Effects of Muscle Contraction Frequency on Blood Glucose Control, Insulin Sensitivity, Endothelial Function and Blood Pressure Among Obese Males

by

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ABSTRACT

Cardiovascular disease and diabetes are major health burdens. Diabetes is a primary risk factor of cardiovascular disease, and there is a strong link between obesity and risk of developing diabetes. With the prevalence of prediabetes highest among overweight/obese individuals, investigation into preventative strategies are needed. Aerobic exercise is a potent stimulus for both insulin and non-insulin dependent glucose uptake into the skeletal muscle. A single exercise session can improve insulin sensitivity within hours after exercise. The effects of intensity, type, and volume of exercise on glucose homeostasis have been studied extensively; however, controlling for muscle contraction frequency with a constant exercise intensity and workload has not been examined. The purpose of this study was to compare muscle contraction frequency during aerobic exercise by altering cycling cadence on insulin sensitivity and vascular health. Eleven obese males (age=28yr, BMI=35kg/m^2) completed three conditions in random order: 1) control-no exercise; 2) 45-min cycling at 45 revolutions per minute (45RPM) at 65-75%VO_{2max}; 3) 45-min cycling at 90RPM at 65-75%VO_{2max}. Glucose control and insulin sensitivity were assessed with oral glucose tolerance tests (OGTT) 4 hours post-exercise. Vascular health was assessed via flow-mediated dilation (FMD) pre-exercise, 1-hr and 2-hr post exercise and ambulatory blood pressure was assessed pre-exercise, and continually every 15 min post-exercise. Linear mixed models were used to compare the mean differences in outcome variables. There were no significant differences found between control and both exercise conditions for all OGTT outcomes and no differences were found between control and exercise in FMD (all, $p>0.05$). Significant effects for exercise were found for both brachial and central blood pressure

measures. Brachial systolic blood pressures were lower at 2- and 4-hr post-exercise by approximately -10 and -8mmHg, respectively ($p<0.001$ and $p=0.004$) versus control. Central systolic blood pressures were lower at 2-, 3-, and 4-hr post-exercise by approximately -8, -9 and -6mmHg, respectively ($p<0.001$, $p=0.021$ and $p=0.004$) versus control. In conclusion, aerobic exercise, regardless of muscle contraction frequency, were unable to effect glucose control and insulin sensitivity. Similarly, there was no effect on vascular function. However, there was a significant effect of aerobic exercise on reducing post-exercise blood pressure.

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CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD) is the number one cause of death in the US, with a death rate of more than 2200 per day (Heron & Anderson, 2016; Mozaffarian et al., 2015). Diabetes is a major risk factor for cardiovascular disease, and there is a strong link between obesity and the risk of developing diabetes. Diabetes mellitus affects one of every 10 Americans, with most of those cases being type 2 diabetes (Mozaffarian et al., 2016). Recently, the National Health and Nutrition Examination Survey analysis revealed that diabetes might represent a greater contribution to total mortality in the United States, estimating that it was the third leading cause of death in 2010 (Stokes & Preston, 2017). Cardiovascular disease and type 2 diabetes (T2D) are both considered obesity-related disorders and are strongly linked to physical inactivity (Eaton & Eaton, 2017). Investigations focusing on diabetes prevention are essential for reducing diabetes and cardiovascular disease morbidity and mortality. Exercise plays an important role in reducing diabetes and heart disease risk due to its systemic metabolic and cardiovascular benefits.

Insulin resistance is a key characteristic among individuals with type 2 diabetes and is observed in the skeletal muscle, liver, and adipose tissue (Accili, 2004). Insulin resistance is a metabolic disease that directly affects glucose metabolism; however, it is frequently observed in non-diabetic individuals. Metabolic and cardiovascular risk factors such as dyslipidemia, hypertension, and visceral obesity are related to impaired insulin action (DeFronzo, 1997). It has long been known that exercise plays a pivotal role in the prevention and treatment of metabolic disorders. This understanding has led to

investigations focusing on which type of exercise has the greatest influence and what physiological changes improve glucose regulation and insulin sensitivity.

