Effects of Eight Weeks of High-intensity Interval Training on Blood Glucose regulation, Endothelial Function, and Visceral Fat in Obese Adults

by

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ABSTRACT

Cardiovascular disease (CVD) is the number one cause of death in the United States and type 2 diabetes (T2D) and obesity lead to cardiovascular disease. Obese adults are more susceptible to CVD compared to their non-obese counterparts. Exercise training leads to large reductions in the risk of CVD and T2D. Recent evidence suggests high-intensity interval training (HIT) may yield similar or superior benefits in a shorter amount of time compared to traditional continuous exercise training. The purpose of this study was to compare the effects of HIT to continuous (CONT) exercise training for the improvement of endothelial function, glucose control, and visceral adipose tissue. Seventeen obese men (N=9) and women (N=8) were randomized to eight weeks of either HIT (N=9, age=34 years, BMI=37.6 kg/m2) or CONT (N=8, age=34 years, BMI=34.6 kg/m2) exercise 3 days/week for 8 weeks. Endothelial function was assessed via flow-mediated dilation (FMD), glucose control was assessed via continuous glucose monitoring (CGM), and visceral adipose tissue and body composition was measured with an iDXA. Incremental exercise testing was performed at baseline, 4 weeks, and 8 weeks. There were no changes in weight, fat mass, or visceral adipose tissue measured by the iDXA, but there was a significant reduction in body fat that did not differ by group (46±6.3 to 45.4±6.6%, P=0.025). HIT led to a significantly greater improvement in FMD compared to CONT exercise (HIT: 5.1 to 9.0%; CONT: 5.0 to 2.6%, P=0.006). Average 24-hour glucose was not improved over the whole group and there were no group x time interactions for CGM data (HIT: 103.9 to 98.2 mg/dl; CONT: 99.9 to 100.2 mg/dl, P>0.05). When statistical analysis included only the subjects who started with an average glucose at baseline > 100 mg/dl, there was a significant improvement in glucose control overall, but no group x

time interaction (107.8 to 94.2 mg/dl, P=0.027). Eight weeks of HIT led to superior improvements in endothelial function and similar improvements in glucose control in obese subjects at risk for T2D and CVD. HIT was shown to have comparable or superior health benefits in this obese sample with a 36% lower total exercise time commitment.

DEDICATION

I dedicate this work to my family. To my wife who supported me in every way possible and was my rock all the way through my education. To my son Soren who kept me having fun everyday no matter how hard it was. To my son that is in his mother's womb I can't wait to meet you little man. To my mother who became a second mother to Soren during this crazy time. To my father who supported us with the "Sawyer scholarship". To my father and mother-in-law who were always there to help when we needed it most.

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Chapter 1

INTRODUCTION

Background: Cardiovascular Disease, Diabetes, and Exercise

Cardiovascular disease (CVD) remains the number one cause of death in the United States (Go et al., 2013). In the year 2009 more than 2150 Americans died of CVD related causes every day, averaging 1 death every 40 seconds (Go et al., 2013). Furthermore, in 2009 8.2 percent of the US population had diagnosed type 2 diabetes (T2D) and 38.2% of the population had pre-diabetes (Go et al., 2013). Both T2D and CVD are obesity related disorders and although the increase in the prevalence of obesity appears to have reached a plateau (Flegal, Carroll, Ogden, & Curtin, 2010), the US prevalence of ~35% is still very high (Flegal, Carroll, Kit, & Ogden, 2012). Finally, despite decades of public health recommendations (Haskell et al., 2007) and research unequivocally showing that exercise improves cardiovascular and metabolic health (Booth, Roberts, & Laye, 2012), estimates suggest that only 2-4% of adults in the US achieve the recommended goal of greater than 150 min/wk of moderate intensity physical activity, even when short 10 minute bouts are included (Troiano et al., 2008).

High-intensity Interval Training

The most commonly cited reason for adults not engaging in regular physical activity is a perceived lack of time (Godin et al., 1994; Stutts, 2002; Trost, Owen, Bauman, Sallis, & Brown, 2002). High-intensity interval training (HIT) is typically much shorter than standard continuous exercise and has recently appreciated a resurgence in the scientific literature showing comparable or superior improvements in fitness and cardiovascular disease (CVD) risk factors compared to conventional continuous moderate

endurance training programs (C. Earnest, 2009; Gibala, 2007; Kemi & Wisløff, 2010; Wisløff, Ellingsen, & Kemi, 2009). Furthermore, HIT has been shown to be more enjoyable than moderate-intensity continuous exercise (Bartlett et al., 2011). HIT may prove to be a time-efficient strategy for improving cardiovascular health, but effectiveness and sustainability of longer-term HIT interventions have not yet been evaluated (Gaesser & Angadi, 2011).

Blood Glucose Control

The treatment and prevention of type 2 diabetes with aerobic exercise is a well-accepted approach (Balducci et al., 2008; Knowler et al., 2002). Hepatic and peripheral insulin resistance coupled with impaired β-cell function occurs long before fasting hyperglycemia and represents the early stages of the progression to T2D (DeFronzo, 2009; S. Kahn, 2003; Lillioja et al., 1993). Obese, otherwise healthy, individuals (BMI $\geq$ 30 kg/m^2) are more susceptible to insulin resistance than their normal weight counterparts (B. B. Kahn & Flier, 2000). A relatively novel method of assessing blood glucose control or regulation, called continuous glucose monitoring (CGM), provides researchers with unprecedented granularity in assessment of blood glucose control (Klonoff, 2005). The CGM device provides blood glucose readings every five minutes for durations up to 72 hours. A recent study showed abnormal glucose control in normal to overweight healthy men with abdominal obesity that was not detected with fasting glucose measurements (Ma et al., 2011). Furthermore studies on the effectiveness of acute high-intensity interval exercise (HIIE) on the control of blood glucose have shown promising results in diabetics (Gillen et al., 2012; S. F. Praet et al., 2006). A short-term (6 sessions) HIT program showed similar effectiveness (Little et al., 2011). There are currently no HIT

interventions longer than 4 weeks assessing changes in glucose control measured via CGM in obese adults.

Endothelial Function

Improvements in traditional cardiovascular disease (CVD) risk factors including blood lipids, blood pressure, diabetes, weight, and hemostatic factors only account for ~59% of the reduction in CVD events associated with exercise (D. H. J. Thijssen et al., 2010). This discrepancy calls for assessment of novel and early markers of the atherosclerosis process. Decline in the capacity of the endothelial cells to produce NO has been shown to be an important indication of early atherosclerosis (Landmesser, Hornig, & Drexler, 2004). The use of high-resolution ultrasound to assess endothelial function via flow-mediated dilation (FMD) began in 1992 (Celermajer, Sorensen, & Gooch, 1992) and is now a popular research tool in assessment of endothelial function (McCully, 2012). The amount of arterial dilation (FMD) that occurs after 5 minutes of blood flow occlusion has been shown to be a strong predictor of cardiovascular events in healthy subjects as well as subjects with known cardiovascular disease (Green, Jones, Thijssen, Cable, & Atkinson, 2011; Inaba, Chen, & Bergmann, 2010).

Continuous and interval exercise training has been shown to improve FMD in healthy and diseased subjects (Clarkson et al., 1999; Hambrecht et al., 2003; Wisloff et al., 2007). Some studies suggest that HIT leads to superior improvements in FMD (Ciolac et al., 2010; Molmen-Hansen et al., 2012; Schjerve et al., 2008; A. Tjonna et al., 2009; A. E. Tjonna et al., 2008; Wisloff et al., 2007) while others show no difference between HIT and continuous training (Currie, Dubberley, McKelvie, & MacDonald, 2013; T. Moholdt

et al., 2011; Rakobowchuk et al., 2008). Therefore, the effectiveness of HIT compared to continuous exercise for improvement of endothelial function is unknown.

Visceral Adipose Tissue

It is well established that visceral adipose tissue (VAT) accumulation is linked to cardiovascular disease (J. P. Despres et al., 1990), hypertension (Rhéaume et al., 2009), endothelial function (Arcaro et al., 1999), hepatic insulin resistance (Gastaldelli et al., 2000), impaired insulin secretion of pancreatic β-cells (S. Kahn, 2003), and peripheral insulin resistance (Mourier et al., 1997; Rhéaume et al., 2011). Aerobic exercise with and without caloric restriction and/or weight loss has proven to be effective in reducing VAT (Ismail, Keating, Baker, & Johnson, 2012; Kay & Singh, 2006). Some studies suggest that vigorous intensity exercise is superior to moderate or light intensity exercise in reducing VAT (Coker, Williams, Kortebein, Sullivan, & Evans, 2009; Irving et al., 2008), while others show no difference between moderate and vigorous intensities (Cho, Lee, Lee, & Kang, 2011; Gutin et al., 2002; Kay & Singh, 2006; Nicklas et al., 2009; Ohkawara, Tanaka, Miyachi, Ishikawa-Takata, & Tabata, 2007; Slentz et al., 2005). A recent investigation found 15-weeks of HIT superior to steady state exercise in reduction of trunk adiposity, but VAT was not measured (E. Trapp, Chisholm, Freund, & Boutcher, 2008). The effectiveness of HIT in the reduction of VAT is largely unknown and since some studies suggest that VAT reduction may be intensity dependent, research on HIT and VAT reduction is needed.

Purpose, Specific Aims, and Hypotheses

Purpose: Due to the lack of published long term studies using high-intensity interval training for the improvement of glucose control, artery function, and visceral

adiposity the purpose of this study was to compare the effectiveness of eight weeks of HIT to continuous exercise training in the improvement of these cardiovascular disease risk factors.

Aim #1: To determine the efficacy of a novel, 8 week high-intensity aerobic interval exercise training program for improving blood glucose regulation in obese individuals.

Hypothesis #1: I hypothesize that high-intensity interval training will yield superior improvements in blood glucose control compared to the continuous training.

Aim #2: To determine the efficacy of a novel, 8 week high-intensity aerobic interval exercise training program for improving endothelial function in obese individuals.

Hypothesis #2: I hypothesize that high-intensity interval training will yield superior improvements in endothelial function compared to the continuous training.

Aim #3: To determine the efficacy of a novel, 8 week high-intensity aerobic interval exercise training program for decreasing visceral adipose tissue in obese individuals.

Hypothesis #3: I hypothesize that high-intensity interval training will yield superior reductions in visceral adipose tissue compared to the continuous training.

Definition of Terms

High-intensity interval training: Exercise training characterized by short (6 seconds to 6 minutes) periods of exercise at an intensity just below maximum separated by low-intensity recovery periods.

Continuous exercise training: Exercise training characterized by maintaining a constant workload for a sustained period of time.

Continuous glucose monitoring: A method for assessing glucose control or regulation over 24-72 hours that utilizes a micro dialysis catheter inserted into adipose tissue that records blood glucose values every 5 minutes from the interstitial fluid.

Endothelial function: Ability of the cells lining arteries to produce and release vasodilators, including nitric oxide, that in response to shear stress stimulate smooth muscle relaxation and vasodilation.

Flow-mediated dilation: Used as a test of endothelial dependent vasodilation, artery diameter and blood flow are measured at rest, then again at peak flow and diameter after a five minute period of blood flow occlusion.

Visceral adipose tissue: adipose tissue that accumulates around organs in the abdominal region and has a strong positive association with cardiovascular disease.

Delimitations

The delimitations of the study for men and women include: body mass index of 30 kg/m^2 or greater, sedentary, non-smoking, not taking blood pressure, cholesterol, thyroid, or heart medications, no personal history of cardiovascular disease, and no physical disabilities or injuries not allowing them to engage in physical activity. Individuals must also have a clear acoustic window for optimal imaging of the brachial artery. Men must be between the age of 18 and 45 years and women between 18 and 55 years. Women must not be pregnant or become pregnant and have less than 8 days of variation in their menstrual cycle from month to month.

Limitations

The major limitation of this study is the lack of ability to control for dietary changes. We ask all participants to not intentionally change their diet, but we cannot be sure of their compliance. Similarly, we ask all participants to not engage in any regular vigorous physical activity outside of the intervention, but we cannot be sure of their compliance. We chose to not use a sedentary control group and to use the continuous exercise as a standard of care control due to metabolic deterioration typically seen in sedentary control groups (Patel, Slentz, & Kraus, 2011). Finally, only including 10 subjects in each group provides sufficient statistical power for our primary outcome (glucose area under the curve), but may not provide statistical power for all of the outcomes measured.

Chapter 2

BACKGROUND LITERATURE

Endothelial Function

Atherogenesis and the Progression of Atherosclerosis

It is now well recognized that the first step in the progression of atherosclerosis is endothelial cell dysfunction (Vanhoutte, 2009). Endothelial cell dysfunction is caused by direct injury to the vascular endothelium (Croce & Libby, 2007). The endothelial injury is caused and/or worsened by high levels inflammatory mediators and cytokines, high levels of low-density lipoproteins (LDL), hypertension, high blood glucose, high homocysteine, genetic changes, or free radicals caused by smoking (Croce & Libby, 2007; Ross, 1999; Vanhoutte, 2009). Endothelial cells are formed early in development and remain quiescent for many years until advanced age and programmed cell death cause their turnover. After turnover the endothelial cells are quickly regenerated, but unfortunately the regenerated endothelial cells are dysfunctional. This turnover and replacement of healthy endothelial cells with dysfunctional cells is part of the natural ageing process, but it is accelerated when cardiovascular disease risk factors are elevated as in hypertension, hypercholesterolemia, and hyperglycemia (Vanhoutte, 2009). Dysfunction of the endothelium is characterized by a decrease in the ability of endothelial cells to produce (via endothelial nitric oxide synthesis [eNOS]) and release nitric oxide (NO). NO has many protective roles in the vascular system including: preventing abnormal constriction of the coronary arteries, inhibition of the expression of vascular and intracellular adhesion molecules (VCAMs and ICAMs) on the endothelium, which lead to adhesion and penetration of monocytes from the immune system (macrophages),

and inhibition of endothelin-1 (which has been shown to cause vasoconstriction and lymphocyte transformation into macrophages). The normal protective release of NO is stimulated by the presence of thrombin and other platelet aggregators, but in endothelial dysfunction the presence of these substances does not lead to adequate NO release.

Inadequate NO release starts the process of atherogenesis by allowing platelet aggregation, increased permeability of the endothelium to leukocytes, a shift toward the domination of pro-coagulation substances, increased oxidation of LDL particles by the uncoupling of eNOS, increased release of pro-inflammatory cytokines, and the release of growth factors (Ross, 1999).

Leukocytes are stimulated to migrate through the endothelial layer into the arterial intima where they differentiate into macrophages. The macrophages then release many inflammatory mediators leading to the formation of a pro-inflammatory milieu in the intimal layer of the artery. This pro-inflammatory milieu leads to the migration and proliferation of smooth muscle cells and the engulfing of accumulated oxidized LDL particles by macrophages which initiates their transition into foam cells and the formation of the fatty streak (Croce & Libby, 2007). Also extracellular matrix proteins such as collagen and elastin accumulate in the region causing the plaque to grow and harden. Eventually the inflammatory milieu transitions from an active growth site to a thick collagenous formation that is characterized by hypoxia which leads to necrosis and apoptosis. Cell death eventually leads to thinning of the fibrous cap and eventual rupture of the plaque thereby exposing the collagenous interior of the plaque as well as tissue factor to the blood stream. Both tissue factor and collagen lead to rapid coagulation which stays locally around the plaque. The coagulation leads to further growth of the

plaque and therefore further narrowing of the vessel lumen. Eventually a plaque rupture will lead to coagulation that blocks that particular vessel or an embolus will form leading to the blockage of a distant blood vessel resulting in myocardial infarction or stroke (Croce & Libby, 2007; Ross, 1999; Vanhoutte, 2009). The overview above outlined the pathogenesis of atherosclerosis which is the root of the number one cause of death in the United States (Go et al., 2013). Much research has focused on clinical assessment tools to recognize the earliest sign of atherosclerosis: endothelial dysfunction. The most widely used technique is the assessment of flow-mediated dilation (FMD) (McCully, 2012).

First Flow-mediated Dilation Study

In 1992 Celermajer and colleagues (1992) published their landmark study in *Lancet* showing the assessment of endothelial function and dysfunction via measurement of the femoral and brachial arteries with high resolution ultrasound. This study was the first to utilize a non-invasive measurement of endothelial function and therefore provided potential insight into the early stages of atherosclerosis (Vanhoutte, 2009). This elegant new measurement was grounded in a long line of experiments that took place over the 12 years prior to this seminal publication.

Importance of Endothelial Cells

The health of endothelial cells lining arteries is one of the most important factors in the onset and progression of atherosclerosis leading to CVD (Vanhoutte, 2009). The importance of vascular endothelial cells for normal vasodilation was first recognized in the early-1980s with a series of Nobel Prize winning experiments using excised animal arteries (Furchgott & Zawadzki, 1980). In these experiments excised rabbit aortas were stimulated by acetylcholine with the endothelium intact or with the endothelial cells

destroyed by mechanical rubbing of the interior surface of the artery. Under the intact endothelium condition normal vasodilation via relaxation of smooth muscle occurred. Conversely, under the destroyed endothelium condition no relaxation of smooth muscle occurred. The authors concluded that a healthy endothelial cell lining is obligatory for vasodilation to occur (Furchgott & Zawadzki, 1980). Furchgott and others confirmed their findings in rabbit aortas with follow-up studies in many mammal species (1983).

Discovery of Nitric Oxide

Furchgott speculated that a potent vasodilator substance or substances termed endothelium derived relaxing factors (EDRFs) were produced and released by healthy endothelial cells to lead to vasodilation (1983). Many subsequent experiments were conducted exploring different substances (Prostanoids, acetylcholine, adenosine diphosphate, thrombin, and others) that could play the role of the EDRFs. In the mid-eighties Palmer and colleagues (1987) provided strong evidence that NO was the most dominant EDRF. Their first study used endothelial cell free rabbit aortas and stimulated vasodilation by either infusing NO or by infusing EDRF from porcine endothelial cells. Interestingly, both stimuli led to very similar vasodilatory responses. Furthermore, they showed that the amount of NO released by the porcine endothelial cells could quantitatively account for the amount of vasodilation that occurred (R. Palmer et al., 1987). Therefore, it became clear that NO was the major EDRF (Moncada, Radomski, & Palmer, 1988). Palmer and colleagues were also the first group to show the amino acid L-arginine as the direct precursor to NO (1988).

Blood Flow-mediated Vasodilation

From the early studies of endothelial cells and EDRFs it was clear that certain chemicals (i.e. acetylcholine) could stimulate the release of EDRFs, but it was not until the mid-1980s that the role of blood flow in the modulation of vasodilation came into focus (Pohl, Holtz, Busse, & Bassenge, 1986; Smiesko, Kozik, & Dolezel, 1985). By inserting arterial catheters into canine arteries to measure diameter while blood flow was meticulously increased or decreased it was clear that increases in blood flow led to vasodilation and decreases in flow led to constriction (Pohl et al., 1986). These authors coined the term "flow mediated dilation" or FMD. In these same experiments the authors destroyed the endothelial cells by a balloon catheter and found that without endothelial cells no FMD occurs. Therefore, it was concluded that endothelial cells play the key regulatory role in flow mediated dilation.

Shortly after these early FMD studies a series of elegantly designed experiments led to the discovery of mechano-receptors on the luminal surface of endothelial cells (Cooke, Rossitch Jr, Andon, Loscalzo, & Dzau, 1991; Lansman, Hallam, & Rink, 1987; Olesen, Claphamt, & Davies, 1988). When stimulated by shear stress, due to an increase in blood flow, these mechano-receptors lead to the opening of ion channels causing hyperpolarization of the endothelial cells. Hyperpolarization of endothelial cells leads to the phosphorylation of endothelial cell nitric oxide synthase (eNOS) which leads to an increase in the production of NO (Boo et al., 2002; Dimmeler et al., 1999; Fisslthaler, Dimmeler, Hermann, Busse, & Fleming, 2000). Follow-up studies provided strong evidence to solidify the link between blood flow, shear stress, eNOS activation, and NO levels (Berdeaux et al., 1994; Tuttle et al., 2001).

Is the Flow-mediated Dilation Technique in Large Conduit Arteries NO Dependent?

Since most of the early studies that led to the conclusion that arteries respond to shear stress by vasodilation were conducted in excised animal vessels or smaller resistance vessels it was necessary to justify the assessment of flow-mediated dilation in conduit arteries. Two early studies provided this justification by showing a clear FMD response to ischemic conditions in brachial arteries (E. A. Anderson & Mark, 1989) and large coronary vessels (Nabel, Selwyn, & Ganz, 1990). Still the critical assumption that underlies the FMD assessment technique utilized by Celermajer et al (1992) is that the vasodilation that occurs in these conduit arteries is NO dependent. Evidence for this assumption was found by two studies utilizing a non-selective inhibitor of nitric oxide synthase (NG monomethyl-L-arginine [L-NMMA]). Joannides et al (1995) measured radial artery blood flow before and after 3 minutes of blood flow occlusion once under normal physiologic conditions then again during infusion of L-NMMA. There was a significant increase in blood flow observed without L-NMMA infusion that was completely abolished with infusion of L-NMMA. These results were replicated and expanded in a subsequent study that showed 5 minutes of blood flow occlusion led to completely NO dependent FMD, but 15 minutes of occlusion led to an FMD response that was not blocked by infusion of L-NMMA (Mullen et al., 2001). Therefore, it is now generally well accepted (see (K. Pyke et al., 2010) for one study with conflicting results) that the FMD response seen in conduit arteries after five minutes of blood flow occlusion is nitric oxide dependent.

Brachial Artery Flow-mediated Dilation: Clinical Relevance

Assessment of brachial artery flow-mediated dilation (BAFMD) has become an extremely popular measurement in cardiovascular research. In 2011 alone 199 peer reviewed papers were published using BAFMD (McCully, 2012). Soon after the first BAFMD paper was published the clinical relevance of the ability of the brachial artery to dilate was questioned. In an elegant study by Anderson and colleagues (1995) the relationship between BAFMD and coronary artery FMD was explored. Patients first underwent a coronary catheterization with direct measurement of flow-mediated vasodilation via infusion of acetylcholine. Next, within 24 hours of the catheterization procedure subjects underwent measurement of BAFMD. The authors found a very close relationship between BAFMD and coronary artery FMD. The ability of the BAFMD test to predict coronary FMD was 95%. Therefore, the health of the endothelial cells in large peripheral conduit arteries like the brachial artery is closely related to that of the coronary arteries. Furthermore, measurement of BAFMD is a well-established technique in the assessment of CVD risk in healthy and clinical populations (Inaba et al., 2010; D. H. J. Thijssen et al., 2010). A recent meta-analysis including 14 cohort studies totaling 5,547 subjects clearly showed the importance of FMD assessment in prediction of future CVD events. The compiled evidence showed that for every 1% or 1 standard deviation decline in FMD there is an 8% or 22% increase in the risk of a future CVD event, respectively (Inaba et al., 2010).

Brachial Artery Flow-mediated Dilation Methodological Considerations

Due to its ease of use, cost-effectiveness, and non-invasive nature BAFMD is now commonly assessed by use of high resolution B-mode ultrasound. The assessment of

BAFMD is conducted by imaging the brachial artery at rest for one minute to establish a baseline reading. The baseline reading is followed by occlusion of blood flow in the forearm by a blood pressure cuff inflated to 250 mmHg for five minutes. After four minutes of occlusion imaging begins again and continues for three to five minutes after cuff release (at 5 minutes) (D. H. J. Thijssen et al., 2010). The recorded images are then analyzed using specialized analysis software which detects artery diameter and blood flow velocity then calculates shear rate and blood flow (Woodman et al., 2001). The resulting FMD value is arrived at by calculating the percent increase in artery diameter from the pre-cuff occlusion baseline video to the peak diameter measured post cuff-release. BAFMD has been shown to be a stable and reliable assessment tool in many different laboratories (Hijmering et al., 2001; D. H. J. Thijssen et al., 2010; Welsch, Allen, & Geaghan, 2002).

Conversely, some authors have questioned the reliability, standardization, and validity of flow-mediated dilation due to the high variability seen within subjects (Peretz et al., 2007) and the fact that small methodological changes can lead to large changes in the outcome (Black, Cable, Thijssen, & Green, 2008; Mullen et al., 2001). Hijmering et al (2001) has pointed out the high variability of BAFMD response within subjects and concluded that it is useful to see group differences but not sensitive enough to detect individual changes. This critique is likely overly critical due to the many studies that have accurately assessed changes in FMD on an individual level (D. H. Thijssen et al., 2010). Nonetheless, BAFMD assessment in the research setting needs more standardization (R. A. Harris, Nishiyama, Wray, & Richardson, 2010; Hijmering et al., 2001; Peretz et al., 2007; D. H. J. Thijssen et al., 2010).

It has recently been demonstrated that the quantification of shear stress in the FMD response is likely very important to capture the whole picture of endothelial function (Padilla et al., 2008; K. E. Pyke, Dwyer, & Tschakovsky, 2004; K. E. Pyke & Tschakovsky, 2005; K. E. Pyke & Tschakovsky, 2007). Shear stress is the physiological stimulus that leads to the production and release of NO leading to the FMD response. Since shear stress is quantified from the product of blood flow velocity and artery diameter, assuming that blood viscosity remains constant, it has been shown that shear stress decreases significantly with increasing artery diameter (D. H. J. Thijssen et al., 2010). Average, peak, and area under the curve shear stress have all been recommended to normalize the FMD measurement. Due to conflicting results (G. Birk et al., 2012; Green et al., 2013; K. E. Pyke & Tschakovsky, 2007) and statistical errors elicited by improper use of normalization ratios (Atkinson et al., 2009) the current guidelines recommend reporting shear rate AUC from cuff release to peak diameter, but normalization is not recommended.

Other important technical considerations include occlusion duration, cuff placement, pre- or post-occlusion baseline diameter assessment, time-course measurement of artery diameter and blood flow velocity, and sonographer training. It has been shown that longer than 5 minutes of cuff occlusion leads to a non-NO dependent vasodilation (Mullen et al., 2001), therefore 5 minutes of occlusion is recommended for measurement of endothelium dependent dilation. Some early FMD studies utilized proximal (to the ultrasound probe) cuff placement instead of distal. One study showed a 7% FMD in response to distal cuff placement that was completely blocked by L-NMMA, but a 12% FMD in response to proximal cuff placement that was only partially blocked

by L-NMMA (Doshi et al., 2001). Some authors have reported FMD using post-cuff occlusion baseline diameter instead of the true resting diameter (Magen et al., 2005). The problem with using post-occlusion baseline diameter is that it has been shown that the artery responses to occlusion differ depending on the age of the subjects (D. H. Thijssen et al., 2008). Young subjects tend to slightly dilate during occlusion therefore lowering the calculated FMD percentage whereas older subjects do not respond to occlusion. These heterogeneous results to cuff occlusion call for standardization in the use of pre-cuff occlusion baseline in all studies (D. H. J. Thijssen et al., 2010). Due to the lack of sophisticated edge detection software and continuous image capture technology the early FMD studies (Celermajer et al., 1992) assessed baseline and peak diameter at set time points (i.e. 60 or 90 seconds). This technique of peak diameter assessment has been shown to potentially lead to the underestimation of FMD by 25-40% due to missing true peak diameter (Black et al., 2008). Logically, it is now recommended to measure artery diameter over the full time course of 180 seconds post-occlusion using advanced edge detection software (D. H. J. Thijssen et al., 2010; Woodman et al., 2001). The most challenging aspect of the FMD technique is acquisition of a clear image of the brachial artery walls. The most current methodological guidelines suggest that sonographers obtain at least 100 practice FMD assessments before their participation in research using this technique (R. A. Harris et al., 2010; D. H. J. Thijssen et al., 2010).

Many subject preparation considerations need to be addressed when using the FMD technique. FMD can be significantly affected by dietary intake (Tyldum et al., 2009), recent exercise (G. Birk et al., 2012), caffeine (Papamichael et al., 2005), alcohol (Hijmering, De Lange, Lorsheyd, Kraaijenhagen, & Van De Wiel, 2007), vitamin

supplements (Eskurza, Monahan, Robinson, & Seals, 2004), medications (Magen et al., 2005), and menstrual cycle stage (Hashimoto et al., 1995) therefore it is necessary to adequately control for all of these potential confounders when using the FMD technique.

Recent literature on endothelial function and psychological factors suggests that some of the high variability seen with BAFMD could be due to uncontrolled variables such as stress, anger, and happiness (Ghiadoni et al., 2000; Gottdiener et al., 2003; C. W. Harris et al., 2000; Miller et al., 2006; Shimbo et al., 2007; Spieker et al., 2002). Stress and anger have been shown to negatively affect BAFMD (Ghiadoni et al., 2000; Gottdiener et al., 2003; C. W. Harris et al., 2000; Miller et al., 2006; Shimbo et al., 2007; Spieker et al., 2002). Shimbo et al (2007) showed complete abolishment of BAFMD due to anger provocation (from 7.5% at baseline to 0.6% 90 minutes after anger provocation). Another study by Spieker et al (2002) showed that BAFMD decreased from 8.0% at baseline to 4.1% after induction of mental stress. Conversely, laughter brought on by watching a comical film has been shown to improve BAFMD by up to 22%, whereas watching a stressful movie can decrease BAFMD by 35% (Miller et al., 2006; Sugawara, Tarumi, & Tanaka, 2010). These psychological factors can be partially controlled by the recommended standard procedure of having subjects lay quietly in a dark room for 20 minutes before the BAFMD assessment begins (D. H. J. Thijssen et al., 2010).

