history for T2DM on a subjects' phenotype may predispose an individual towards an increase in body weight and a tendency for obesity and visceral adiposity (Bianco et al 2013, Boyko et al 2000, Hamaguchi et al 2004, Hayashi et al 2003, Morales et al 1997, Tuomi et al 1999). In addition, a positive family history of T2DM is a significant risk factor leading to the metabolic syndrome, including the chronic conditions of insulin resistance, hypertension, and impaired glucose tolerance (Bianco et al 2013, Carstens et al 2013, Florez et al 1999, Grill et al 1999, Groop et al 1996, Morales et al 1997, Srinivasan et al 1998, Valdez 2009). Moreover, the results of a previous study (Townsend 2007) demonstrated significant insulin resistance in some, but not all, healthy normoglycemic college-age individuals with a family history of T2DM. Although these subjects had a normal response to a glucose challenge, it appears as though they achieved it by producing more insulin compared to those who were considered more insulin sensitive. Perhaps even more interesting, many of these healthy, college-aged students that had signs of insulin resistance also demonstrated significant signs of endothelial dysfunction which is considered by many as one of the earliest, measureable signs of atherogensis (Townsend 2007).

The regulation of plasma levels of low-density lipoprotein (LDL), such as its precursor of very low-density lipoprotein (VLDL), is complex in individuals with insulin resistance and T2DM (Ginsberg et al 2005). In the presence of hypertriglyceridemia, dense, cholesteryl ester-depleted, triglyceride-enriched LDL are present, and the basis for small dense LDL in insulin resistance is derived in large part from action of cholesteryl

ester transfer protein (CETP) (Ginsberg et al 2005). Small dense LDL is present in insulin resistance and T2DM patients, even though their TG levels appear normal (Ginsberg et al 2005).

While the benefits of engaging in regular physical activity are well known, the results of a recent Centers for Disease Control and Prevention (CDC) survey (Jaslow 2013) indicates that 80% of American adults do not meet the weekly-recommended amount of exercise. Not surprisingly, many of these individuals claim that they simply do not have enough time to participate in a regular exercise training program because of their busy lifestyle. However, there may be an alternative approach to exercise training, which would allow individuals to improve or maintain their level of physical fitness, while at the same time spending relatively little time actually exercising. Interval training, in one form or another, has been utilized by coaches and fitness experts for many years, but the concept of high intensity interval training (HIIT) has received considerably more attention recently. This may due, at least in part, to the reported findings of improved exercise tolerance, cardiopulmonary function, and metabolic adaptations, including significant weight loss, with as little as 10 minutes of exercise each day (Tabata 1996). According to the results of recent research, an effective HIIT program may require a participant to perform as few as 10 bouts of near-maximal effort exercise for as little as 60 seconds that are interspersed with 60 seconds of rest or recovery between each bout (Gibala et al 2012, Rakobowchuk et al 2008).

There is considerable evidence to suggest that many of the chronic diseases associated with obesity such as T2DM and CVD may be preventable if individuals were to participate in regular physical activity. If the idea is to prevent chronic diseases such

as T2DM and CVD, how HIIT is beneficial based upon a clear understanding of both the barriers (i.e. a lack of time) and underlying causative factors (i.e. genetic predisposition and lifestyle risk factors) must be established. The primary purpose of this study was to determine whether two weeks of HIIT would result in a significantly greater improvement in insulin sensitivity in healthy individuals with a family history of T2DM compared to healthy individuals without a family history of T2DM. The second purpose of this study was to characterize the relationship between insulin sensitivity and lipid profile. We hypothesized that change in insulin sensitivity in healthy individuals with a family history of T2DM would be higher than that of healthy individuals without a family history of T2DM after two weeks of HIIT. Second, we hypothesized that there would be a significant relationship between insulin sensitivity and lipid profile (a known risk factor associated with T2DM) in healthy individuals with a family history of T2DM compared to healthy individuals without a family history of T2DM.

4.2 Methods

4.2.1 *Participants*

Nineteen participants with or without a family history of T2DM between 18-55 years of age (Table 4.1 and Table 4.2) were recruited through local YMCAs, a local public school system, and through the use of on-campus poster advertisements. Individuals between the ages of 18-55 years were recruited to participate in the study. According to upon American Diabetes Association (ADA) recommendations, participants did not have T2DM, such as a fasting plasma glucose less than 100 mg/dl, and/or two hours oral glucose tolerance test blood glucose concentration less than 140

mg/dl, and/or HbA1c of less than 5.7%. Subjects had no overt signs, symptoms, or known diagnoses of any cardiovascular or pulmonary disease, end-stage liver or kidney diseases, peripheral neuropathy, retinopathy, or hypertension that could not be controlled by standard medication. Individuals with musculoskeletal injuries, currently smoked or who had quit smoking more than six months prior to their participation in the study were excluded from the participating in the study. The study protocol was approved by Institutional Review Board at the University of Toledo and was carried out in accordance with guidelines set forth in the Declaration of Helsinki.

4.2.2 *Experimental Design*

Participants reported to the Cardiopulmonary and Metabolism Research Laboratory at the University of Toledo on ten separate occasions over a four week period. Once enrolled in the study, subjects were asked to refrain from any strenuous exercise for the remainder of their participation in this study. Each participant was instructed to consume only a light meal and to abstain from vigorous exercise and caffeinated beverages for ≥ 12 hours prior to arriving for testing. The experimental design consisted of 1) medical clearance, 2) pre-testing, 3) a two-week HIIT intervention, 4) post-testing.

4.2.3 *Medical clearance*. During the first session, the experimental protocol and all known risks associated with participating in this study were discussed with each participant; each subject provided written and informed consent prior to participating in any testing. Study participants were asked to complete a medical history questionnaire that was used to assess inclusion/exclusion criteria for this study.

4.2.4 *Pre-testing*. Anthropometric data (body height, body mass, and body composition) was measured using standard approaches. Body fat (% body fat, %BF) was measured by using BodPod (COSMED USA, Chicago, IL) which utilizes an air displacement method to measure body mass and to calculate lean body mass and fat mass. An oral glucose tolerance test (OGTT) was performed during the second visit. All OGTT sessions were performed in the morning after an overnight fast. Participants ingested a 75 g glucose load over five-minute period. A venous blood sample was obtained from an antecubital vein at baseline and 120 minutes following the ingestion of the glucose challenge (Bianchi et al 2012). Participants were asked to remain in the examination and remain inactive until the second blood sample had been obtained. During the third visit, participants performed a maximal exercise test on an electromagnetically braked cycle ergometer for the determination of peak O_2 uptake (VO_2peak), which was used as an overall indication of cardiorespiratory fitness level. Pulmonary gas exchange (including O_2 uptake, CO_2 output and minute ventilation, VE) was measured breath-by-breath using a commercially available metabolic measurement system (Vmax, Sensormedics, Yorba Linda, CA). The metabolic measurement system was calibrated prior to each maximal exercise test according to the specifications provided by the manufacturer. The participant was required to breathe through a rubber mouthpiece while his or her nose was occluded with a nose clip. Electrocardiography (ECG) (Hewlett Packard, Palo Alto, CA) was recorded by placing three electrodes over specific skin surfaces on the chest to determine heart rate response to exercise. To obtain an optimal ECG signal, the chest was prepped by shaving (if necessary) any hair in the area of the electrode placement, slightly abrading with gauze and cleaning with an

alcohol wipe prior to electrode placement. All exercise tests were performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Subjects were instructed to maintain a pedal cadence of 80 rpm during each the maximal exercise test. The maximal exercise test included four minutes of warm-up with the subject pedaling at constant load exercise of 20 W to obtain baseline measures. Following the four minute lead-in, the work rate increased linearly as a ramp function at a rate of 15-25 W/min, depending upon the initial fitness level which was determined by surveying each subject on their level of engagement in regular physical activities. The exercise trial was terminated either upon request by the participant, volitional fatigue had been reached, or when the participant could no longer maintain pedal cadence above 40 revolutions per minute despite strong verbal encouragement by the investigator.

4.2.5 *High Intensity Interval Training (HIIT)*. Nineteen participants were separated into two groups; healthy individuals without a family history of T2DM and healthy individuals with a family history of T2DM. The HIIT program consisted of a low intensity warm-up followed by 10 bouts of exercise on a cycle ergometer for 60 seconds followed by 60 seconds of recovery involving pedaling against a light exercise intensity of 30 W. The participants performed the training protocol for three days a week for two weeks, similar to the protocol in several recent studies (Currie 2013, Currie et al 2013, Currie et al 2012, Gibala et al 2012, Little et al 2011). Participants started at an exercise intensity equivalent to 85% of peak work rate achieved during their initial fitness test during visit two. After the first two training sessions (visits four and five), the intensity increased to 90% in the third training session (visit six) and to 95% in the fourth training

session (visit seven) followed by 100% in the fifth and six sessions (visits eight and nine). Heart rate was monitored during each training session. In addition, the plasma glucose was measured before and after each training session of HIIT using a glucose meter (HOMEdiagnostics, Fort Lauderdale, FL). Subjects were asked to record their meals and snacks in a food diary over the two week training session from which kilocalorie equivalent, carbohydrate, fat, protein, sugar, and sodium were determined using readily available software (www.myfitnesspal.com).

4.2.6 *Post-testing*. Following completion of the HIIT program, (visit ten), all of the measurements and tests performed during visit one (body mass and %BF) and visit two (OGTT) were repeated during this session within 48 hour following the last training session.

4.2.7 *Venous Blood Sample*

Venous blood samples for the measurement of plasma glucose concentration ([glucose]), A1c, and plasma concentrations of lipids were collected into syringes containing heparin via venipuncture of an antecubital vein. Plasma [glucose] was measured using a glucose meter (HOMEdiagnostics, Fort Lauderdale, FL), A1c was measured by A1c Now+ (Polymer Technology Systems, Inc., Indianapolis, IN), and lipid profile, including high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, and cholesterol, were measured using CardioCheck® Brand Analyzers (Polymer Technology Systems, Inc., Indianapolis, IN) immediately after the samples were obtained. The remaining blood samples were centrifuged at 1,500 g for 7 minutes,

and the plasma was stored at -80°C until insulin was analyzed at a later time (EMD Millipore Corporation, Billerica, MA).

4.2.8 *Insulin Analysis*

On the first day, six glass tubes were labeled and 1.0 mL assay buffer was added to each of six tubes in order to prepare the standard. A serial dilution was performed by adding 1.0 mL of the 200 µU/mL standard to tube 1, mixed well and transferred 1.0 mL of tube 1 to tube 2, and continued to use the same mixture for subsequence tube 2 to tube 6. After serial dilution was completed, 300 µL of assay buffer was added to the non-specific binding tubes (3-4), 200 µL to reference tubes (5-6), and 100 µL to tubes 7 through the end of the assay. Second, 100 µL of standards and quality controls in duplicate were pipetted. Third, 100 µL of each sample was prepared in duplicate. Fourth, 100 µL of hydrated ^{125}I-Insulin was added to all tubes followed by the addition of 100 µL of human insulin antibody to all tubes, except for tubes (1-2) and non-specific binding tubes (3-4). Finally, samples were vortexed, covered, and allowed to incubate overnight (20-24 hours) at room temperature (22-25 °C). On the second day, 1.0 mL of cold (4 °C) precipitating reagent was added to all tubes, vortexed and incubated for 20 minutes at 4 °C prior to centrifuge all tubes at 4 °C. After these steps, the tubes were decanted and the supernatant of all tubes, with the exception of total count tubes (1-2), were drained for 15 to 60 seconds, blotted for excess liquid from the lip of tubes. In the last step, all tubes in a gamma counter for one minute were counted, and the uU/mL of human insulin in the unknown samples was calculated using automated data reduction procedures.

4.2.9 *Insulin Sensitivity*

Insulin sensitivity was measured according to previously established methods (Gutt et al 2000) using the equation; $ISI_{0, 120}$ = MCR/log MSI = m/MPG/log MSI was used, including m (mg/min) = (75,000 mg + (0 min glucose value – 120 min glucose value) x 0.19 x body weight)/120 min, MCR (metabolic clearance rate) = m/MPG (mean plasma glucose), and MSI (mean serum insulin, mU/l). If 0 min plasma glucose value is higher than that of 120 min plasma glucose value, $ISI_{0, 120}$ will be increased. If 0 min and 120 min serum insulin values are increased, $ISI_{0, 120}$ will be lower.

4.2.10 *Statistical Analyses*

Differences between pre- and post-experimental values were analyzed using either an analyses of variance (ANOVA) with two repeated measures such as fasting plasma glucose, A1c, 2-hour plasma glucose, lipid profile, insulin sensitivity in OGTT compared healthy individuals without a family history of T2DM to healthy individuals with a family history of T2DM. Differences between pre- and post-intervention values were also analyzed using either an analyses of variance (ANOVA) with two repeated measures such as plasma glucose in each training session compared healthy individuals without a family history of T2DM to healthy individuals with a family history of T2DM. In addition, the relationship between insulin sensitivity and lipid profile was analyzed using multiple linear regression in two groups separately. All data are presented as the group mean ± SEM. Statistical significance was set *a priori* $P \leq 0.05$. All statistical analyses were performed using Sigma Stat 3.0 (Systat Software, San Jose, CA).