Exercise is often prescribed using the FITT principle: frequency, intensity, type, and time. Numerous studies have been conducted evaluating the most appropriate prescription for improving insulin sensitivity with a training program. When evaluating studies of the effects of training programs, employing exercise of various intensities and ranging in duration from 12 weeks to 6 months, on improvements in glucose control, it becomes clear that duration of exercise may play a more powerful role than intensity (Dube, Allison, Rousson, Goodpaster, & Amati, 2012; Hansen et al., 2009; Houmard et al., 2004; Li et al., 2012; Sigal et al., 2007). The lower intensity groups spent more total time exercising, resulting in more muscle contractions during the exercise session. Acute aerobic exercise trials reveal a similar pattern when comparing varying intensities on insulin sensitivity (Deschenes et al., 2000; Manders, Van Dijk, & van Loon, 2010; Newsom, Everett, Hinko, & Horowitz, 2013; Rynders et al., 2014; Wojtaszewski et al., 2000). The total amount of exercise time is longer in the low-intensity conditions, which supports the role for total amount of muscle contractions during exercise in the improvement of glucose control.

Ranges of cycling cadence have been shown to regulate physiological responses during exercise of the same intensity. In a simply designed study, recreationally active men who underwent a cross-over design cycling at 50-55% VO_{2peak} at either 40 or 80 revolutions per minute (RPM) revealed significant alteration in glucose levels from the 80 RPM condition and significantly higher glucose and plasma insulin measured 5 and 15 minutes post exercise (Deschenes et al., 2000). Most investigations with varying pedal

rates are primarily focused on cycling cadence and their physiological responses which may provide information for exercise performance and efficiency. This includes effects on VO_2 slow component (i.e., excess VO_2 associated with intense exercise), muscle recruitment patterns, perceived exertion and various metabolic and cardiovascular measures during and acutely post exercise (Barstow, Jones, Nguyen, & Casaburi, 1996; Gotshall, Bauer, & Fahrner, 1996; Kounalakis & Geladas, 2012; Lollgen, Graham, & Sjogaard, 1980; Migita & Hirakoba, 2006; Pringle, Doust, Carter, Tolfrey, & Jones, 2003; Vercruyssen, Missenard, & Brisswalter, 2009). While these studies provide physiological insight at a wide range of cycling cadences (35-120 RPM), they do not demonstrate the effects of the muscle contraction frequency on glucose uptake and insulin sensitivity in a prolonged acute period. Moreover, they did not utilize populations outside of active healthy individuals.

Longer durations of exercise have been shown to improve glucose control and insulin sensitivity (Brestoff et al., 2009; Dube et al., 2012; Houmard et al., 2004; Li et al., 2012; Manders et al., 2010; Newsom et al., 2013) suggests that more muscle contractions during exercise sessions may be the driver for improvements. Using pedaling cadence to simulate muscle contraction frequency could help elucidate the effects of improved glucose regulation with longer duration exercise bouts. The time point measured after exercise, when improved insulin sensitivity is pronounced, appears to be important. A time point of 3- to 4-hours after exercise has been identified for exercise induced increase in glucose uptake (Frosig & Richter, 2009).

Exercise effects on diabetes and cardiovascular disease risk reduction occur systemically in the body. Essentially, every tissue, organ, and organ system benefits from

the effects of exercise. If exercise were to be prescribed like medicine it would be considered a ‘polypill’, with potential for greater impacts on glucose tolerance, blood pressure and endothelial function than drugs (Fiuza-Luces, Garatachea, Berger, & Lucia, 2013; Gaesser, Tucker, Jarrett, & Angadi, 2015). Understanding the influence on vascular function and blood pressure following exercise among at-risk populations is still ongoing.