The Impact of Aerobic Exercise on Endothelial Function

The arterial response to exercise training is characterized by immediate and short-lived functional improvements in vasodilatory capacity in response to increased shear stress brought on by aerobic exercise. These improvements begin within the first week or two of training and reach a plateau by the fourth week. After the fourth week of training

the arteries show marked structural changes leading to increased artery diameter that may plateau by the 8th week of training. Once the artery diameter is increased the shear stress levels will normalize thus allowing vasodilatory function to return to baseline levels (Tinken, Thijssen, Black, Cable, & Green, 2008).

Clarkson et al (1999) assessed FMD in 25 healthy male military recruits before and after ten weeks of aerobic exercise training. The exercise training elicited a 78% improvement in FMD showing that the vasculature of young healthy men of average fitness levels can be improved by aerobic exercise training. Conversely, this effect may not be seen in older healthy subjects. Eight weeks of cycle training led to no improvement in FMD in older men (D. Thijssen, De Groot, Smits, & Hopman, 2007). Although there is some controversy regarding the effects of exercise training on FMD in healthy populations (Green, Maiorana, O'Driscoll, & Taylor, 2004; Green et al., 2013), most studies using aerobic exercise interventions in patients with CVD, diabetes, or risk factors for these diseases show improvements in FMD (D. H. Thijssen et al., 2010).

A host of recent studies have used exercise training in patients with severe CVD and seen drastic improvements in endothelial function. An elegant study by Hambrecht and colleagues (2003) exemplified the powerful effect that exercise training can have on endothelial function in CVD patients and elucidated potential mechanisms for these improvements. They examined the effects of 4 weeks of exercise (60 min moderate intensity 7d/wk) on the left internal mammary artery (LIMA) of patients scheduled for coronary bypass surgery. The exercise intervention improved endothelium dependent vasodilation in the LIMA as assessed by acetylcholine (~100% increase) and adenosine (~150% increase; suggesting improvement in flow dependent dilation) responses in vivo.

The authors also assessed dilatory responses in vitro finding large improvements in dilation that were coupled with increases in eNOS mRNA (100%) and eNOS protein expression (200%). This was the first study to confirm the positive effects of exercise on NO expression and availability that was most likely stimulated by the shear stress induced via exercise. This study brought to light the mechanism of upregulation of eNOS in response to exercise training. Wisloff et al (Wisloff et al., 2007) randomized 27 men and women with post-infarction heart failure to 12 weeks of 3 d/wk of continuous exercise (47 min @ 70% VO_2peak), interval training (4x4' intervals at 90% VO_2peak), or a control group. After the training both exercise groups improved FMD greater than the control group. Interestingly the interval-training group improved FMD to a greater extent (4-11%) than the continuous group (4-8%) suggesting that higher intensity exercise may be better for FMD improvement. A study in patients with peripheral artery disease showed that 6 weeks of aerobic exercise 3 days/wk led to an improvement in FMD from 7.6 to 10.3% (Andreozzi, Leone, Laudani, Deinite, & Martini, 2007). A recent study by Hermann et al (2011) compared the effects of 8 weeks of high-intensity interval training versus a control group on FMD in stable heart transplant patients. The exercise group realized a significant improvement in FMD compared to the control group (3% improvement vs. no change).

The studies discussed above clearly show that patients with advanced cardiovascular disease can show drastic improvements in endothelial function from exercise training interventions. Endothelial function has also been shown to improve in subjects with increased cardiovascular disease risk factors such as hypercholesterolemia, hypertension, diabetes, and obesity. Twenty-two hypercholesterolemic patients

underwent 8 weeks of combined aerobic and resistance training or a control condition (Walsh et al., 2003). The subjects in the exercise group showed significant improvements in both FMD and the forearm blood flow response to acetylcholine demonstrating improvements in both resistance and conduit vessels that were not seen in the control group. Fifty-four elderly hypertensive patients with systolic 24-hour ambulatory blood pressure above 140 mm Hg were randomized to either 12 weeks of treadmill exercise or a control condition (Westhoff et al., 2007). The subjects in the treatment group had a significant improvement in FMD (5.6 to 7.9%). Diabetics have also been shown to greatly improve endothelial function in response to exercise training. Fifteen type 2 diabetic subjects underwent 12 weeks of combined aerobic and resistance exercise training and realized improvements of endothelial function in both conduit (FMD increased from 1.7 to 5%) and resistance vessels (forearm blood flow response to acetylcholine). Schjerve et al (2008) randomized 40 obese adults to either moderate exercise training, high-intensity interval training, or strength training for 12 weeks and assessed endothelial function before and after the intervention. Flow-mediated dilation was significantly improved in all three groups, but improved to a greater extent in the high-intensity interval training group.

Taken together the above studies show that aerobic exercise training can elicit drastic improvements in endothelial function in populations ranging from young healthy men to and men and women with severe heart failure and CVD.

High-intensity Interval Training in the Improvement of Endothelial Function

A thorough review of the literature on high-intensity interval training and endothelial function yields a limited number of studies. Only eight human subject

exercise interventions that directly compared HIT to another form of exercise training for the improvement of endothelial function could be found (Ciolac et al., 2010; Currie et al., 2013; T. Moholdt et al., 2011; Molmen-Hansen et al., 2012; Schjerve et al., 2008; A. Tjonna et al., 2009; A. E. Tjonna et al., 2008; Wisloff et al., 2007). Of these eight exercise interventions six showed HIT to be superior to moderate or continuous exercise training for the improvement in endothelial function (Ciolac et al., 2010; Molmen-Hansen et al., 2012; Schjerve et al., 2008; A. Tjonna et al., 2009; A. E. Tjonna et al., 2008; Wisloff et al., 2007) and two showed improvements in both groups with no group difference (Currie et al., 2013; T. Moholdt et al., 2011). Interestingly six of the eight articles were written by the same research group (T. Moholdt et al., 2011; Schjerve et al., 2008; A. Tjonna et al., 2009; A. E. Tjonna et al., 2008; Wisloff et al., 2007). One study compared sprint interval training (4-6 wingates, 3 times/wk) to continuous endurance exercise (40-60 min at 65% of VO_{2max}) in young healthy subjects and saw similar improvements in FMD in both groups (Rakobowchuk et al., 2008). Two other studies using heart transplant patients (Hermann et al., 2011) and post-stent procedure patients (Munk, Staal, Butt, Isaksen, & Larsen, 2009) that did not use a comparison or a control group showed substantial improvements in endothelial function in response to HIT.

The most robust of the randomized trials comparing HIT to other interventions is perhaps the RCT conducted by Wisloff et al in heart failure patients (2007). The most striking finding of this study was the fact that the drastic improvement in FMD elicited by the continuous exercise (2 fold increase) was actually surpassed by the improvement in the HIT group (2.75 fold increase). The only study directly comparing HIT with moderate exercise and strength training in obese adults for the improvement in

endothelial function was a 12-week intervention conducted in Norway (Schjerve et al., 2008). Forty subjects were randomized to each of the three groups for 3 days/wk of supervised exercise. After the 12-week intervention FMD improved in all three groups, but the HIT groups improved significantly more than the other two exercise groups. Similarly, 35 metabolic syndrome patients were randomized to 16 weeks of either HIT or moderate aerobic exercise training for 3 days a week (A. E. Tjonna et al., 2008). Both groups had significant improvements in FMD, but the HIT group (5 to 14%) improved more than the moderate group (4-8%). HIT has also been shown to be more effective for the improvement in endothelial function than a multi-treatment centered approach (consisting of moderate exercise, group meetings, as well as physical, psychological, and nutritional therapy in overweight and obese adolescents (A. Tjonna et al., 2009). After the 12-month intervention the HIT group improved FMD from 6-12% whereas the multi treatment group did not significantly improve FMD. Another study randomized 44 healthy women who had a family history of hypertension to16 weeks of HIT or continuous exercise (Ciolac et al., 2010). After the 16-week intervention the HIT group significantly lowered their endothelin-1 levels and significantly increased their NO levels to a greater extent than the continuous group, both factors demonstrating improvements in endothelial cell health. The only study from the Norwegian group that did not show HIT to be superior for the improvement in endothelial function was a 12-week RCT comparing usual cardiac rehabilitation to HIT in post myocardial infarction patients (T. T. Moholdt et al., 2009). Both the HIT and the usual care groups had improvements in FMD of approximately 2.5 to 3.0% in absolute terms. The authors explained that the usual care was more intense than the moderate exercise training in their previous studies

and that may have been the reason for the lack of dominance in the HIT group. Lastly, the most recent study comparing HIT to continuous exercise randomized 22 subjects with documented coronary artery disease to 12 weeks of 2 days/week of HIT (10-1 minute intervals at 89% of peak power separated by 1 minute of recovery) or continuous cycling (30-50 min at 58% of peak power) plus each group completed one day/week of continuous exercise on their own (Currie et al., 2013). After the 12-week intervention both groups improved FMD significantly (~1.5-2% absolute increase), but there was no difference between HIT and continuous exercise.

The results from the available data on the role of HIT in the improvement of endothelial function suggest that HIT may be superior to continuous exercise training. The potential mechanism for a greater improvement in endothelial function is the higher level of shear stress induced by HIT compared to moderate intensity exercise leading to greater stimulus on the endothelial cells. Twelve weeks of HIT was shown to increase antioxidant capacity and reduce oxidized LDL in heart failure patients more than moderate exercise (Wisloff et al., 2007). Similarly, three weeks of interval exercise improved NO availability more than continuous exercise (Deljanin Ilic et al., 2009). Lastly, a metabolic syndrome rat model study showed that HIT improved endothelial function, HDL, eNOS, endothelial cell caveolae, insulin action, free fatty acid uptake, and lipolysis more than continuous exercise (Haram et al., 2009). Therefore, potential mechanistic studies do exist to explain a superior improvement with HIT, but much more research is needed in this area. The small number of direct comparison studies in humans shows the need for more research of this type as well. The fact that only one study has been conducted in obese subjects comparing HIT to continuous exercise for the

improvement in endothelial function signifies a great need for research in obese subjects. The next section will focus on the potential physiological mechanisms for the improvement in endothelial function with exercise training with a special consideration for high-intensity interval training.

Mechanisms for the Improvement of Endothelial Function with Exercise

The improvements in endothelial function realized by exercise training are hypothesized to be largely induced by the increases in shear stress brought on by exercise (G. K. Birk et al., 2012; Dimmeler et al., 1999; Green et al., 2004; Green, 2009; Lenk, Uhlemann, Schuler, & Adams, 2011; Ribeiro, Alves, Duarte, & Oliveira, 2010; Tinken et al., 2009). Acutely shear stress in the form of increased anterograde flow was shown to improve BAFMD in response to heating, handgrip exercise, and cycling (Tinken et al., 2009). Interestingly, in this same study the other arm had the shear rate manipulated by inflating a blood pressure cuff to 60 mmHg during each of the three interventions, therefore causing an increase in retrograde flow. Instead of increasing BAFMD like in the non-cuffed arm, the FMD response in the cuffed arm was actually decreased. A similar effect was seen with eight weeks of exercise training (G. K. Birk et al., 2012). In this elegantly designed study young healthy men underwent eight weeks of cycle exercise with a blood pressure cuff on one arm during each exercise session. Each two weeks of the intervention BAFMD was assessed in each arm. A significant increase in BAFMD was observed at 2 weeks in the non-cuffed arm, but the cuffed arm did not improve FMD at any time point over the 8 week intervention. These two studies underscore the importance of shear stress in the adaptations that occur in endothelial function in response to exercise training. Besides the direct role of increased shear stress, exercise

has been shown to improve multiple risk factors that induce an indirect effect on endothelial function (Haram et al., 2009; Ribeiro et al., 2010). The subsequent sections will be dedicated to discussing the potential mechanisms by which increased shear stress and reduced CVD risk factors lead to improvements in endothelial function. The main theory for the increased endothelial cell function due to exercise revolves around increased nitric oxide availability (Green et al., 2004). Increased nitric oxide availability can occur by one or more of the following mechanisms: 1) Decreased reactive oxygen species (ROS), 2) Improved antioxidant capacity, 3) Increased eNOS activation and expression, 4) Decreased endothelial cell death, or 5) Improvements in endothelial progenitor cell level and function.

Antioxidant effect of exercise. Exercise training has been shown to exert an antioxidant effect (Adams et al., 2005; Edwards et al., 2004; Goto et al., 2007; Rush, Laughlin, Woodman, & Price, 2000; Wisloff et al., 2007). A hallmark of endothelial dysfunction is the endothelial expression of angiotensin II which leads to vasoconstriction and activation of a multi-enzyme complex known as NAD(P)H oxidase which causes a major increase in reactive oxygen species (ROS) formation (Adams et al., 2005). In the presence of high levels of ROS, NO is degraded into an inactivated molecule ($ONOO^-$). Furthermore, high levels of ROS lead to the oxidation of LDL particles in the artery intima. Oxidized LDL are readily engulfed by macrophages which eventually turn into foam cells leading to the inflammatory milieu of atherosclerosis which further decreases endothelial function (Ribeiro et al., 2010).

Perhaps the most elegant study evaluating the antioxidant effects of exercise training involved 22 CAD patients with a history of at least 10 myocardial infarctions

each (Adams et al., 2005). Half of the patients were randomized to four weeks of 60 minutes/day rowing or cycling in the hospital and half were randomized to the control group. After the intervention *in vivo* endothelial function improved by ~100% in the exercise group and did not change in the control group. Since all of the subjects had elected to undergo coronary artery bypass surgery the researchers were able to harvest a portion of their left internal mammary artery (LIMA) before surgery (after the 4 week intervention). In vitro measurement of NAD(P)H oxidase subunits showed a 69% reduction in $gp91^{phox}$ mRNA (similar to statin use: 60% reduction), as well as decreases in other important subunits including $p22^{phox}$ and Nox4 which led to a 58% reduction in ROS formation. Also subjects in the exercise group showed a 77% lower level of AT_1-R mRNA and 46% lower protein expression compared to the control group (AT_1-R is the major activation pathway between Ang II and NAD(P)H oxidase). Furthermore, AngII mediated vessel contraction decreased by 49%. Importantly, correlations between changes in *in vivo* endothelial function and protein expression and ROS generation were moderate to high ($r = 0.63$ to 0.80). This study clearly showed the powerful antioxidant effect of exercise in the reduction of ROS as well as vasoconstriction (Adams et al., 2005). Another study showed that 12 weeks of aerobic exercise (3 days/wk, cycling or walking) led to increased FMD (7.9 to 11.1%), nitrite/nitrate, superoxide dismutase, and decreased oxidative stress in a group of CAD patients (Edwards et al., 2004). Another important mechanistic study showed that exercise training in pigs led to increases in super-oxide dismutase enzyme levels and activity in coronary vessels which would improve antioxidant capacity (Rush et al., 2000).

Exercise increases endothelial nitric oxide synthase. Increases in eNOS expression and activity have also been shown to be linked to the exercise induced increases in NO availability (Davis, Cai, Drummond, & Harrison, 2001; Delp & Laughlin, 1997; Haram et al., 2009; Sessa, Pritchard, Seyedi, Wang, & Hintze, 1994). The first study linking increases in shear stress to up-regulation and increased activation of eNOS was a well-designed study by Sessa and colleagues (1994). Ten days of two hours/day of exercise in dogs led to significant increases in NO release via acetylcholine infusion to the coronary arteries and micro vessels. The authors discovered the potential mechanism of this increase in NO by measuring an increase eNOS gene expression in the coronary vessels. These findings were confirmed and expanded on by others showing that the activation of eNOS from shear stress occurs via the akt dependent phosphorylation pathway (Dimmeler et al., 1999) and another study outlining the time course of these adaptations (Delp & Laughlin, 1997). This work was followed up in 2001 by a study that showed the short term effect of shear stress on eNOS is the upregulation of eNOS transcription and the long-term effect is the stabilization of eNOS mRNA (Davis et al.). A landmark study discussed above was the first to show the upregulation of eNOS in humans by use of the LIMA model in coronary artery bypass patients (Hambrecht et al., 2003). In brief exercise training elicited a 100% increase in eNOS mRNA and a 200% increase in eNOS protein expression. Therefore there is strong and clear evidence that increases in shear stress via exercise lead to improvements in NO bioavailability via upregulation of eNOS.

Endothelial Progenitor Cells. A relatively new area of research relating to improvements in endothelial function involves the reservoir of tissue specific stem cells

in the bone marrow and general circulation called the endothelial progenitor cells (EPCs) (Lenk et al., 2011). Individuals with cardiovascular disease have been shown to have decreased number and functional ability of these cells (Hoetzer et al., 2007; Lenk et al., 2011; Steiner et al., 2005). Interestingly, it has been shown that shear stress in the bone marrow leads to activation of eNOS which leads to the activation, stimulation, and migration of EPCs to regenerate damaged vessels (Aicher et al., 2003). Therefore, the common mechanism of improved endothelial cell function via increases in shear stress also applies to the generation of new endothelial cells in the bone marrow (Lenk et al., 2011). Consequently, it has been shown that physical activity increases the production and circulating number of EPCs via a partially NO-dependent pathway (Laufs et al., 2004). Exercise has also been shown to increase the migratory ability of EPCs to the damaged endothelium (Lenk et al., 2011).

EPCs are stimulated by acute exercise via release of PGC-1 α, hypoxia inducible factor-1, vascular endothelial growth factor, stromal cell derived factor-1, erythropoietin, and oxidative stress (which is essential for hypoxia or EPO induced mobilization of EPCs) (Lenk et al., 2011). Finally, it has been shown that the shear stress induced by exercise leads to inhibition of apoptosis of endothelial cells (Dimmeler, Haendeler, Rippmann, Nehls, & Zeiher, 1996) and EPCs (Melino et al., 1997). A study that used human umbilical venous endothelial cells showed the apoptosis induced by tumor necrosis factor α was completely blocked by shear stress (Dimmeler et al., 1996). It has also been shown that shear stress related release of NO in the bone marrow is crucial in regulating the balance between EPC apoptosis and necrosis and therefore producing healthy and functional EPCs (Melino et al., 1997). This exciting and new field of

research relating to endothelial function shows promise of possible pharmacological targets as well as another prominent mechanism by which exercise can improve artery function.

Improvement in CVD Risk Factors Indirectly Effecting Endothelial Function. Finally, the last potential mechanism in which exercise can improve endothelial function is the indirect mechanism of improving cardiovascular disease risk factors that have an influence on endothelial cell health (Ribeiro et al., 2010). Exercise is known to improve inflammatory cytokines and markers of inflammation (Gaesser, Angadi, Ryan, & Johnston, 2012), increase high density lipoprotein (HDL) levels (Kodama et al., 2007), decrease LDL (Snowling & Hopkins, 2006), and improve glucose control (Snowling & Hopkins, 2006). Reduced LDL particles leads to less oxidized LDL in the arterial intima. High serum HDL levels have been shown to prevent the oxidation of LDL and increase eNOS activity thereby protecting endothelial cells (Young, Karas, & Kuvin, 2004). A reduction in inflammatory mediators causes a reduction in cellular adhesion molecules on the surface of endothelial cells leading to less inflammation in the artery walls and improved endothelial function (Ribeiro et al., 2010). Shear stress due to exercise has also been shown to directly reduce VCAM expression on endothelial cells (Ando et al., 1994). Lower glucose levels leads to lower oxidative stress which is directly related to NO availability and therefore endothelial function (De Vriese, Verbeuren, Van de Voorde, Lameire, & Vanhoutte, 2000).

Section 2: Blood Glucose Control

Pathogenesis of Impaired Glucose Tolerance and Type 2 Diabetes

The control of normal blood glucose levels depends on the dynamic interplay between three primary components and many other secondary components (DeFronzo, 2009; S. Kahn, Suvag, Wright, & Utzschneider, 2012; Stumvoll, Goldstein, & van Haeften, 2005). The three primary components that dictate blood glucose levels are endogenous glucose release from the liver, glucose uptake by the tissues, and insulin release by the β-cells of the pancreas.

The more complete picture of the determinants of glucose control includes the additions of adipocytes, pancreatic α-cells, the brain, the kidneys, and the digestive tract. These eight crucial components of glucose control have been called the ominous octet (DeFronzo, 2009). The natural history in the progression from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) to type 2 diabetes (T2D) involves a reduction in insulin sensitivity in the liver that leads to increased glucose production and secretion, a reduction in insulin sensitivity in the peripheral tissues (muscle, fat, and others), and a decline in pancreatic β-cell function (Jallut et al., 1990; Weyer, Bogardus, Mott, & Pratley, 1999). Both obesity (B. B. Kahn & Flier, 2000) and physical inactivity (Knowler et al., 2002) lead to insulin resistance which puts extra strain on the β-cells to produce and release more insulin to control blood glucose. Eventually the β-cells fail to compensate for the insulin resistance and the result is abnormal glucose levels.

The description above is an over-simplified explanation of the progression of T2D since it has been clearly shown that β-cell dysfunction occurs early in the onset of IGT long before hyperglycemia ensues (S. Kahn, 2003). Furthermore, the sequence of events

moving from NGT to IGT to T2D differs by ethnicity (M. A. Abdul-Ghani et al., 2007), but the onset of diabetes is typically highly dependent on β-cell function (M. A. Abdul-Ghani, Tripathy, & DeFronzo, 2006; DeFronzo, 2004; DeFronzo, 2009; S. Kahn, 2003).

Pancreatic B-Cell Function

In order to truly assess β-cell function the change in glucose and insulin in response to a meal (oral glucose tolerance test: OGTT) plus the level of insulin resistance must all be accounted (DeFronzo, 2009). Simply measuring the plasma insulin response to a glucose challenge does not provide a valid measure of β-cell function. Therefore, the gold standard for measuring β cell function involves the measurement of the insulin secretion/insulin resistance index, also known as the disposition index (Delta Insulin/Delta Glucose ÷ Insulin resistance). In the upper range of NGT (120-139 mg/dl) for the 2 hour glucose level during an OGTT the disposition index reveals that even though glucose levels are considered "normal" these individuals have lost approximately two-thirds of their β-cell function. Furthermore, individuals in the upper end of IGT (180-199 mg/dl at the 2 hour OGTT mark) have already lost 80-85% of their β cell function. Therefore before diabetes is even diagnosed the individual could have drastically impaired β-cell function (M. A. Abdul-Ghani et al., 2006; M. Abdul-Ghani, Jenkinson, Richardson, Tripathy, & DeFronzo, 2006; DeFronzo, 2009; Ferrannini et al., 2005; Gastaldelli, Ferrannini, Miyazaki, Matsuda, & DeFronzo, 2004).

Evidence from deceased individuals also shows the importance of β-cell function in the early onset of IGT. Data from 124 autopsies showed that β-cell volume is ~50% lower in IGT subjects compared to NGT. The rate of β-cell apoptosis was 10 fold higher in lean diabetics and three-fold higher in obese diabetics compared to their non-diabetic

counterparts (Butler et al., 2003). Data from the Pima Indians in Arizona showed that the difference between individuals with IGT that transitioned to T2D and those that did not was their β-cell function (Weyer et al., 1999). In a sample of 150 overweight Latino children (mean age = 11 years) with a family history of T2D it was shown that 28% already had IGT and those with IGT had a 16% lower disposition index suggesting they already had impaired β-cell function (M. Goran et al., 2004). Therefore, β-cell dysfunction is not a part of T2D pathogenesis that occurs later in the disease progression, but it is seen very early in the onset of "Pre-Diabetes" and may represent the determining factor in the progression of the disease.

Factors affecting β cell function include: advancing age, genetics, insulin resistance, lipotoxicity, glucotoxicity, amylin, and incretins. B-cell function is found frequently in families and first degree relatives of individuals with T2D are much more likely to have diabetes (Stumvoll et al., 2005). Although the mechanistic basis is not yet well developed it is hypothesized that insulin resistance leads to progressive β-cell failure due to an "over-working" of the β-cells. Lipotoxcity can ensue when adipocytes become maximally filled with free fatty acids (FFAs) and they start to release FFAs into the circulation and these FFAs are absorbed and stored by many tissues around the body including skeletal muscle, heart, pancreas, liver, and the brain. This excess build-up of FFAs in non-adipocyte tissue leads to insulin resistance in those tissues as well as impaired insulin release from the pancreas (DeFronzo, 2009). Similarly, glucotoxicity from long-standing hyperglycemia will eventually lead to β-cell damage. Amylin or Islet-Amyloid Poly Peptide (IAPP) which is co-secreted with insulin, has been found to build up in the β-cells and lead to decreased function. The evidence for this in humans is still in

its infancy, but amyloid area of the pancreas in baboons has been shown to predict β-cell function well (Guardado-Mendoza et al., 2009). Finally, very important stimulating agents for the release of insulin in response to food intake (account for 2/3 of insulin release) are the gut derived hormones known as incretins. The two dominant incretins are glucose-dependent insulinotropic polypeptide or GIP and glucagon-like peptide 1 or GLP-1. Normally release of incretins stimulates insulin release and blocks glucagon release. T2D causes a reduction in GLP-1 and resistance to GIP in the β-cells (Holst & Gromada, 2004).

Insulin Resistance and Glucose Release in the Liver

During the fasting state the brain accounts for 50% of the glucose uptake. This demand is met mostly by glucose production in the liver. Normal fasting glucose production is 2 mg/kg/min, but in individuals with T2D it is 2.5 mg/kg/min (amounting to ~25-30 g extra/night). This high fasting glucose occurs in the presence of 2.5-3 fold higher insulin levels, therefore it is clear that hepatic glucose production and release are responsible for the higher fasting glucose levels (Consoli, Nurjhan, Reilly Jr, Bier, & Gerich, 1990; DeFronzo, Ferrannini, & Simonson, 1989). When the liver is not insulin resistant it responds to insulin by inhibiting the production and release of glucose, but in the insulin resistant state this mechanism is deficient leading to excess glucose release and production in the liver (DeFronzo et al., 1989). The extra glucose release from the liver is also caused by increased glucagon and enhanced hepatic sensitivity to glucagon (Baron, Schaeffer, Shragg, & Kolterman, 1987), lipotoxicity leading to increases in the rate limiting enzymes of hepatic gluconeogenesis (Gastaldelli et al., 2000), and glucotoxicity leading to increases in the rate limiting enzyme for glucose release from the

liver (Clore, Stillman, & Sugerman, 2000). All of these factors together lead to excess endogenous glucose production and release into the blood stream by the liver. Interestingly, it has been shown by use of insulin receptor knock-out models in mice that only when liver and β-cell insulin receptors are knocked out does it lead to hyperglycemia. The knock-out models for the muscle, adipocytes, and neurons led to no change in glucose control. Therefore, the insulin sensitivity of the liver is extremely important in the pathogenesis of T2D and may be the major factor in the control of blood glucose during fasting (Stumvoll et al., 2005).

Insulin Resistance in the Skeletal Muscle

Skeletal muscle insulin resistance could account for approximately 85-90% of the total body impairment of glucose uptake (DeFronzo, 2009). The most prominent aspect of insulin resistance in the muscle is the impairment of the post-binding effects of insulin (DeFronzo, 2004). The pathway of insulin leading to glucose transport into the muscle cell starts with insulin binding to receptor then phosphorylating key tyrosine portions of the β chain on the receptor. Phosphorylation results in insulin receptor substrate-1 (IRS-1) moving to the cell membrane where is binds to the insulin receptor and PI 3-kinase and Akt which results in glucose being transported into the cell, but also activation of nitric oxide synthase leading to vasodilation and activation of lipid, protein, and glycogen synthesis (DeFronzo, 2009). In T2D the ability of IRS-1 to activate PI 3-kinase is severely impaired leading to not only the inability of GLUT 4 to transport glucose into the cell, but impaired activation of NOS leading to endothelial dysfunction, and impairment in glucose metabolism and glycogen synthesis. Interestingly, insulin normally inhibits mitogen activated protein kinase (MAP kinase), but in insulin resistance

MAP kinase does not become inhibited which leads to inflammation, atherosclerosis, lying down of collagen, and smooth muscle proliferation. Since there is so much insulin being released MAP kinase gets hyper-stimulated and this may explain a large portion of the connection between insulin resistance and atherosclerosis (DeFronzo, 2004; DeFronzo, 2009).

Secondary Determinants of Blood Glucose Control

The ability of the gastrointestinal tract to release incretins in response to a meal as well as the sensitivity of the pancreas to these molecules has also been shown to play a key role in insulin secretion and glucose control. For example GLP-1 is a strong inhibitor of glucagon release therefore inhibiting endogenous glucose release after a meal. GLP-1 deficiency in T2D leads to a subsequent rise in blood glucose due to endogenous glucose release even after a meal. GIP is a potent stimulator of insulin release, but in T2D the β-cells become resistant to its effects (Holst & Gromada, 2004). The pancreatic α-cells are responsible for the production and release of glucagon and fasting levels have been shown to be elevated in T2D. This slight glucagon elevation coupled with hypersensitivity to the hormone in the liver may be largely responsible for increases in fasting glucose levels (Baron et al., 1987). The kidneys of non-diabetic individuals have a certain level of glucose that they can filter out of the blood each day then reabsorb into the blood stream. Glucose that exceeds this maximal reabsorption rate is excreted in the urine. Animal models and in vitro kidney studies have shown drastically increased ability of the kidneys to reabsorb glucose in T2D. This is most likely an adaptation of the body to conserve glucose since the lack of glucose in cells signals a starvation state. Therefore the extra reabsorption of glucose by the kidneys also adds to hyperglycemia (DeFronzo,

2009). Finally, the brain has recently been implicated as a key player in the hyperglycemia octet. It is hypothesized that the normal cerebral response to increasing insulin levels in the blood is appetite suppression, increased glucose uptake, and decreased gluconeogenesis stimulation in the liver. Conversely, in T2D there may be insulin resistance in the brain leading to a higher level of impaired glucose control.