4.3 Results

4.3.1 *Descriptive Characteristics of Participants*

Participants did not change their weights and body fat percent over two weeks of high intensity interval training (HIIT) in healthy individuals without a family history of T2DM and healthy individuals with a family history of T2DM groups (Table 4.1). Progressive ramp exercise shows that the peak oxygen uptake among healthy individuals without a family history of T2DM was higher than that of healthy individuals with a family history of T2DM (Table 4.2). Oral glucose tolerance test and lipid profile show in Table 4.3 and Table 4.4). Subjects' peak heart rate during each training session reached over 95% of their maximal heart rate from progressive ramp exercise (Table 4.5). In addition, nutritional assessment was performed. The consumption of calories, carbohydrates, fat, protein, sugar, and sodium in each session of 3-meal prior, 2-meal prior, and 1-meal prior was not statistically significant in both groups ($P > 0.05$), besides carbohydrate 1-meal prior in sessions ($P < 0.05$) (Figure 4-1). However, %BF was significantly different in pre-test ($P < 0.05$). A maximal exercise test, including time to exhaustion, peak watts, and peak heart rate did not show any significant difference between both groups ($P > 0.05$). Peak oxygen uptake in pre-test was statistically significant between two groups (H-H > H-FHx) ($P < 0.05$).

4.3.2 *Oral Glucose Tolerance Test (OGTT) and Insulin Sensitivity*

Insulin sensitivity did not show any significance between groups either before or after over two weeks of HIIT ($P > 0.05$) (Figure 4-2). An OGTT was administered again after over two weeks of HIIT; the results indicated that there was no change in plasma

[glucose] in healthy individuals without a family history of T2DM or in healthy individuals with a family history of T2DM (Figure 4-3). Since there was no difference between groups, an independent t-test was performed that included all 19 participants to investigate the effectiveness of two weeks of HIIT in insulin sensitivity. However, the results of this analysis did not show any statistically significance ($P > 0.05$). In addition, power analysis was performed, and it was less than 0.80 for group (healthy individuals with a family history of T2DM and healthy individuals without a family history of T2DM) and time (pre-training and post-training). In addition, A1c (Figure 4-4) did not change after over 2 week of HIIT in both groups ($P > 0.05$). Insulin concentration showed statistically significance between pre- and post- tests in two groups ($P < 0.05$), but there was no interaction between groups ($P > 0.05$) (Figure 4-5). Similarly, glucose insulin index (= [glucose]/[insulin]) was significantly different between pre- and post-tests in two groups ($P < 0.05$), but there was no interaction between groups ($P > 0.05$) (Figure 4-6).

4.3.3 *Lipid Profile*

High density lipoprotein (HDL) was not significantly different between groups either pre or post over two weeks of HIIT ($P > 0.05$). Other variables, such as low density lipoprotein (LDL), triglycerides, and total cholesterol did not improve before and after over two weeks of HIIT in both groups ($P > 0.05$).

4.3.4 *Relationship between Insulin Sensitivity and Lipid Profile*

Overweight and obesity affects insulin sensitivity and lipid profile that potentially plays an important role in causing metabolic syndrome. Reduced insulin sensitivity

causes pre-diabetes and T2DM, if it is not managed properly. Increased LDL, triglyceride, and total cholesterol effects cardiovascular diseases with decreased HDL. T2DM and cardiovascular diseases are associated with endothelial dysfunction. Thus, insulin sensitivity and lipid profile need to have a link to some extent. In the current study, the results of the multiple regression analysis indicated that insulin sensitivity was predicted from lipid profile, such as a linear combination of HDL and LDL, in the group that had a family history of T2DM, [ISI=54.678 – (0.901*HDL) + (0.507 *LDL) + (0.109*Triglyceride) $R^2 = 0.755$, $P < 0.05$]. However, any other variables of lipid profile in healthy individuals without a family history of T2DM did not predict insulin sensitivity ($P > 0.05$).

Table 4.1. Subject Demographics

Group	H-H Pre (N=9)	H-H Post (N=9)	H-FHx Pre (N=10)	H-FHx Post (N=10)
Age (y/o)	30.0 ± 3.1	30.0 ± 3.1	33.2 ± 3.6	33.2 ± 3.6
Height (cm)	169.2 ± 9.5	169.2 ± 9.5	170.7 ± 1.8	170.7 ± 1.8
Weight (kg)	72.9 ± 5.3	72.6 ± 5.1	79.2 ± 5.5	79.1 ± 5.6
BMI (kg/m^2)	25.5 ± 1.9	25.4 ± 1.8	27.2 ± 1.8	27.1 ± 1.8
Body Fat Percent (%)	21.8 ± 2.9*	21.8 ± 2.8	31.3 ± 3.4	30.6 ± 3.5

Note:
H-H: Healthy Individuals without a Family History of Type 2 Diabetes Mellitus
H-FHx: Healthy Individuals with a Family History of Type 2 Diabetes Mellitus
Mean ± SEM
*, Significant difference between H-H and H-FHx within Pre-Test ($P < 0.05$)

Table 4.2. Progressive Ramp Exercise

Group	H-H Pre (N=9)	H-FHx Pre (N=10)
Time to Exhaustion (s)	839 ± 41.1	762.7 ± 22.7
Peak Watts	234 ± 23.6	194.1 ± 7.6
Peak Heart Rate (bpm)	177.6 ± 4.3	176.6 ± 3.8
Peak Oxygen Uptake (L/min)	3.0 ± 0.3	2.5 ± 0.1
Peak Oxygen Uptake (ml/kg/min)	42.0 ± 4.6*	31.9 ± 1.9

Note:
H-H: Healthy Individuals without a Family History of Type 2 Diabetes Mellitus
H-FHx: Healthy Individuals with a Family History of Type 2 Diabetes Mellitus
Mean ± SEM
*, Significant difference between H-H and H-FHx within Pre-Test ($P < 0.05$)

Table 4.3. Oral Glucose Tolerance Test

Group	H-H Pre (N=9)	H-H Post (N=9)	H-FHx Pre (N=10)	H-FHx Post (N=10)
OGTT (mg/dL) - Fasting Glucose	80.7 ± 2.7	82.6 ± 2.4	77.4 ± 3.1	79.4 ± 2.5
OGTT (mg/dL) - 2 Hours Glucose	72.7 ± 6.9	70.0 ± 5.8	71.7 ± 7.5	73.1 ± 5.6
A1c (%)	5.0 ± 0.3	5.2 ± 0.3	5.3 ± 0.1	5.2 ± 0.1
OGTT (μU/ml) - Insulin Baseline	15.3 ± 1.8	18.7 ± 3.7	21.1 ± 3.0	19.0 ± 2.6
OGTT (μU/ml) - Insulin - 2 Hours	75.7 ± 26.9	66.4 ± 20.9	70.5 ± 26.4	51.4 ± 12.0
ISI	56.9 ± 5.8	55.4 ± 4.7	58.6 ± 6.4	58.2 ± 5.5

Note:
H-H: Healthy Individuals without a Family History of Type 2 Diabetes Mellitus
H-FHx: Healthy Individuals with a Family History of Type 2 Diabetes Mellitus
Mean ± SEM

Table 4.4. Lipid Profile

Group	H-H Pre (N=9)	H-H Post (N=9)	H-FHx Pre (N=10)	H-FHx Post (N=10)
HDL (mg/dL)	65.6 ± 6.4	58.6 ± 6.3	58.6 ± 6.3	53.8 ± 4.8
LDL (mg/dL)	66.0 ± 9.4	78.6 ± 13.0	78.6 ± 13.0	78.4 ± 9.3
Triglyceride (mg/dL)	68.8 ± 3.5	72.7 ± 7.6	72.7 ± 7.6	81.6 ± 10.9
Choresterol (mg/dL)	141.6 ± 5.8	146.8 ± 11.4	146.8 ± 11.4	150.0 ± 11.9

Note:
H-H: Healthy Individuals without a Family History of Type 2 Diabetes Mellitus
H-FHx: Healthy Individuals with a Family History of Type 2 Diabetes Mellitus
Mean ± SEM

Table 4.5. Percentage (%) of Max Heart Rate

Group	**H-H** (N=9)	**H-FHx** (N=10)
Session 1	96.4 ± 2.6	95.9 ± 1.2
Session 2	98.5 ± 2.8	97.2 ± 1.0
Session 3	98.9 ± 2.0	96.9 ± 0.9
Session 4	98.1 ± 2.6	96.2 ± 1.4
Session 5	98.9 ± 1.8	98.4 ± 1.6
Session 6	100.2 ± 1.9	98.0 ± 0.9

Note:
H-H: Healthy Individuals without a Family History of Type 2 Diabetes Mellitus
H-FHx: Healthy Individuals with a Family History of Type 2 Diabetes Mellitus
Mean ± SEM

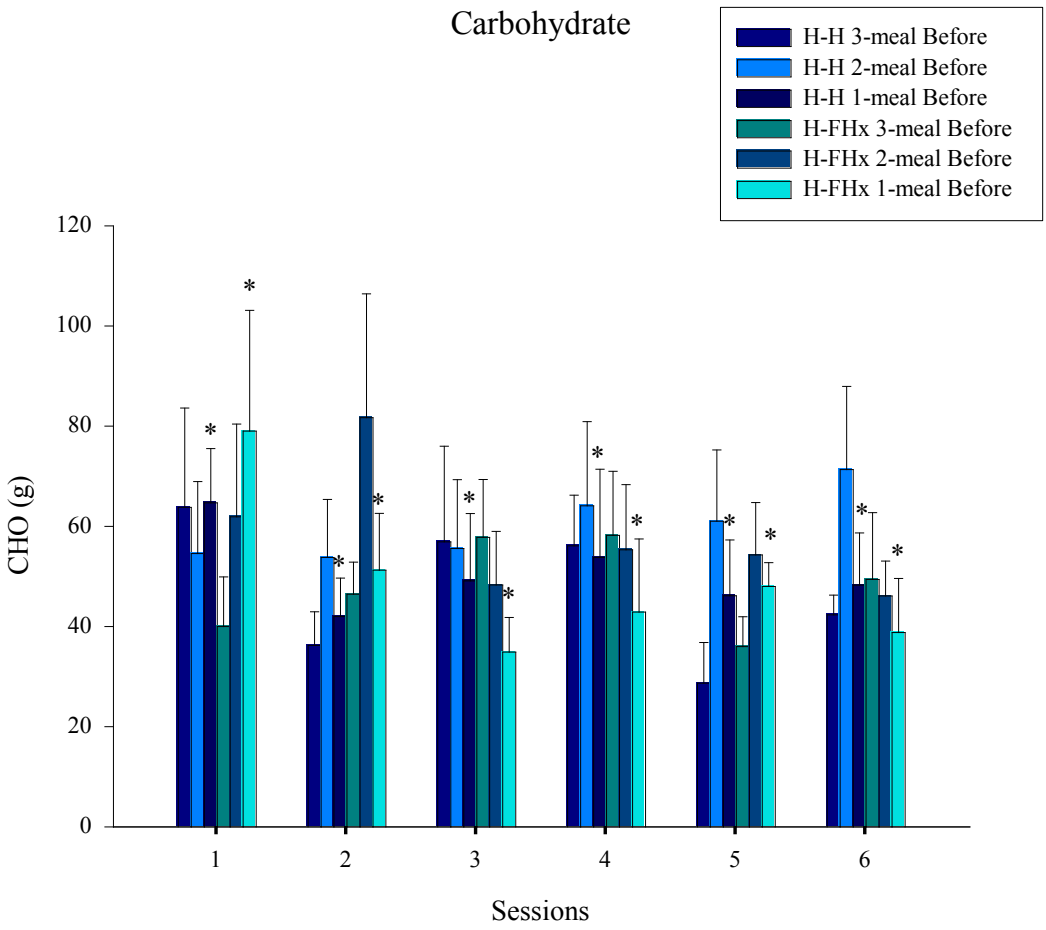

Figure 4-1. The carbohydrate consumption was assessed 3-meal, 2-meal, and 1-meal before each training session during two weeks of high intensity interval training (HIIT) in H-H (N =9) and H-FHx (N = 10).
No significant difference between and groups ($P > 0.05$).
*, Significant difference between sessions ($P < 0.05$).
Data was represented by mean ± SEM.
H-H: Healthy individuals without a family history of type 2 diabetes mellitus.
H-FHx: Healthy Individuals with a family history of type 2 diabetes mellitus.