Vascular function is an important assessment of health due to its representation as a risk factor gap that can be altered by exercise. That is, there may be exercise-mediated effects on the vasculature responsible for reduction in cardiovascular risk. This is significant because traditional cardiovascular risk markers (e.g. blood pressure, blood lipids) explain less than half of the cardioprotective benefits of exercise training (Green, Hopman, Padilla, Laughlin, & Thijssen, 2017). The earliest measurable indicator of atherosclerosis is endothelial dysfunction, which occurs well before any clinical symptoms of cardiovascular disease (Vanhoutte, 2009). Flow-mediated dilation (FMD) has become a common non-invasive method to assess endothelial (dys)function. A frequently cited meta-analysis found that, independent of groups studied, a 1% decrease in FMD was associated with a 13% increase in cardiovascular disease risk (Inaba, Chen, & Bergmann, 2010). The most common FMD measurement method is conducted on the brachial artery, which correlates with coronary artery vasomotor function (Anderson et al., 1995; Takase et al., 1998). Although some data suggests that the brachial artery may not provide an index of systemic endothelial function (Thijssen, Rowley, Padilla, Simmons, Laughlin, Whyte, Cable, & Green, 2011a), there are many studies demonstrating that brachial artery FMD may be predictive of cardiovascular events (Gokce et al., 2002; Neunteufl et al., 2000; Perticone et al., 2001).

Exercise training has clear beneficial effects on vascular health (Green, Spence, Halliwill, Cable, & Thijssen, 2011). Increased blood flow during exercise results in a shear stress stimulus to the endothelial cells lining the blood vessels. This increased blood flow creates greater friction along the inner lining of the vessels, which stimulates the release of vasoactive hormones including nitric oxide (NO) to induce vasodilation. Repeated bouts of exercise are likely to play a role in the functional and or structural changes in the vascular system in response to training (Laughlin, Newcomer, & Bender, 2008). There is clear evidence to support the role of exercise training and improvements in vascular health; however, the acute effects of exercise on vascular function are still not clearly characterized.

The acute effects of aerobic exercise on endothelial function are unclear. This is in part due to the large variation in methodologies and populations studied (Dawson, Green, Cable, & Thijssen, 2013; Padilla, Harris, & Wallace, 2007). Studies have investigated differing exercise intensities, durations, modes, and timing of post exercise evaluation of endothelial function. These studies also vary in the health, levels of activity, and fitness of the participants. Moreover, many researchers have chosen not to include a no-exercise condition to control for the effects of diurnal variation in vascular function (Gaenzer et al., 2000). Failure to do so may have contributed to the disparate conclusions that endothelial function assessed by FMD acutely post exercise can result in improvement, impairment, or no change. Particularly, among obese individuals, acute effects of aerobic exercise on vascular function has yet to be well established (Hallmark et al., 2014; Harris, Padilla, Hanlon, Rink, & Wallace, 2008a).

Resting blood pressure is a common measure used to estimate CVD risk. Exercise training has been well recognized for its blood pressure lowering effects (Chobanian et al., 2003). Post exercise hypotension (PEH) has been investigated as a means to establish the magnitude and duration of the acute hypotensive effect following a single exercise bout. PEH responses have been shown to vary, but this may be explained by the population studied, use of medications, training status, age and how blood pressure was measured. Additionally, the type, duration, and intensity of exercise bouts, as well as the time of day the exercise in performed, may all influence the PEH effect. A recent meta-analysis on the acute effects of exercise on blood pressure found that regardless of the participant characteristics and the exercise mode, blood pressure was reduced in the hours following an acute exercise bout (Carpio-Rivera, Moncada-Jimenez, Salazar-Rojas, & Solera-Herrera, 2016). However, that meta-analysis is lacking investigations among obese populations and the acute effects of exercise on blood pressure. Since over a third of the US population is obese, insight into the hypotensive effects of exercise in this population is important.

Purpose, Specific Aims, and Hypotheses

Purpose: The purpose of the current study was to determine the effect of muscle contraction frequency during acute bouts of moderate-vigorous intensity cycling at 45 or 90 revolutions per minute on glucose control, insulin sensitivity, endothelial function, and blood pressure control in obese inactive males.

Aim #1: To determine the effects of acute bouts of cycling at 45 or 90 RPM at moderate-vigorous intensity on glycemic control.