The Role of Inflammation in the Pathogenesis of Impaired Glucose Control

Inflammation in the pancreas as well as systemically has been shown to be related to IGT and the onset of T2D (Donath & Shoelson, 2011; Pickup, 2004). One of the earliest inflammatory markers that was observed to be associated with IGT was tumor-necrosis factor α (TNF-α). It was originally hypothesized that the enlarged adipocytes were the direct source of TNF-α, but now it is well accepted that most of the TNF-α comes from macrophages around adipocytes, hepatocytes, β-cells, and myocytes (Donath & Shoelson, 2011; Weisberg et al., 2003). Other pro-inflammatory cytokines and chemokines that are released from the macrophages and found to accumulate during obesity, IGT, and T2D are interleukin-1β (IL-1β), IL-6, and chemokine ligands 2, 3, and 8 (CCL2, CCL3, and CXCL8) (Donath & Shoelson, 2011; Weisberg et al., 2003).

Although macrophages are the most prevalent immune cell found in adipose tissue other studies have shown increases in mast cells and T-cells in patients with T2D. The increase in macrophages around adipocytes is most likely caused by hypoxia and cell death. Hypoxia in adipose tissue is mostly likely caused by rapid fat cell growth and proliferation in which angiogenesis cannot keep up with. Therefore, the expanding adipose tissue becomes slightly hypoxic. Hypoxia stimulates macrophages to accumulate in the area which eventually lead to angiogenesis, but in the early term leads to a pro-

inflammatory state. This hypoxia also leads to cell death in which macrophages aggregate around dead adipocytes forming crown-like structures and releasing many pro-inflammatory mediators (Donath & Shoelson, 2011). Furthermore, activation of the NF-kβ and JNK pathways brought about by toll-like receptors (TLRs), FFAs, and advanced glycation end-products associated with IGT and T2D also leads to release of more pro-inflammatory cytokines which cause insulin resistance in the tissues from which they originate (liver, adipocytes, etc) as well as affect other tissues including blood vessels, cardiac and skeletal muscle, and leukocytes. Therefore, the immune system involvement in IGT and T2D is well documented, but extremely complex. The mechanisms involve many different pathways, cytokines, chemokines, and adipokines that are all involved in the causes of pancreatic β cell dysfunction as well as central and peripheral insulin resistance (Donath & Shoelson, 2011; Pickup, 2004).

The Use of Continuous Glucose Monitoring to Assess Glucose Control

Early continuous glucose monitoring technology began in the 1960s with intravenous catheters that recorded blood glucose levels every 1 second to every 15 minutes. This method was associated with significant risk of infection as well as thrombosis. Improvements made over the past 20 years have led to the development of glucose devices that sample from the interstitial space, are less invasive, and have much lower incidence of complications. Modern continuous glucose monitors use a micro-dialysis catheter that is inserted subcutaneously. The sensor contains an enzyme-based electrode (glucose oxidase) that detects current created by the oxidation of glucose and oxygen to create hydrogen peroxide. The oxidation of glucose via glucose oxidase removes one electron from each glucose molecule which results in a measureable

electrical current that can be converted into a blood glucose value (Penfornis, Personeni, & Borot, 2011).

The MiniMed continuous glucose monitor was the precursor to Medtronic iPro2 device which is being used in this study and was released in March of 2012. The validity of the MiniMed device to accurately assess glucose has been shown to be good (Wallace et al., 2006). A recent review pooled all of the studies that compared blood glucose values and hemoglobin A1c values to those obtained by the MiniMed device and found good correlations for blood glucose r = 0.73-0.92 and for hemoglobin A1c (HBA1c) r = 0.53–0.59. The review also looked at controlled studies that used CGM devices to improve glucose control in diabetics and found a significant reduction in HBA1c. Furthermore, the authors stated that the use of these devices could lead to substantial reductions in morbidity and mortality associated with diabetes (Tavris & Shoaibi, 2004). CGM technology has shown great utility in helping diabetic patients detect periods of hyper- or hypoglycemia (Hay, Wilmshurst, & Fulcher, 2003; S. Praet et al., 2006). There has been some concern about the lag time between glucose levels in the blood compared to the interstitial space (Cengiz & Tamborlane, 2009; Riddell & Perkins, 2009). Most studies show that a 5-10 minute lag time in the change in blood glucose compared to interstitial glucose exists (Boyne, Silver, Kaplan, & Saudek, 2003). Due to greater interstitial space glucose utilization, CGM use during exercise exaggerates this lag (Riddell & Perkins, 2009). With these problems in mind all CGM devices account for this time lag with their built in algorithms, but measurement during exercise may still be problematic (Penfornis et al., 2011).

The recent application of CGM devices in exercise studies has opened up a new realm of possibilities for exercise physiologists to obtain a more complete picture of glucose control with a relatively non-invasive measurement. Recent studies using CGM before and after exercise interventions have shown promising results in its utility in exercise research (Cauza et al., 2005; Gillen et al., 2012; Little et al., 2011; Mikus, Oberlin, Libla, Boyle, & Thyfault, 2012). Also CGM may give an insightful picture of glucose control in subjects who have normal fasting glucose levels, but may be in the early phases of progression to IGT (Ma et al., 2011).

Finally, the assessment of glycemic variability has shown importance in the prediction of CAD severity and diabetic complications (Monnier, Colette, & Owens, 2008; Monnier & Colette, 2011; Su et al., 2011). Even more so than sustained hyperglycemia, high glycemic variability is associated with increases in oxidative stress which accounts for many of the diabetic complications due to advanced glycation end-products in T2D (Monnier et al., 2008). CGM represents the most accurate means of assessing glycemic variability. Therefore, the use of continuous glucose monitoring in exercise and diabetes research is a valid and meaningful method of assessing glucose control in subjects anywhere on the spectrum from NGT to T2D.

The Effect of Exercise on the Various Aspects of Glucose Control

It has become quite clear that high levels of physical fitness as well as physical activity help to prevent the occurrence of IGT and T2D (Helmrich, Ragland, Leung, & Paffenbarger Jr, 1991; LaMonte, Blair, & Church, 2005). Data from the Aerobics Center Longitudinal Study showed that being in the lowest fitness quartile of a group of 8633 men led to a 1.9 fold higher risk of developing IGT and a 3.7 fold higher risk of

developing T2D after 6 years of follow-up (Wei et al., 1999). This protection from the onset and progression of T2D afforded by exercise is most likely due to a multitude of different effects encompassing all aspects of the pathophysiology of impaired glucose control discussed above (Booth et al., 2012). The following sections will focus on the improvements in the three main components of glucose control (β-cell function, hepatic insulin resistance, and peripheral insulin resistance) due to exercise. Each section will also include a discussion on the research conducted using HIT on each specific parameter.

The Effect of Exercise on β-Cell Function. Only seven studies to date have been published on the effects of exercise on β-cell function in humans (Bloem & Chang, 2008; Dela, von Linstow, Mikines, & Galbo, 2004; S. Kahn et al., 1990; Krotkiewski et al., 1985; Malin & Kirwan, 2012; Slentz et al., 2009; T. P. Solomon et al., 2010). The first study published in this area showed that 3 months of aerobic exercise in T2D patients lead to improvements in insulin secretion during an OGTT (Krotkiewski et al., 1985). Another early study used six months of intensive endurance exercise in 13 healthy older men and saw a 36% increase in insulin sensitivity. Interestingly, the authors stated that β-cell function decreased because they observed a reduction in the acute phase insulin response (AIR) to the OGTT. The reduction in AIR was most likely caused by the improvement in insulin sensitivity and without the use of the disposition index to assess β-cell function we cannot be sure of the results (S. Kahn et al., 1990). A more recent study in healthy sedentary elderly men and women showed that 7 consecutive days of one hour per day of aerobic exercise led to a 29% improvement in the disposition index along with a 57% improvement in insulin sensitivity. Similar to the study above AIR

significantly decreased, but the use of the disposition index clearly shows that this decrease was likely due to the large increases in insulin sensitivity and β-cell function (Bloem & Chang, 2008). Another recent study showed that three months of five days/week of exercise at 75% of VO_{2max} in T2D subjects led to improvements in β-cell function in the subjects who started out as moderate insulin secretors, but those that started out as low secretors did not improve at all. The findings suggest that there may be a point of no return in β-cell dysfunction (Dela et al., 2004). A study in 76 pre-diabetic adults used 12 weeks of 5 days/week aerobic exercise for 60 minutes at 85% of maximum heart rate and found improvements in β-cell function across all pre-diabetes subtypes (Malin & Kirwan, 2012).

The only study in the literature to assess different doses and titrations of exercise for the improvement of β-cell function was the STRRIDE trial. 237 subjects were randomized into either the 1) high amount vigorous exercise group (equivalent to 20 miles/week of jogging); 2) low-amount vigorous intensity group (equivalent of jogging 12 mile/week); or 3) low-amount moderate intensity (equivalent of 12 miles/week of walking). The results from the intravenous glucose tolerance test showed that the moderate group had significantly greater improvements in insulin sensitivity (24 hours after the last session (Houmard et al., 2004) and 14 days after (Bajpeyi et al., 2009)) and β-cell function (Slentz et al., 2009), but no reduction in the AIR. The authors hypothesized that the moderate exercise led to more fat oxidation (evidenced by a greater decrease in triglycerides in the moderate group (Slentz et al., 2007)) than the vigorous exercise and this may have caused subsequent decreases in liver, adipocyte, and

pancreatic lipid accumulation leading to overall insulin sensitivity improvements as well as β-cell improvements (Slentz et al., 2009).

Animal model studies may also shed some light on the effectiveness of exercise to improve β-cell function since the true gold standard technique in β-cell function can be used (β-cell mass, which cannot be utilized in live humans). Exercise training in diabetic rats leads to improvements in β-cell function and mass by 40-60% (Beaudry & Riddell, 2012). A recent diabetic rat model study showed that exercise in diabetic rats led to increased insulin release, which was coupled with upregulation of insulin mRNA and insulin storage in the β-cell islets (Delghingaro-Augusto et al., 2012). This study provides a potential mechanism via the upregulation of insulin production and storage by the β-cells in response to exercise training. Other potential mechanisms in the improvement of β-cell function via exercise include reductions in pancreatic lipotoxicity and glucotoxicity leading to improved insulin secretion (DeFronzo, 2009; T. P. J. Solomon et al., 2010). Therefore, the evidence that exercise improved β-cell function is limited, but the available studies suggest that exercise training has beneficial effects on β-cell function especially in diabetic subjects.

The Effects of Exercise on Hepatic Glucose Secretion. As discussed above hepatic glucose secretion is a major determinant of blood glucose control. Insulin sensitivity of the liver dictates the ability of the liver to respond to a meal and/or current glucose levels (DeFronzo, 2009). Hepatic lipid accumulation may be a primary cause of reduced insulin sensitivity in the liver (Kotronen, Seppaelae-Lindroos, Bergholm, & Yki-Jaervinen, 2008; Magkos, 2010; Ryysy et al., 2000; Seppälä-Lindroos et al., 2002). This buildup of lipids in the liver is most likely due to increased circulating FFAs released from expanded

visceral (Nielsen, Guo, Johnson, Hensrud, & Jensen, 2004) and subcutaneous (Horowitz, Coppack, & Klein, 2001) fat depots. When large amounts of lipids accumulate in the liver in the form of FFAs they are easily converted into glucose and then released into the blood stream. The extra glucose production combined with insulin signaling dysfunction that occurs with hepatic lipid accumulation and the inflammatory status of IGT and T2D leads to the inability of insulin to inhibit hepatic glucose release (Bergman et al., 2006; Kabir et al., 2005). Promising results in the ability of exercise alone (Johnson et al., 2009; Kirwan, Solomon, Wojta, Staten, & Holloszy, 2009; Shojaee-Moradie et al., 2007; Sullivan, Kirk, Mittendorfer, Patterson, & Klein, 2012) and exercise in combination with diet (Haus et al., 2010; Schäfer et al., 2007; T. P. J. Solomon et al., 2009; T. P. J. Solomon et al., 2010; Tamura et al., 2005; Thamer et al., 2012) to reduce hepatic insulin resistance with or without reductions in hepatic fat have begun to surface.

The most robust study to look at the effects of exercise alone on hepatic fat and hepatic insulin resistance used six weeks of aerobic exercise (n = 10) or a control condition (n = 7) in sedentary men. The aerobic exercise consisted of 3 days/week of aerobic exercise at 60-85% of VO_{2max} for at least 20 minutes. Magnetic resonance specroscopy (MRS) was used to measure liver and skeletal muscle fat and a two-step hyperinsulinaemic-euglycaemic clamp was used to assess hepatic and peripheral insulin sensitivity. After the intervention there were significant reductions in fasting non-esterified fatty acids, glycerol, and palmitate, but no reductions were seen in muscle or liver fat. The small n may account for the lack of significance in the liver fat reduction. Most importantly the exercise group showed improvements in both hepatic and peripheral insulin sensitivity measured 72 hours after the last exercise session (Shojaee-

Moradie et al., 2007). The authors speculated that the improvement in hepatic insulin sensitivity may have been due to reductions in FFAs, reductions in visceral fat, and/or reductions in hepatic fat that were not detected due to the small subject number. Another exercise only study used 7 days of exercise training (30 min/day at 70% of VO_{2max}) in obese type 2 diabetics. Hepatic and peripheral insulin sensitivity, assessed via the hyperinsulinemic-euglycemic clamp, were improved after the intervention (Winnick et al., 2008). The results of this study may be completely due to the acute effect of exercise since the clamp was done 24 hours after the last exercise bout and it has been shown that the effects of exercise on insulin sensitivity last at least 48 hours (Eriksson, Taimela, & Koivisto, 1997). The authors speculated that the improvements in hepatic insulin sensitivity may have been due to activation of adenosine monophosphate-activated protein kinase (AMPK) in the liver, which results in the inhibition of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase and therefore inhibits gluconeogenesis. This hypothesis is backed by the evidence that exercise activates AMPK (Carlson & Winder, 1999). Two other exercise only studies that resulted in no weight loss showed decreases in hepatic fat using either four weeks of aerobic exercise in obese men (Johnson et al., 2009) or 16 weeks of aerobic exercise in obese adults with non-alcoholic fatty liver disease (Sullivan et al., 2012). Unfortunately neither of those studies measured hepatic insulin resistance. The four-week study did measure fasting glucose and insulin and reported no changes in HOMA-IR scores, but that is not the ideal method of assessing peripheral or hepatic insulin resistance (Shaibi, Davis, Weigensberg, & Goran, 2011).

Short-term (Tamura et al., 2005) and long-term (Haus et al., 2010; Schäfer et al., 2007; T. P. J. Solomon et al., 2010; Thamer et al., 2012) exercise and diet interventions in obese subjects, subjects with IGT, NGT, and T2D have shown positive results in the reduction of liver fat and/or improvements in liver insulin sensitivity. An exemplary study used 12 weeks of aerobic exercise (1 hour/day, 5 days/week, at 85% of max heart rate) plus either a low-glycemic diet (n = 10) or a high glycemic diet (n = 12). Measurements were done via euglycemic-hyperinsulinemic clamp, oral glucose insulin and incretin reponses, dual-energy x-ray absorptiometry (DXA) and computed tomography (CT) scans. After the intervention both groups showed marked improvements in both peripheral (76%) and hepatic (27%) insulin sensitivity with no differences between groups (T. P. J. Solomon et al., 2010). Another study compared exercise alone to diet plus exercise and found improvements in hepatic insulin sensitivity in both treatments, but only the diet plus exercise group improved their insulin sensitivity response to infused FFAs (Haus et al., 2010).

The only study comparing the effects of HIT to continuous exercise in the improvement in hepatic insulin resistance was a training study conducted with rats. After eight weeks of exercise in rats with metabolic syndrome there were increases in insulin receptor phosphorylation in the skeletal muscle and liver cells. The HIT showed to be more effective at improving insulin receptor phosphorylation in the skeletal muscle, but the effect was the same in both groups for the liver insulin receptors (Haram et al., 2009).

A recent review of the literature on exercise and the reduction of hepatic fat stated that a 20-40% reduction in hepatic fat is typically observed after exercise interventions (Magkos, 2010). Since lipid accumulation in the liver is the most likely reason for

reduced hepatic insulin sensitivity it is likely that these improvements would be translated into improved hepatic responses to insulin. With the limited data available in the improvements in hepatic insulin sensitivity in response to exercise it is conceivable that exercise alone and very convincing that exercise with diet can reduce hepatic insulin resistance and hepatic fat.

The Effects of Exercise on Peripheral Insulin Sensitivity. The first suggestion that exercise training may increase the muscle's sensitivity to insulin came in 1972 with a simple cross-sectional study comparing insulin sensitivity in trained versus untrained men (Björntorp et al., 1972). This study was followed by multiple studies showing that trained individuals have a lower insulin response to an oral glucose challenge compared to untrained, but the glucose levels of the trained individuals still stays lower (Seals et al., 1984). Next via use of the hyperinsulinemic-euglycemic clamp technique it was shown that trained people have a higher rate of insulin stimulated glucose disposal than untrained (Hollenbeck, Haskell, Rosenthal, & Reaven, 1985). During this time of early exercise and insulin resistance research there was some controversy over the question of the long vs. short term effects of exercise (Goodyear & Kahn, 1998). One study showed that after 10 days of inactivity the insulin response to a glucose load was the same in trained and untrained men (Heath et al., 1983). However, by use of the hyperinsulinemic-euglycemic clamp it was shown that the maximal insulin-stimulated glucose disposal rate was higher even after 10 days of inactivity suggesting a sustained training effect on insulin responsiveness (King et al., 1988). Therefore, it is now well accepted that exercise stimulates both acute effects and chronic adaptations in insulin sensitivity and responsiveness (Goodyear & Kahn, 1998). Whereas, insulin stimulated GLUT4

translocation is dependent on rapid phosphorylation of the insulin receptor, insulin receptor substrate-1/2 (IRS-1/2) on tyrosine residues, and the activation of phosphatidylinositol 3-kinase (PI3-K), exercise acutely stimulates GLUT4 translocation without use of any of these mechanisms. Acute exercise increases glucose uptake by stimulating the translocation of GLUT4 via the Ca^{2+} dependent activation of calmodulin, the AMP:ATP ratio driven activation of AMPK, and atypical protein kinase C activation all of which are insulin dependent pathways (Röckl, Witczak, & Goodyear, 2008).

The adaptations to exercise training that induce increased insulin sensitivity and glucose disposal include: 1) muscle fiber type changes, 2) increases in mitochondrial activity and content, and 3) increases in GLUT4 protein expression. It is well known that endurance training elicits increases in mitochondrial content of skeletal muscle which are associated with, but not completely indicative of muscle fiber type. Type I fibers have more mitochondrial mass than type II and type IIa have greater mitochondrial mass than IIx and finally type IIb have the lowest mitochondrial mass. Muscle fiber typing done specifically on the myosin heavy chain isoforms shows that endurance training leads to fiber type switching from type IIb to IIx to IIa and even in rare cases a complete switch to type I (Pette & Staron, 2001). The more oxidative the fibers the more glucose disposal they perform. As muscle fiber type moves towards the more oxidative fibers they have higher GLUT4 expression and higher mitochondrial mass. Furthermore, independent of fiber type endurance training leads to increased expression of GLUT4. The effects of endurance training induce three very important adaptations in skeletal muscle that lead to greater insulin sensitivity and greater glucose disposal (Röckl et al., 2008).

Due to the large volume of studies published on the improvements in insulin sensitivity and exercise (Web of Science search for “Aerobic Exercise” AND “Insulin Sensitivity” yielded ~900 articles), the section below will be spent discussing large impactful studies looking at the effects of exercise on insulin sensitivity.

A study of 1467 women with impaired glucose tolerance measured physical activity via questionnaire and insulin sensitivity via the frequently sampled intravenous glucose tolerance test. The authors showed that women who participated in vigorous exercise 5 days/week had insulin sensitivity that was 75% better than those who did no vigorous exercise at all. Even reporting vigorous exercise 1-3 times/month yielded a 22% increase in insulin sensitivity (Mayer-Davis et al., 1998). Results from the HERITAGE study including 316 women and 280 men showed an approximate 10 percent improvement in insulin sensitivity (assessed via intravenous glucose tolerance test) after 20 weeks of three days/week of aerobic exercise (Boulé, Haddad, Kenny, Wells, & Sigal, 2001). A large (n = 102), well-designed study evaluating the effectiveness of exercise on improvements in muscle oxidative capacity and insulin resistance showed that subjects of all ages (21-87 years) improved muscle markers of oxidative capacity, GLUT4 expression, aerobic capacity, and visceral fat, but only the young (under 40 years) showed improvements in insulin sensitivity suggesting an age related effect (Short et al., 2003).

Another large intervention study evaluated the effectiveness of six-months of endurance training in 119 NGT and 47 IGT individuals for improving insulin sensitivity assessed via OGTT (Jenkins & Hagberg, 2011). Interestingly, the individuals who started out with NGT did not significantly improve in any glucose levels during the OGTT, but

did significantly reduce insulin and HOMA levels. The IGT subjects significantly reduced all variables during the OGTT and most of those improvements were greater than the NGT subjects. Although there was a significant improvement in insulin sensitivity the IGT subjects still did not attain the NGT levels at baseline. Therefore, subjects with IGT will most likely improve insulin sensitivity to a greater extent than NGT individuals in response to exercise training, but they may not become completely NGT even after long-term exercise training. Complete normalization of glucose tolerance has been seen in T2D subjects and IGT subjects in response to training of a much higher intensity (Holloszy, Schultz, Kusnierkiewicz, Hagberg, & Ehsani, 1986). Twelve months of exercise (5 days/week at ~90% of VO_{2max} for 60 minutes each session) led to complete normalization of glucose tolerance in all IGT subjects and most T2D subjects. Therefore it may be that exercise of higher intensity and duration is necessary to completely restore glucose tolerance (Jenkins & Hagberg, 2011). Similarly, a study in 25 healthy older, inactive women used 3 randomly selected exercise prescriptions for nine-months. All three groups were matched for caloric expenditure and varied by intensity and duration (80%, 65%, and 50% VO_{2peak}). Insulin sensitivity was measured via the euglycemic-hyperinsulinemic clamp conducted 72 hours after the last exercise session. The higher intensity group showed significant improvements in both glucose utilization at the high-insulin dose and insulin-stimulated suppression of adipose tissue lipolysis at the low-insulin dose. These results suggest that long-term higher intensity exercise may be superior for improvements in both fat and muscle insulin sensitivity (DiPietro, Dziura, Yeckel, & Neufer, 2006).

The most robust study to date examining the effects of different exercise doses in the improvement of insulin sensitivity is the study already described in detail above known as the STRRIDE trial (Bajpeyi et al., 2009; Houmard et al., 2004). An intravenous glucose tolerance test was used to measure insulin action and calculate the insulin sensitivity index in 154 sedentary, overweight/obese subjects before and after six months of exercise training. All three exercise groups (high volume, moderate intensity; low volume, moderate intensity; and the low volume, high-intensity) improved insulin sensitivity, but the two groups that exercised for longer durations each day (low volume, moderate intensity and high volume, high-intensity) improved to a greater extent (85% improvement versus 40% improvement (Houmard et al., 2004). This same pattern was seen when insulin sensitivity was measured again 15 days after the cessation of exercise indicating that the effect of greater exercise time per session may elicit more sustainable changes (Bajpeyi et al., 2009).

Many studies have shown improvements in insulin sensitivity with high and low intensity, volume, and duration of exercise therefore the findings of the STRRIDE trial are difficult to explain (Babraj et al., 2009; Kang et al., 1996; Larsen, Dela, Madsbad, & Galbo, 1999; S. F. Praet & van Loon, 2007; S. F. Praet & van Loon, 2009; Snowling & Hopkins, 2006). For example a study used 6 weeks of three days per week of sprint interval exercise which totaled 10 minutes per session and observed a 28% improvement in insulin sensitivity measured 3 days after the last exercise session (Metcalfe, Babraj, Fawkner, & Vollaard, 2011). Conversely, Larsen's group in Denmark has used high and low intensity exercise in T2D patients and has observed improvements with both modes,

bringing them to a similar conclusion of the STRRIDE trial that total exercise time may be the most important factor (Larsen, Dela, Kjær, & Galbo, 1997; Larsen et al., 1999).

The most comprehensive findings in the issue of exercise dose in improving insulin sensitivity may be found in a recent meta-analysis (Snowling & Hopkins, 2006). After pooling the results of 13 well-designed studies there was no clear effect of exercise intensity or time in the improvement of insulin sensitivity. The major difference in intensity related improvements in insulin sensitivity may not be found in moderate exercise versus vigorous, but possibly in HIT versus continuous (C. P. Earnest, 2008). Some recent, small, studies without comparison groups in T2D and healthy subjects show the drastic effects that HIT can have on insulin sensitivity in a short period of time or even after one bout of exercise (Babraj et al., 2009; Gillen et al., 2012; Hood, Little, Tarnopolsky, Myslik, & Gibala, 2011; Little et al., 2011; Metcalfe et al., 2011; Whyte, Gill, & Cathcart, 2010). Two weeks of 30 minutes of HIT per week led to a 369% improvement in GLUT4 protein content as well as improved 24-hour glucose control in T2D patients (Little et al., 2011). Similarly, two weeks of HIT led to significantly improved markers of muscle mitochondrial function, glucose transport, as well as insulin sensitivity in seven sedentary adults (Hood et al., 2011). A small RCT found that 15 weeks of HIT versus steady state exercise led to significant reductions in fasting insulin only in the HIT group (E. Trapp et al., 2008). Therefore, there is much work to be done in the area of comparing the effects of HIT to continuous exercise for improvements in insulin sensitivity, but the early observations are suggesting that HIT may yield superior benefits.

The Effects of Exercise on Glycemic Control. After the review of literature above it is clear that exercise can improve the three major determinants of glucose control. The ability to control blood glucose levels in subjects with impaired glucose tolerance as well as individuals with T2D is of paramount importance in regards to delaying or halting the progression and onset of cardiovascular disease associated with poor glucose control (Selvin et al., 2004). The most common method of assessing long-term blood glucose control is via measurement of glycosylated hemoglobin (HbA1c). A non-enzymatic reaction between glucose and hemoglobin in red blood cells leads to glycosylated hemoglobin. The higher the blood glucose has been over the past four weeks to three months then the higher percentage of hemoglobin will be glycosylated.

Both aerobic and resistance exercise have been shown to improve HbA1c (Snowling & Hopkins, 2006). Many large scale clinical trials have been conducted evaluating the effects of exercise on the improvement of glucose control measured via HbA1c. Due to the large number of studies in this area multiple meta-analyses have been conducted that adequately pool the results of all studies. The first meta-analysis pooled the clinical trials published up until December of 2000. The authors included only studies in T2D that involved durations of 8 weeks or more. The authors located 12 aerobic exercise studies and two resistance exercise studies and found that overall the exercise interventions yielded a reduction in HbA1c of 0.66% (Boulé et al., 2001). The next meta-analysis published five years later included 27 exercise studies that used multiple outcomes for glucose control improvement. Most relevant to this section was the HbA1c data which showed the following reductions in HbA1c%: -0.37 aerobic, -0.29 resistance, and -0.43 combined aerobic and resistance. The authors concluded that combined

strength and endurance training as well as higher intensity exercise (-0.29% greater improvement) may both lead to greater improvements in HbA1c (Snowling & Hopkins, 2006). Another meta-analysis published in 2006 included 14 RCTs including 377 T2D patients. The authors found a -0.6% reduction in HbA1c that was associated with a -45.5 cm^2 reduction in visceral fat (Thomas, Elliott, & Naughton, 2006). Therefore, from these comprehensive reviews it is clear that exercise has a small but significant effect on glucose control assessed via HbA1c.

Another relatively novel approach to assessing glucose control is via use of continuous glucose monitoring (CGM). Instead of obtaining average glucose values over weeks to months CGM gives glucose values every 1-15 minutes. A few studies have used CGM to monitor changes in glucose control after an intervention period. The first study published in this area used four months of strength or endurance training in 15 patients with T2D and conducted continuous glucose monitoring before and after the intervention (Cauza et al., 2005). The authors did not mention any steps taken to control food intake during the testing periods, which is a serious limitation to the study. Interestingly, the authors did not see a significant improvement in glucose control in the endurance training group but the strength training group improved significantly. A seven day aerobic exercise study in T2D patients used continuous glucose monitoring to assess glucose control before and during the last 3 days of the exercise intervention. The subjects significantly decreased maximum blood glucose, difference between minimum and maximum blood glucose, and the number of low excursions each day during the intervention period (Mikus et al., 2012). Results from this study must be taken in the light of the fact that the subjects were exercising while wearing the CGMs, therefore the

improvements in glucose control were due to the acute effects of exercise. Finally, a two-week exercise study using HIT in T2D patients utilized CGM to assess glucose control. After only 6 sessions of exercise the subjects decreased their average 24-hour glucose values and the sum of the 3 hour postprandial glucose areas under the curve for each meal assessed by CGM 48-72 hours after their last exercise session (Little et al., 2011). Therefore, there are very limited exercise interventions using CGM as the method of assessing glucose control, but the studies conducted so far show encouraging results that align with the large body of evidence showing the exercise can improve glucose control in subjects with IGT or T2D.