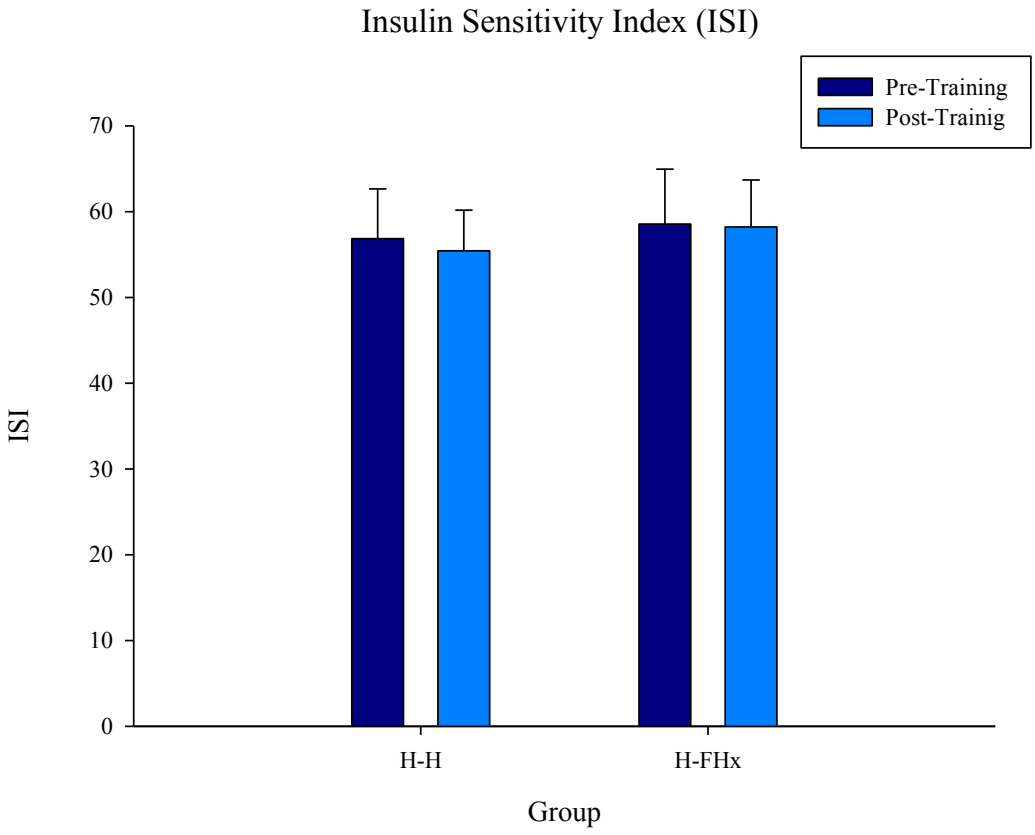

Figure 4-2. H-H (N = 9) and H-FHx (N = 10) of Insulin Sensitivity Index (ISI) were assessed before and after two weeks of high intensity interval training (HIIT).
No significant difference between pre- and post-training in H-H ($P > 0.05$).
No significant difference between pre- and post-training in H-FHx ($P > 0.05$).
No significant difference between H-H and H-FHx ($P > 0.05$).
Data was represented by mean ± SEM.
H-H: Healthy individuals without a family history of type 2 diabetes mellitus.
H-FHx: Healthy Individuals with a family history of type 2 diabetes mellitus.

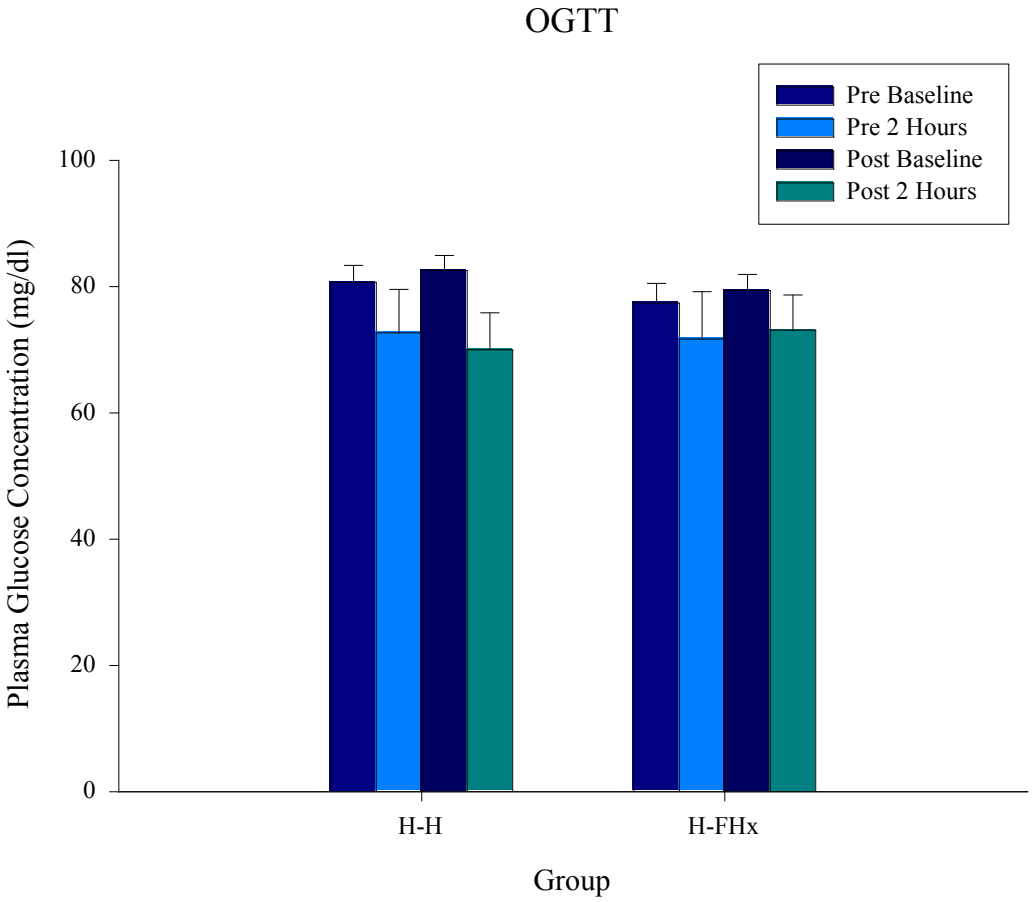

Figure 4-3. H-H (N = 9) and H-FHx (N = 10) of plasma glucose concentration in OGTT were assessed before and after two weeks of high intensity interval training (HIIT).
No significant difference between pre- and post-training in H-H ($P > 0.05$).
No significant difference between pre- and post-training in H-FHx ($P > 0.05$).
No significant difference between H-H and H-FHx ($P > 0.05$).
Data was represented by mean ± SEM.
OGTT: Oral Glucose Tolerance Test.
H-H: Healthy individuals without a family history of type 2 diabetes mellitus.
H-FHx: Healthy Individuals with a family history of type 2 diabetes mellitus.

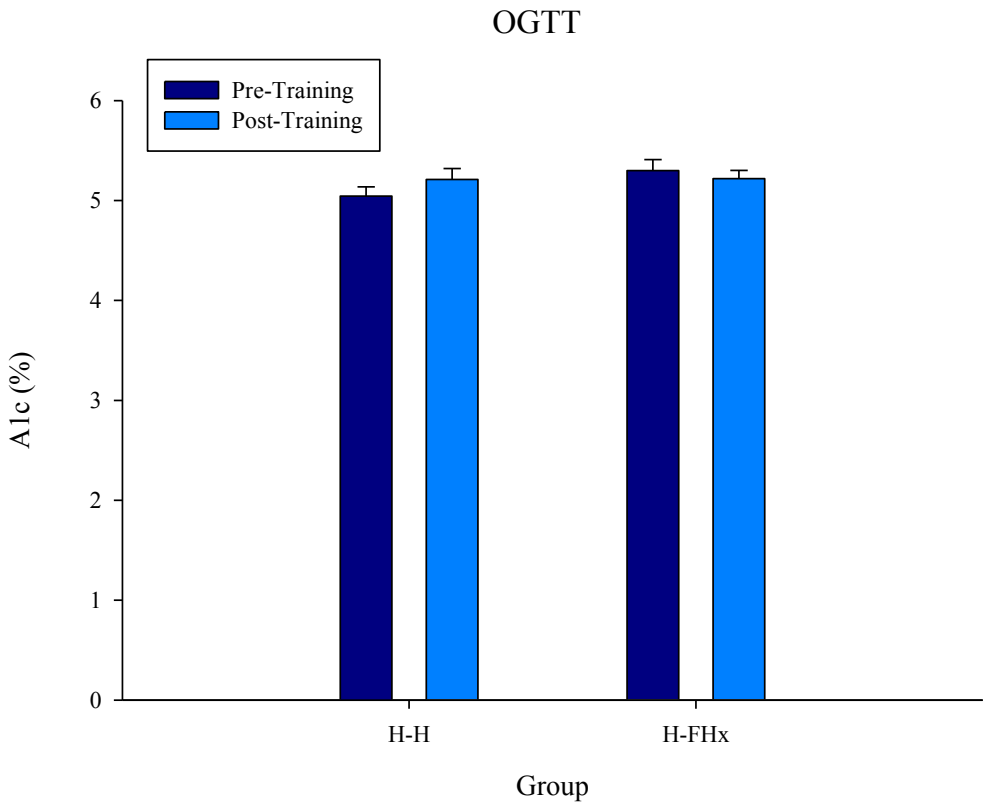

Figure 4-4. H-H (N = 9) and H-FHx (N = 10) of A1c Hemoglobin in OGTT were assessed before and after two weeks of high intensity interval training.
No significant difference between pre- and post-training in H-H ($P > 0.05$).
No significant difference between pre- and post-training in H-FHx ($P > 0.05$).
No significant difference between H-H and H-FHx ($P > 0.05$).
Data was represented by mean ± SEM.
OGTT: Oral Glucose Tolerance Test.
H-H: Healthy individuals without a family history of type 2 diabetes mellitus.
H-FHx: Healthy Individuals with a family history of type 2 diabetes mellitus.

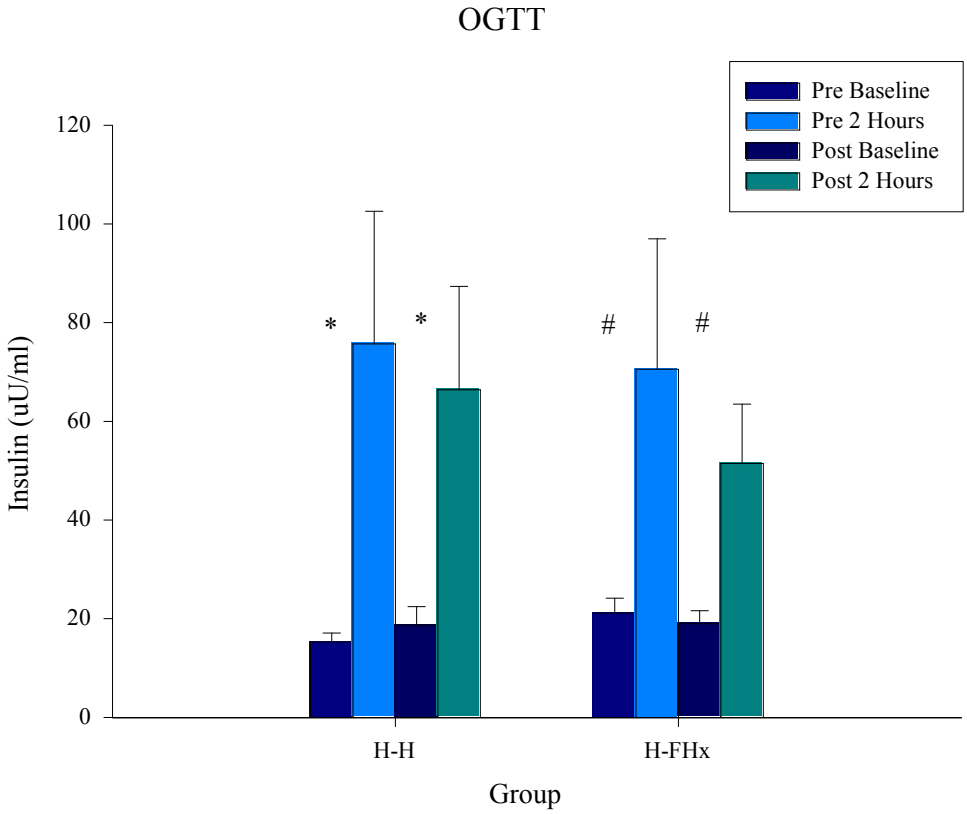

Figure 4-5. H-H (N = 9) and H-FHx (N = 10) of insulin concentration in OGTT were assessed before and after two weeks of high intensity interval training.
*, significant difference between baseline and 2-hour insulin concentration before two weeks of high intensity interval training (HIIT) in both groups ($P < 0.05$).
#, significant difference between baseline and 2-hour insulin concentration after two weeks of high intensity interval training (HIIT) in both groups ($P < 0.05$).
No significant difference between H-H and H-FHx ($P > 0.05$).
Data was represented by mean ± SEM.
OGTT: Oral Glucose Tolerance Test.
H-H: Healthy individuals without a family history of type 2 diabetes mellitus.
H-FHx: Healthy Individuals with a family history of type 2 diabetes mellitus.