Section 3: Visceral Adipose Tissue

Correlation between Visceral Adipose Tissue and Cardiometabolic Diseases

The interest in abdominal fat first started in the early 1980s when Kissebah and colleagues (1982) published their landmark study showing that women with upper body obesity had a higher likelihood of having glucose/insulin disturbances than those with lower body obesity. A large epidemiological study of 15,532 obese women followed that report and showed that those with upper body obesity had a 10-fold increased risk of having T2D compared to those with lower body obesity (Hartz, Rupley, Kalkhoff, & Rimm, 1983). Another landmark study from Japan showed that even in non-obese men high levels of visceral adipose tissue (VAT) were predictive of CAD, hyperlipidemia, hyperglycemia, and hypertension (Nakamura et al., 1994). Many other large epidemiological studies confirmed these findings by showing that anthropometrically measured abdominal obesity is related to T2D, IGT, and CVD (J. P. Despres et al., 1990).

After the development of computed tomography (CT) for the quantification of VAT, multiple studies confirmed the findings of the earlier studies that only used anthropometric measurements. These findings clearly confirmed that the amount of VAT quantitatively measured by CT was inversely associated glucose tolerance (Després et al., 1989; Fujioka, Matsuzawa, Tokunaga, & Tarui, 1987; Sparrow, Borkan, Gerzof, Wisniewski, & Silbert, 1986). Since then many large studies have shown strong correlations between high levels of VAT and hyperlipidemia, hypertension, impaired vascular function, cardiovascular disease, IGT, and T2D (Arcaro et al., 1999; Bergman et al., 2006; J. P. Despres et al., 1990; Després et al., 1989; Després, 2007; Tchernof & Despres, 2013).

Pathophysiology of Visceral Adipose Tissue

The internal adipose tissue, adipose tissue that is not subcutaneous, includes the intrathoracic and intra-abdominopelvic regions. Intrathoracic adipose includes pericardial adipose tissue and is not part of what is considered VAT. Intra-abdominopelvic adipose includes intraperitoneal and extraperitoneal adipose. Intraperitoneal adipose tissue is made up of two regions: the omentum and mesentery. Extraperitoneal adipose tissue includes preperitoneal and retroperitoneal adipose tissues. Even though the intra- and extraperitoneal adipose tissues are not distinguished when VAT is measured only the intraperitoneal adipose tissue drains its circulation into the portal vein (Tchernof & Despres, 2013). The proximity of the adipose tissue to the portal vein is central to one of the main hypotheses of the pathophysiology of VAT (Bergman et al., 2006; Björntorp, 1990).

The adipocytes found in the mesenteric and especially the omentum regions have been shown to have distinct morphologies (less organized and larger), metabolic activities, neurological innervations, and blood flow patterns that make them unique and potentially pathological compared to other sites of adipose tissue. Furthermore, it is clear that during times of adipose tissue expansion (weight gain) premenopausal women tend to store more lipids in their subcutaneous abdominal and leg regions through hyperplasia whereas men and postmenopausal women tend to expand their visceral adipose regions via hypertrophy (Tchernof & Despres, 2013). This gender difference in preferential lipid storage may in part explain the earlier onset of CVD for men compared to women.

Adipocyte size is positively correlated with insulin resistance and T2D, partly because of a high correlation of cell size and lipoprotein lipase (LPL) activity leading to increased blood lipids (Tchernof & Despres, 2013; Weyer, Foley, Bogardus, Tataranni, & Pratley, 2000). Since adipocytes in the omental and mesenteric regions are typically larger the increased LPL activity of these cells is strongly linked to a higher release of FFAs which can be taken up by the liver through the portal circulation. Studies using a high fat feeding dog model have given molecular evidence for this hypothesis. Twelve weeks of a high fat diet in dogs led to increases in the visceral to subcutaneous ratio for mRNA expression of lipolytic enzymes (LPL and hormone sensitive lipase despite higher insulin levels) and lipid accumulation enzymes (sterol regulatory element-binding transcription factor-1) suggesting expansion of the VAT as well as high release of FFAs. Furthermore the authors found increased gluconeogenic enzymes (glucose-6-phosphate and phosphoenolpyruvate carboxykinase) and triglyceride content of the liver, suggesting

increased hepatic glucose production and release (even in the face of hyperinsulinemia) and hepatic fat accumulation (Kabir et al., 2005).

Another pathological finding with larger adipocytes, especially in the abdominal region, is that they have been shown to be resistant to hyperplasia which leads to lipids spillover into surrounding organs including the liver, the heart, the pancreas, and muscle. This spillover leads to "ectopic fat" accumulation in these other organs which is highly linked to insulin resistance in each of those sites (Gray & Vidal-Puig, 2007; Unger, 2002; Virtue & Vidal-Puig, 2010). An elegant of example of this is shown by the use of PPAR-γ agonists (thiazolidinediones; TZDs) which stimulate adipocyte hyperplasia and lead to the formation of small insulin sensitive adipocytes and have been shown to improve glucose tolerance (Giannini, Serio, & Galli, 2004). It has also been shown that visceral adipose tissue is highly resistant to the suppression of lipolysis compared to subcutaneous tissue. This resistance is likely to lead to a higher release of FFAs from visceral fat compared to subcutaneous fat, especially in pre-diabetic conditions where hyperinsulinemia may be present (Zierath et al., 1998). Studies have shown that a large proportion of splanchic FFA release ends up accumulating in the liver in obesity (Nielsen et al., 2004). Hepatic triacylglycerol content measured with proton spectroscopy is closely correlated with hepatic insulin sensitivity in type 2 diabetic patients (Ryysy et al., 2000) and in non-diabetic men (Seppälä-Lindroos et al., 2002).

Another hypothesis regarding the link between VAT and cardiometabolic disease deals with the release of cytokines and adipokines from visceral adipocytes as well as macrophages that have infiltrated the fat regions. Leptin and adiponectin are both adipokines that are known to be protective against cardiometabolic disease. Leptin is

involved in regulating food intake and energy expenditure. Leptin resistance may play a role in increasing adiposity and decreasing energy expenditure during obesigenic states. Adiponectin is another adipokine that has anti-inflammatory and insulin sensitizing properties. Visceral fat is known to release lower levels of leptin and adiponectin and has been shown to be inversely related to both of these adipokines. Furthermore, high levels of the pro-inflammatory cytokine IL-6 have been shown to be related to VAT accumulation which may lead to insulin resistance and atherogenesis (Tchernof & Despres, 2013).

The potential mechanisms of visceral fat leading to cardiometabolic disease discussed above can be summarized into three main hypotheses: 1) The portal theory: stating that differences in metabolism and the proximity of VAT to the portal circulation lead to excessive accumulation of FFAs in the liver, contributing to abnormal liver metabolism resulting in: high levels of apolipoprotein B-containing lipoprotein production and release, increased liver glucose production and release, and reduced hepatic insulin sensitivity (Bergman et al., 2006; Björntorp, 1990). 2) The inflammatory profile of enlarged visceral adipocytes with infiltrated macrophages leads to low levels of protective cytokines (leptin and adiponectin) and high levels of pro-inflammatory cytokines (IL-6, IL-1β) which contribute to systemic inflammation, insulin resistance, and possibly an inflammatory state of the liver leading to further degradation of liver metabolism (Donath & Shoelson, 2011). 3) The inability of subcutaneous fat to act as a the primary storage depot of excess caloric intake leads to excessive VAT accumulation as well as ectopic fat accumulation and lipotoxicity of the heart, liver, kidneys, pancreas,

and muscle which may lead to dysfunction of these organs and further insulin resistance (Tchernof & Despres, 2013).

Use of the iDXA to Measure Visceral Adipose Tissue

The gold standard method of quantifying visceral adipose tissue is via computed tomography (Tchernof & Despres, 2013). Magnetic resonance imaging and ultrasound are both also used as viable alternatives to CT scans. All three assessment tool mentioned above are expensive and labor intensive. There is a need to identify automated and accessible methods of quantifying VAT that do not induce radiation on the patient. Recent technological advances in the imaging quality and algorithms used in the newest version of the General Electric dual-energy x-ray absorptiometry (DXA) system (iDXA) allow for the quantification of visceral adipose tissue (Ergun & Rothney, 2012). The patented CoreScan software uses the region of the scan known as the android region to quantify VAT. The android region is about 10 cm in height and as wide as the individual. The android region is between the iliac crest and a line that is 20% of the distance from the iliac crest to the base of the mandible. Since the fat in this region consists of subcutaneous and visceral fat the software uses an algorithm consisting of the width of the subcutaneous fat as the far lateral regions of body where the thickness of the fat layer actually represent the thickness of the subcutaneous fat and the anterior-posterior thickness of the abdomen. The anterior-posterior thickness can be attained using the tissue attenuation image obtained by the iDXA. Finally, the subcutaneous fat is subtracted from the total fat to obtain VAT. The CoreScan algorithm was developed using paired iDXA and CT images from approximately 350 subjects with a wide range of BMI and age, located in three different sites (Ergun & Rothney, 2012).

The iDXA measurement of VAT was validated in two publications in the summer of 2012 (Kaul et al., 2012; Micklesfield, Goedecke, Punyanitya, Wilson, & Kelly, 2012). The first study compared CT and iDXA scans in 131 black and white South African women. The authors found good correlation (r = 0.93) and low error rates (SEE = 16 cm^2) when comparing the iDXA measurement of VAT to expertly read CT scans. This study also compared the iDXA scans to a clinical read of the CT scans and found that the iDXA performed just as well as the clinical read of the CT scans. The iDXA predicted 86% of the variance (R^2) in the expert read of the CT scan (Micklesfield et al., 2012). Another study conducted an iDXA and a CT scan in a sample of 124 men and women ranging in age from 18-90 years and BMI from 18.5-40 kg/m^2. The R^2 value between the VAT measured from the iDXA and the CT was 0.96. The iDXA bias calculated by using Bland-Altman plots was +56cm^3 (Kaul et al., 2012). The authors of both studies concluded that the iDXA is a precise and valid tool in the measurement of VAT. Another study used 32 clinical subjects as well as phantom abdomen with measured quantities of VAT and conducted iDXA scans with 11 different iDXA devices. Each clinical subject completed 2 scans whereas the phantom abdomens underwent 5 scans each. The combined intra- and inter-device variation was less than 5% for both phantom and human scans (Rothney et al., 2013). Finally, the iDXA measurement of VAT in a sample of 939 subjects was shown to correlate well with increased odds of hypertension, impaired fasting glucose, metabolic syndrome, and T2D (Rothney et al., 2012).

No studies have been conducted that assess the ability of the iDXA system to accurately detect changes in VAT. The DXA has been shown to accurately detect small

changes in fat mass induced by addition of a 1 kg fat packet on individuals, but these measurements were for total body fat and not specific to the VAT (Warolin et al., 2012).

The newly developed software used with the iDXA device seems to be a precise and valid assessment tool for the quantification of VAT. Unfortunately, all of the studies published so far on the use of the iDXA device to quantify VAT included GE employees with obvious conflicts of interest in the success of their product as authors on the papers. Therefore, no truly independent studies have been conducted on the iDXA's ability to quantify VAT.

The Effects of Exercise on Visceral Adipose Tissue

Aerobic Exercise to Reduce VAT. The role of exercise in the reduction of visceral adipose tissue has been studied extensively with many different doses of exercise and in many different populations. Multiple studies have shown that reductions in VAT are significantly related to improvements in glucose tolerance and insulin sensitivity (Mourier et al., 1997; O'Leary et al., 2006). One of the first long-term studies to assess the ability of aerobic exercise to reduce VAT involved 14 months of exercise at 55% of VO_{2max}, 4-5 days/week in premenopausal obese women (J. Despres et al., 1991). After the 14 month intervention the women lost 4.6 kg of fat, improved VO_{2max}, and significantly reduced VAT measured via CT. Therefore, the earliest study in this area indicated that aerobic exercise alone with total body fat loss reduces VAT. Many studies followed this early study so the rest of this section will be spent discussing the large impactful studies, meta-analyses, and the issues of exercise intensity and HIT for the reduction of VAT.

A classic study evaluating many cardiovascular disease risk factors and their improvements with exercise training was the HERITAGE study. This study included 557

men and women who exercised at 55-75% of VO2max 50 minutes/day, 3 days/week for 20 weeks. CT scans were conducted pre and post and there was a significant reduction in VAT (77.9 to 73.3 cm^2). The study showed that men reduced VAT by 7% whereas women only reduced VAT by 4.5% (Wilmore et al., 1999). Another large scale RCT including 173 overweight and obese postmenopausal women assessed visceral fat with CT scan before and after 12 months of aerobic exercise (5 days/week of moderate intensity exercise 45 minutes each session) or a control condition. After the intervention the exercise group significantly decreased VAT by 8.6 g/cm^2 and the control group increased by .1 g/cm^2 (Irwin et al., 2003). A more recent large scale RCT involved ~100 men and 100 women in 12 months of aerobic exercise or control. The exercise consisted of 3 days/week supervised and 3 days/week home-based moderate to vigorous activity for 60 minutes each session. Subjects in the exercise group lost weight, but even though the VAT seemed to go down the reduction was not significant (McTiernan et al., 2007).

A very comprehensive meta-analysis published in 2006 found 10 RCTs that used advanced imaging techniques to measure VAT before and after an aerobic exercise intervention. Seven of the 10 RCTs showed reductions in VAT compared to the controls (Kay & Singh, 2006). A very recent meta-analysis limited their inclusion of studies to subjects who were overweight or obese, performed aerobic exercise, measured VAT with MRI or CT. The authors found 15 RCTs that met their inclusion criteria. The pooled data showed that in as little as 12 weeks a reduction of 30-40 cm^2 in VAT is likely (Vissers et al., 2013). Therefore, the results from pooled data and meta-analytic techniques show a strong probability that exercise without diet can lower VAT in overweight and obese individuals.

Exercise Intensity and HIT. A systematic review attempted to calculate the exercise dose relationship for the reduction of VAT. Using nine RCTs and seven non-randomized studies the authors concluded that exercise volume and not intensity was the most important variable in reduction of VAT (Ohkawara et al., 2007). Conversely, both meta-analyses discussed above stated that there were not enough studies directly comparing exercise intensities to draw conclusions about the ideal exercise intensity to reduce VAT (Kay & Singh, 2006; Vissers et al., 2013).

Multiple studies have not detected differences in exercise intensity in the reduction of VAT (Cho et al., 2011; Gutin et al., 2002; Slentz et al., 2005). The STRRIDE trial (previously described in detail) evaluated the effects of different doses of exercise on the reduction in VAT. The study randomized 175 men and women with dyslipidemia to control or exercise at three different doses. Interestingly, the only group that lost a significant amount of visceral fat was the group that did high-intensity exercise for a long duration (equivalent of jogging 20 miles/week). The moderate intensity low volume and the high-intensity high volume groups did not lose VAT, suggesting that exercise volume and not intensity is the key factor in VAT reduction (Slentz et al., 2005). Unfortunately the STRRRIDE trial did not include a high volume moderate intensity group to truly evaluate the effects of differing intensities on VAT reduction. Similarly another RCT with 45 Korean women showed no difference in VAT reduction between low (40-50%VO_{2max}) and high (70-75% VO_{2max}) intensity exercise for 12 weeks, but both group significantly reduced VAT (Cho et al., 2011).

Conversely, a few studies have shown that exercise at a vigorous intensity is superior to moderate exercise in reducing VAT (Coker et al., 2009; Irving et al., 2008; E.

Trapp et al., 2008). An RCT in obese women with the metabolic syndrome involved 16 weeks of exercise (5 days/week) above (high-intensity group) or below (low intensity group) the lactate threshold. Interestingly, only the women in the high-intensity group significantly reduced VAT, suggesting that intensity of exercise may play a role in the reduction of VAT (Irving et al., 2008). Similarly, a small comparison randomized elderly overweight adults to either moderate (50% VO_{2max}) or high (75% of VO_{2max}) intensity exercise. After the 12-week intervention VAT the high-intensity group reduced VAT by 39 cm^2 and the moderate and control groups did not change (Coker et al., 2009). Finally, another RCT randomized 45 young normal weight women to either control, steady state exercise at 60% of VO_{2max}, or high-intensity interval training 3 days/week for 15 weeks. Abdominal fat percentage was measured via DXA, therefore true VAT assessment was not conducted. Intriguingly the HIT group reduced abdominal fat significantly more than the other two groups (E. Trapp et al., 2008). This is the only study found comparing HIT to continuous exercise in the reduction of VAT.

Mechanisms. Even though weight loss via diet without exercise has been shown to reduce VAT (Nicklas et al., 2009) the physiological effects of exercise may play a key role in the reduction of VAT. For example one study randomized 33 obese postmenopausal women with T2D into a diet only, exercise only, or a diet plus exercise group for 14 weeks. Percent body fat was reduced with all three interventions, but only the exercise groups showed significant reductions in VAT (Giannopoulou et al., 2005). An interesting physiological difference between fat cells in differing regions of the body could partially explain these differences. Studies conducted in the late 1980s to the early 1990s by Peter Arner and colleagues (Arner, Kriegholm, Engfeldt, & Bolinder, 1990;

Arner, 1995; Wahrenberg, Lönnqvist, & Arner, 1989) measured the lipolytic activity of various adipose regions under the stimulation of various chemicals. Lipolytic activity can be measured in vivo via insertion of a microdialysis catheter which simultaneously infuse stimulatory chemicals (catecholamines) and measure glycerol release. This allowed the authors to discovery that the regulation of lipolysis during exercise occurs via catecholamine release (Arner et al., 1990). This technique limits the measurements to be of subcutaneous fat only, due to the location of visceral fat. Conversely, these authors also measured lipolytic activity *in vitro* after adipose tissue samples were removed from subjects (Wahrenberg et al., 1989). These studies showed that in subcutaneous adipose tissue the abdominal adipocytes are 10-20 times more sensitive to catecholamines via stimulating lipolysis compared to gluteal adipocytes (Wahrenberg et al., 1989).

Shortly after these studies were conducted another group obtained visceral (omental) and subcutaneous (abdominal and gluteal) adipose tissue samples from subjects undergoing abdominal surgery. The researchers stimulated the cells via epinephrine and observed a 500% greater lipolysis in occur in the VAT cells compared to the subcutaneous cells. Furthermore, the omental cells were resistant to the normal antilipolytic effects of insulin (Richelsen, Pedersen, Møller-Pedersen, & Bak, 1991). Together these findings not only give credence to the portal theory in the high levels of FFAs released from VAT cells, but also show that VAT cells may be more responsive to the outpouring of catecholamines induced by exercise.

Therefore, the likely mechanism of exercise reducing visceral fat even in the absence of weight loss is the high sensitivity of VAT cells to catecholamines. Catecholamines are released during exercise of all intensities and could stimulate

lipolysis to occur in the VAT cells, therefore reducing VAT. This same theory gives credence for an intensity dependent effect of exercise in the reduction of VAT. As exercise intensity increases, catecholamine release increases in a proportional manner (Pritzlaff et al., 1999; Pritzlaff et al., 2000). High-intensity interval training has recently been shown to acutely elevate catecholamines as well as glycerol levels in the blood suggesting that acute HIT leads to lipolysis (E. G. Trapp, Chisholm, & Boutcher, 2007). Furthermore it has been shown that more fat oxidation occurs after vigorous intensity exercise compared to moderate or light (Pritzlaff et al., 2000).

Chapter 3

METHODOLOGY AND DATA ANALYSIS

Participants

We enrolled 22 healthy, obese (BMI ≥ 30) individuals between 18-45 years of age (men) and 18-55 (women). This age range was chosen based on the risk stratification guidelines published by the American College of Sports Medicine (Gordon, 2009). Subjects were screened by completion of the Physical Activity Readiness Questionnaire (PAR-Q) and excluded by any "yes" answers.

Using change in glucose AUC from pre- to post-intervention (ΔAUC) as the primary outcome measure, **sample size** was calculated to detect a 5% difference between groups for ΔAUC. This seems reasonable based on previously published data (Little et al., 2011). We planned for a 15% dropout rate and enrolled 11 subjects in each group which would have provided 90% power at a 0.05 α level of significance (two-sided). Based on these parameters, the recruitment goal was 11 subjects per group. Only 9 subjects per group completed the study and this gave us ~83% power to detect a 5% group difference in ΔAUC.

Participant Screening

After completing telephone or email screening all subjects visited the laboratory for a one-hour screening visit. During this visit subjects read and signed the informed consent form, filled out the PAR-Q, and discussed the details of the study with one of the investigators. Next we performed a brachial artery ultrasound in order to verify that a suitable acoustic window was present. Finally, each subject had their height and weight

measured to verify a BMI of 30 kg/m^2 or above and rode a stationary bicycle for 5 minutes to ensure this mode of exercise was suitable.

Exercise Training

All subjects completed three supervised exercise sessions per week for eight weeks for a total of 24 exercise sessions. Subjects were randomized to either HIT or continuous training (CONT) upon enrollment in the study. All exercise was conducted on cycle ergometers and the maximum heart rate achieved during either phase of the maximal exercise test (ramp or verification, at baseline and at four weeks) was used for exercise intensity prescription. Heart rate was continuously monitored by Polar heart rate monitors and recorded by research technicians. Each HIT training session consisted of a 5-minute warm up at 50-60% of HR_{peak} followed by 10 one-minute intervals at 90-95% of HR_{peak} separated by 1 minute of cycling at a low intensity (~25-50 W), followed by a five minute cool down at 50-60% of HR_{peak}. Each CONT training session consisted of a 5-minute warm up at 50-60% of HR_{peak} followed by 30-minutes of exercise at 70-75% of HR_{peak}, followed by a five minute cool down at 50-60% of HR_{peak}. Each session lasted 40 minutes for the CONT group and 29 minutes for the HIT group.

Testing Overview

Baseline testing was conducted after abstaining from vigorous exercise and vitamins for 48 hours as well as alcohol and caffeine for 24 hours. The FMD at four weeks was ~48-72 hours after their last exercise session. Eight week testing was conducted with the 24 hour monitoring occurring ~48 after the last exercise session and the FMD and maximal exercise test occurring ~72 hours after the last exercise session.

Day 1: For both the pre- and post-intervention testing subjects arrived at the laboratory in the afternoon at least two-hours after their last meal. At this time the continuous glucose monitor was inserted. Next, we went through detailed instructions on how to use and care for the CGM. Over the next day subjects ate controlled meals provided by the research team. Breakfast and lunch were given to the participants to take with them, dinner was provided via gift card to a local sandwich restaurant, and snacks were provided (See Appendix 2 for detailed dietary information). The CGM was inserted the day before the 24 hour monitoring period to ensure device initialization was complete by the next morning. Subjects were asked to record the exact time and quantity eaten of each food item so that precise timing and content matching could be performed for the pre- and post-testing.

Day 2: After the 24 hour monitoring and controlled diet period subjects returned to the laboratory the next morning after at least a 10 hour fast. During this visit we removed the CGM, took anthropometric measurements, performed the brachial artery FMD, Dual-energy X-ray Absorptiometry (DXA), and the maximal exercise test.

Anthropometrics and Blood Pressure

Height was assessed on a standing wall mounted stadiometer (SECA, Birmingham, United Kingdom). We ensured that subjects removed shoes and had their heels against the wall during the height measurement. Height was only measured at baseline. Weight was measured at baseline, 4 weeks, and 8 weeks while fasting in the morning with minimal clothing and all jewelry removed on an electronic scale that was calibrated daily (Cosmed, Rome, Italy). Abdominal circumference, sagittal diameter, and blood pressure were all assessed at baseline and 8 weeks in the morning while subjects

were fasting. Abdominal circumference was measured with subjects standing, at the level of the umbilicus using a standard Gulick tape measure. Sagittal diameter was measured using Holtain-Kahn calipers (Holtain, Crymych, United Kingdom) at the level of the superior iliac crests while subjects were standing. All anthropometric measurements were taken by the same researcher on all subjects. Blood pressure was assessed after 20 minutes of quiet rest in a dimly lit room using a Dinamap Pro series automated blood pressure monitor (GE, Helsinki, Finland). Two blood pressure measurements were taken with 5 minutes between the measurements.

Maximal Exercise Test

All subjects performed a ramp style maximal exercise test on a cycle ergometer at baseline, after four weeks of exercise, and after the 8-week intervention. Subjects were equipped with a mask attached to a hose, and Polar heart rate monitor for the metabolic measurement device (Parvo Truemax 2400TM, Parvomedics, Sandy, Utah) to measure ventilation and respiratory gas exchange data and heart rate continuously. The Parvo Truemax 2400TM has shown high validity and reliability (Bassett et al., 2001). We performed the standard three point calibration before each test. After collecting resting data for 2 minutes, subjects pedaled on a stationary cycle ergometer at a cadence of their choice at 50 watts (males) or 25 watts (females) for 5 minutes for the warm-up phase. After the warm-up phase, load increased continuously by 30 watts/min every minute (males) or 15 watts/min every minute (females) until the subject could not continue. Verbal encouragement was given to all subjects throughout the entire test. The average of the two highest consecutive 15 second oxygen uptake averages during the test was taken as the peak VO_2. In order to verify attainment of VO_{2max}, after a 5 to 10-minute

cool down period, in which subjects pedaled at the warm up work load, each subject performed an all-out test on the cycle ergometer at a constant-load of 100% of the peak load reached during the incremental exercise test. Oxygen uptake was monitored as in the incremental exercise test, and the highest VO_{2peak} was recorded as described above. If a subject achieved a higher heart rate or VO_2 during the verification phase the verification results were used as the "peak" value for that test.

Continuous Glucose Monitoring

Continuous glucose monitoring was conducted by a Medtronic iPro2 continuous glucose monitor (CGM; Medtronic, Northridge, CA) both pre- and post-intervention. This device has been shown to correlated well with blood glucose (r = 0.73-0.92) (Tavris & Shoaibi, 2004). Furthermore, 97% of sensor readings have been shown to fall within the clinically acceptable zone for accuracy (Sachedina & Pickup, 2003). Subjects had a small micro-dialysis catheter inserted subcutaneously in their abdomen, via a spring loaded insertion device, to continuously monitor blood glucose levels. After insertion the glucose sensor was given 15 minutes to hydrate before the iPro2 recorder was connected. After the iPro2 was connected the device is covered with an adhesive, transparent dressing (Tegaderm, 3M) to hold the device in place and to protect the device from moisture. Subjects were instructed on how to use and care for the device and the catheter insertion site. Subjects were asked to keep the monitor in place until their return to the laboratory after the 24-hour monitoring period. CGM devices were calibrated using a standard glucometer (One-Touch, Ultra 2, Lifescan, Inc., Milpitas, California) 4 times throughout each 24-hour period (one hour after insertion, three hours after insertion, before each meal and before bed time). This calibration was conducted by the participants

using a standard finger prick. Subjects were asked to record their glucose values and time of each reading in the provided glucose log. The dietary and the glucose logs are then inputted upon uploading the CGM data.

Brachial Artery Ultrasound

Flow-mediated dilation (FMD) was measured with a Terason t3000 high resolution ultrasound machine (Terason Ultrasound, Burlington, MA) with a 10-MHz multi-frequency linear array probe. We followed the criteria set forth by the Brachial Artery Reactivity Task Force and the latest guidelines (Corretti et al., 2002; D. H. J. Thijssen et al., 2010). Participants were asked to lie quietly in a dimly lit room for 20 minutes on a vascular imaging table before a sonographer (who has performed this procedure approximately 400 times) obtained baseline images for 60 seconds from the participant's left arm. After the baseline images were completed the sonographer inflated a blood pressure cuff on the participants forearm to a pressure of 250 mmHg for five minutes. Sixty seconds prior to cuff release the sonographer began recording the image to ensure that a full one-minute baseline image was captured during occlusion. After five minutes of occlusion, cuff deflation occurred while the sonographer continued to record images for the next five minutes. In order to minimize error between the three measurement periods and so that exact matching of settings and conditions could be ensured the distance of the probe from the medial epicondyle of the humerus was measured and all ultrasound settings were noted in the subject's file. Images were analyzed using a previously validated, brachial artery edge-detection software (Woodman et al., 2001) to detect baseline and peak diameter and blood flow velocity as well as the percent difference between the two diameters (FMD). We analyzed 20 of our FMD

videos two separate times in a blinded fashion to evaluate the reproducibility of our laboratory analysis of the FMD videos. Intra-class correlation coefficients were calculated using Cronbach's alpha. Coefficients of variation were calculated by dividing the standard deviation of the duplicate measurements by the mean of the duplicate measurements then multiplying by 100 to obtain a percent. Cronbach alpha values between our duplicate measurements were 0.998, 0.998, and 0.997 for baseline diameter, peak diameter, and FMD%, respectively. The CV values were 0.44%, 0.41%, and 6.05% for baseline diameter, peak diameter, and FMD%, respectively. Therefore, we have demonstrated that the reproducibility of the FMD outcomes is high in our laboratory.

Female subjects completed the ultrasound measurements during days 1-7 of the follicular phase of their menstrual cycle (immediately after menstruation begins) for each measurement period. Female subjects were asked to notify the investigators when they began menstruation at each of the three measurement points in order to schedule their testing visits during the follicular phase. The timing of this visit is important due to the large variation in FMD measurements caused by the menstrual cycle (D. H. J. Thijssen et al., 2010).

Dual-energy X-ray Absorptiometry

Dual-energy X-Ray Absorptiometry (DXA) was used to determine percent body fat, regional fat distributions, and visceral fat. The use of the new iDXA to quantify visceral fat has recently shown excellent validity by correlating very well with the gold standard (computed tomography) for visceral adipose tissue assessment (Kaul et al., 2012; Micklesfield et al., 2012). Subjects lied face up in the DXA bed for 7-12 minutes while the DXA arm passed over the entire body. Females conducted a urine pregnancy

test in our laboratory (negative test was required before DXA could take place) prior to each DXA measurement. All DXA scans were performed by a certified radiology technician.

Activity Monitoring

In order to assess if subjects changed their overall physical activity level in response to the exercise interventions we used the Actigraph GT3x+ tri-axial accelerometer (Actigraph, Pensacola, FL). We used the device to measure both physical activity and sleep. The actigraph was given to each participant to be worn for a total of three weeks; baseline (a 7 day period within one month prior to the training), week 5, and week 8 of the training protocol. Participants were given an elasticized waist band to wear on the hip during the day and wristband to fix to the wrist at night and instructed on how to properly place the device during these times. Participants also completed a daily accelerometer/sleep log that captured information about their physical activity and sleep (e.g., bed time, wake time, time not worn, exercise bouts). The Actigraph sampling rate was set to 60 Hz during data collection periods. We defined a valid day as wear time of 10 hours or more. Periods of at least 60 minutes with consecutive zero counts were considered non-wear time. We used the Freedson 1998 cut-points for identifying and quantify time spent in sedentary, light, lifestyle, moderate, vigorous, and very vigorous intensity activity zones (Freedson, Melanson, & Sirard, 1998). The cut points are as follows: Sedentary: 0 – 99 counter/minute (CPM), Light: 100 – 759 CPM, Lifestyle: 760 – 1951 CPM, Moderate: 1952 – 5724 CPM, Vigorous: 5725 – 9498 CPM, Very Vigorous: 9499 - ∞ CPM.

Data Analysis

Descriptive statistics (Means, SD) for the study participants were calculated across gender and intervention groups (i.e., HIT and CONT). Two-way ANOVA was used to test baseline mean differences between groups and gender. The Shapiro-Wilk tests were used to check normality assumptions for all outcome measures. Two-way repeated measures ANOVA (RMANOVA) was used to test mean differences for each outcome measure across group, time, and group x time interaction factors. Time and group were used as the fixed factors. The sphericity assumption was justified using the Greenhouse-Geisser Epsilon (ε) test. If the sphericity assumption was violated, the adjusted critical F value (multiplied ε by the degrees of freedom) was used to test the research hypotheses. The Bonferroni post-hoc tests were also used to detect mean differences for groups and times, respectively, when appropriate. Area under the curve analyses were conducted with the CGM data using the trapezoidal method. Physical activity enjoyment scales and baseline data were checked for group differences with independent samples t-tests. Levene's test for equality of variance was used for each t-test and adjusted *P* values were used if necessary. Pearson correlations were used to investigate the relationships between the changes (delta values) in outcome variables.

For the brachial artery ultrasound data, we utilized an allometric scaling technique recently described (Atkinson, Batterham, Thijssen, & Green, 2013) that accounts for the effect that baseline diameter has on FMD %. The scaling was conducted by transformation of both the baseline and peak diameters for pre- and post-intervention via the natural log function, then calculating the difference between peak diameter and baseline diameter. See equation below:

ln(Peak Diameter) – ln(Baseline Diameter) = ln Diameter Difference

Next, we used linear mixed models with ln Diameter Difference as the dependent variable, group and time as the fixed factors, time as a repeated factor, and ln(baseline diameter) as a covariate to yield adjusted diameter change (from baseline to peak) values for each group at each time point. Finally, the adjusted changes in diameters were back transformed into FMD % values. All *p*-values were two-tailed, and values of less than 0.05 were considered to indicate statistical significance. All statistical procedures were performed by using SPSS software (IBM, Armonk, NY).

Chapter 4

RESULTS

Subjects

Twenty-one adults were enrolled in the study and 17 completed all the requirements of the study. Of those who completed the study, there were 4 males and 4 females in the CONT group and 5 males and 4 females in the HIT group. As shown in Table 1 there were no statistical differences between groups in any variable at baseline.. Adherence to the exercise program by the 17 completers was 100%. All 17 completers attended all 24 exercise sessions and all testing visits over a period of nine to ten weeks. The Shapiro-Wilk tests justified normality assumptions in all main outcome variables. There was no statistical difference for physical activity enjoyment scale between HIT and CONT groups (CONT: 82 ± 22, HIT: 88 ± 19; $P = 0.540$).

Table 1: Baseline descriptive characteristics for the completers in each group split by gender. Values represent mean ± standard deviation.

	Continuous		High-Intensity	
	Males (N=4)	**Females (N=4)**	**Males (N=5)**	**Females (N=4)**
Age (years)	35 ± 5.8	33 ± 10.4	32.8 ± 9.3	39 ± 8.1
Height (cm)	174.5 ± 9.61	164.45 ± 3.51	180.8 ± 3.35	162.93 ± 10.22
Weight (Kg)	104.73 ± 14.16	93.77 ± 5.29	127.58 ± 25.23	94.16 ± 14.48
BMI (kg/m^2)	34.5 ± 4.8	34.7 ± 1.9	39.1 ± 8.1	35.3 ± 2.0
Body Fat (%)	41.7 ± 8.71	51.05 ± 1.81	42.64 ± 5	49.35 ± 2.82

** Height and weight were significantly higher in the males compared to the females. Body fat was significantly higher in the females compared to the males.

Body Composition and Anthropometrics

There were significant reductions in abdominal circumference (1.8%, $P < 0.001$), sagittal diameter (2.6%, $P = 0.015$), body fat % (1.3%, $P = 0.025$), leg fat % (2.1%, $P = 0.009$), and gynoid fat % (2.4%, $P < 0.001$) from pre to post with all subjects combined. The sagittal diameter was not measured on two subjects in the HIT group because they were out of the range of measurement for the calipers used in the study. There was also a non-significant trend for an increase in lean mass from pre to post with all subjects together ($P = 0.071$). There were no significant changes in weight, BMI, fat mass, arm fat, trunk fat, gynoid fat, android fat, or visceral fat (all P values were > 0.05). There were no statistical differences between HIT and CONT groups for all outcome measures

(all *P* values were > 0.05). There were non-significant trends for greater reductions in gynoid fat (*P* = 0.099) and visceral fat mass (*P* = 0.162) in the HIT group compared to the CONT (See Table 2).

Table 2: Descriptive and anthropometric data Pre- and Post-intervention. Values represent: Mean ± Standard deviation

	All (N=17)		Continuous (N=8)		High-intensity Interval (N=9)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Weight (kg)	106.38 ± 21.44	106.44 ± 21.14	99.25 ± 11.50	99.48 ± 12.15	112.73 ± 26.59	112.63 ± 25.96
Abdominal Circumference (cm)	115.7 ± 14.6	113.6* ± 14.8	113.8 ± 8.7	112.8 ± 8.3	117.4 ± 18.8	114.7 ± 19.4
Sagittal Diameter (cm)+	30.2 ± 2.4	29.4* ± 2.8	30.6 ± 2.9	29.8 ± 3.6	29.8 ± 1.8	28.9 ± 1.4
Body Fat (%)	46.0 ± 6.3	45.4* ± 6.6	46.4 ± 7.7	46.0 ± 7.7	45.6 ± 5.3	44.8 ± 5.8
Body Fat (kg)	48.82 ± 11.77	48.23 ± 11.74	45.81 ± 8.51	45.57 ± 8.58	51.49 ± 14.02	50.60 ± 14.06
Lean (kg)	57.57 ± 13.53	58.21 ± 13.55	53.43 ± 11.35	53.91 ± 11.62	61.23 ± 14.88	62.03 ± 14.64
Arm Fat (%)	40.7 ± 8.5	40.4 ± 8.8	41.6 ± 9.5	41.4 ± 9.9	39.9 ± 8.0	39.4 ± 8.2
Leg Fat (%)	42.6 ± 7.5	41.7* ± 7.7	43.6 ± 7.2	43.0 ± 7.2	41.7 ± 8.2	40.6 ± 8.4
Trunk Fat (%)	51.0 ± 6.7	50.4 ± 6.9	51.1 ± 8.6	50.8 ± 8.6	50.9 ± 5.0	50.0 ± 5.5
Android Fat (%)	55.0 ± 6.9	54.5 ± 7.2	55.2 ± 8.9	55.0 ± 9.0	54.8 ± 5.1	54.0 ± 5.7
Gynoid Fat (%)	45.3 ± 7.0	44.2* ± 7.1	46.1 ± 7.5	45.5 ± 7.6	44.6 ± 7.0	43.1 ± 6.9
Visceral Fat (cm^3)	2118 ± 1172	2131 ± 1171	1762 ± 953	1797 ± 944	2434 ± 1309	2428 ± 1323

*Significant change from pre to post all subjects together; $^{+}$N=15 for this variable only.

Blood Pressure and Maximal Oxygen Uptake

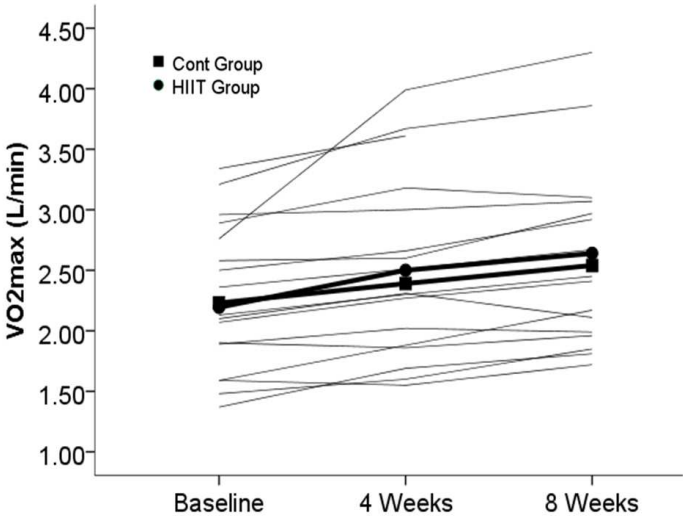
As shown in Table 3, there were no significant changes in resting systolic or diastolic blood pressure by time, group, or group x time interaction factors. One subject in the HIT group did not perform the post VO_{2max} test. VO_{2max} significantly increased from baseline to 4 weeks (2.21 ± 0.57 to 2.44 ± 0.72, $P = 0.023$) and again from 4 weeks to 8 weeks (2.44 ± 0.72 to 2.56 ± 0.75, $P = 0.008$) with all subjects combined. There were no statistical differences in VO_{2max} between the HIT and CONT groups ($P = 0.870$) or group x time interaction ($P = 0.477$). Similar results were seen with VO_{2max} expressed relative to body weight (See Table 3 and Figure 1).

Table 3: Resting blood pressure, continuous glucose monitoring data, and maximal oxygen uptake. Values represent mean ± Standard deviation.

	All (N=17)		**Continuous (N=8)**		**High-Intensity Interval (N=9)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Systolic Blood Pressure (mmHg)	130 ± 10	129 ± 10	128 ± 6	128 ± 7	132 ± 13	129 ± 12
Diastolic Blood Pressure (mmHg)	82 ± 9	86 ± 10	80 ± 5	84 ± 7	85 ± 11	88 ± 12
Glucose Average 24 hours (mg/dl)[+]	101.7 ± 8.1	99.3 ± 12.2	99.9 ± 9.3	100.3 ± 10.7	103.9 ± 6.5	98.2 ± 14.6
Glucose Area Under the Curve 24 hours (mg/dl)[+]	140192 ± 12885	137240 ± 17983	136864 ± 13005	137874 ± 17079	143995 ± 12585	136515 ± 20326
VO_{2max} (L/min)*	2.21 ± 0.57	2.56 ± 0.75	2.23 ± 0.51	2.54 ± 0.65	2.19 ± 0.65	2.64 ± 0.88
VO_{2max} (ml/kg/min)*	21.35 ± 4.37	24.85 ± 5.21	22.37 ± 3.85	25.33 ± 4.81	20.35 ± 4.86	24.38 ± 5.87

[+] N = 15 for All, 7 HIT and 8 CONT. *Significant change from pre to post all subjects together. N = 8 for HIT Post VO_{2max} data.

Figure 1: Maximal oxygen uptake (L/min) baseline, after 4 weeks of exercise, and after 8 weeks of exercise. There was a significant time effect ($P = 0.001$), but no group x time interaction ($P = 0.474$). There were significant time effects between baseline and 4 weeks ($P = 0.023$), baseline and 8 weeks ($P = 0.003$), and 4 weeks and 8 weeks ($P = 0.008$). Individual data in lighter lines and mean data in bold lines. HIT group: circles, CONT group: squares.



Continuous Glucose Monitoring

Due to missing data we removed the glucose results from three subjects in the HIT group therefore the whole group glucose results are on 6 subjects in the HIT group and 8 subjects in the CONT group. With these 13 subjects there were no statistical differences for glucose AUC or average 24 hour glucose between the group and time factors and the group x time factors (All *P* values > 0.40, See Table 3 and Figure 2). Subjects were split according to their baseline 24-hour average glucose value into two groups: > 100 mg/dl or < 100 mg/dl. There were 4 subjects from each intervention group (HIT and CONT) in the >100 mg/dl group. In the < 100 mg/dl group there were 2 subjects from the HIT group and 4 from the CONT group. In the <100 mg/dl group there

were no significant changes from pre to post in average glucose (94.2 ± 4.7 to 103.6 ± 12.4 mg/dl, *P* = 0.176) and glucose AUC (131336 ± 8814 to 144445 ± 15219, *P* = 0.203). In the >100 mg/dl group there were significant reductions pre to post in average glucose (107.8 ± 4.7 to 94.3 ± 10.8 mg/dl, *P* = 0.027) and glucose AUC (148684 ± 9777 to 130310 ± 17068, *P* = 0.007). There were no statistical differences between the HIT and CONT groups or group x time interaction when the sample was split by average baseline glucose value (All P values > 0.30). There was a significant group x time interaction observed using the baseline glucose groups (AUC: *P* = 0.007, Average glucose: *P* = 0.002) as shown in Figures 2-4.

Figure 2: Average glucose values for all subjects (each 5 minute glucose reading) pre- (solid line) and post-intervention (dotted line). **A)** Continuous exercise group (CONT, N = 8), **B)** High-intensity Interval Training Group (HIT, N = 6). Error bars represent ± 1 standard error.

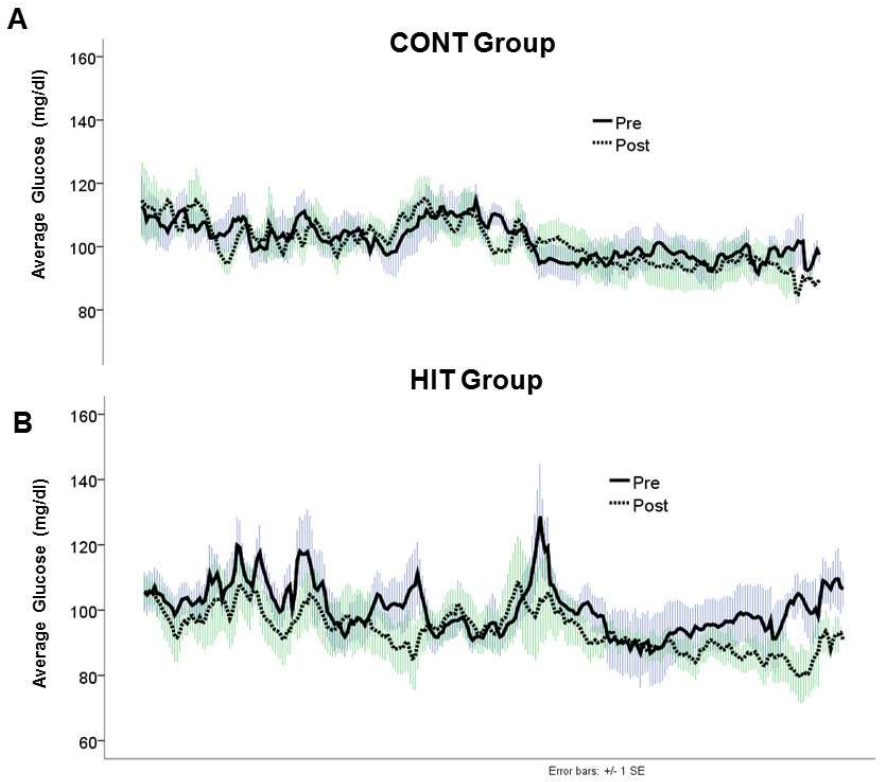


Figure 3: Average glucose values (each 5 minute glucose reading) pre- (solid line) and post-intervention (dotted line) for **A)** Subjects with baseline average 24 hour glucose of < 100 mg/dl (N = 8) and **B)** Subjects with baseline average 24 hour glucose of > 100 mg/dl (N=6). Intervention groups are together. Error bars represent ± 1 standard error. Significant reduction in average glucose in the >100 mg/dl group ($P = 0.027$), but not in the <100 mg/dl group ($P = 0.176$).

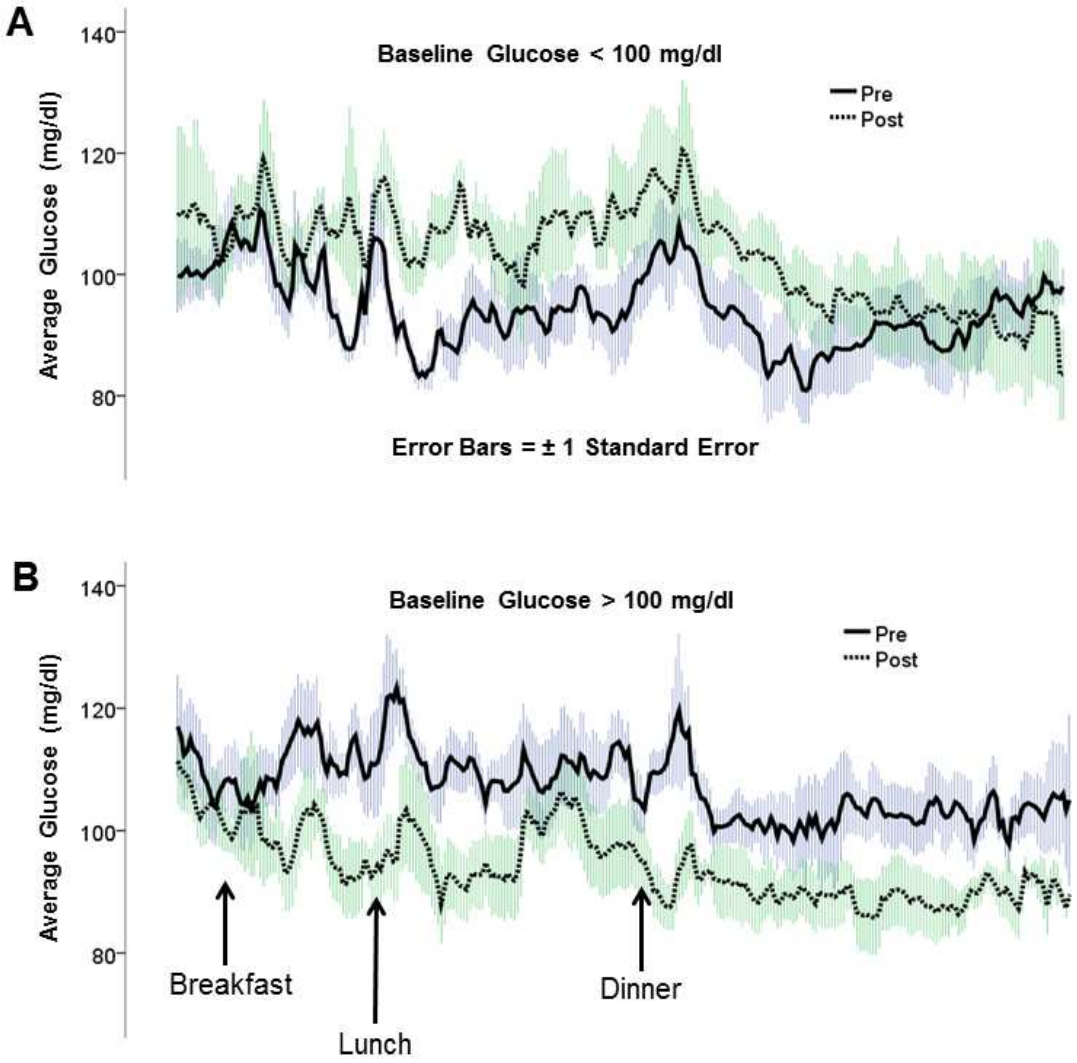
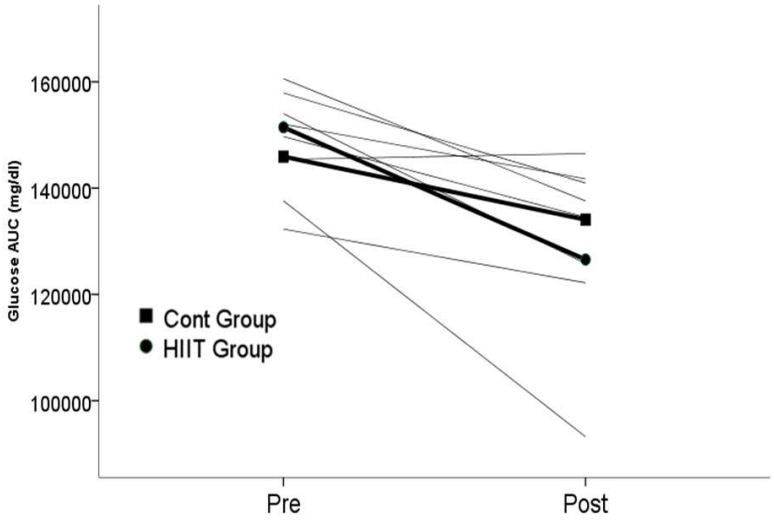


Figure 4: Glucose area under the curve (AUC) pre- and post-intervention for the subjects whose baseline average 24-hour glucose was greater than 100 mg/dl. There was a significant time effect ($P = 0.027$), but no group x time interaction ($P = 0.388$). Individual data in lighter lines and mean data in bold lines. HIT group: circles, CONT group: squares.



Flow Mediated Dilation

There were no significant time, group or group x time effects for any measurements of blood flow, velocity, shear rate, peak diameter, or time to peak diameter when all three time points were considered. When all three time points were included the HIT group had a greater baseline diameter (HIT = 0.407 ± 0.067, CONT = 0.375 ± 0.060 mm; $P = 0.100$) and had significantly larger peak diameter (HIT = 0.434 ± 0.066, CONT = 0.392 ± 0.058 mm; $P = 0.027$) and FMD response (HIT = 6.91 ± 4.03, CONT = 4.70 ± 3.92%; $P = 0.047$) than did the CONT group. There were no significant time effects on any of the FMD variables. There was a significant group x time interaction in the FMD % ($P = 0.043$) as shown in table 4 and Figures 5-7.

When the baseline and 8 week data were considered only and the data was split by group, the CONT group had a significant increase in baseline diameter (0.367 to 0.386 cm, $P = 0.001$), no change in peak diameter (0.388 to 0.396 cm, $P = 0.301$), and a significant decrease in FMD % (5.91 to 2.94%, $P = 0.018$). Conversely, the HIT group had no change in baseline diameter (0.404 to 0.409 cm, $P = 0.542$), a significant increase peak diameter (0.422 to 0.442 cm, $P = 0.009$), and a significant improvement in FMD % (4.82 to 8.62%, $P = 0.045$). Furthermore, with only the baseline and 8 week data included there was a significant group x time interaction for FMD % ($P = 0.001$).

Since there were small non-significant differences in artery size between groups as well as differences in the pattern of diameter changes between groups it was appropriate to employ the allometric scaling technique described in the methods. Furthermore the slope of the ln adjusted change in baseline diameter (pre to post) between groups was only 0.89 (95% confidence interval = 0.81-0.97). The recommendations in the literature deem it necessary to employ allometric scaling if the slope is not close to 1.0 or if the 95% CI does not include 1 (Atkinson et al., 2013). Therefore the allometrically scaled FMD % between groups (results shown are after back transformation) was significantly higher in the HIT group compared to the CONT (See Figure 8).

Table 4: All outcomes from the brachial artery ultrasound measurements at baseline, 4 weeks, and 8 weeks. Values represent: Mean ± Standard deviation.

	Continuous			HIT		
	Baseline	**4 Weeks**	**8 Weeks**	**Baseline**	**4 Weeks**	**8 Weeks**
Diameter Baseline (cm)	0.367 ± 0.062	0.374 ± 0.063	0.386 ± 0.062	0.404 ± 0.07	0.408 ± 0.07	0.409 ± 0.07
Diameter Peak (cm)	0.388 ± 0.065	0.392 ± 0.059	0.396 ± 0.056	0.422 ± 0.068	0.436 ± 0.067	0.442 ± 0.07
Flow Mediated Dilation %	5.91 ± 4.32	5.35 ± 4.03	2.84 ± 3.09	4.82 ± 2.74	7.29 ± 2.94	8.62* ± 5.33
Average Velocity (cm/s)	25.32 ± 6.78	24.44 ± 11.37	26.71 ± 9.38	26.31 ± 9.41	19.99 ± 7.59	20.09 ± 6.26
Average Blood Flow (ml/min)	2.86 ± 1.16	2.77 ± 1.44	3.14 ± 1.41	3.31 ± 1.16	2.78 ± 1.3	2.87 ± 1.27
Integral/min Flow (ml/min)	171.53 ± 69.61	166 ± 86.19	188.41 ± 84.84	198.51 ± 69.56	166.6 ± 78.4	172.42 ± 76.13
Integral Anterograde Flow (ml/min)	171.6 ± 69.53	166.4 ± 86.05	188.68 ± 84.65	199.19 ± 69.16	166.8 ± 78	172.85 ± 75.64
Integral Retrograde Flow (ml/min)	-0.09 ± 0.18	-0.45 ± 0.85	-0.39 ± 0.9	-0.73 ± 1.17	-0.3 ± 0.5	-0.52 ± 0.63
Average Shear Rate (1/s)	278.22 ± 96.53	267.05 ± 144.01	286.81 ± 123.75	278.13 ± 151.03	200.91 ± 106.77	198.17 ± 89.26
Integral/min Shear Rate	16688.59 ± 5791.75	16019.53 ± 8633.52	17215.57 ± 7425.56	16684.72 ± 9058.04	12051.01 ± 6389.11	11891.1 ± 5353.33
Integral Anterograde Shear Rate	16694 ± 5781	16052 ± 8616	17234 ± 7401	16749 ± 9067	12073 ± 6386	11920 ± 5336
Integral Retrograde Shear Rate	-7.46 ± 13.99	-34.31 ± 64.63	-30.83 ± 70.67	-66.63 ± 120.71	-23.54 ± 41.49	-34.03 ± 45.23
Time to Peak (sec)	64.78 ± 13.19	68.36 ± 16.17	68.98 ± 20.37	88.61 ± 56.69	68.71 ± 18.46	71.54 ± 19.53

* Significant group x time interaction, $P < 0.05$.

Figure 5: Brachial artery baseline (resting) diameter at baseline, 4 weeks, and 8 weeks in the **A)** Continuous exercise group and **B)** High-intensity exercise group. Individual subject and group values are shown. No significant changes over time.