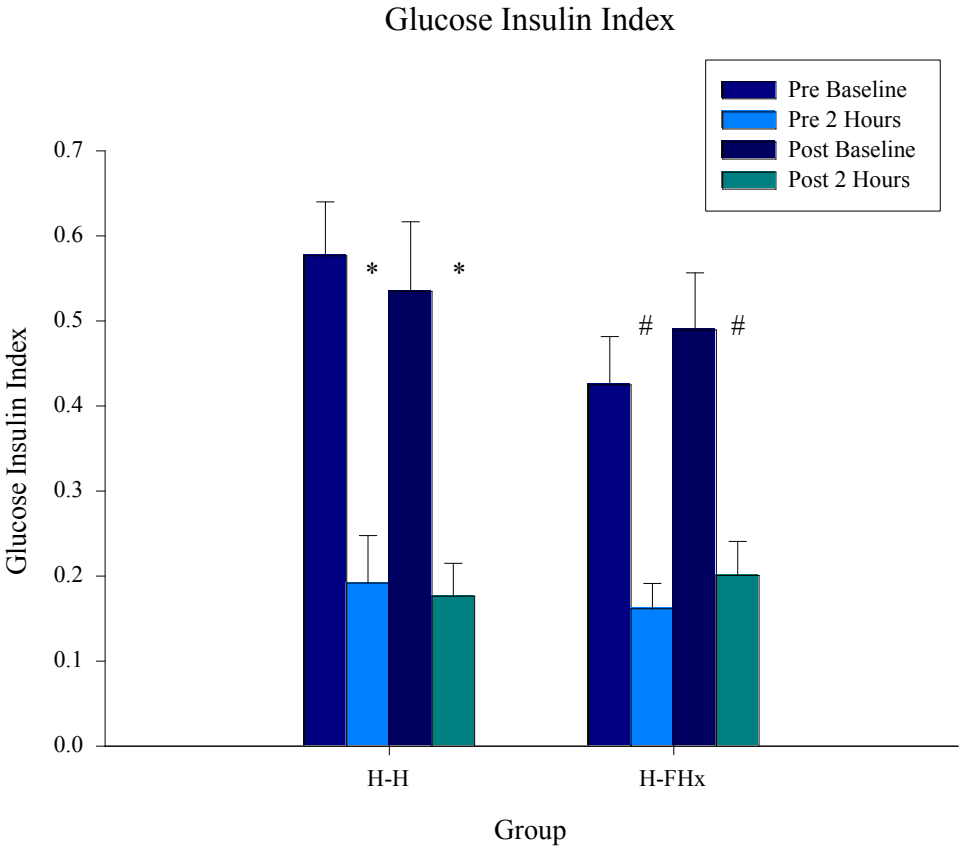

Figure 4-6. H-H (N = 9) and H-FHx (N = 10) of glucose insulin index in OGTT were assessed before and after two weeks of high intensity interval training.
*, significant difference between baseline and 2-hour glucose insulin index before two weeks of high intensity interval training (HIIT) in both groups ($P < 0.05$).
#, significant difference between glucose insulin index after two weeks of high intensity interval training (HIIT) in both groups ($P < 0.05$).
No significant difference between H-H and H-FHx ($P > 0.05$).
Data was represented by mean ± SEM.
OGTT: Oral Glucose Tolerance Test.
H-H: Healthy individuals without a family history of type 2 diabetes mellitus.
H-FHx: Healthy Individuals with a family history of type 2 diabetes mellitus.

4.4 Discussion

The results of the present study demonstrated that healthy individuals both with and without a family history of T2DM did not improve insulin sensitivity following two weeks of HIIT. The significance of examining healthy individuals with a family history of T2DM is that it is becoming increasingly clear that metabolic and physiological changes associated with the onset of diabetes may occur very early in the progression of the disease, prior to the appearance of symptoms and may not necessarily be appear as impaired glucose regulation (Nigro et al 2006, Townsend 2007). This association is corroborated by the findings that insulin resistance in young adults is associated with cardiovascular disease risk factors in a very predictable manner (Andersen et al 2006, Townsend 2007). It may be that the progression of cardiovascular complications and "silent" atherosclerosis hides behind a façade of good health, especially in young adults who are typically asymptomatic (Andersen et al 2006, Townsend 2007). In fact, insulin resistance can be detected in young adults who appear to be non-diabetic and considered to be in good health but have one or more parents with T2DM (Straczkowski et al 2003). In addition, at the microvascular level, an impaired conduit vessel response to shear stress stimuli has been observed in college-aged participants with a family history of T2DM, in spite of having normal oral glucose tolerance test response (Townsend 2007). The benefits of regular physical activity for individuals with or without T2DM are well established in the literature. Since HIIT can be used as an effective alternative to traditional endurance training, resulting in similar or even better changes in a range of physiological, performance, and health-related biomarkers (Gibala et al 2012, Hwang et

al 2011, Tjonna et al 2009, Wisloff et al 2007), the present investigation sought to examine the impact of HIIT on healthy individuals with a family history of T2DM.

4.4.1 *A Family History of T2DM and Insulin Sensitivity*

The results of a previous investigation (Townsend 2007) indicated that college-aged healthy individuals with a family history of T2DM demonstrated signs of insulin resistance, in spite of the observation that response to the OGTT was normal. Further analysis of these findings indicated that the subjects with a family history of T2DM typically had a higher insulin response in response to the glucose challenge. Furthermore, these apparently healthy college age students also had signs of endothelial dysfunction consistent with early signs of atherogenesis (Townsend 2007). Thus, we hypothesized that healthy individuals with a family history of T2DM would have improved insulin sensitivity greater than that of healthy individuals without a family history of T2DM due to improvement of fasting glucose and 2-hour plasma glucose of an oral glucose tolerance test (OGTT). However, the current study did not show that fasting glucose and 2-hour plasma glucose improved over two weeks of HIIT, although there are several other studies that showed significant differences (Babraj et al 2009, Burgomaster et al 2007, Burgomaster et al 2006, Burgomaster et al 2008, Burgomaster et al 2005, Gibala et al 2006, Heilbronn et al 2007, Hughes et al 1993, Rakobowchuk et al 2008, Richards et al 2010, Simoneau et al 1995). Interestingly, the findings of another study (Whyte et al 2010) observed that the change to insulin sensitivity was lost after 72 hours post-intervention assessment (Richards et al 2010), which appears to be a common finding with many exercise interventions that have reported relatively transient improvements in insulin sensitivity (Boule et al 2005, Burstein et al 1985). In the present study, the OGTT

was conducted within 48 hours of completion of the last training session. Additional sampling such as 30 minutes, 60 minutes, and 90 minutes to examine the change in insulin sensitivity following HIIT may prove beneficial, instead of only baseline and 2 hours plasma glucose concentration in OGTT.

In the current study, VO_{2peak} in healthy individuals without a family history of T2DM was 42.0 ± 4.6 ml/kg/min, so our participants (30 ± 3.1 years old) were fairly active prior to over two weeks of HIIT, and their regimen was potentially not long enough to improve insulin sensitivity. Body fat percentage of our participants was 21.8 ± 2.9%. In contrast, VO_{2peak} with healthy individuals with a family history of T2DM was 31.9 ± 1.9 ml/kg/min. Previous research (Hood et al 2011) found GLUT 4 protein content increased ~260% and insulin sensitivity improved by ~35% in participants with VO_{2peak} of 30.0 ml/kg/min. However, their age was 45 years old and their body mass index (BMI) was 27 kg/m^2, while our participants were 33.2 ± 3.6 years old with 27.2 ± 1.8 kg/m^2 in a family history of T2DM. Potentially, individual's fitness level, body composition (%BF), and/or age may play an important role in determining insulin sensitivity.

A key physiological mechanism in linking obesity with T2DM is that increased general and abdominal obesity is strongly correlated with insulin resistance, that represents the factor leading to T2DM (Stewart 2009). Consequently, there is a gradual rise in insulin production that eventually cannot compensate for increasing levels of insulin resistance. Insulin resistance can cause a complete halt in the ability to produce insulin among patients who do not exercise or lose weight to prevent their insulin resistance (Stewart 2009). The present study did not show any significant differences in

body fat percentage before and after over two weeks of HIIT in both groups, so it did not contribute to improvement of insulin sensitivity. Nonetheless, a family history of T2DM did not show any differences in insulin sensitivity.

4.4.2 *A Family History of T2DM: Relationship between Insulin Sensitivity and Lipid Profile*

We hypothesized that the significant relationship between insulin sensitivity and lipid profile in healthy individuals with a family history of T2DM compared to healthy individuals without a family history of T2DM. We found that the linear combination of HDL and LDL variables predicts insulin sensitivity in a family history of T2DM group, but there was no significant relationship between insulin sensitivity and lipid profile in healthy individuals without a family history of T2DM. It is a new finding on effects of HIIT in healthy and diseased populations.

Insulin resistance is a central pathogenic factor for the metabolic syndrome and is related to both generalized obesity and the accumulation of fat in the omental and intramyocellular compartments (Esler et al 2001, Stewart 2009). The accumulation of intramyocellular lipids may be due to reduced lipid oxidation capacity (Esler 2000, Stewart 2009). The triglyceride (TG) in low density lipoprotein (LDL) can be lipolyzed by lipoprotein lipase (LPL) or hepatic lipase (HL), generating small, dense LDL, which is present in insulin resistance and T2DM patients, even though their TG levels are normal (Ginsberg et al 2005). In addition, a previous study (Steinberger et al 1995) indicated that the abnormal glucose uptake correlated with an abnormal lipid profile in obese individuals. There are at least two explanations for the observed relationship between insulin resistance and lipid abnormalities: 1) insulin resistance and lipid abnormalities are

two distinct abnormalities that co-exist in many obese adolescents but are not mechanistically related or 2) insulin resistance is directly related to the lipid abnormalities observed in obese adolescents. Therefore, researchers concluded not only that obese adolescents have lipid abnormalities (elevated serum LDL and triglycerides, and reduced HDL), but also these lipid abnormalities correlate with the degree of insulin resistance (Steinberger et al 1995). In the present study, healthy individuals with a family history of T2DM show the important relationship between insulin sensitivity, LDL, and HDL in order to prevent chronic diseases in the future, although our participants were overweight individuals based upon BMI.

The current study did not show any significant differences in lipid profile either before or after two weeks of HIIT between groups. To date, there is limited data examining the change in lipid profile following a HIIT program (Kessler et al 2012), including TG responses following aerobic interval training (Ciolac et al 2010, Moholdt et al 2009, Schjerve et al 2008, Thomas et al 1984, Tjonna et al 2008, Tjonna et al 2009, Tsekouras et al 2008, Wallman et al 2009, Wisloff et al 2007). There were no studies that evaluated LDL reported changes in response to either aerobic interval training or moderate intensity exercise (Ciolac et al 2010, Kessler et al 2012, Moholdt et al 2009, Nybo et al 2010, Wallman et al 2009). A previous study (Nybo et al 2010) showed a decrease in the TC:HDL ratio in the moderate intensity exercise group only because of an index reflecting a relative improvement in HDL, but it was not in the high intensity exercise group (Kessler et al 2012).

4.5 Limitations

There are a number of limitations in the present study. First, the participants in a healthy, no family history of T2DM were considerably more active, based upon VO_{2peak} and percent body fat, than those subjects who were included in the family history of T2DM group, Therefore, the fact that relatively healthy individuals were recruited to participate in this study, the potential to observe differences between groups may have been reduced. While the subjects were asked to maintain a food diary, the comparisons were made on those days when they exercised in the laboratory and consumption of alcohol was not considered. Therefore, between inconsistencies in the reporting of food diaries along with not knowing alcohol consumption, the effect of these factors on the observed responses in plasma [glucose] both during training and the OGTT test cannot be adequately addressed. OGTT will be performed within 24 hours instead of 48 hours, so plasma [glucose] lowers than that of 48 hours based upon a previous study (Little et al 2011). In an effort to avoid diurnal variations which may affect plasma [glucose], subjects were asked to perform the testing and training sessions at the same time of the but this was not always possible given student schedules and obligations. In addition, sleeping patterns were not recorded in this study which may also lead to variations in plasma [glucose] throughout the day (add reference here). A post-training maximal VO_2 test was not performed, so there is no objective measurement whether each participant improved their cardiovascular fitness level over two weeks of HIIT. However, the results of studies using similar training programs have found either improved or no difference in VO_{2pk} making any conclusions based only on this variable suspect to other possibilities including the familiarity of testing or other conditions.

4.6 Conclusions

The results of a previous study (Townsend 2007) demonstrated that college-aged healthy individuals with a family history of T2DM develop early endothelial dysfunction, although their responses to an OGTT are quite normal. However, due to their insulin resistance, their insulin sensitivity was lower and insulin production higher compared to those with healthy individuals without a family history of T2DM. If these individuals do not engage in regular physical activity along with making changes to improve their diets, they will progress towards becoming pre-diabetics, type 2 diabetics, and in all likelihood, cardiovascular disease will ensue. The results of the current investigation indicated that healthy individuals with a family history of T2DM did not have any difference in their insulin sensitivity (or plasma [glucose]) compared to healthy individuals without a family history of T2DM. Insulin sensitivity did not change either pre- to post-training over two weeks of HIIT between the groups. The relationship between insulin sensitivity and lipid profile was not statistically significant those individuals without a family history of T2DM however, multiple regression analyses indicated that HDL and LDL predicts insulin sensitivity in healthy individuals with a family history of T2DM group. Further investigations into the role that body composition (%BF), lipid profile and cardiovascular fitness on insulin sensitivity, insulin resistance and glucose tolerance in relatively healthy individuals with a family history of T2DM are warranted.

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Chapter 5

Concluding Remarks

5.1 Summary

There is a growing body of evidence demonstrating that HIIT can be considered as and effective, efficient and safe alternative to traditional endurance training, (Currie et al 2013, Currie et al 2012, Gibala et al 2012, Gillen et al 2012, Hwang et al 2011, Little et al 2011, Tjonna et al 2009, Tjonna et al 2008, Wisloff et al 2007).

In the first study, only two variables, such as time to exhaustion and peak watts, which were statistically significant during the progressive ramp exercise both in over two weeks of HIIT with L-arginine and over two weeks of HIIT groups. HIIT with L-arginine did not help improve peak oxygen uptake, deoxygenation in a microvasculature level, and FMD. Potentially, healthy individuals without a family history of T2DM may not need extra L-arginine in order to improve cardiovascular function.

In the second study, a family history of T2DM was not associated with a difference of insulin sensitivity after two weeks of HIIT compared to healthy individuals without a family history of T2DM. Insulin sensitivity did not change between groups either before or after over two weeks of HIIT. Thus, no benefits of HIIT were observed. No difference in A1c was observed between groups or either pre or post over two weeks

of HIIT as expected given the relative short duration of the study. The relationship between insulin sensitivity and lipid profile was not statistically significant in healthy individuals without a family history of T2DM, although multiple regression analysis indicated HDL and LDL variables predicted insulin sensitivity in a family history of T2DM group.

5.2 Future Direction

Previously, the marked improvements in cardiovascular fitness, glucose tolerance, and exercise endurance as well as the lowering of systolic blood pressure put emphasis on the potential benefits of high intensity training and its ability to improve certain physiological health parameters (Nybo et al 2010). However, the intense low-volume training regimen had limitations, and it was less effective than prolonged training for treating hyperlipidemia and obesity (Nybo et al 2010). Moreover, 12 weeks of high intensity training had no impact on muscle mass or leg bone mass, while strength training also provided a significant osteogenic stimulus besides increasing the muscle mass that may have both acute and prolonged effects for musculoskeletal health (Nybo et al 2010). Thus, we do not know how much more effective it will be for long-term health using high intensity training, since we used only two weeks of HIIT for our studies.

Here are several considerations for future studies. First, it is necessary to control food and alcohol consumptions, because plasma glucose concentration changes very frequently. Second, oral glucose tolerance test (OGTT) needs to be performed within 24 hours after the last session of HIIT to capture training effect on insulin sensitivity. It is also better to record multiple samples such as 30 minutes, 60 minutes, and 90 minutes during OGTT. Third, it is always challenging to recruit participants, which is why future

studies could rely on physicians that will help refer obese and pre-diabetes individuals that potentially prevent chronic diseases, such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Fourth, if HIIT is used to prevent from chronic diseases, pre-diabetes individuals will be likely to respond differently compared to healthy individuals. Fifth, the perceived barriers to exercise in obese and pre-diabetes individuals need to be investigated because of difficulties to recruit participants to exercise study. Sixth, it is important to examine psychological responses based upon improvement in physiological responses before and after two weeks of HIIT. It has been shown that feeling well-being is improved. Seventh, we consider metabolic syndrome.

The International Diabetes Federation has developed a new definition based upon the World Health Organization and the National Cholesterol Education Program's Adult Treatment Panel III in 2005. The new definition highlighted both central obesity and insulin resistance (Brennan et al 2009). The metabolic syndrome/insulin-resistance syndrome was defined as central obesity plus two or more of the following four factors that must be present such as A) Raised concentrations of triglycerides: 150 mg/dl (1.7 mmol/l) or specific treatment for this lipid abnormality, B) Reduced concentration of high-density lipoprotein-c (HDL-cholesterol): <40 mg/dl (1.03 mmol/l) in men and <50 mg/dl (1.29 mmol/l) in women or specific treatment for this lipid abnormality, C) Raised blood pressure: systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or treatment of previously diagnosed hypertension, D) Raised fasting plasma glucose concentration ≥100 mg/dl (5.6 mmol/l) or previously diagnosed type 2 diabetes (Brennan et al 2009, IDF 2005). Since metabolic syndrome includes developing insulin resistance, HIIT might affect changes in insulin sensitivity over two weeks of HIIT.

Eighth, our studies did not examine potential differences between genders, but it will be interested to investigate women that tend to be more fatigue resistance and potentially they respond differently over two weeks of HIIT compared to men. Ninth, in terms of device, continuous glucose monitor will be helpful to record plasma glucose concentration before, during, and after high intensity interval training (HIIT).

5.2.1 *Continuous Glucose Monitor (CGM)*

Glycemic control in individuals with diabetes is important to avoid long-term complications of hyperglycemia, such as cardiovascular disease, retinopathy, neuropathy, and acute adverse events related to hypoglycemia, that may cause seizures, coma, and death (Lane et al 2013). A continuous glucose monitor (CGM) typically consists of a sensor, a wireless transmitter, and a receiver that allows a user to follow his or her interstitial glucose measurements either retrospectively or in real time based upon the system and the settings (Lane et al 2013). Currently, the sensor is electrochemical and consists of a wire inserted subcutaneously into the back, buttocks, or most commonly abdomen. The receiver may be a dedicated handheld device or an insulin pump designed to integrate with CGM, such as a sensor augmented pump (Lane et al 2013). It also alerts the user, when glucose levels are (or are predicted to soon be) below or above a certain glycemic range (Lane et al 2013). Compared with traditional self-monitoring of blood glucose (SMBG) alone, CGM use has been shown to improve glycemic control in measuring the HbA1c levels and the time spent in hypoglycemia (Lane et al 2013, Pickup et al 2011). Previous study (Vigersky et al 2012) showed that subjects with T2DM not on prandial insulin, who used real-time CGM (RT-CGM) intermittently for 12 weeks, significantly improved glycemic control at 12 weeks and sustained the improvement

without RT-CGM during the 40-week follow-up period, compared with those who used only SMBG.

The fear of hypoglycemia along with the consequences associated with very low blood glucose levels has been identified as the greatest barrier to exercise in people with T1DM (Brazeau et al 2008), and it is likely that the same holds true for insulin and secretagogue dependent individuals with T2DM (Younk et al 2011). Thus, measurement of blood glucose levels before, during, immediately, and several hours after exercise is necessary for patients treated with insulin or insulin secretagogues in order to avoid extreme excursions in blood glucose levels (Younk et al 2011). It is important to monitor blood glucose continuously, especially if a patient changes diet, body weight, and/or exercise duration and intensity (Younk et al 2011). In people with well-controlled diabetes treated with insulin or insulin secretagogues, moderate intensity exercise can induce hypoglycemia, and the American Diabetes Association (ADA) has advised that supplemental carbohydrates should be consumed if blood glucose levels are less than 100 mg/dl (5.6 mmol/l) prior to the start of exercise (Younk et al 2011)(ADA 2010). Patients should not exercise during periods of hypoglycemia (Younk et al 2011). In addition, exercise should be postponed for 24 hours after an episode of hypoglycemia, since antecedent hypoglycemia severely blunts counter-regulatory responses to exercise, and it increases the risk of recurrent hypoglycemia (Galassetti et al 2003, Younk et al 2011). Moreover, ADA's newest recommendations do not restrict exercise in the presence of any level of hyperglycemia, but exercise should be postponed, irrespective of glycemic level, for example, if ketosis is detected (ADA 2010, Younk et al 2011).

Previous research (Iscoe et al 2006) investigated the efficacy of RT-CGM during

and after prolonged cycling exercise in T1DM for 48 hours in total, since they were concerned about late-on-set hypoglycemia occurring 1 to 36 hours after exercise. Five subjects participated in a 60 min spin class that maintained their intensity above 60% of age-predicted max heart rate. Researchers defined hypoglycemia as < 4 mM and hyperglycemia > 11mM, and three out of five subjects had late-on-set hypoglycemia. The other two decreased to 4 mM. A strong correlation was found between SMBG and RT-CGM.

Several previous studies (Bussau et al 2006, Bussau et al 2007, Guelfi et al 2005a, Guelfi et al 2005b, Guelfi et al 2007a, Guelfi et al 2007b) reported that a brief sprint or a series of intermittent high intensity exercise bouts, such as typical form of sprints, attenuates the usual decrease in blood glucose concentration normally associated with continuous moderate intensity aerobic exercise (Iscoe & Riddell 2011). Although intermittent high intensity exercise may protect against acute exercise-induced hypoglycemia, the effects of intermittent high intensity exercise on glucose levels in recovery is not well-described (Iscoe & Riddell 2011). When intermittent high intensity exercise uses muscle glycogen as a predominant fuel source, recovery blood glucose levels may decline even more with this form of exercise due to an increased need for muscle glycogen restoration (Iscoe & Riddell 2011). On the other hand, the increase in glucose counter-regulatory hormones associated with intermittent high-intensity exercise, such as catecholamine and cortisol, or elevations in other metabolites, such as free fatty acid, may lower glucose concentrations in recovery (Iscoe & Riddell 2011).

The primary purpose of a primary study was to examine the trend of absolute change in plasma glucose before and after each training session of HIIT, and our results

show (Figure 5.1.) that acute glucose concentration tends to be lower after each training session. Potentially, CGM will help understand the mechanism of acute glucose response before, during, after HIIT in the future.

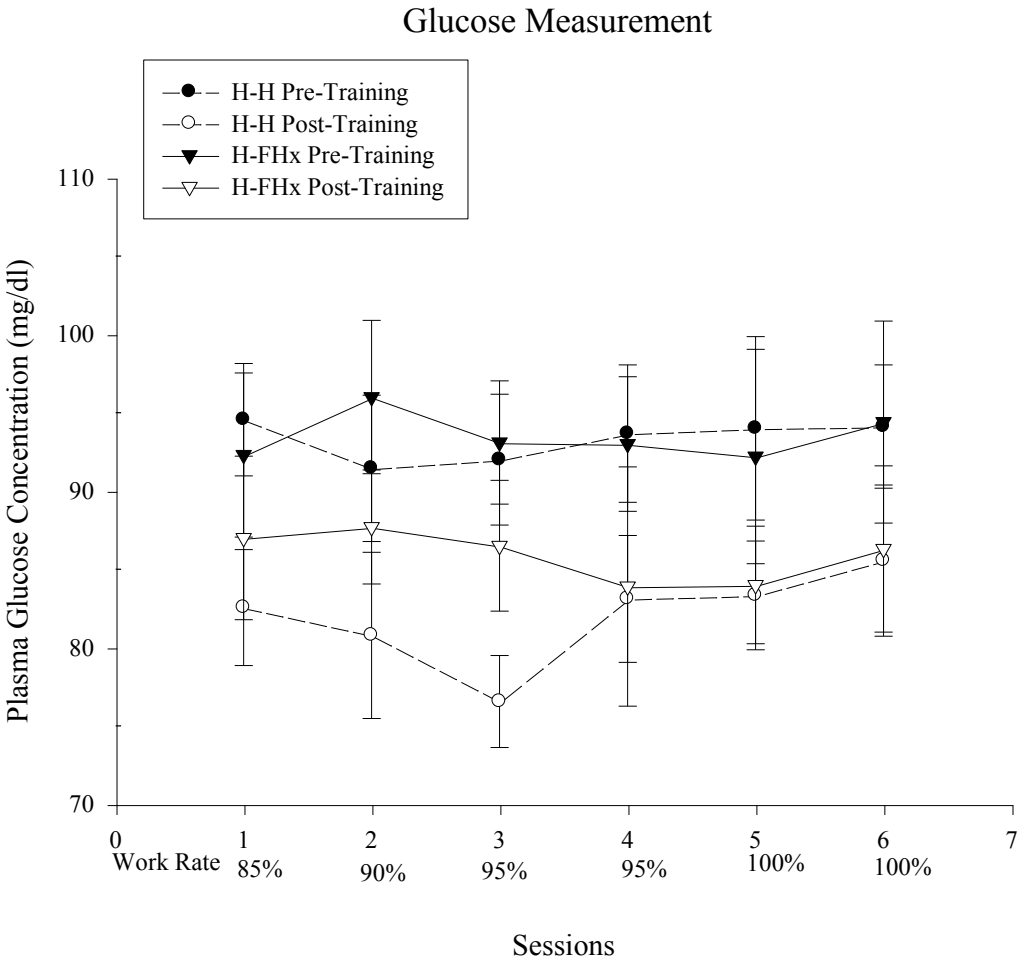

Figure 5.1. Plasma glucose concentration was assessed before and after each training session during two weeks of high intensity interval training (HIIT).
H-H (N = 9) and H-FHx (N = 10).
No significant difference between sessions and group ($P > 0.05$).
Data was represented by mean ± SEM.
H-H: Healthy individuals without a family history of type 2 diabetes mellitus.
H-FHx: Healthy Individuals with a family history of type 2 diabetes mellitus.