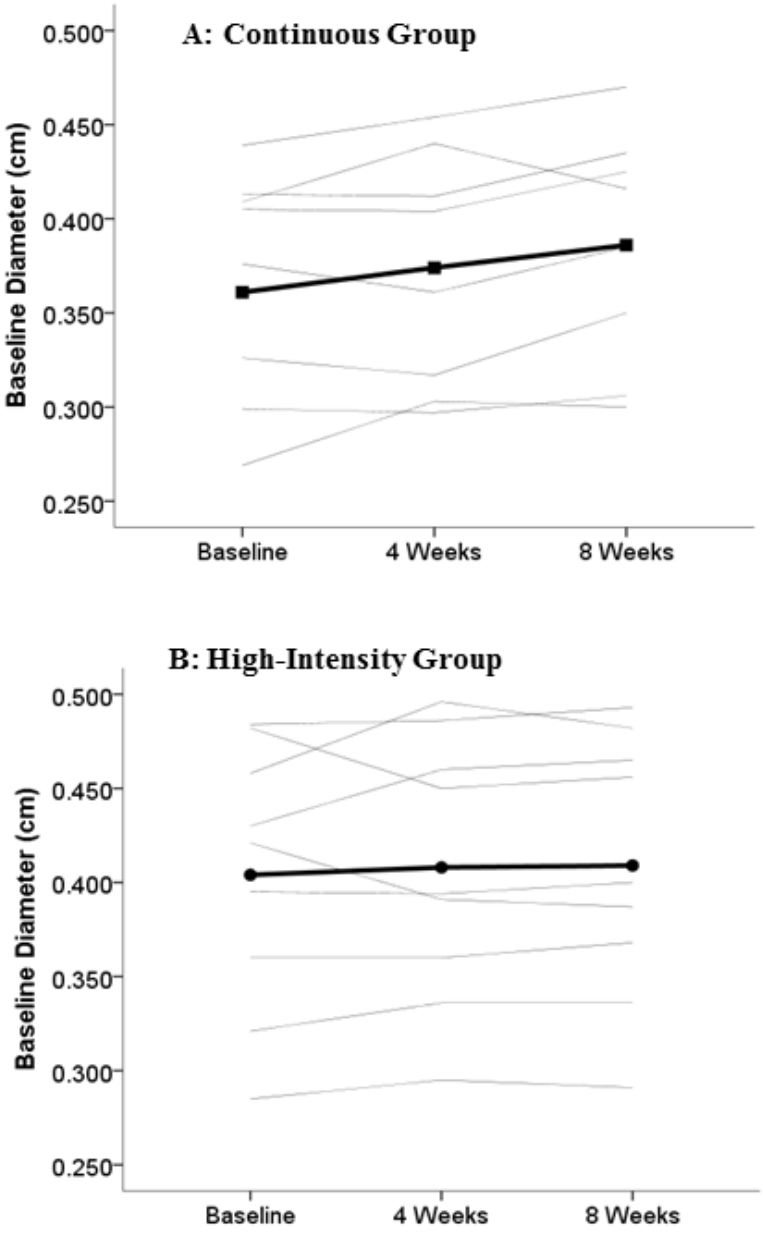


Figure 6: Brachial artery peak (after cuff release) diameter at baseline, 4 weeks, and 8 weeks in the **A)** Continuous exercise group and **B)** High-intensity exercise group. Individual subject and group values are shown. No significant changes over time, significant group difference ($P = 0.027$).

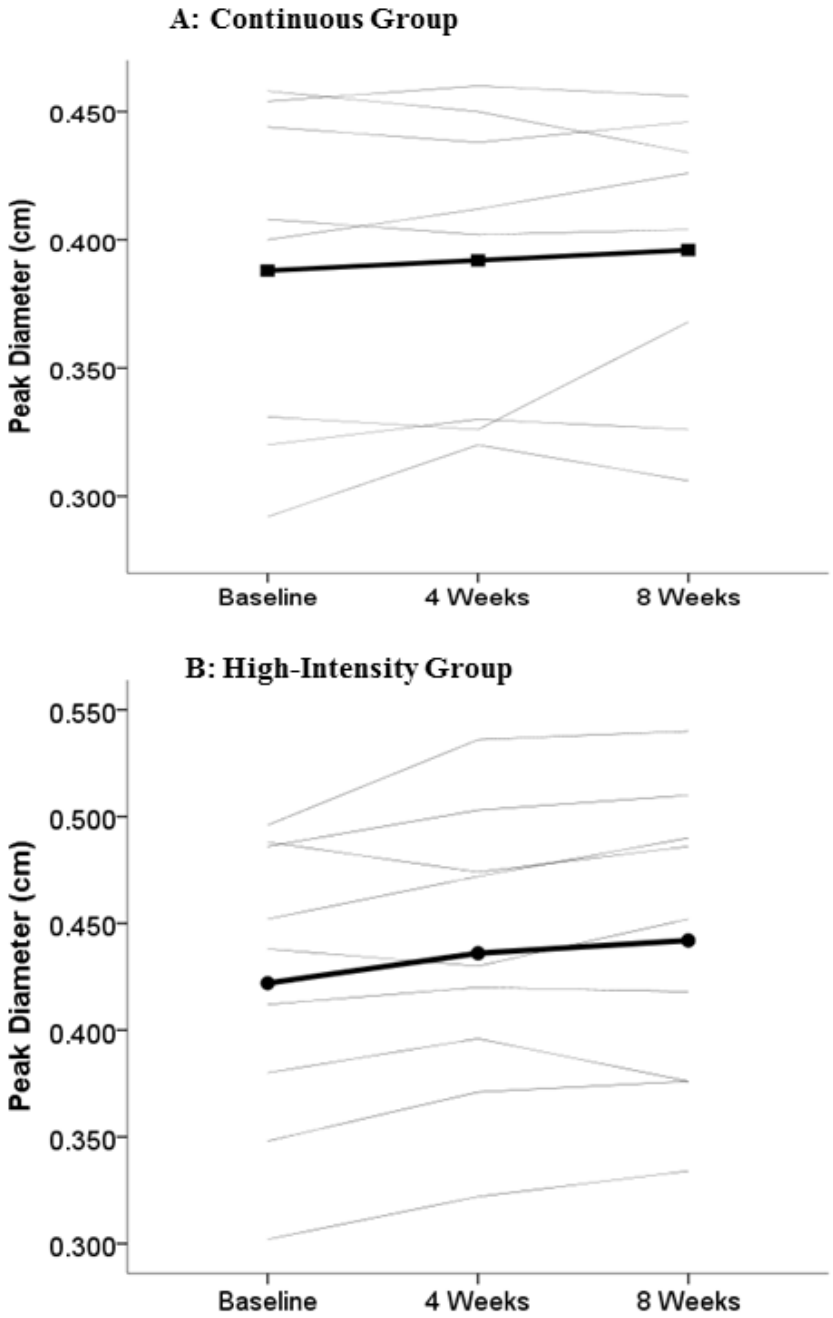


Figure 7: Percent increase in diameter from baseline to peak at baseline, 4 weeks, and 8 weeks in the **A)** Continuous exercise group and **B)** High-intensity exercise group. Individual subject and group values are shown. No significant changes over time. Significant group difference ($P = 0.047$) and group x time interaction ($P = 0.043$).

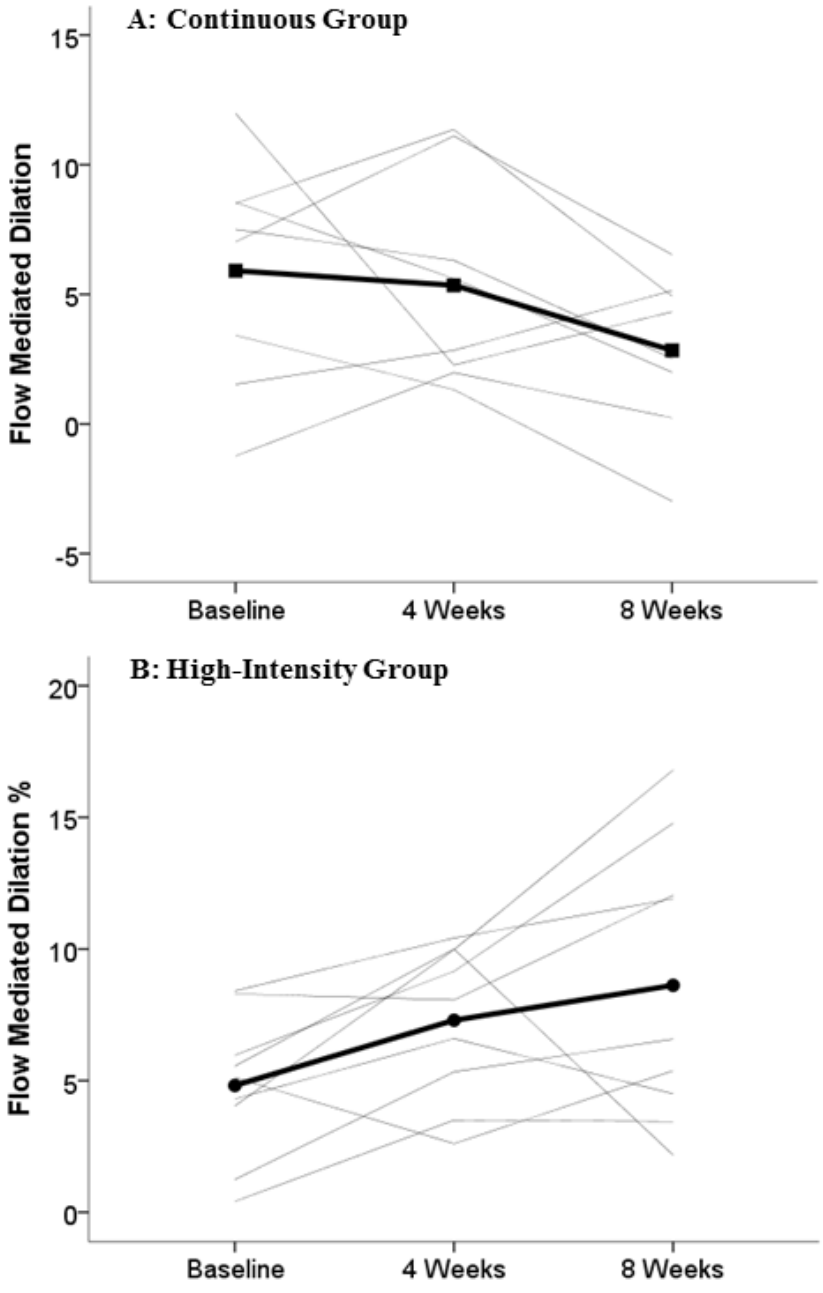
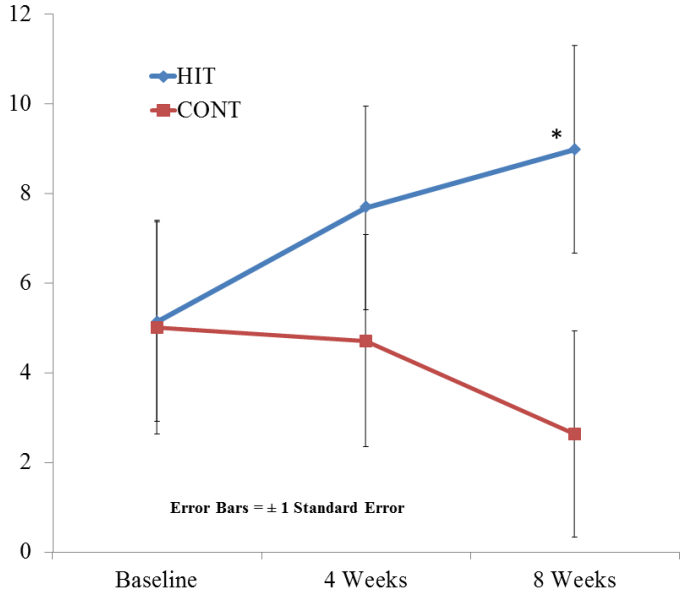


Figure 8: Allometrically scaled flow mediated dilation at baseline, 4 weeks, and 8 weeks in the continuous exercise group (CONT, N = 8), high-intensity interval training group (HIT, N = 9). *Significant group x time interaction (*P* = 0.006).



Activity Monitoring

The acclerometer results are shown in Table 6. We obtained reliable data on 16 subjects for baseline and 5th week testing and on only 9 subjects for the 8th week testing. There were no significant mean differences between the groups and time factors and the group x time interaction factor in any of the accelerometer outcomes.

Table 6: Outcomes from the accelerometers at baseline, 4 weeks, and 8 weeks. There were no significant differences between groups at baseline. Also using only the baseline and 5th week data there were no significant group, time, or group x time differences. Statistical analyses were not performed on the 8th week data due to small sample size. Values represent: Mean ± Standard deviation.

	Continuous			**HIT**		
	Baseline (N=7)	**5th Week (N=7)**	**8th Week (N=4)**	**Baseline (N=9)**	**5th Week (N=9)**	**8th Week (N=5)**
Sedentary Time (min/day)	534.1 ± 93.6	511.3 ± 47.7	525.7 ± 76.1	571.4 ± 69.8	554.2 ± 111.8	621.7 ± 61.5
Light Activity Time (min/day)	230.8 ± 61.1	212.7 ± 56.1	194.1 ± 35.4	194.7 ± 62.7	173.7 ± 61.2	164.5 ± 76.3
Lifestyle Activity (min/day)	92.4 ± 37.0	81.6 ± 27.3	64.4 ± 18.8	70.8 ± 16.9	72.7 ± 24.1	64.7 ± 21.8
Moderate Activity (min/day)	24.8 ± 9.2	26.1 ± 13.4	20.5 ± 10.2	27.1 ± 11.5	23.8 ± 11.9	22 ± 8.4
Vigorous Activity (min/day)	1.0 ± 0.9	0.2 ± 0.3	1.1 ± 2.2	0.6 ± 0.8	0.7 ± 1.3	1.4 ± 1.8
Vertical Axis (Counts/day)	273863 ± 72225	251259 ± 72299	213979 ± 65087	237587 ± 48744	225546 ± 67217	209838 ± 80668
Vertical Axis (Counts/min)	294 ± 121	296 ± 128	275 ± 136	276 ± 114	284 ± 152	238 ± 110
Steps/day	6062 ± 1713	6536 ± 1873	5754 ± 1248	5819 ± 1836	5578 ± 2059	5519 ± 2233

Correlations

The change in FMD % was significantly correlated with the change in baseline artery diameter (r = -0.58, *P* = 0.015). The change in glucose AUC was significantly correlated with the change in body fat % (r = 0.59, *P* = 0.025). The change in baseline artery diameter was significantly correlated with the change weight (r = 0.53, *P* = 0.027) and lean mass (r = 0.62, *P* = 0.008). Abdominal circumference and sagittal diameter

measurements pre and post correlated moderately with VAT (r = 0.68, P <0.001 r = 0.52, P = 0.003, respectively), but the changes in each did not correlate well (sagittal diameter: r = 0.10, P = 0.712; waist circumference: r = 0.202, P = 0.416). The change in visceral fat was strongly associated with the change in fat mass (r = 0.80, P < 0.001) and weight (r = 0.72, P = 0.002). The change in sedentary time between baseline and the 5^{th} week was significantly related to physical activity enjoyment (r = -0.53, P = 0.036).

Chapter 5

DISCUSSION

The current study compared the effects of high-intensity interval training to continuous exercise on endothelial function, glucose regulation, and visceral adipose tissue in obese individuals. The total exercise time in the HIT group including warm-up and cool-down times was only 72% of the time spent by the CONT group (11.6 vs. 16 total hours). Despite the lower time commitment the interval training led to superior improvements in endothelial function in obese individuals. This study also showed that HIT and continuous exercise for eight weeks led to improvements in glucose regulation in the individuals whose average 24-hour glucose was above 100 mg/dl at baseline. Lastly, we showed no reduction in visceral adipose tissue measured by the iDXA even though there were significant reductions in waist circumference and sagittal diameter.

Endothelial Function

It is well known that exercise training in individuals with impaired vascular function leads to improvements in endothelial function (Green, 2009). Recent evidence suggests that HIT may lead to superior improvements in endothelial function (Ciolac et al., 2010; Molmen-Hansen et al., 2012; Schjerve et al., 2008; A. Tjonna et al., 2009; A. E. Tjonna et al., 2008; Wisloff et al., 2007) while other studies show similar improvements between continuous and interval training (Currie et al., 2013; T. Moholdt et al., 2011; Rakobowchuk et al., 2008). The only study conducted in obese individuals directly comparing HIT to CONT exercise training showed that 12 weeks of HIT led to superior improvements in FMD compared to CONT exercise (Schjerve et al., 2008). Our study used a similar sample with a mean BMI of ~36 kg/m^2 and a slightly shorter training

duration (8 vs. 12 weeks), but the study by Schjerve and colleagues has several limitations. Firstly, Schjerve and colleagues measured artery diameter every 30 seconds using calipers instead of the recommended time-course measurement using advanced edge detection software (D. H. J. Thijssen et al., 2010). The use of calipers to measure artery diameter has been shown to produce large variability in FMD% (CV: 15.9%) compared to edge detection software (R. A. Harris et al., 2010). Furthermore, it is likely that the true peak diameter could have been missed using the 30 second windows of diameter measurement instead of the full time-course (Black et al., 2008). Conversely, we used the recommended time-course measurement of artery diameter using an externally validated (Woodman et al., 2001) advanced edge detection software in which we have shown our own high levels of reliability.

Despite these methodological differences we found similar but not identical results. Using the un-scaled FMD % HIT led to an absolute increase of 3.8% while CONT exercise led to a decrease of 3.1%. Schjerve and colleagues (2008) showed an absolute increase in FMD % of 7.3 in the HIT and only 4.2 in the continuous group. Much of the drastic increase in FMD seen in the Schjerve et al study could have been driven by the fact that baseline diameter decreased in the HIIT group (0.402 to 0.391 cm), but did not change in the continuous group (0.400 to 0.401 cm). Interestingly, peak diameter increased in both groups by the same amount after the intervention (0.018 cm). Conversely, we observed divergent changes in artery diameter in response to the two exercise interventions. Peak diameter significantly increased in the HIT group, but not in the CONT group and baseline diameter significantly increased in the continuous group, but not in the HIT group. It has been recently shown that up to 64% of the variability in

FMD% can be explained by variation in baseline diameter (Atkinson & Batterham, 2013). In our study the correlation between change in baseline diameter and change in FMD % was -0.61 indicating that 37% of the variance in FMD change was due to change in baseline diameter. Due to the divergent changes in artery diameter and the differences in baseline diameter at the onset of the study (HIT = 0.404 vs. CONT = 0.367, although not statistically different possibly impactful, see (Senn, 2006)) between groups the allometric scaling technique recently described (Atkinson et al., 2013) was appropriate in the current study. The allometrically scaled FMD values showed a similar FMD at baseline between groups followed by an absolute increase of 3.85% in the HIT group and an absolute decrease in the CONT group of 2.39% (Figure 19). The apparent reduction in FMD % in the CONT group while the HIT group increased using the un-scaled FMD is therefore partially explained by adjusting for baseline diameter. Therefore, the allometric scaling of FMD confirms our findings of a superior improvement in endothelial function in response to HIT and shows that CONT exercise may have led to structural remodeling and increased brachial artery diameter.

The reason for the larger increase in brachial artery diameter and decrease in FMD in the CONT group compared to the HIT group is not apparent. Interestingly peak artery diameter increased at both 4 and 8 weeks in the CONT group, but the baseline diameter increased more, leading to a lower FMD. Other studies using 8 weeks of continuous cycling exercise at a similar intensity to our study showed no increase in FMD (Maiorana et al., 2001; D. Thijssen et al., 2007). Recent cross-sectional studies have shown that masters-level and professional athletes have much larger arteries (especially in the active limb for their sport) compared to age matched controls (Green et

al., 2013). Furthermore, it has recently been suggested that increases in artery diameter may be a function of time spent exercising (Spence, Carter, Naylor, & Green, 2013). Since the exercise intervention for the CONT group was 38% longer (in total exercise time) compared to the HIT intervention the differential changes in artery diameter may be partially explained by the longer total exercise time. It has also been proposed that in young normal weight individuals FMD may peak after 2 weeks of exercise training then start to decline as artery diameter increases and the stimulus for FMD (shear stress) declines (Tinken et al., 2008). Subsequently in our study we may have missed the peak in FMD if it occurred around 2 weeks in the CONT group. These structural adaptations have not been shown in an obese population, but could lead to improvements in vascular health despite decreasing FMD (Brown, 2003). Differing modulations and patterns of shear stress have been shown to cause differing effects on endothelial function and it is possible that HIT caused a different shear stress pattern compared to CONT exercise training (D. H. Thijssen, Dawson, Tinken, Cable, & Green, 2009; Tinken et al., 2009). One possibility is that the sustained duration of shear stress with continuous exercise led to more rapid vascular remodeling when compared to the transient nature of the shear stress with HIT in this population.

Most studies that have directly compared the effects of HIT to continuous exercise with FMD as an outcome have not reported baseline diameter (T. T. Moholdt et al., 2009; Molmen-Hansen et al., 2012; A. Tjonna et al., 2009; A. E. Tjonna et al., 2008; Wisloff et al., 2007). Furthermore, many of the direct comparison studies used calipers or non-time course analysis for measurement of artery diameter which may not be precise enough to detect small changes in artery diameter (T. T. Moholdt et al., 2009; Molmen-

Hansen et al., 2012; Schjerve et al., 2008; A. Tjonna et al., 2009; A. E. Tjonna et al., 2008; Wisloff et al., 2007). A recent study comparing 12 weeks of HIT to continuous exercise in CAD patients showed similar improvements in FMD % and a small non-significant increase in brachial artery diameter in the continuous group (0.430 to 0.432 mm) and with no change in the HIT group (0.452 to 0.452 mm) (Currie et al., 2013). Our study is the first to show divergent changes in artery diameter between HIT and continuous exercise. More studies directly comparing HIT with continuous exercise utilizing larger samples, more frequent FMD measurement, and automated edge detection software are needed to confirm and expand on our findings of differential changes in artery diameter.

The main proposed mechanism for increases in FMD in response to exercise training is the shear stress elicited by increased blood flow (G. K. Birk et al., 2012; Dimmeler et al., 1999; Green et al., 2004; Green, 2009; Lenk et al., 2011; Ribeiro et al., 2010; Tinken et al., 2009). The increase in shear stress has been shown to lead to increased NO availability (Deljanin Ilic et al., 2009; Green et al., 2004) via increases in eNOS (Delp & Laughlin, 1997; Hambrecht et al., 2003; Sessa et al., 1994), increases in number and functional ability of endothelial progenitor cells (Dimmeler et al., 1999; Laufs et al., 2004; Lenk et al., 2011), increases in antioxidant capacity (Adams et al., 2005; Edwards et al., 2004; Goto et al., 2007; Rush et al., 2000; Wisloff et al., 2007), and improvements in other CVD risk factors related to endothelial cell health (Ribeiro et al., 2010).

Reasons for the greater increase in FMD% and allometrically scaled FMD in the HIT group compared to the CONT group could be due to the larger levels of shear stress

induced by the higher exercise intensity with the interval training. Higher levels of shear stress theoretically lead to greater stimulus for the above mentioned effects of shear stress. This has been shown in humans and animals by greater increases in eNOS expression and phosphorylation, antioxidant capacity, NO availability, and greater decreases in oxidized LDL, in response to HIT compared to continuous exercise (Deljanin Ilic et al., 2009; Haram et al., 2009; Wisloff et al., 2007). Therefore, this study confirms previous findings that HIT leads to superior improvements in endothelial function when directly compared to continuous exercise and we extend the findings of Schjerve et al (2008) showing that the effects hold true in an obese sample with more stringent FMD methodology.

Interestingly, the change in FMD was inversely correlated with the change in abdominal circumference ($r = -0.50$, $P = 0.041$), but not with VAT ($r = 0.004$, $P = 0.989$). Cross-sectional studies have shown that abdominal circumference, waist to hip ratio, and VAT measured via CT scan are good predictors of endothelial function (Arcaro et al., 1999; Brook, Bard, Rubenfire, Ridker, & Rajagopalan, 2001; Hashimoto et al., 1998). Furthermore, the change in VAT in response to a dietary weight loss program in healthy obese individuals has been shown to be inversely related the change in endothelial function (Park & Shim, 2005). We can speculate that the reductions in visceral fat could have led to reduced inflammation, oxidative stress, and increased NO availability, but we did not measure these markers (Ritchie & Connell, 2007). The reason for the iDXA measured VAT not correlating with FMD may be a methodological limitation of the iDXA which will be discussed in the VAT section.

Glucose regulation

The ability of aerobic exercise to improve glucose regulation in individuals with impaired glucose tolerance as well as type 2 diabetes is well recognized (Boulé et al., 2001; Snowling & Hopkins, 2006; Thomas et al., 2006). In the present study the time, group, or group x time effects for glucose regulation assessed via continuous glucose monitoring were not significant. The lack of effect may have been due to the fact that impaired fasting glucose was not an inclusion criteria for the study and many of the subjects started with normal glucose tolerance. Some studies have shown that subjects with normal glucose tolerance do not improve insulin sensitivity, glucose regulation, or insulin release in response to exercise interventions (Jenkins & Hagberg, 2011; Krotkiewski et al., 1985). To examine this hypothesis we divided our sample between individuals who started with average 24-hour glucose of greater than 100 mg/dl and those who started with average glucose below 100 mg/dl. The individuals who started with lower glucose levels did not improve glucose regulation but those who started with higher glucose levels decreased their average 24 hour glucose and glucose AUC values by 12.5% (See Figure 4). There were four subjects from each intervention group with baseline average glucose above 100 mg/dl, but no significant intervention group x time interactions were observed.

The improvements in glucose regulation seen in the subjects with higher average glucose levels at the onset of the study could have been due to reductions in hepatic fat and improvements in liver insulin sensitivity leading to improved control of hepatic glucose release (Haram et al., 2009; Magkos, 2010; Shojaee-Moradie et al., 2007), improvements in pancreatic β-cell function via reductions in lipotoxicity and

improvements in insulin production (Bloem & Chang, 2008; Delghingaro-Augusto et al., 2012; Malin & Kirwan, 2012), or improvements in peripheral insulin sensitivity via improved glucose uptake and insulin responsiveness in the peripheral tissues (DiPietro et al., 2006; Jenkins & Hagberg, 2011; Röckl et al., 2008). Interestingly, the change in body fat % was directly correlated with the change in glucose AUC ($r = 0.59$, $P = 0.025$). Reductions in body fat have been shown to be related to improvements in glucose regulation in some studies (Knowler et al., 2002; Pi-Sunyer et al., 2007), but improvements in glucose regulation can be realized without reductions in body fat (Gaesser, Angadi, & Sawyer, 2011). Since some of the variance in glucose regulation improvement can be accounted for by the change in body fat it is possible that the reductions in body fat were related to reductions in hepatic, pancreatic, and skeletal muscle fat accumulation leading to improved liver and peripheral insulin sensitivity as well as pancreatic β-cell function (Unger, 2002; Virtue & Vidal-Puig, 2010). Unfortunately without direct measurements of these lipid accumulation sites, insulin sensitivity and β-cell function we can only speculate.

Conversely, other studies have shown significant improvements in insulin sensitivity (assessed via OGTT) in healthy normal glucose tolerant individuals of all ages (Babraj et al., 2009; Irving, Short, Nair, & Stump, 2011; Metcalfe et al., 2011; Short et al., 2003). The difference between our study and the results of other studies that have shown improvements in insulin sensitivity in healthy subjects is the fact that we did not measure insulin sensitivity. Insulin sensitivity is only one aspect of the determinants of glucose regulation. In young healthy individuals improvements in insulin sensitivity may not be detected via continuous glucose monitoring because the tissues were already

sensitive enough to keep glucose levels stable during normal dietary intake. The studies that showed improvements in insulin sensitivity employed the OGTT. The OGTT is unique in that individuals are given a large load of glucose which leads to larger levels of stress on the insulin-glucose system than normal dietary intake (Muniyappa, Lee, Chen, & Quon, 2008). It may only be under conditions of high glucose intake that the glucose uptake system is stressed enough to notice the small changes that occur in individuals with NGT.

Our results agree with most studies showing that improvements in glucose regulation are not dependent on exercise intensity (Bajpeyi et al., 2009; Houmard et al., 2004; Larsen et al., 1997; Larsen et al., 1999; Slentz et al., 2009; Snowling & Hopkins, 2006). The results from the STRRIDE trial showed that walking 12 miles/week or jogging 20 miles/week were both better for improving insulin sensitivity than jogging 12 miles/week suggesting total volume of exercise is the key factor (Houmard et al., 2004). The STRRIDE trial also showed that moderate exercise improved β-cell function to a greater extent than vigorous exercise (Slentz et al., 2009). Likewise multiple studies from Denmark show that light intensity exercise is just as effective in improving glucose regulation in diabetics as vigorous (Larsen et al., 1997; Larsen et al., 1999). Conversely, a few studies show superior benefits from vigorous or high-intensity exercise in improving insulin sensitivity (DiPietro et al., 2006; Haram et al., 2009), fasting insulin (E. Trapp et al., 2008), and markers of skeletal muscle mitochondrial function and glucose transport (Hood et al., 2011; Little et al., 2011). With the conflicting results in the literature, more large scale studies directly comparing HIT to continuous exercise need to be conducted.

The present study is the first HIT study to date utilizing continuous glucose monitoring to measure glucose regulation in non-diabetic obese subjects. Implementing time efficient exercise interventions that may improve glucose regulation in obese individuals who are prone to developing impaired glucose tolerance and T2D is a high priority. A recent cross-sectional study showed that abdominally obese men had 10% higher 24-hour average glucose levels, assessed via CGM, compared to non-abdominally obese men (Ma et al., 2011). The first exercise intervention using CGM in T2D found that 4 months of strength training improved glucose control, but endurance exercise did not. Those results must be taken with caution because there was no mention of dietary control during the CGM use (Cauza et al., 2005). A recent study using just six sessions of HIT over a two week period in type 2 diabetics showed significant reductions in glucose AUC and average glucose measured via CGM (Little et al., 2011). Another study showed that 7 days of exercise training in T2D improves glucose control and glycemic variability (Mikus et al., 2012). Due to the fact that the CGMs in our study were worn 48-72 hours after the last exercise session we cannot rule out the possibility that the reduction in glucose AUC that we observed was due to the acute effects of exercise (Goodyear & Kahn, 1998). Conversely, this timing was chosen specifically because most studies show that the acute effect of exercise is mostly gone by 48 hours and usually completely gone by 72 hours (Eriksson et al., 1997). Therefore the present study is the first to show improvements in glucose regulation assessed via CGM in obese subjects without diagnosed impaired glucose tolerance.