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Appendix A

ADULT RESEARCH SUBJECT INFORMATION AND CONSENT FORM

The Effects of High Intensity Interval Training (HIT) versus HIT with L-Arginine Supplement on Cardiovascular Function

Principal Investigator:	Barry W. Scheuermann, Ph.D.
Other Staff (identified by role): investigator)	Shinichiro Sugiura, MS (Graduate student Erin Garmyn, BS (Graduate student investigator) Trent Cayot, BS (Graduate student investigator) Chris Silette, BS (Graduate student investigator) David L. Weldy, M.D., Ph.D.
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 examining the effects of high intensity interval training (HIT) along with a commercially available nutritional supplement on blood vessel function and exercise performance. You will be asked to perform a maximal exercise test to determine your level of fitness both before and after 5 days of HIT. There will be two different subject groups; you will be randomly assigned to participate in either the HIT only group or the HIT with nutritional supplement group. If you are assigned to the HIT and supplement group, you will be provided with a drink mix containing a nutritional supplement called L-arginine that is available commercially.

You were contacted since you expressed an interest in taking part in this investigation. A maximum of 30 volunteers (15 in each of two groups) will take part in this investigation.

To participate in this study, you must be a male between 18 and 35 years of age, be free of any known cardiovascular or pulmonary disease or have any musculoskeletal (muscle or joint) injuries as determined by the medical history questionnaire. Individuals who quit smoking less than 6 months ago or do not meeting these criteria will be excluded from participating in this study.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT

If you decide to take part in this investigation, you will be asked to visit the Cardiopulmonary and Metabolism Research Laboratory (CMRL), which is located in the Department of Kinesiology on the main campus of the University of Toledo. You will be asked to visit the CMRL on 9 separate occasions over a period of approximately 2 weeks (4 sessions of testing and 5 sessions of exercise training). Each of the visits to the CMRL for blood vessel function and exercise testing is expected to last approximately 1½ to 2½ hours while the days that you peform HIT will require less than 30 minutes for a total of not more than 12-13 hours of your time.

Study Design/Protocol

Visit 1:

All of the procedures and the exercise protocols will be explained to you. You will be asked to read and sign this informed consent form and complete a medical history questionnaire during the first session. You will be instructed to consume only a light

meal and to abstain from vigorous exercise and caffeinated beverages for at least 12 hours prior to arriving at the CRML.

The following procedures will also be conducted during the first visit;

- Your anthropomentric characteristics will be measured including your height, weight, and body composition (percent body fat).
- You will be asked to lie down on a table and after a brief resting period, you will have two separate tests performed, one involving your left upper arm and one test involving your left lower leg. This test measures how well your blood vessels function using a procedure called flow-mediated dilation (FMD). The procedures involved in performing this test are described below in detail.

Visit 2:

The following tests or procedures will be conducted during your second visit **(all of the procedures are described in detail below**);

- All exercise tests will be performed on a stationary bike where the exercise intensity can be gradually increased using a computer.
- Prior to performing the exercise test, a plastic device will be placed on your skin over the muscles of your one your thighs to measure muscle oxygenation (near-infrared spectroscopy; NIRS) while a small adhesive device will be placed on the skin over the muscles of your other thigh to measure muscle electrical activity (surface electromyography, EMG).
- An elastic strap with a monitor will be placed around your chest to measure your heart rate and you will be asked to breath through a plastic mouthpiece while wearing a noseclip to measure the amount of oxygen you are using at rest and during exercise. These measurements tell us your current level of physical fitness.
- A small, flexible plastic tube will be inserted into a vein on the back of your hand and your hand will be wrapped in a heating pad. This will allow us to collect venous blood samples at rest and during exercise.
- Once you have been prepared, you will be asked to pedal the bike at a rate of 80 revolutions each minute during each test. The exercise test will be preceded by 4 minutes of a constant load exercise at 40 W to obtain baseline measures and provide you with a warm-up period. Following the 4-min lead-in, the work rate will progressively increase as a ramp function (a smooth, steady increase in exercise intensity) at a rate of 15-25 W/min. The rate at which the exercise intensity will increase depends on your current level of physical activity.
- The exercise trial will be stopped either upon request by you or volitional fatigue has been reached which is defined as the point where you are no longer able to maintain pedaling rate at the desired revolutions of 40 revolutions per minute in spite of strong verbal encouragement by the investigator at the very end of the test.

Visits 3 to 7 (High Intensity Interval Training Protocol):

On the first day, we will randomly place you in one of two groups. Group 1, which will perform high intensity interval training (HIT) only or Group 2, which will perform the

same high intensity interval training but will also be provided with a drink mix containing the nutritional supplement L-Arginine (HIT+L-Arg). If you are assigned to Group 1, you will be provided with a placebo drink mix containing maltodextrin (a carbohydrate manufactured by breaking down starches found naturally in corn, wheat, rice or starchy vegetables with little nutritional value). If you are assigned to the Group 2, you will be asked to drink 6 grams per day of L-Arginine supplement mixed in 500 ml of water for 5 days.

The HIT protocol consists of 10 repeated bouts of exercise at an intensity corresponding to 90% of maximal heart rate each bout lasting for 60 seconds with 60 seconds of recovery between each bout. You will be asked to perform this HIT exercise training protocol on 5 consecutive days.

Visit 8:
During visit 8, which will be scheduled two days following the last training session, all of the measurements and tests peformed during Visit 2 will be repeated.

Visit 9:
During visit 9, which will be scheduled three days following the last training session, all of the measurements and tests peformed during Visit 3 will be repeated.

Study Procedures:
Flow Mediated Dilation (FMD); Endothelial-Dependent Function:
This test will last approximately 1½ hours. You will be asked to lie quietly for 20 minutes on a standard treatment table.

- A blood pressure cuff will be placed around your left forearm, just below the elbow.
- A plastic sensor (Doppler ultrasound) with gel will be placed on the skin of your upper arm just above your elbow in order to obtain 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 minutes mark, the cuff around your arm will be rapidly deflated and an image of your artery in your arm will be measured for another 4 minutes.
- After measuring your arm response, you will be asked to lay face down for 20 minutes and your leg will be prepared, similar to your arm. A blood pressure cuff will be placed around your leg below your knee. A plastic sensor will gel will be placed on the skin of leg just above the knee in order to obtain an image of your artery.
- The cuff around your leg will be inflated to a high pressure for five minutes. After five minutes has passed, the cuff will be rapidly deflated and an image of your artery will be measured for another 4 minutes.

Maximal Oxygen Uptake (VO_{2MAX} or Overall Fitness) Assessment:

This procedure will last approximately 1 hour to complete.

- You will be asked to sit comfortably on the stationary electrically braked cycling ergometer.
- You will be required to breathe through a rubber mouthpiece while your nose is occluded with a nose clip.
- You will be asked to perform a maximal exercise test where the exercise intensity will gradually increase until you reach volitional fatigue which is the point at which you can no longer maintain the desired pedaling revolutions.
- You will be asked to pedal at approximately 80 revolutions each minute (rpm) for the entire duration of the test.
- The exercise protocol will consist of four minutes of warm-up cycling at a resistance of 40 watts. Following the warm-up, the resistance on the pedals will gradually increase at a rate of 15-25 watts each minute until you reach volitional fatigue. As mentioned above, the rate at which the exercise increases will depend on current level of physical activity.
- During the exercise test, you will breathe through a plastic mouthpiece and we will sample the air you breathe out for the measurement of oxgen uptake, carbon dioxide output and minute ventilation. We will use a commercially available metabolic measurement system during this test which will provide you with information about your overall fitness level.

Measurement of Muscle Oxygen Delivery and Utilization (NIRS):

- Near infrared spectroscopy (NIRS) is a safe, non-invasive method that uses different types of light to measure how much oxygen is bound to the hemoglobin in the small vessels of your muscles. Using this device, we are able to determine how much oxygen is being used by your muscles to provide energy during exercise.
- We will shave and clean a small area of skin and then place a small plastic sensor on the surface of the skin over your left thigh muscles.
- The sensor will be secured on your thigh using a Velcro-strap, adhevise tape and athletic stretch wrap.
- In order to compare your results with other participants in this study, we ask you to complete a standardization test where we increase the pressure in the cuff around your thigh to 240 mm Hg for 5 minutes and measure the change in oxygen delivery to your muscle while sitting comfortably in a chair.

Venous Blood Sample:

- An experienced individual will insert a small plastic tube into a vein located on the back of your hand so that a small amount of venous blood (3 mL each sample) can be obtained at rest and throughout the exercise. You may feel slight discomfort, which will only last for a few seconds.
- A heating pad will be placed upon your hand and forearm which helps to maintain the flow of venous blood throughout the exercise.

- We will collect venous blood samples at rest and at 2 minute intervals during exercise. The number of samples collected will depend on how long it takes you to reach volitional fatigue but typically we obtain between 6 and 8 samples resulting in approximately 18 to 24 milliliters of blood (or between 4 and 6 teaspoons) being collected during Visit 2 and Visit 9.

Surface Electromyography (EMG):

- Small round plastic adhesive monitors will be placed along two muscles on the front of your upper leg or thigh muscles aftern cleaning and shaving the skin surface. This will be used to measure the small electrical activity that your muscles generate when they are asked to contract during movement (surface electromyography).
- Additional tape and athletic stretch wrap will be used to reduce movement of the surface monitors during exercise. The surface monitors will be utilized during maximal ramp exercise to fatigue during Visit 2 and Visit 8.

Continuous Heart Rate:

- Heart rate will be measured continuously during all exercise tests and training sessions using a small monitor placed on your chest.

Body Composition:

- Your body composition will be determined using a BodPod, which uses the air displacement method order to calculate your distribution of lean body weigtht versus body fat mass distribution.

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

Risks and Discomforts Associated with Exercise:

The most common risks and discomforts that you may experience as a result of exercising are muscle cramping, strain, or soreness either during or following exercise that may last for 24 to 48 hours. Heart rate and blood pressure will be closely monitored throughout each exercise test requiring maximal effort. Heart rate will be monitored during all submaximal exercise training sessions will be supervised by individuals with a background in exercise testing and programming and are trained in both first aid and CPR.

Flow-mediated Dilation: You may also experience numbness, tingling sensation, or bruising in the upper extremeties during the duration of the blood flow restriction. Numbness and tingling sensation will stop immediately upon the release of the pressure in the blood pressure cuffs.

Near Infrared Spectroscopy: There are no known risks and/or discomfort associated with measuring oxygen delivery using near-infrared spectroscopy (NIRS) techniques.

Venipuncture is a common procedure with minimal risk. The use of sterile needles for each venous puncture and using proper sterile echniques will reduce the risk of infection at the site. Bruising around the site of insertion into the vein sometimes occurs because of blood sampling or when the needle is removed. Bruising and any sorness associated with the venipuncture generally fades within one day of the procedure.

Electromyography: There are no known risks and/or discomfort associated with measuring the nerve conduction using electromyography (EMG).

L-arginine: It is an amino acid that is used in our body to make proteins. Supplements containing L-arginine may cause some side effects such as abdominal pain, bloating, diarrhea, gout, allergic reaction, airway inflammation, worsening of asthma, and low blood pressure.

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 volunteer to participate in this study will be provided with an opportunity to learn more about current areas of research and laboratory techniques that may be of benefit to their educational experience.

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 form of payment or compensation for participating in this study nor will you be provided with "extra credit" in any academic courses that you are enrolled.

ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH

There are no reasonable alternative procedures or treatments available for this research project. This research study does not involve any treatments that may be of therapeutic benefit to the study participant.

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.

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 study as described in the research consent/authorization form.

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. 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 W. Scheuermann, Department of Kinesiology (419-530-2692). 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, you may contact at any time, day or night:
Dr. Barry W. Scheuermann (419-530-2692 - office) or (567-288-9732 - available 24 hrs)
or
Dr. David L. Weldy (330-620-3329 - cell phone) or (419-218-2040 -pager).

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.

Appendix B

ADULT RESEARCH SUBJECT INFORMATION AND CONSENT FORM

The Effects of High Intensity Interval Training (HIIT) on Glucose Regulation in Pre-diabetes Patients

Principal Investigator:	Barry W. Scheuermann, Ph.D.
Other Staff (identified by role): investigator)	Shinichiro Sugiura, MS (Graduate student
	Erin Garmyn, BS (Graduate student investigator)
	Zakaria Alyousif, BS (Graduate student investigator)
	Chris Silette, BS (Graduate student investigator)
	David L. Weldy, M.D., Ph.D.
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 examining the effects of high intensity interval training (HIIT) on glucose regulation. You will be asked to perform a maximal exercise test to determine your level of fitness, and an oral glucose tolerance test will be conducted before 6 days of HIIT (3 times a week for 2 weeks). There will be two different subject groups including a healthy control and pre-diabetic group based upon the results of a glucose tolerance test. You will wear a continuous glucose monitor continuously for 2-week of HIIT.

You are contacted since you expressed an interest in taking part in this investigation. A maximum of 100 volunteers (50 in each of the two groups) will take part in this investigation.

To participate in this study, you must be a male or a female between 18 and 55 years of age, be free of any known cardiovascular or pulmonary disease or be free of any musculoskeletal (muscle or joint) injuries as determined by the medical history questionnaire. Individuals who quit smoking less than 6 months ago or do not meet these criteria will be excluded from participating in this study. In addition, you will be excluded from this study, if you have been already diagnosed with Type 1 or Type 2 Diabetes Mellitus.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT

If you decide to take part in this investigation, you will be asked to visit the Cardiopulmonary and Metabolism Research Laboratory (CMRL), which is located in the Department of Kinesiology on the main campus of the University of Toledo (HH1407). You will be asked to visit the CMRL on 10 separate occasions over a period of approximately 4 weeks (4 sessions of testing and 6 sessions of exercise training). Each of the visits to the CMRL for glucose level and exercise testing is expected to last approximately 45 minutes to 3 hours while the days that you perform HIIT will require less than 30 minutes for a total of not more than 15 hours of your time.

Study Design/Protocol

Visit 1:

All of the procedures and the exercise protocols will be explained to you. You will be asked to read and sign this informed consent form and complete a medical history questionnaire during the first session. You will be instructed to consume only a light meal and to abstain from vigorous exercise and caffeinated beverages for at least 12 hours prior to arriving at the CRML.

The following procedures will also be conducted during the first visit;

- Your height, weight, and body composition (percent body fat) will be measured.
- You will be asked to lie down on a table and after a brief resting period, you will have two separate tests performed, one involving your left upper arm and one test involving your left lower leg. This test measures how well your blood vessels function using a procedure called flow-mediated dilation (FMD). The procedures involved in performing this test are described below in detail.

Visit 2:

The following tests or procedures will be conducted during your second visit **(all of the procedures are described in detail below**);

- All exercise tests will be performed on a stationary bike where the exercise intensity can be gradually increased using a computer.
- An elastic strap with a sensor will be placed around your chest to measure your heart rate and you will be asked to breathe through a plastic mouthpiece while wearing a nose-clip to measure the amount of oxygen you are using at rest and during exercise. These measurements tell us your current level of physical fitness.
- Once you have been prepared, you will be asked to pedal the bike at a rate of 80 revolutions each minute during each test. The exercise test will be preceded by 4 minutes of a constant load exercise at 20 Watts to obtain baseline measures and provide you with a warm-up period. Following the 4-min lead-in, the work rate will progressively increase as a ramp function (a smooth, steady increase in exercise intensity) at a rate of 15-25 Watts/min. The rate at which the exercise intensity will increase depends on your current level of physical activity.
- The exercise trial will be stopped either upon request by you or volitional fatigue has been reached which is defined as the point where you are no longer able to maintain pedaling rate at the desired revolutions of 40 revolutions per minute in spite of strong verbal encouragement by the investigator at the very end of the test.

Visit 3 (Oral Glucose Tolerance Test)

We will take a blood sample from your elbow vein for baseline. You will drink a liquid containing 75 g of sugar within 5 minutes, and we will draw your blood 30 minutes, 1 hour, and 2 hours later to see how well your body is able to reduce this sugar load. This is a test that is routinely used in many clinical facilities to test your ability to regulate your blood sugar level.

Visits 4 to 9; High Intensity Interval Training (HIIT) Protocol:

Based upon oral glucose tolerance test, we will place you in a healthy control or a pre-diabetic group.
The HIIT protocol consists of 10 repeated bouts of exercise at an intensity possibly corresponding to 90% of maximal heart rate with each bout lasting for 60 seconds with 60 seconds of recovery between each bout. You will be asked to perform this HIIT exercise training protocol 3 times a week for 2 weeks. We calculate your peak exercise intensity based upon the test on Visit 2. You will start 80% of your peak exercise intensity on Visit 4 and 5, 85% on Visit 6 and 7, followed by 90% on Visit 8 and 9. On Visit 4 and 9, we will measure your capillary function during HIIT. In addition, you will wear a continuous glucose monitor continuously for 2 weeks of HIIT, and you will complete food diaries. Moreover, we will provide referrals for individuals in the pre-diabetic group to ensure they will receive proper counseling and treatment based upon oral glucose tolerance test.

Visit 10:
During visit 10, which will be scheduled two days following the last training session, all of the measurements and tests performed during Visit 1 and 3 will be repeated.

Study Procedures:
Oral Glucose Tolerance Test (OGTT):
This test will last approximately 3 hours.

- A trained individual (a registered respiratory therapist or a physician) will insert a small plastic tube into a vein located on the front of your elbow so that a small amount of venous blood (3 mL each sample) can be obtained. You may feel slight discomfort, which will only last for a few seconds.
- We will collect venous blood samples before you drink 75g of sugar-drink and will collect venous blood samples at 30 minutes, 1 hour, and 2 hours resulting in approximately 24 milliliters of blood total (approximately 6 teaspoons total) being collected during Visit 3 and Visit 10.

Continuous Glucose Monitor (CGM)
This test will last over 2 weeks.

- An experienced individual will help you insert a sensor into the side of your abdomen and will help calibrate the device. It will take 2 hours to start to record your glucose level every 5 minutes for 7 days. The calibration needs to be performed every 12 hours.
- An experienced individual will help remove a sensor and insert a new one after the first 7 days, and you will continue to wear this device for another 7 days. After the completion of high intensity interval training, an experienced individual will remove the sensor.

Flow Mediated Dilation (FMD); Endothelial-Dependent Function:

This test will last approximately 1½ hours. You will be asked to lie quietly for 20 minutes on a standard treatment table.

- A blood pressure cuff will be placed around your left forearm, just below the elbow.
- A plastic sensor (Doppler ultrasound) with gel will be placed on the skin of your upper arm just above your elbow in order to obtain 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 minutes mark, the cuff around your arm will be rapidly deflated and an image of your artery in your arm will be measured for another 4 minutes.
- After measuring your arm response, you will be asked to lay face down for 20 minutes and your leg will be prepared, similar to your arm. A blood pressure cuff will be placed around your leg below your knee. A plastic sensor with gel will be placed on the skin of leg just above the knee in order to obtain an image of your artery.
- The cuff around your leg will be inflated to a high pressure for five minutes. After five minutes has passed, the cuff will be rapidly deflated and an image of your artery will be measured for another 4 minutes.

Maximal Oxygen Uptake (VO_{2MAX} or Overall Fitness) Assessment:

This procedure will take approximately 1 hour to complete.

- You will be asked to sit comfortably on the stationary electrically braked cycling ergometer.
- You will be required to breathe through a rubber mouthpiece while your nose is occluded with a nose clip.
- You will be asked to perform a maximal exercise test where the exercise intensity will gradually increase until you reach volitional fatigue which is the point at which you can no longer maintain the desired pedaling revolutions.
- You will be asked to pedal at approximately 80 revolutions each minute (rpm) for the entire duration of the test.
- The exercise protocol will consist of four minutes of warm-up cycling at a resistance of 20 watts. Following the warm-up, the resistance on the pedals will gradually increase at a rate of 15-25 watts each minute until you reach volitional fatigue. As mentioned above, the rate at which the exercise increases will depend on current level of physical activity.
- During the exercise test, you will breathe through a plastic mouthpiece and we will sample the air you breathe out for the measurement of oxygen uptake, carbon dioxide output and minute ventilation. We will use a commercially available metabolic measurement system during this test which will provide you with information about your overall fitness level.

Measurement of Muscle Oxygen Delivery and Utilization (NIRS):

- Near infrared spectroscopy (NIRS) is a safe, non-invasive method that uses different types of light to measure how much oxygen is bound to the hemoglobin in the small vessels of your muscles. Using this device, we are able to determine how much oxygen is being used by your muscles to provide energy during exercise.
- We will shave and clean a small area of skin and then place a small plastic sensor on the surface of the skin over your left thigh muscles.
- The sensor will be secured on your thigh using a Velcro-strap, tape and athletic stretch wrap.
- In order to compare your results with other participants in this study, we ask you to complete a standardization test where we increase the pressure in the cuff around your thigh to 240 mm Hg for 5 minutes and measure the change in oxygen delivery to your muscle while sitting comfortably in a chair.

Continuous Heart Rate:

- Heart rate will be measured continuously during all exercise tests and training sessions using a small monitor placed on your chest.

Body Composition:

- Your body composition will be determined using a BodPod, which uses very small changes in air pressure inside a small chamber, in order to calculate your lean body weight versus body fat mass.

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

Risks and Discomforts Associated with Exercise:

The most common risks and discomforts that you may experience as a result of exercising are muscle cramping, strain, or soreness either during or following exercise that may last for 24 to 48 hours. Although you are being screened with a medical history questionnaire to reduce the probability that you might a heart problem from the exercise, very rarely there are heart problems occurring with high intensity physical activity, that include abnormal heart rhythm, sudden cardiac arrest (heart stops), or heart attack. Heart rate and blood pressure will be closely monitored throughout each exercise test requiring maximal effort. Heart rate will be monitored during all submaximal exercise training sessions and will be supervised by individuals with a background in exercise testing and programming and are trained in both first aid and CPR. In addition, with an ingestion of any substance, such as the very sweet liquid participants will drink for the oral glucose tolerance testing, there is the possibility of a participant becoming nauseous.

Continuous Glucose Monitor: The sensor is electrochemical and consists of a wire inserted subcutaneously into the back, buttocks, or the abdomen, but there are only minor local adverse events, such as hypersensitivity, itching, pain, redness, burning, and subcutaneous hemorrhage. It is a minimum risk of infection or no complications.

Flow-mediated Dilation: You may also experience numbness, tingling sensation, or bruising in the upper extremities during the duration of the blood flow restriction. Numbness and tingling sensation will stop immediately upon the release of the pressure in the blood pressure cuffs.

Near Infrared Spectroscopy: There are no known risks and/or discomfort associated with measuring oxygen delivery using near-infrared spectroscopy (NIRS) techniques.

Venipuncture from Oral Glucose Tolerance Test is a common procedure with minimal risk. The use of sterile needles for each venous puncture and using proper sterile echniques will reduce the risk of infection at the site. Bruising around the site of insertion into the vein sometimes occurs because of blood sampling or when the needle is removed. Bruising and any soreness associated with the venipuncture generally fades within one day of the procedure.

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 from the University of Toledo area community. Possible students from the Department of Kinesiology who volunteer to participate in this study will be provided with an opportunity to learn more about current areas of research and laboratory techniques that may be of benefit to their educational experience.

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 form of payment or compensation for participating in this study nor will you be provided with "extra credit" in any academic courses that you are enrolled.

ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH

There are no reasonable alternative procedures or treatments available for this research project. This research study does not involve any treatments that may be of therapeutic benefit to the study participant.

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 the information we collect, 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 an injury, you may contact at any time, day or night:
Dr. Barry W. Scheuermann (419-530-2692 - office) or (419-258-0283 - available 24 hrs)
or
Dr. David L. Weldy (330-620-3329 - cell phone) or (419-218-2040 -pager).

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.

Appendix C

Appendix A – Medical History Questionnaire

Age: _____ **Gender: M/F Subject ID #:** _____

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

*Do you have any of the following health conditions?**	*Yes*	*No*
Family history of heart disease? i.e. Heart attack, bypass, stroke, or sudden death before age 55 in 1st degree male relative (father, brother, son) or before age 65 in 1st degree female relative (mother, sister, daughter)		
Smoking habit? i.e. Current cigarette smoker *or* one who has quit within the previous 12 months		
High blood pressure? i.e ≥140/90 on two separate occasions *or* currently on antihypertensive medication		
Abnormal cholesterol levels? i.e. Total Cholesterol >200mg/dL, *or* LDL >130 mg/dL, *or* HDL <35 mg/dL, *or* currently on lipid lowering medication		
High fasting glucose? i.e Fasting blood glucose ≥110 on two separate occasions		
Are you inactive? i.e. Accumulate <30 minutes of moderate physical activity on most days of the week		
If you can answer *yes* to *2 or more* above please obtain *medical clearance* for exercise from your *personal physician*.		