Visceral Adipose Tissue

This was the first study to our knowledge attempting to use the iDXA to quantify changes in visceral adipose tissue in response to exercise training. Many studies have shown the effectiveness of aerobic exercise training in reducing VAT especially in obese subjects (Kay & Singh, 2006; Vissers et al., 2013). Conversely, after eight weeks of aerobic exercise training we observed no changes in VAT measured by the iDXA. Most studies evaluating VAT reductions with exercise use longer durations than the current study, but shorter term studies have shown reductions in VAT (Boudou, Sobngwi, Mauvais-Jarvis, Vexiau, & Gautier, 2003; Johnson et al., 2009; Mourier et al., 1997; Shojaee-Moradie et al., 2007). A study using only 4 weeks of cycling 3 days/week at a similar or lower intensity than our study in obese women led to a 12% reduction in VAT measured by MRI (Johnson et al., 2009). Another study using 3 days/week of exercise at 60-85% of VO_{2max} in overweight women showed a 17% reduction in VAT measured via CT scan (Shojaee-Moradie et al., 2007). Two studies using 8 weeks of aerobic exercise similar to our study showed VAT reductions of approximately 45% (Boudou et al., 2003; Mourier et al., 1997). It is not readily apparent why there was no reduction in VAT in our study.

The iDXA has never been validated against CT or MRI for the ability to accurately quantify changes in VAT and it is possible that it is not sensitive enough to detect small changes. The iDXA has been shown to accurately track an increase in fat mass of 1 kg by placement of exogenous fatty tissue on humans (Warolin et al., 2012). In

that study the mean increase in fat mass assessed by the DXA was only 0.93 kg even though the mass of the added fat was 1.0 kg. There was no statistical difference between measured and added fat mass, but a mean difference of 0.07 kg while measuring exogenous fat could indicate larger variation while attempting to quantify deep visceral fat. Since the iDXA is a two-dimensional imaging system it relies on the width of the lateral compartments of abdominal subcutaneous fat to detect and extrapolate the width of the subcutaneous fat in the anterior region. This approach may lead to inaccurate estimates of VAT since individuals most likely have great variability in the level of subcutaneous fat in the lateral compartments compared to the anterior. Furthermore the total depth of the abdomen is assessed using the tissue attenuation levels measured by the iDXA which could also lead to error. In support of this argument our data show significant decreases in both abdominal circumference and sagittal diameter between pre- and post-intervention, but no change in visceral fat. Many studies have shown that abdominal circumference and sagittal diameter are significantly related to VAT (Pouliot et al., 1994; Rankinen, Kim, Perusse, Despres, & Bouchard, 1999; Van Der Kooy, Leenen, Seidell, Deurenberg, & Visser, 1993). Conversely, some studies have shown that changes in abdominal circumference and sagittal diameter are better predictors of changes in subcutaneous fat than VAT (Van Der Kooy et al., 1993). Interestingly, in our study abdominal circumference ($r = 0.68$, $P < 0.001$) and sagittal diameter ($r = 0.52$, $P = 0.003$) correlated moderately with VAT both pre and post, but the changes in each did not correlate well (Sagittal diameter: $r = 0.10$, $P = 0.712$; waist circumference: $r = 0.202$, $P = 0.416$). These findings may imply that the iDXA is precise enough to quantify VAT at discrete time points, but not precise enough to quantify small changes in VAT which

could be less than 0.5 kg of fat. Furthermore, the change in VAT was highly correlated with the changes in fat mass ($r = 0.80$, $P < 0.001$) and weight ($r = 0.72$, $P = 0.002$). VAT reductions are commonly observed without weight loss (Boudou et al., 2003; Johnson et al., 2009; Shojaee-Moradie et al., 2007), but the high correlations between weight and fat mass change with iDXA measured VAT may shed light on the fact that the iDXA visceral fat measurements are more related to total mass. Alternatively, subjects in our study could have lost subcutaneous fat and not lost visceral fat to elicit the reduction in abdominal measurements. Without more direct measurement of visceral fat we cannot be certain. Studies validating the iDXA's ability to accurately measure change in VAT by comparison to MRI or CT scan are needed.

Some studies suggest that higher intensity exercise may lead to superior reductions in VAT (Coker et al., 2009; Irving et al., 2008), while others show no difference between moderate and vigorous intensities (Cho et al., 2011; Gutin et al., 2002; Kay & Singh, 2006; Nicklas et al., 2009; Ohkawara et al., 2007; Slentz et al., 2005). In our study both groups did vigorous intensity exercise and there were no reductions in VAT with either HIT or CONT exercise training. A recent study showed that 15 weeks of HIT led to superior reductions in trunk adiposity compared to steady-state exercise training, but actual VAT was not measured (E. Trapp et al., 2008). The ability of HIT to reduce VAT is still largely unknown. More direct comparison studies using HIT versus continuous exercise and VAT measured with validated methods are needed.

Maximal Oxygen Uptake

Many studies to date have compared HIT to continuous exercise for the improvement of maximal oxygen uptake. Some studies show that HIT elicits greater improvements in VO_{2max} compared to continuous exercise (Rognmo, Hetland, Helgerud, Hoff, & Slørdahl, 2004; Schjerve et al., 2008; A. E. Tjonna et al., 2008; Wisloff et al., 2007), but others have shown similar improvements (Ciolac et al., 2010; Currie et al., 2013; Poole & Gaesser, 1985). VO_{2max} is widely known as the criterion measure of cardiorespiratory fitness and has been shown to be a strong predictor of all-cause and cardiovascular disease mortality rates across all BMI levels (Blair et al., 1989; Kavanagh et al., 2002; Wing et al., 2007). Furthermore, it has been shown that improvements in physical fitness significantly lower mortality risk independent of BMI and health status (Blair et al., 1995; Myers et al., 2002). The participants in our study had significant improvements in VO_{2max} (15.8% overall). The approximate 3.5 ml/kg/min increase in VO_{2max} seen in this study has been shown to reduce the risk of mortality by 11% (Myers et al., 2002). The HIT group improved by 21% and the CONT improved by 14%, but there was no difference between the groups. Many HIT studies in populations with low VO_{2max} values have shown improvements of 30-50% (Schjerve et al., 2008; A. E. Tjonna et al., 2008; Wisloff et al., 2007). Our study may have been too short in duration to see improvements in VO_{2max} to the extent that other studies observed. Although the 21% improvement in our HIT group in 8 weeks is similar or greater to what some other studies have reported (Ciolac et al., 2010; Currie et al., 2013; T. Moholdt et al., 2011; T. T. Moholdt et al., 2009; Molmen-Hansen et al., 2012).

Resting Blood Pressure

We observed no reductions in resting blood pressure in response to the intervention. This is not surprising due to the fact that our subjects did not start out with hypertension. The baseline mean blood pressures were slightly in the pre-hypertensive range, but most subjects started with normal blood pressure. A meta-analysis showed that the expected reduction in systolic blood pressure in response to exercise training in normotensive individuals is only ~2-3 mmHg (Fagard, 2001). Overall we observed a 1 mmHg (not significant) reduction in resting systolic blood pressure which would require a much larger sample size to reach statistical significance.

Body Composition and Anthropometrics

This study led to no significant changes in body weight, body fat mass or lean mass. There was a significant reduction in body fat % measured by the DXA. The significant decrease in body fat % was caused by the 0.6 kg reduction in fat mass and the 0.6 kg increase in lean mass neither of which were large enough to reach statistical significance on their own, but together they led to a small decrease in body fat %. We did not invoke a large enough calorie deficit to cause weight loss. The recommended amount of exercise to elicit weight loss is 200-300 min/week (Donnelly et al., 2009). Our participants only exercised for 90 to 120 minutes/week, therefore we did not expect weight loss to occur. This study is a good example of the health benefits of exercise (improved vascular function, structure, and glucose regulation) without weight loss. Even though all subjects were still considered obese at the conclusion of the study the improvements in health outcomes and VO_{2max} show that they improved their cardiometabolic health without reductions in weight.

We did observe significant decreases in leg fat %, gynoid fat %, sagittal diameter and waist circumference with all subjects together. There were no differences between HIT and CONT for these parameters. The decreases in leg and gynoid fat % may be due to increased muscle mass in the legs and hips since the exercise was done completely with the legs. These changes in regional fat distributions are common in studies that involve cycling exercise (Wallman, Plant, Rakimov, & Maiorana, 2009). Some evidence suggests that HIT may be superior to continuous exercise for fat loss (Boutcher, 2010; E. Trapp et al., 2008). Evaluating methods of fat loss was not a goal of this study and our findings do not support this hypothesis.

Activity Monitoring

We did not observe any down regulation of physical activity in response to the exercise intervention in this group of subjects. Some studies have shown that individuals who start an exercise program tend to decrease their non-exercise activity thermogenesis throughout the rest of the day when they are not exercising (Colley, Hills, King, & Byrne, 2010; M. I. Goran & Poehlman, 1992; Manthou, Gill, Wright, & Malkova, 2010). The evidence is still unclear as to whether or not exercise compensation occurs regularly, but it may be one of the many reasons individuals do not lose as much weight as expected in response to an exercise program (Gomersall, Rowlands, English, Maher, & Olds, 2013). The lack of down regulation in our study provides internal validity to our results because we know our subjects did not drastically change their activity patterns outside of the intervention over the course of the study. Interestingly we observed a moderate inverse correlation between the changes in sedentary time from pre-intervention to the 5^{th} week of the intervention with physical activity enjoyment. Therefore, the less individuals

enjoyed the exercise in the study the more sedentary they became. This finding underscores the need for individualized exercise programs that allow subjects to choose their preferred mode of exercise if the desired outcome is sustained physical activity and decreased sedentary time.

Strengths and Limitations

The largest limitation of this study is the lack of dietary control during the course of the intervention. We cannot rule out the possibility that some of the changes we saw in our outcomes were related to dietary changes the subjects made over the course of the study. To control for this factor we continually reminded subjects of their commitment to maintain their current dietary habits. The lack of weight change is a good indicator that subjects did not start a hypocaloric diet over the course of the study. We chose to not use a sedentary control group and to use the continuous exercise as a standard of care control due to metabolic deterioration typically seen in sedentary control groups (Patel et al., 2011). A strength of this study is the fact that compliance to the intervention was 100%. All subjects completed all 24 exercise sessions and each session was supervised by research technicians while heart rate was being monitored. Furthermore, we confirmed that subjects were not engaging in extra activity or down regulating other activity outside of the study by accelerometers. Therefore, it is likely that the changes we observed were due to our exercise intervention and not other changes the subjects made to their lifestyle.

Conclusions

In conclusion we showed that high-intensity interval training for eight weeks in obese individuals led to superior improvements in endothelial function when compared to

continuous vigorous exercise. Furthermore, by way of continuous glucose monitoring we showed that HIT and CONT exercise led to improvements in glucose regulation in individuals who had slightly impaired glucose regulation. Finally, visceral adipose tissue measured by the iDXA showed no changes. It is unclear whether this is due to methodological issues with the iDXA itself or if VAT did not change in our study. The strongest deterrent of participating in regular exercise is the time commitment required (Godin et al., 1994; Stutts, 2002; Trost et al., 2002). The time commitment for the subjects in the HIT group was 38% less, but these individuals realized superior or equal benefits in the health related outcomes. This study adds to the mounting evidence that high-intensity interval training is a time efficient strategy for improving health. We extend this evidence to show that obese adults can participate in HIT and improve their health without weight loss.

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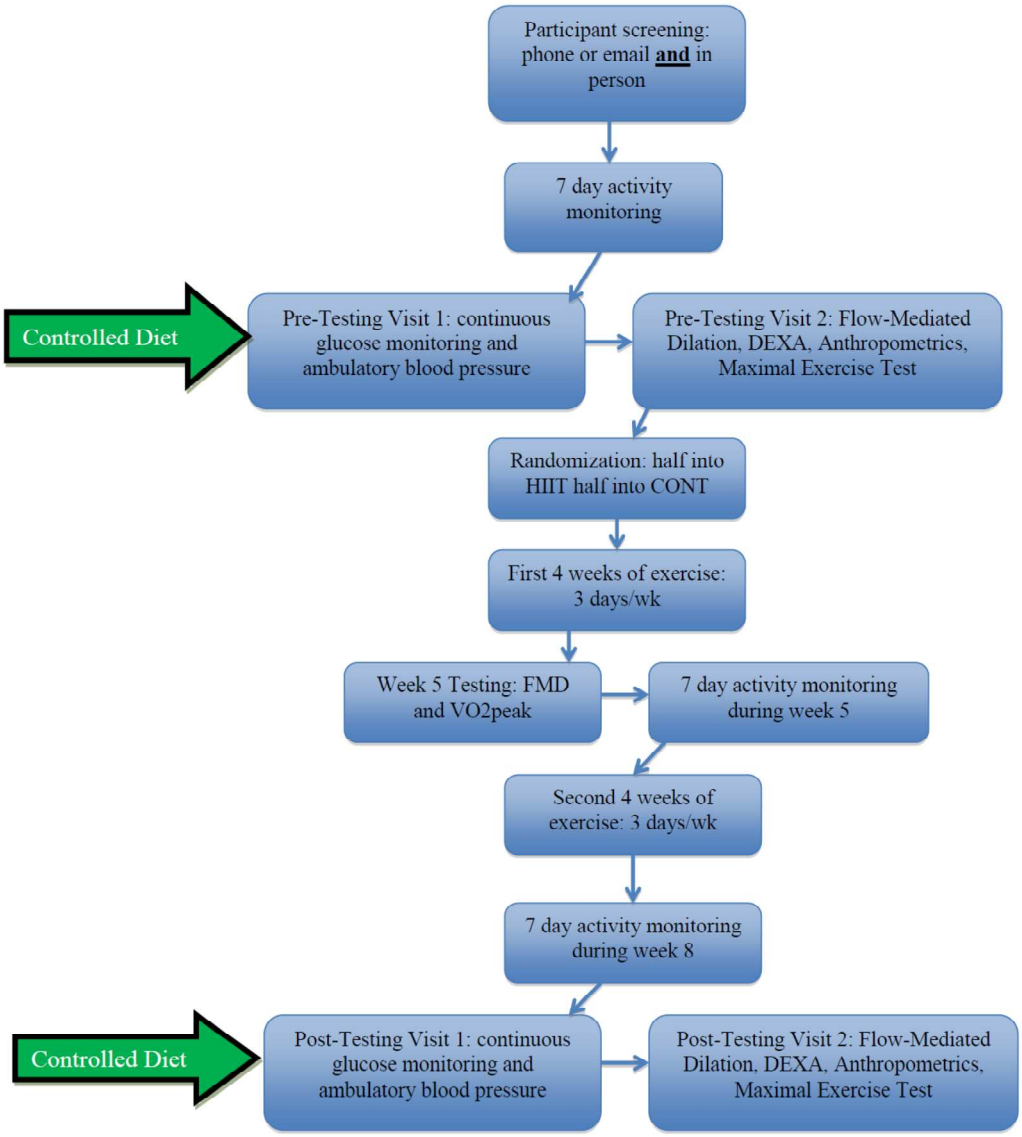
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APPENDIX A

STUDY FLOW DIAGRAM


Participant screening: phone or email and in person
7 day activity monitoring
Controlled Diet
Pre-Testing Visit 1: continuous glucose monitoring and ambulatory blood pressure
Pre-Testing Visit 2: Flow-Mediated Dilation, DEXA, Anthropometrics, Maximal Exercise Test
Randomization: half into HIIT half into CONT
First 4 weeks of exercise: 3 days/wk
Week 5 Testing: FMD and VO2peak
7 day activity monitoring during week 5
Second 4 weeks of exercise: 3 days/wk
7 day activity monitoring during week 8
Controlled Diet
Post-Testing Visit 1: continuous glucose monitoring and ambulatory blood pressure
Post-Testing Visit 2: Flow-Mediated Dilation, DEXA, Anthropometrics, Maximal Exercise Test

APPENDIX B

DIETARY INFORMATION: FOOD CONSUMED DURING CONTROLLED DIET PERIODS

	Kcal	Fat g (Kcal)	Carbohydrate g (Kcal)	Protein g (Kcal)
Breakfast				
Cinnamon Raisin Bagel	265	1 (9)	54 (216)	10 (40)
Cream Cheese (2 T)	79	7 (63)	2 (8)	2 (8)
Kern's Nectar (12 oz)	164	0	41 (164)	0
Lunch				
Lean Cuisine	300	8 (72)	39 (156)	18 (72)
Sun Chips	218	10 (90)	29 (116)	3 (12)
Dinner				
Subway: Sweet Onion Chicken Teriyaki Sandwich	12": 761 6": 380	9 (81) 4.5 (40.5)	118 (472) 59 (236)	52 (208) 26 (104)
Doritos	253	13 (117)	30 (120)	4 (16)
Powerade (12 oz)	76	0	19 (76)	0
Snacks				
Nature Valley Sweet and Salty Peanut	1: 86.5 2:173	1: 4.5 (40.5) 2: 9 (81)	1: 9.5 (38) 2: 19 (76)	1: 2 (8) 2: 4 (16)
Total Males % of Total Kcal	**2375.5**	**57 (513) 23%**	**332 (1328) 61%**	**93 (372) 16%**
Total Females % of Total Kcal	**1909**	**43.5 (391.5) 24%**	**263.5 (1054) 62%**	**65 (260) 14%**

APPENDIX C

STAMPED/APPROVED INFORMED CONSENT LETTER

CONSENT FORM

Effects of 8 Weeks of Either High-Intensity Interval Exercise Training or Continuous Steady-State Exercise Training on Glucose Control and Cardiovascular Disease Risk Factors in Obese Men and Women

INTRODUCTION
The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

RESEARCHERS
Glenn Gaesser, PhD, a professor, Matt Buman, PhD, Brandon Sawyer, Dharini Bhammar, and Wesley Tucker, doctoral students, in the Exercise and Wellness Program in the School of Nutrition and Health Promotion, Ginger Hook and Lindsay Pilbeam, laboratory technicians for the School of Nutrition and Health Promotion, and Amy Woof, a masters student in Exercise and Wellness, have requested your participation in a research study.

STUDY PURPOSE
Our primary objective is to determine the effects of both high-intensity interval exercise training and moderate-intensity continuous exercise training on blood sugar control and peripheral artery function in healthy individuals considered obese by body mass index standards (BMI of 30 or greater).

Our secondary objectives are to determine the effects of both types of exercise training on other cardiovascular disease (CVD) risk factors including blood pressure (BP), intra-abdominal fat (i.e., deep belly fat), blood cholesterol levels, fasting blood sugar and insulin, your body's ability to handle blood sugar, antioxidant level of your blood, and aerobic fitness level (VO_{2peak}).

DESCRIPTION OF RESEARCH STUDY
If you decide to participate, then as a study participant you will join a study involving research on the effects of 8-weeks of either high-intensity interval exercise training or continuous steady-state exercise training on blood glucose control and CVD risk factors.

You are being asked to participate in this study because you are considered obese by current public health guidelines (i.e. have a BMI of 30 kg/m^2 or above) and are, 18 - 45 years of age (for men), or 18-55 years of age (for women) in good health, and capable of performing vigorous physical activity.

Females must not be pregnant or become pregnant throughout the course of the study. Also in order to accommodate the measurement of artery function females must have a history of a regular menstrual cycle (variation of 8 days or less).

As a study participant you will have 30 total visits to the Health Lifestyles Laboratory in ISTB3 room 181 on the Polytechnic campus of ASU.

Visit 1 (screening):
Your first visit will involve coming to the test site on the ASU Polytechnic campus and filling out a physical activity readiness questionnaire that consists of 7 questions designed to assess whether participation in this study is appropriate for you. All aspects of the study will be explained to you, and we will answer any questions you may have. If you decide to participate

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after signing this consent form we will conduct the flow mediated dilation (FMD) procedure (see below) to make sure we can adequately image your brachial artery. This visit will take approximately 1 hour.

Testing:

All testing will be completed at baseline and after 8-weeks of exercise training. Each measurement period will consist of 2 visits over two consecutive days (day 1 will be fasting). In addition, the VO_{2peak} fitness test and the FMD measurement will also be conducted after 4 weeks of training in order to adjust exercise training intensity and track monthly changes in artery function.

Measurement of Physical Activity and Sleep Quality

We will be measuring your movement and sleep quality at different times throughout the study by using a small movement sensor called an accelerometer. We will have you wear this device for a week before you start the exercise as well as during the 5th and 8th weeks of the study for a total of 3 weeks of wear time. This device is attached to an elastic belt that you wear around your waist during the day. While you are sleeping you will wear the device on your wrist using an elastic wrist band. You will need to wear the device for 24 hours a day (except while in water, and bathing) for 7 days during each 7 day measurement periods. The device is very light, easy to wear, and easy to conceal. While you are wearing the accelerometer we will have you fill out an activity/sleep quality log.
Also to assess your sleep quality we will have you fill out a 24-question sleep quality questionnaire before and after the 8 weeks of exercise.

Testing during Day 1 of the two-consecutive-day visits before and after 8-weeks of training:

*__Note:__ Females will be asked to complete this testing during the follicular phase of the menstrual cycle (immediately after menstruation begins) for both pre and post testing. This allows for control of the large variation in artery function seen throughout different stages of the menstrual cycle. This means the training could last slightly longer or shorter than the prescribed 8 weeks.

- You will need to arrive at the laboratory in a fasted state (no food for the past 12 hours; nothing but water to drink for the past 12 hours)

- Height and weight will be measured using a standard scale.

- Dual-Energy X-ray Absorptiometry (DEXA)
  Your body composition (relative amounts of fat and lean tissue) will be determined by using an FDA-approved bone density measurement machine. The procedure is called Dual-energy X-ray Absorptiometry (DEXA). You will be asked to lie face up, on a padded table for 7 minutes while the scanner arm of the DEXA machine passes over your entire body. The scanner will not enclose you or touch you, and you can wear my regular clothing (no metal allowed).

  You will be exposed to minimal radiation (1-4 microSieverts) that is within an acceptable range as provided by the US FDA. Anytime you are exposed to radiation there is potential risk. The amount of radiation (1-4 microSieverts) that you would be exposed to

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is quite minimal. For example, you would receive radiation exposure of approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x-ray.

If there is ANY chance of being pregnant then you should not undergo DEXA scanning. You will be asked to take a urine pregnancy test immediately before each DEXA scan. If you become pregnant during the course of the study, you must immediately inform the staff, and withdraw from the investigation. A certified X-ray technician will complete all DEXA scans. This test takes about 15 minutes.

- Resting blood pressure will be measured using an automated blood pressure machine.

- Brachial Artery Flow-Mediated Dilation:
  This procedure involves taking ultrasound images of an artery in your upper arm before, during, and after a blood pressure cuff is inflated around your forearm. All measurements are made on your non-dominant arm. After lying quietly on a padded ultrasound table for 20 minutes, a blood pressure cuff will be positioned on your forearm. After recording baseline ultrasound measures on your upper arm, the blood pressure cuff will be inflated to a pressure of 240 mmHg (enough to stop blood flow to your wrist and hand), and kept in place for 5 minutes. You may experience a tingling feeling in your hand, which is normal. The blood pressure cuff will then be deflated rapidly and ultrasound measures will be taken for 5 minutes.

- A small amount of blood will be drawn from one of the veins in your forearm for measurement of blood lipids, glucose, insulin, insulin sensitivity, and antioxidant capacity.

24-Hour Monitoring

- After your blood draw you will be fitted with the following monitors and instructed on their use.

- Ambulatory Blood Pressure Monitoring: You will be fitted with an ambulatory blood pressure monitor that fits around your upper arm in the same way that a typical blood pressure cuff fits around your arm. This ambulatory blood pressure monitor also has a small device, about twice the size of a typical cell phone, which you will have to wear attached to an adjustable waistband you wear around your waist. The cuff automatically inflates every 15 minutes during waking hours and every 60 minutes during bedtime hours, and records and stores your blood pressure. You will be asked to refrain from any other moderate/intense activities for at least 24 hours prior to your scheduled appointment. You will be given instructions on how to use the ambulatory blood pressure monitor, and will be asked to wear the monitor for 24 hours (except while bathing).
  - Blood pressure diary: On the two days that you wear the ambulatory blood pressure monitor for 24 hours, you will be instructed as to what to do while your blood pressure is being taken, and you will be asked to fill out a diary (that we provide to you) in which you write down the time, place, and what you are doing when the blood pressure cuff inflates.

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- Continuous Glucose Monitoring: You will have a small micro-dialysis catheter, which is a small tube, inserted just under the skin of your abdomen in order to continuously monitor your blood glucose levels. You will be instructed on how to use and care for the device and the catheter insertion site on your skin. You will be asked to keep the monitor in place until you return to the laboratory after the 24-hour period. During this 24-hour period you will have to conduct 4 finger pricks by yourself (upon awakening, before lunch, breakfast, and dinner) to measure your blood glucose with a standard blood glucose monitor.
- Standardization of meals: After fitting with the monitoring devices you will be given a meal in the laboratory. You will also be given gift cards for dinner that night and breakfast the next morning, as well as snacks in order to ensure you eat the exact same food following the exact same timing before and after the 8-weeks of training.
    - Dietary Record: We will ask you to write down everything you eat in detail (using a form we give you) for each of the two days that you wear the 24-hour monitors.

Total time for testing visit 1 both pre and post is approximately 90-120 minutes

Testing during Day 2 of the two-consecutive-day visits before and after 8-weeks of training:

- You will return to the laboratory after the 24-hour monitoring period to return the monitoring devices and have the following testing completed:

- Maximal Exercise Test: For this test, you will be wearing a mask attached to a hose that collects the air you breathe out. You will also wear a heart rate monitor that consists of an elastic strap that wraps around your chest to measure your heart rate. These devices will measure your breathing and heart rate continuously. You sit quietly on the stationary cycle ergometer for 2 minutes then you will be asked to pedal at a speed of your choice at 50 watts (light resistance) for 5 minutes for the warm-up phase. After the warm-up phase the resistance will increase continuously by 30 watts per minute every minute (for males) or 15 watts per minute every minute (for females) until you cannot continue. We will encourage you to push yourself as hard as you feel comfortable in order to obtain an accurate measurement (This first part of the test usually lasts between 8 and 15 minutes). After a 10 minute rest period you will perform an "all-out" bout of exercise at the same resistance you ended at on the previous test. Again you will push yourself as hard as you feel comfortable with until you are exhausted. This test is used to make sure you reached your true maximum on the previous test and usually last about 2-3 minutes. The whole test will take about 30 minutes.

Total time for testing visit 2 both pre and post is approximately 60 minutes

Exercise Training

You will be asked to complete 3 exercise sessions per week for 8 weeks for a total of 24 exercise sessions. You will be randomized to either the high-intensity interval exercise training group or to the continuous steady-state exercise training group upon enrollment in the study. All exercise will be conducted on a stationary bicycle.

- High-intensity Interval Exercise Training:

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This exercise protocol lasting 30 minutes consists of the following:

- 5-minute warm up at 50-60% of your maximum heart rate
- Ten 1-minute intervals at 90-95% your maximum heart rate separated by 1 minute of cycling at a low intensity (~50 W)
- 5 minute cool down at 50-60% of your maximum heart rate

- Continuous Steady-state Exercise Training:

  This exercise protocol lasting 40 minutes consists of the following:
  - 5-minute warm up at 50-60% of your maximum heart rate
  - 30-minutes of exercise at ~70% of your maximum heart rate
  - 5 minute cool down at 50-60% of your maximum heart rate

Total time for each training visit is approximately 45 minutes.
Total time commitment for completion of the study is approximately 26 hours over 30 visits.

RISKS

Research studies often involve some risks. The risks of exercise include local muscle soreness, abnormal changes in blood pressure, nausea, faintness, dizziness, irregular heartbeats (rare), and, in very rare instances, heart attack.

You will be monitored by trained investigators and if there are any adverse effects, the exercise testing or the exercise session will be halted. All exercise testing procedures will comply with the guidelines for exercise test administration as recommended by the American College of Sports Medicine and required by the Healthy Lifestyles Research Center at Arizona State University. You will be asked not to attempt any exercise that you feel is beyond your physical abilities. If you experience discomfort, feel you are unable to continue or wish to stop an exercise at any point, you are requested to inform the investigator immediately.

The blood pressure cuff may feel uncomfortable to start with especially while you are sleeping, but people who have undergone this procedure claim that this discomfort isn't bothersome after you get used to it.

The blood glucose assessment and the blood draw involve skin pricks, insertion of the micro-dialysis catheter, as well as a needle puncture in your forearm and hence may lead to some discomfort as well as a slight risk of infection. These will be minimized by using standard procedures for controlling blood-borne pathogens as well as properly cleaning the finger prick site, the catheter insertion site as well as the blood draw site. The lancets, needles, and catheters used will be sterile. Other possible risks of a blood draw include dizziness, fainting, nausea, and vomiting. All blood draws will be conducted while you are seated to ensure your safety in case any of these possible side effects occur.

Since pregnancy tests are only 99% accurate there is a slight risk of a false negative reading on a test leading to radiation exposure to your fetus. This risk is minimal due to the high accuracy rate of the pregnancy tests used.

As with any research, there is some possibility that you may be subject to risks that have not yet been identified.

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BENEFITS
Although there may be no direct benefits to you, the possible benefits of your participation in the research are that this study will provide valuable information regarding the effect of 8-weeks of exercise training on CVD risk factors.

NEW INFORMATION
If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY
All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Gaesser will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

WITHDRAWAL PRIVILEGE
It is ok for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time.
Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.
Your participation is voluntary and if you decide not to participate or decide to withdraw from the study it will not affect your grade, treatment, care, employment status.