Other Health Related Questions:
Prescription Medications: _____ _____
Allergies:
Do you have any orthopedic conditions/arthritis that may limit your activities? _____ _____
Are you pregnant? If yes, how many weeks? _____
Do you have any other problems or medical conditions not addressed on this form including any disorders that might affect the ability of your blood to clot normally? _____
How long have you had your medical condition(s)? _____

*Do you current have any of the following?**	Yes	No
Pain in the chest, neck, jaw, or arms?		
Shortness of breath at rest or with mild exertion?		
Dizziness or fainting?		
Awakened by a shortness of breath?		
Swelling in your ankles?		
Rapid heart rate while at rest?		
Leg pain or cramping while walking; stops with rest?		
Heart murmur?		
Unusual fatigue or shortness of breath with usual activities?		
If you can answer *yes* to *any* of the above please obtain *medical clearance for exercise from your personal physician*.		

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

Signature: _____ **Date:** _____

***Adapted from *ACSM's Guidelines for Exercise Testing and Prescription, 6th Edition*.**

Appendix D

December 2, 2014

Shinichiro Sugiura
2677 Whiteway Rd., Apt. 6
Toledo, OH 43606

Dear Shinichiro,

Thank you for submitting your grant titled, *The Effects of High Intensity Interval Training (HIIT) on Glucose Regulation in Individuals with a Family History or Diabetes,* to the Ohio Athletic Trainers' Association Research and Grant Committee. I am pleased to inform you that your grant has been approved for funding. Many quality submissions were received and in order to best support research efforts by our members, partial awards have been granted. The amount of your award is **$500.00** to offset the cost the glucose and insulin test kits. As soon as you let me know that you accept this award, a check will be mailed to you. **Please respond to me by December 10, 2014 by email (**spayne@otterbein.edu**) with your decision.**

The subcommittee also requires that you present your work at the annual OATA symposium upon completion of your research. The call for free communications will be in March, so please refer to www.oata.org for further instructions.

The members of the committee wish you the best of luck in completing your research. If you have specific questions or concerns regarding the grant at any time, please feel free to contact us by email research_chair@oata.org or by phone at 614-823-1974. Thank you again for your support of the OATA and the Research & Grants Committee.

Sincerely,

Shelley Payne, DHS, AT, PT
Sub-Chair
OATA Research and Grants Subcommittee

Appendix E

VITAE
SHINICHIRO SUGIURA

Campus
The University of Toledo
Department of Kinesiology
2801 W. Bancroft, MS#119
Toledo, OH 43606
(email)

Home
2677 Whiteway Rd. Apt. 6
Toledo, OH 43606
(207) 807-7119 ©
shinboatc@hotmail.com

Educational Background

Ph.D.	The University of Toledo, Toledo, Ohio. May, 2015. Major: Exercise Science *Dissertation title: The Effects of High Intensity Interval Training on Insulin Sensitivity in Individuals with a Family History of Type 2 Diabetes Mellitus*
M.S.	Indiana State University, Terre Haute, Indiana. August, 2002. Major: Athletic Training
B.S.	Indiana State University, Terre Haute, Indiana. May, 2001. Major: Athletic Training
B.A.	Chuckyo University, Toyota, Aichi, Japan. March, 1997. Major: Leisure Sports with emphasis on Sports Psychology

Professional Experience

August 2011-Present	**The University of Toledo**, Toledo, OH *Doctoral Graduate Assistant* Teaching Responsibilities: KINE2520-001/003: Human Anatomy Laboratory, Spring 2015 KINE2520-005/006: Human Anatomy Laboratory, Fall 2014

KINE2520-001/003/004: Human Anatomy Laboratory, Spring 2014
KINE2520-004/005/006: Human Anatomy Laboratory, Fall 2013
KINE2520-001/003/004: Human Anatomy Laboratory, Spring 2013
KINE2520-001/002/003: Human Anatomy Laboratory, Fall 2012
KINE2520-004/005: Human Anatomy Laboratory, Spring 2012
KINE2460-013: Anatomy and Physiology I Laboratory, Spring 2012
KINE2520-001/002: Human Anatomy Laboratory, Fall 2011

Assisted Teaching:
KINE4840: Fitness Internship II, Spring 2015
American Heart Association BLS re-certification, Fall 2014, Fall 2012
KINE4140: Fitness Internship I, Fall 2014
KINE2470: Anatomy and Physiology II Lecture, Summer 2012
KINE3630: Therapeutic Modalities for Athletic Trainers, Fall 2011
HEAL1500: First Aid, Spring 2014, Spring, 2013, Fall, 2012, Spring 2012, Fall 2011

July 2007-
June 2011

Indiana University, Bloomington, IN
Assistant Athletic Trainer for cross-country and track and field
Responsibilities include: provided health care, treat and referred illness/injury to team physician, coordinated practice and meet coverage, supervised CAATE undergraduate students and Post-Professional graduate students, assisted the institutional drug testing. Hosted 2007 and 2009 NCAA Regional Cross Country Championships, 2009 Big Ten Indoor Track and Field Women's Championships, 2010 and 2011 USATF Indoor Combined Events, 2010 Big Ten Outdoor Track and Field Championships, and 2011 NCAA Division I Track and Field East Preliminary Round.

August 2003-
June 2007

University of Southern Maine, Gorham, ME
Assistant Athletic Trainer
Responsibilities include: provide health care, administered the wrestling's weight certification program. Supervised CAATE undergraduate students. Helped establish the concussion protocol. Helped exercise-induced asthma study. Presented stress issue on certified athletic trainers and NCAA drug testing policy and procedure. Taught First Aid, CPR, AED to coaches and administrators with other athletic trainers. Hosted 2003 NCAA New England Cross Country Championships and 2004 Little East Conference Men's Tennis Tournament.

August 2002-

Back in Motion Physical Therapy, LLC, Gorham, ME

August 2003	*Athletic Trainer/PT Assistant* Responsibility includes: Assisted patients with clinical support directed by physical therapists. Worked as head athletic trainer in Gorham high school. Established emergency action plan in Gorham High School. Instructed on/off season strength and conditioning to athletes. Hosted 2003 Western Maine Track and Field Championships.
July 2001- June 2002	**Indiana State University**, Terre Haute, IN *Graduate Assistant* Responsibilities include: Worked for Terre Haute North High School as a graduate assistant. Assisted faculty in Research Injury Laboratory. Supervised CAAHEP-ATEP undergraduate athletic training students and checked off proficiencies as ACI. Taught First Aid, CPR, and Community Health with another instructor.

Research Poster Presentation

Sugiura, S. Alyousif, ZA, Silette, CR, Garmyn, EG, Scheuermann, BW. *The Effects of High Intensity Interval Training (HIIT) with L-arginine versus HIIT on Cardiovascular Function*. A poster presentation at 2015 NATA convention, St. Louis, MO. Abstract was accepted on February 16, 2015.

Sugiura, S. Stemmans, CL, Storsved, JR. Ingersoll, CD (June, 2003). *Predictive Factors of Perceived Stress and Work-Related Strain Among Collegiate Athletic Trainers*. A poster presentation at 2003 NATA convention, St Louis, MO

Grant/Sponsorship

2015: Sponsorship: Sonia M. Najjar, Ph.D. in Center for Endocrine and Diabetes Research
2014: Ohio Athletic Trainers' Association Research Assistance Grant
2002: Indiana State University Graduate Student Research Assistance Grant

Committees

2014: Assistant/Associate Tenure-Track Professor Search Committee in the Department of Kinesiology, The University of Toledo, Toledo, OH
2012-2013: Representative for College of Health Science and Human Services at Graduate Student Association, The University of Toledo, Toledo, OH
2012-2013: Midwest Graduate Research Symposium Committee, The University of Toledo, Toledo, OH
2007-2010: Alumni Award Committee in the Athletic Training Department, Indiana State University, Terre Haute, IN
2004: Men's Soccer Head Coach Search Committee, University of Southern Maine, Gorham, ME

Certifications

February, 2015: Healthcare Provider: American Heart Association
June 2014: CPR/AED for the Professional Rescuer: American Red Cross
January 2012: Emergency Response Instructor: American Red Cross
July 2010: NASM Corrective Exercise Specialist
August 2009: Functional Movement Screening (Level 1) certification
July 2009: NASM Performance Enhancement Specialist
October 2008: Graston Instrument Manual Technique Certification
July 2007: State of Indiana Athletic Trainer's License, 36001406A
April 2007: Basic Life Support (BLS) Instructor: American Heart Association
March 2007: US Permanent Resident
July 2006: LifeFlight of Maine Ground Safety Certificate
February 2006: National Provider Identifier Number 1467427104
February 2002: Professional Rescuer Instructor: American Red Cross
February 2002: Lay Responder Instructor: American Red Cross
August 2001: National Athletic Trainers' Association Board of Certification, Certification Number
080102099
May 2000: Preventing Disease Transmission: American Red Cross
May 2000: Oxygen Administration: American Red Cross
March 1997: Teaching Licenses of Physical Education for High School and Junior High School in Japan

Past Certifications

September 2006-March 2013: National Registry Emergency Technician-Basic
Certification Number B1775955
December 2009-December 2011: Wilderness and Remoter First Aid Instructor: American Red Cross
December 2009-December 2011: Blood-borne Pathogens (PDT) Instructor: American Red Cross
January 2007-December 2008: Emergency Response Instructor: American Red Cross
May 2006-May 2009: Human Participation Protections Education for Research Certificate

Advanced Seminars and Workshops

September 2011: Kinesio-taping-KT-3: Healing Art Institute; Perrysburg, OH,
October 2010: Kinesio-taping-KT-1/KT-2: Rehab Education, LLC; Indianapolis, IN
July 2009: Functional Movement Screening: PerformBetter; Indianapolis, IN
October 2008: Graston Instrument Manual Technique: Indiana University; Bloomington, IN
June 2007: Sport Psychology Seminar: University of Southern Maine; Portland, ME
June 2007: Pre-hospital Trauma Life Support: Bridgton Hospital; Bridgton, ME
April 2007: Current Trends in the Management of Head Injuries in Athletics: University of New England; Biddeford, ME

January 2007: Myofascial Release: State of the Art Tissue Mobilization: Cross Country Education, Inc.; South Portland, ME
June 2006: USM Sports Nutrition Symposium: University of Southern Maine; Gorham, ME
June 2005: Incorporating the NATA Concussion Position Statement into Clinical Practice: NAT convention; Indianapolis, IN
June 2003: Therapeutic Ultrasound and Diathermy: NATA convention; St. Louis, MO
March 2003: When Heads Don't Heal: Health South; Portland, ME

Award

2014 NATA Research and Education Foundation: GLATA Living Memorial Scholarship

Acknowledgement

Remaley DT, Fincham B, McCullough B, et al. Surface electromyography of the forearm musculature during the windmill softball pitch. *The Orthopaedic Journal of Sports Medicine* 2015;3(1):1-8

Professional Memberships

American Physiological Society, American College of Sports Medicine, Ohio Athletic Trainers' Association, Great Lakes Athletic Trainers' Association, National Strength and Conditioning Association, Golden Key National Honor Society, National Athletic Trainers' Association

Volunteer Experience

July 2014-Present: American Heart Association Instructor, Toledo Flower Hospital, Toledo, OH
April 2013: Midwest Graduate Research Symposium, The University of Toledo, Toledo, OH
July 2012-Present: American Red Cross Instructor, Greater Toledo Area Chapter, Toledo, OH
June 2012-June 2014: American Heart Association Instructor, The University of Toledo, Division of EMS Education, Toledo, OH
December 2007-July 2011:American Heart Association Instructor, Bloomington Hospital CTC, Bloomington, IN
August 2007-July 2011: American Red Cross Instructor, Monroe County Chapter, Bloomington, IN
May 2007-June 2007: Emergency Medical Consultants, Falmouth, ME
September 2006-June 2007: Southern Maine EMS, South Portland, ME
August 2002-June 2007: American Red Cross Instructor, Portland Chapter, Portland, ME
July 2003: Orthopedic Associates of Portland, Portland, ME
May 1999-2001: NCAA Division I Men's Tennis Regional Tournament, Terre Haute, IN
June 2000: Sports + Clinic at Regional Hospital, Terre Haute, IN

May 2000: Sycamore Sports Medicine at Union Hospital, Terre Haute, IN
March 1999: Special Olympic Basketball, Terre Haute, IN
August 1999: Indiana State University Football Camp, Terre Haute, IN