COSTS AND PAYMENTS
All study procedures will be provided to you at no cost to you.
You will be paid $250 for completion of the study by either check, cash, or gift certificate. You will also receive 6 free meals (2 in the lab and 4 via gift cards).
Partial payment will be made in the following manner if you only complete some of the visits.

Completion of All Baseline Testing and less than 4 weeks of training (i.e., less than 12 exercise sessions): $25

Completion of 4 weeks of training (i.e., between 12 and 24 exercise sessions without post-testing procedures): $50

Completion of the entire study (i.e., baseline testing, 24 exercise sessions, and all post-testing procedures): $250

COMPENSATION FOR ILLNESS AND INJURY
If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury. In the event of a medical emergency first aid will be administered and if necessary, 911 will be called.

VOLUNTARY CONSENT
Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Glenn Gaesser, 500 N 3rd ST, Phoenix, AZ 85004; 602-827-2283; 480-727-1884.

_____
Initial here

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study

Subject's Signature	Printed Name	Date

Contact phone number	E-mail

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

ASU IRB APPROVED FOR
CAROL JOHNSTON, CHAIR
7/18/2012-6/12/2013

_____
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APPENDIX D

INSTITUIONAL REVIEW BOARD APPLICATION

Arizona State University
Office of Research Integrity and Assurance
660 S. Mill Avenue Suite 315
Arizona State University
Tempe AZ 85287-6111
(Mail Code 6111)
Phone: 480-965-6788
Fax: (480) 965-7772

For Office Use Only:
H.S. #:

Pre-Reviewer:

BIOSCIENCE APPLICATION HUMAN SUBJECTS

PROTOCOL INFORMATION

1.
Protocol Title: Effects of 8 Weeks of Either High-Intensity Interval Exercise Training or Continuous Steady-State Exercise Training on Glucose Control and Cardiovascular Disease Risk Factors in Obese Men and Women

Date of Request: May 25, 2012

PRINCIPAL INVESTIGATOR (PI)

Please note that the PI's CV and human subject's protection training certification must be attached with this application.
2.
Name and Degree(s): Glenn Gaesser, PhD

Department/Center: Exercise and Wellness

Mailing Address: 500 N. 3rd Street Phoenix, AZ 85004

Email: glenn.gaesser@asu.edu Phone #: 480-727-1884 Fax: 480-727-5233

University Affiliation:
☒ Professor
☐ Associate Professor
☐ Assistant Professor
☐ Instructor
☐ Other: Please specify. ("Other" categories may require prior approval. Students cannot serve as the PI)

CO-INVESTIGATORS (CO-I)

- A Co-I is anyone who has responsibility for the project's design, implementation, data collection, data analysis, or who has contact with study participants.
- If the project involves medical procedures or patient care that the PI is not certified or licensed to conduct, a responsible physician or other certified or licensed professional must be included as a Co-I. The application must include a copy of supporting documentation for this individual (CV, license, board certification etc).

3. Name	Study Role	Affiliation	Department	Email/Tel/Fax	Student (yes/no)
Brandon Sawyer	Research Assistant	ASU	EXW	bjsawyer@asu.edu	Yes
Dharini Bhammar	Research Assistant	ASU	EXW	dbhammar@asu.edu	Yes
Wesley Tucker	Research Assistant	ASU	EXW	wjtucker@asu.edu	Yes

Ginger Hock	Registered Nurse	ASU	NUT	ghock@asu.edu	no

PROJECT FUNDING

4a) How is the research project funded? (A copy of the grant application must be provided prior to IRB approval)

- ☐ Research is **not funded** (Go to question 5)
- ☐ Funding decision is pending
- ☒ Research is **funded**

b) What is the source of funding or potential funding? (Check all that apply)

- ☐ Federal
- ☐ Private Foundation
- ☐ Department Funds
- ☐ Subcontract
- ☐ Fellowship
- ☒ Other See below

c) Please list the name(s) of the sponsor(s): Medtronic Diabetes: Equipment Donation (CGM devices and supplies)

d) What is the grant number and title? N/A

e) What is the ASU account number/project number? N/A

f) Identify the institution(s) administering the grant(s): N/A

SUMMARY OF PROTOCOL

Please include a summary for each of the questions. Use as much space as necessary **AND** attach a copy of the study proposal or protocol. If you attach a copy of the full proposal, place page and paragraph numbers from the proposal next to each question in this section to show precisely where information pertaining to each question can be found. Please note that information should be consistent between the proposal, consent form, and IRB application. **FOR ALL OF THE QUESTIONS, WRITE YOUR ANSWERS ON THE APPLICATION RATHER THAN SAYING SEE ATTACHED.**

5a) What is the hypothesis?

Our primary objective is to determine the effects of 8 weeks of either high-intensity aerobic interval training or continuous steady-state exercise training on glucose control and endothelial function in healthy, obese individuals.

Our secondary objectives are to determine the effects of both training programs on other cardiovascular disease (CVD) risk factors including ambulatory and resting blood pressure (BP), visceral fat, blood lipids, fasting glucose and insulin, insulin resistance, antioxidant capacity, and VO_{2peak}.

We hypothesize that glucose control, ambulatory and resting blood pressure, endothelial function, visceral fat, blood lipids, fasting glucose and insulin, insulin resistance, antioxidant capacity, and peak aerobic capacity (VO_{2peak}) will be improved to a greater extent with the high-intensity interval exercise program.

b) Describe study procedures and methodologies.

The study will consist of thirty total visits to the Healthy Lifestyles Research Center Laboratory in ISTB3 room 181 on the Polytechnic campus of ASU.

Visit 1 (screening and obtaining written consent):

The purpose and nature of the study as well as the procedures will be explained to the subjects in detail. Questions and concerns will be answered and subject written informed consent will be obtained. Subjects will be screened for readiness to participate in exercise (See attached PAR-Q). The PAR-Q will be completed to verify the absence of contraindications to a maximal oxygen consumption test ($V0_{2peak}$) and the exercise training. Subjects will also have

the flow mediated dilation (FMD) technique conducted in order to ensure a suitable acoustic window is available for the procedure.

Subsequent visit: for Outcome measurement: (before and after 8 weeks of exercise training):

All outcomes will be measured at baseline and after 8-weeks of exercise training. Each measurement period will consist of visits on 2 consecutive days (day 1 will be fasting). The VO_{2peak} test will also be conducted at 4 weeks to ensure changes in fitness level are reflected in training intensity. Furthermore, the FMD procedure will be conducted at 4 weeks to track changes in artery function mid-way through the study.

Testing during Day 1 of each pre-training and post-training testing visit:

- Subjects must come to the laboratory in a fasted state (no food for 12 hours; nothing but water to drink for the past 12 hours)
- Anthropometrics: Weight will be measured on a standard Beam scale. Height will be measured on a stadiometer.
- Body Composition Assessment: Dual-energy X-Ray Absorptiometry (DEXA) will be used to determine percent body fat, regional fat distributions, and visceral fat. Subjects will lie face up in the DEXA bed for 7 minutes while the DEXA arm passes over the entire body. Females will conduct a urine pregnancy test in our laboratory (negative test is required before DEXA can take place) prior to each DEXA measurement. All DEXA scans will be performed by a certified radiology technician.
- Resting blood pressure will be measured using a Dynamap automated sphygmomanometer.
- Flow-mediated dilation (FMD) will be measured following the criteria set forth by the Brachial Artery Reactivity Task Force (1). Participants will be asked to lie quietly for 15 minutes on a vascular imaging table before a sonographer (co-investigator Brandon Sawyer, who has performed this procedure approximately 100 times) obtains baseline images for 60 seconds from the participant's non-dominant arm. After the baseline images are complete the sonographer will inflate a blood pressure cuff on the participants forearm to a pressure of 240 mmHg for 5 minutes. 60 seconds prior to cuff release the sonographer will begin to record images. After 5 minutes of occlusion, cuff deflation occurs while the sonographer continues to record images for the next 5 minutes. Images will be analyzed using a previously validated, brachial artery edge-detection software. Female subjects will be asked to complete this visit during the follicular phase of their menstrual cycle (immediately after menstruation begins) both before and after the training. After the screening visit, female subjects will be asked to notify the investigators when they begin menstruation in order to schedule their first testing visit during the follicular phase. Likewise, at the end of the 8-week training period the females subjects will be asked to notify the investigators when menstruation begins, within one-week of the eighth week of training, in order to schedule their first testing visit of the post-testing period during the follicular phase. The timing of this visit is important due to the large variation in FMD measurements caused by the menstrual cycle (2).
- A blood draw will be conducted via one of the common antecubital fossa veins (cephalic, median cubital, or basilic vein) for measurement of blood lipids, glucose, insulin, and antioxidant capacity. All blood draws will be performed by a certified phlebotomist or a registered nurse.

1. Corretti M, Anderson T, MD, Benjamin E, Celermajer D, Charbonneau F et al. Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow Mediated Vasodilation of the Brachial Artery. *Journal of the American College of Cardiology* 2002; 39(2): 257-265.
2. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ. Assessment of flow-mediated dilation in humans: a

methodological and physiological guideline. *Am J Physiol Heart Circ Physiol* 2011; 300(1): H2–H12.

24-Hour Monitoring

- Immediately following the blood draw above, subjects will be fitted with the following monitors and instructed on their use.

- Continuous glucose monitoring will be conducted by a Medtronics iPro 2 continuous glucose monitor (CGM). Subjects will have a small micro-dialysis catheter inserted subcutaneously in their abdomen to continuously monitor blood glucose levels. Subjects will be instructed on how to use and care for the device and the catheter insertion site. Subjects will be asked to keep the monitor in place until their return to the laboratory the next morning after a 24-hour period. CGM devices will be calibrated using a standard glucometer 4 times throughout the 24-hour period (upon awakening, before lunch, breakfast, and dinner). This calibration will be conducted by the participants using a standard finger prick.

- 24-hour ambulatory blood pressure will be monitored by a Sun Tech Oscar 2 blood pressure monitor. Subjects will be fitted with the monitor, instructed on its use, and instructed to wear it until return of the device the next morning after the 24-hour period. The blood pressure monitor will be set to record blood pressure every 15 minutes during waking hours and every 60 minutes during sleeping hours.
    - Blood Pressure Diary: Subject will be asked to keep a diary that records their activity each time a BP measurement was taken.

- Standardization of meals will be conducted during this 24-hour period to ensure the same intake and timing of intake occurs during each testing period. Subjects will eat one meal in the laboratory after placement of the 24-hour monitoring devices. Subjects will also be given snacks to eat at specified times (mid-afternoon and evening) as well as gift cards for dinner and breakfast (the next morning).

    - Dietary Record: Food intake will be recorded using a standard 24-hour food record to ensure similar intake during each testing period.

Testing during Day 2 of each pre-training and post-training testing visit:

- Subjects will arrive at the laboratory after the 24-hour monitoring period and have the following testing completed:

- Return and removal of 24-hour monitoring equipment.

- VO_{2peak} testing: Subjects will be equipped with a mask attached to a hose, and Polar heart rate monitor for the metabolic measurement device (Parvo Truemax 2400TM) to measure ventilation and respiratory gas exchange data and heart rate continuously. After collecting resting data for 2 minutes, subjects will pedal on a stationary cycle ergometer at a cadence of their choice at 50 watts for 5 minutes for the warm-up phase. After the warm-up phase, load will increase continuously by 30 watts/min every minute (males) or 15 watts/min every minute (females) until the subject cannot continue. Verbal encouragement will be given to all subjects throughout the entire test. The highest oxygen uptake during the test will be taken as the peak VO_2. In order to verify attainment of VO_{2peak}, after a 10-minute rest period, each subject will perform an all-out test on the cycle ergometer at a constant-load between 85 and 100% of the peak load reached during the incremental exercise test. Oxygen uptake will be monitored as in the incremental exercise test, and the highest VO_2 will be recorded.

Exercise Training
All subjects will complete 3 exercise sessions per week for 8 weeks for a total of 24 exercise sessions. Subjects will be randomized to either HIIT or continuous training (CONT) upon enrollment in the study. All exercise will be conducted on a cycle ergometer.

- **HIIT Training:**

  This exercise protocol lasting 30 minutes consists of the following:
  - 5-minute warm up at 50-60% of HRpeak
  - Ten 1-minute intervals at 90-95% of HRpeak separated by 1 minute of cycling at a low intensity (~50 W)
  - 5 minute cool down at 50-60% of HRpeak

- **CONT Training:**

  This exercise protocol lasting 40 minutes consists of the following:
  - 5-minute warm up at 50-60% of HRpeak
  - 30-minutes of exercise at ~70% of HRpeak
  - 5 minute cool down at 50-60% of HRpeak

c) What is the participant selection?

Participants will be selected on the basis of inclusion and exclusion criteria. Posters/Flyers, emails, craigslist ads, and newspaper ads will be used to recruit participants. (See attached)

d) What is the statistical design?

Repeated measures ANOVA will be used to evaluate within-subject and between-subject differences pre- and post-intervention.

e) Describe whether the study involves randomization to control/intervention groups.

Subjects will be randomized into either HIIT or CONT groups.

f) How will study results be used?

The study results will be used for Masters Theses, Doctoral Dissertations, abstracts, publications, and conference presentations.

DATA SAFETY MONITORING BOARD (DSMB)

6a) Does the study have a Data Safety Monitoring Board?
☐ Yes (prompt submission of DSMB reports is required) ☒ No

b) If no, what is the structure/plan to report serious adverse events to the ASU IRB?

In case of an adverse event, the ASU IRB shall be promptly notified by the PI within 48 hours.

STUDY DURATION

7a) What is the expected duration of the study through data analysis? (Attach a timeline, if applicable) June 2012 to May 2013

b) What is the expected date that recruitment will begin? (must be after the submission date) Upon IRB Approval

STUDY SITES

8a) Where will the study be conducted? (Check all that apply)
☒ On campus (Please indicate building(s) and room number (s) when known) ASU Polytechnic-ISTB3 Rooms 181 and 183

☐ Off campus (Please provide location and letter of permission, where applicable)

b) Is this study being reviewed by another IRB? ☐ Yes ☒ No

Status of other IRB review: ☐ Approved ☐ Pending ☐ Not yet submitted ☐ Attached

INTERNATIONAL RESEARCH

9a) Does this study include an international site? ☐ Yes (list country) ☒ No

9b) If this is an international study, please provide a statement including the following items:
- The investigator's familiarity with the culture in which the study in taking place.
- Cultural norms and how this study may affect an individual's standing in his/her community.
- The standard of care in the community, how it differs from the proposed research procedures, and a plan for the continuation of care once the research is complete.

RESEARCH PARTICIPANT INFORMATION

10a) What are the inclusion criteria? (Use an expanded list and attach a secondary sheet with explanation, where applicable. If you attach a secondary sheet, reference on which page the information can be found.)
Subjects must be adults between the age of 18 and 45. Subjects must be in good health and willing to comply with all visits required in the study. Females must not be pregnant or become pregnant while enrolled. Females must have a history of regular menstrual cycles (i.e. variation of less than 8 days) in order to accommodate the FMD measurement. All subjects must have a body mass index of 30 kg/m^2 or above. Subjects must also have a suitable acoustic window around the brachial artery in order to accommodate the FMD measurement.

b) What are the exclusion criteria? 'Yes' to any of the 7 questions on the Physical Activity Readiness Questionnaire (PAR-Q) form that would exclude the subject from participating in exercise. Current or previous foot or lower limb injuries, smokers, neuromuscular or cardiovascular disorders, BMI under 30 kg/m^2, pregnancy, or irregular menstrual cycle (greater than 8 days of variation on a regular basis). A poor acoustic window that causes an inability to perform the FMD measurement will also serve as an exclusionary criterion.

c) Please explain recruitment procedures in detail. (A copy of the recruitment materials must be attached.) Subjects will be recruited via email, flyers, and craigslist

d) What is the expected duration of participation of each participant? (total and at each session) Total time commitment at our laboratory is approximately 26 hours: One 1-hr screening visit
- Two 2-hr testing visits: Day 1 of pre-training and post-training testing visits
- Three 1-hr testing visits: Day 2 of pre-training and post-training testing visits, and the VO_{2max} test at 4 weeks
- Twenty-four 45 minute exercise training visits

There will also be time when the subjects are on their own wearing the blood pressure and glucose monitoring equipment totaling 48 hours.

e) What is the expected number of individuals to be screened for enrollment? 80

f) What is the maximum number of individuals to be enrolled? (This includes individuals who drop out) 40

g) What is the approximate number of: 20 Males 20 Females

h) Indicate the age range of the participants that you plan to enroll in your study. 18 to 45

i) What is the race of participants? All

j) Does the study target any of the following participants? ☐ Yes (please check all that apply) ☒ No

- ☐ Children (under 18)
- ☐ Decisionally impaired
- ☐ Prisoners or detainees
- ☐ Fetuses
- ☐ Native Americans
- ☐ Pregnant women
- ☐ Economically disadvantaged
- ☐ Persons at high risk of becoming detained or imprisoned
- ☐ Patients, if yes – what is the status of their health?
- ☐ Non-English speakers (Include copy of all materials in language of participants)

k) If any of the above categories have been checked, please state how you will protect the rights and privacy of these individuals.

l) Does the study involve participants who have **low-literacy**? ☐ Yes ☒ No (If yes, please describe how investigators will ensure the participants' understanding of the research):

m) Does the study involve participants who are **students or faculty** of ASU? ☒ Yes ☐ No (If yes, please state the investigator's involvement in the participant's education/employment): There will be not be any effect on the participants employment or grade if they choose to not participate or withdraw from the study at any time.

COMPENSATION

11. Will any type of compensation be used? (e.g. money, gift, raffle, extra credit, etc)

a) ☒ Yes (Please describe what the compensation is): $250 and 6 free meals ☐ No (go to question 12)

b) Explain why the compensation is reasonable in relation to the experiences of and burden on participants. $250 and 6 free meals is reasonable for the level of time commitment.

c) Is compensation for participation in a study or completion of the study? (Note: participants must be free to quit at any time without penalty).

☒ Participation is: All Baseline testing procedures and less than 12 exercise sessions → $25; All Baseline testing procedures and between 12 and 24 exercise sessions → $50; completion of study → $250 ☐ Completion

d) If some or all participants are economically disadvantaged, explain how the compensation is provided in such as a way that participants cannot refuse the request to participate? N/A

RISKS AND BENEFITS

Please reference the proposal, where applicable and answer the questions below:

12a) What are potential risks to participants? Potential risks include those associated with vigorous physical activity and include soreness, muscle strains, sprains, dizziness, fatigue, nausea and abnormal heart rhythms. There is also slight risk of infection with the glucose monitoring as well as the blood draw. Other possible risks of blood draws include dizziness, fainting, nausea, and vomiting. Possible false negative for the pregnancy test (tests are 99% accurate) leading to radiation exposure to a fetus.

b) What steps will the investigators take to reduce risks? Participants will be screened for suitability of exercise using a standardized questionnaire. All testing will be conducted at Arizona State University where exercise will be carried out with access to life support trained personnel including exercise physiologists. DEXA scans will be conducted by a certified radiology technician and blood drawn by a certified phlebotomist. To prevent infection standard procedures for controlling blood borne pathogens will be used as well as properly cleaning the sites for blood draw and glucose monitor insertion. The needles and lancets used will be sterile. All blood draws will be conducted with the participants seated incase dizziness or fainting occur. Pregnancy tests will be conducted before each DEXA. Radiation dose of a full-body scan for the DEXA is extremely low (1-4 microSieverts).

c) What are any potential benefits to participants? Compensation, free meals, the benefits of exercise, and knowledge of multiple health outcomes.

d) Please note how the results of the study will affect the health and welfare of the general public. Knowledge of the effects of 8-weeks of high intensity exercise could be beneficial to society.

e) What are the types of incentives, if any, will participants receive? $250 and 6 free meals.

f) What are the costs, if any, to participants? (This should be mentioned in the consent form): The subjects will need to present themselves at the testing facilities at the appointed time. Costs incurred for travel will be the only cost incurred by the subjects.

CONFIDENTIALITY

13a) Describe the steps you will take to ensure the confidentiality of the participants and data. Participants will be assigned a unique study ID number to be used on all study documents. The consent will serve as a link between identifiers and study ID number. The consents will be kept separately from data charts and kept under lock and key in ISTB3 room 183.

b) How will you safeguard data that includes identifying or potentially identifying information (e.g. coding)? Subjects will be identified by a study ID number and kept in a locked file cabinet. Electronic data will be stored on password protected computers and files. They will be accessible only to those on the research protocol.

c) When will identifiers be separated or removed from the data? Upon consent

d) Where on campus will you store the data and media and ensure its security (videotapes and/or audiotapes)? ISTB3 room 183

e) How long do you plan to retain the data? 10 years

f) How will you dispose of the data? Paper documents will be shredded and computer data will undergo a secure deletion

g) Is a certificate of confidentiality required? ☐ Yes ☒ No

HIPAA

14a) Are any of the data coming from covered entities under Health Insurance Portability and Accountability Act (HIPAA)? ☐ Yes ☒ No (If yes, please describe):

b) Is a data use agreement required? ☐ Yes ☒ No

c) Is a HIPAA Waiver of Authorization being requested? ☐ Yes ☒ No

DATA SOURCES AND USES

15a) Please check all the ways that you will obtain data: (Copies of written and oral questions must be provided for ASU IRB review and approval prior to implementation.)

- ☐ Interviews
- ☐ Focus Groups
- ☐ Medical Records
- ☐ Registries
- ☒ Questionnaires/Surveys
- ☐ Public Records
- ☒ Biological Specimens
- ☒ Other Exercise testing, DEXA, blood pressure, FMD

b) How will the data be used? (Check all that apply)

- ☒ Dissertation
- ☒ Thesis
- ☒ Results released to participants/parents
- ☒ Publication/journal article
- ☐ Undergraduate honors project
- ☐ Results released to employer or school

☐ Results released to agency or organization ☒ Conferences/presentations
☐ Other *(please describe)*

INFORMED CONSENT

16. Describe the procedures you will use to **obtain and document informed consent and assent**. Attach copies of the forms that you will use. In the case of secondary data, please attach original informed consent or describe below why it has not been included. Fully justify any request for a waiver of written consent or parental consent for minors.
(The ASU IRB website has additional information and sample consent and assent forms.) The PI or a designated member of the research team will initiate contact with individuals as described in the recruitment section. A brief description of the study and procedures will be offered verbally and if interested, and the individual meets general inclusion/exclusion criteria individuals will be invited to the Health Lifestyles Research Center where they will be provided with a more in-depth explanation of the study procedures, opportunity to ask questions, and lastly will be asked to read and sign the informed consent form. Individuals will be given the time to read through the consent and will be asked if she/he has any questions before signing the forms. Written consent will be obtained from subjects before any procedure starts. (See attached consent form)

INVESTIGATIONAL NEW DRUG OR DEVICE

17a) Does this study involve an investigational new drug (within the meaning of 21 U.S.C. 355(i) or 357(d)) or a significant risk device (as defined in 21 CFR 812.3(m))? ☐ Yes ☒ No (If no, go to question 18. If unsure, go to www.fda.gov/oc/ohrt/irbs/).

b) What is the drug or device?

c) Has the 30-day interval required for investigational new drugs and for significant risk devices elapsed, or has the FDA has waived that requirement?

d) If the 30-day interval has expired, has the FDA requested that the drug or device be withheld or restricted for use in human subjects? ☐ Yes ☐ No

DRUGS

18. What are the drugs to be used and in what dosage? (If no drugs will be used, please write N/A.) N/A

RADIATION

19a) Will ionizing radiation (x-rays and or radiopharmaceuticals) be used? ☒ Yes ☐ No (If yes, include a copy of the radiation certification). See attached certificate

b) Will non-ionizing radiation (MRI, ultrasound, lasers, ultraviolet) be used? ☒ Yes ☐ No

BIOLOGICAL MATERIALS

20a) Will biological materials be collected from subjects or given to subjects? ☒ Yes ☐ No (If no, please skip to question 21)

b) Provide a description of the material (blood, tissue, vectors, antibodies, etc.) that will be used: Blood and urine.

c) If the study involves human blood, do you have the required ASU Biosafety disclosure on file? ☒ Yes ☐ No
(If yes, what is the biosafety disclosure number?) #12-467
(If yes, when and how are the samples to be destroyed? Note: an active protocol is required the entire period that the samples are retained) The samples will be kept in a secured restricted access room for 3 years after the completion of the study. After 3 years the samples will be placed in an approved biohazard bag and then discarded properly.
d) Will any of the material being used in the study come from a third party? ☐ Yes ☒ No (If yes, attach copy of the Material Transfer Agreement if required.)

e) Does this study involve transfer of genetic material of animal tissue into humans? ☐ Yes ☒ No
(If yes, please cite the ASU Institutional Biosafety Disclosure number).

GENETIC ANALYSIS

21a) Does this study involve genetic analysis? ☐ Yes ☒ No (If no, please skip to question 22).

b) What sources of genetic material will be studied (blood, tissue, DNA)?

c) Please specify whether the genetic analysis involves pedigree, positional cloning, mutational polymorphism, or gene therapy research.

d) Please specify whether:
☐ Stored samples already exist
☐ Samples will be collected specifically for this study
☐ Stored samples already exist from a previously approved study
☐ Samples collected are part of a routine clinical procedure
☐ Samples are discarded, already existing, and de-identified

e) If stored samples will be used, did participants consent to the use of their stored sample(s)?
☐ Yes ☐ No (If yes, was the consent prospective to collection of the sample, or retrospective of the collection of the sample?)

f) Will any identifiers be maintained? ☐ Yes ☐ No (If yes, please specify)

g) When will samples be destroyed or discarded?

h) Are any of the diseases being studied considered preventable? ☐ Yes ☐ No

i) Is there a possibility of an incidental finding of a genetic condition? ☐ Yes ☐ No
If so, is there a plan to disclose this to the individual?

j) Will this information be kept confidential from third parties such as employers, or insurance companies, or will findings be included in the participant's medical record for clinical treatment?

k) How will other risks (e.g. discovery of information regarding paternity or ancestry) be disclosed to subject?

CONFLICT OF INTEREST AND COMMERCIALIZATION

22a) Does any member of the research team have a potential conflict of interest with this study that could affect study participants and/or study outcome? For more information about examples of conflicts of interests, please visit the ASU objectivity website: http://researchintegrity.asu.edu/coi
☐ Yes ☒ No (If yes, please describe and disclose in the consent form)

b) Have all investigators filed a current annual conflict of interest questionnaire with the ASU Office of Research Integrity and Assurance?
☒ Yes ☐ No (Review ASU's objectivity in research policy: http://www.asu.edu/aad/manuals/rgp/rgp206.html)

c) Among the research team, is there any financial association that could affect any of the following: the study outcome, data analysis, enrollment of subjects, study design?
☐ Yes ☒ No (If yes, please describe and disclose in the consent form)

TRAINING

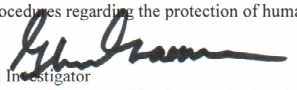
23. The research team must document completion of human subjects training.

Please provide the date that the PI and Co-Investigators completed the training.
Gaesser 9-3-2009
Sawyer 6-13-2011
Bhammar 9-7-2011
Tucker 9-1-2010

(Attach a copy of the human subjects training from the past 3 years: http://researchintegrity.asu.edu/training/humans for the PI and Co-Investigators. Training must be within the past 3 years)

REQUIRED SIGNATURES

24. By signing this application form:
- I agree to accept responsibility for the rights and welfare of the human subjects involved with this study.
- I believe that the benefits outweigh the risks to the participants in this study.
- I agree to comply with Arizona State University IRB policies and procedures.
- I certify that, to the best of my knowledge, I am in compliance with the Department of Health and Human Services policies and procedures regarding the protection of human subjects.



5-25-2012

Principal Investigator Date

Attach a copy of the PI's CV unless one is already on file with the Research Compliance Office.

(If the PI is the Department Chair or Dean, the application must be signed by another authorized Department/ School/College level Administrator)

FOR OFFICE USE:	This application has been reviewed by the Arizona State University IRB: ☐ Full Board Review ☐ Expedite Categories:_____ ☐ Exempt Categories: _____ ☐ Approved ☐ Deferred ☐ Disapproved ☐ Project requires review more often than annual Every months
Signature of IRB Chair/ IRB Member:	Date:

APPENDIX E

INSTITUTIONAL REVIEW BOARD APPROVAL

ASU Knowledge Enterprise Development

Office of Research Integrity and Assurance

To:	Glenn Gaesser Exercise a
From:	Carol Johnston, Chair Bioscience Full Board
Date:	06/20/2012
Committee Action:	Approval
IRB Action Date	06/20/2012
Approval Date	06/13/2012
IRB Protocol #	1206007902
Study Title	Effects of 8 Weeks of Either High-Intensity Interval Exercise Training or Continuous Steady-State Exercise Training on Glucose Control and Cardiovascular Disease Risk Factors in Obese Men and Women
Expiration Date	06/12/2013

The above-referenced protocol has been APPROVED following Full Board Review by the Institutional Review Board.

This approval does not replace any departmental or other approvals that may be required. It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date noted above. Please allow sufficient time for continued approval. Research activity of any sort may not continue beyond the expiration date without committee approval. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol on the expiration date. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study termination.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Bioscience Full Board immediately. If necessary a member of the Committee will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Bioscience Full Board. The new procedure is not to be initiated until the IRB approval has been given.