Hypothesis #1: I hypothesize that both exercise conditions will result in significantly less insulin resistance compared to the control condition as determined by OGTT. I also hypothesize that the 90 RPM condition will result in significantly less insulin resistance compared to the 45 RPM condition.

Aim #2: To determine the effects of acute bouts of cycling at 45 or 90 RPM at moderate-vigorous intensity on endothelial function as assessed by brachial artery flow-mediated dilation at 1hr and 2hr post exercise.

Hypothesis #2: I hypothesize that both exercise conditions will result in significantly improved endothelial function acutely post exercise compared to the control condition. I also hypothesize that the 90 RPM condition, will result in significantly improved endothelial function post exercise comparted to the 45 RPM condition.

Aim #3: To determine the effects of acute bouts on cycling at 45 or 90 RPM at moderate-vigorous intensity on continuous ambulatory blood pressure.

Hypothesis #3: I hypothesize that both exercise conditions will result in significantly reduced post exercise blood pressure. I also hypothesize that there will be no significant differences between 45 and the 90 RPM conditions.

Definition of Terms

Oral Glucose Tolerance Test: A test to determine the body's ability to handle a glucose load. This test is commonly used to define impaired glucose tolerance and type 2 diabetes, but has also been employed to determine insulin sensitivity after exercise.

Matsuda Index: OGTT derived index of whole-body insulin sensitivity, including fasting and mean glucose and insulin concentrations in the equation. This measure is comparable to the insulin clamp method of assessing insulin sensitivity.

Area under the curve (AUC): Glucose and insulin measured during the OGTT can be calculated with the trapezoidal method to determine AUC, which represents glucose and insulin excursion relative to baseline.

Ambulatory blood pressure monitor: A 24-hour blood ambulatory blood pressure monitor that can be set at specific time intervals to measure blood pressure.

Post Exercise Hypotension: A reduction in blood pressure following a session of aerobic exercise compared to resting blood pressure.

Endothelial function: Capacity of the endothelium organ to secrete mediators of vasomotor tone.

Brachial artery flow-mediated dilation: A commonly used non-invasive measure to test endothelium-dependent vasodilation using high-resolution ultrasound. This procedure includes a five-minute occlusion of the brachial artery by applying a blood pressure cuff to the forearm and inflating it to supra-systolic cuff pressures. The cuff deflation induces a brief high-flow state through the artery resulting in shear stress and vasodilation. Flow-mediated dilation is expressed as the change in post-stimulus diameter as a percentage of the baseline diameter.

Delimitations

The delimitations of this study in men include: body mass index (30-45 kg/m^2), age 18 to 45 years old, inactive, non-smoking, not taking medications for the treatment of diabetes, heart disease or high cholesterol, capable of performing moderate-vigorous physical activity.

Limitations

Although dietary intake the day prior to testing was controlled, this study lacked the ability to control for dietary changes throughout the time between conditions. Participants were asked to not change their habitual diet, but compliance between conditions was not measured. All participants were asked to not engage in any regular moderate or vigorous physical activity outside of the study, but compliance outside of our accelerometer data the day prior to the condition visit cannot be confirmed.

Chapter 2

BACKGROUND LITERATURE

Measurements of Insulin Resistance

Insulin action in the body encompasses a wide range of important metabolic processes. This includes, but not limited to, regulating glucose uptake, glycogen synthesis, gluconeogenesis, lipid metabolism, gene expression and protein synthesis, cell growth and division and vasoreactivity (Beale, 2013). Regarding glucose metabolism, the biological effects of insulin occur after the beta cells of the pancreas release this peptide hormone and it passes through the liver, and then from the circulation to reach target tissues. The insulin molecule must interact with insulin receptor substrates on the cell membrane, and this will trigger a cascade of intracellular interactions ultimately resulting in glucose transporters to translocate to the cell membrane which prompts facilitated diffusion of glucose into the cell (Scheen, Paquot, Castillo, & Lefebvre, 1994). This allows for glucose utilization in the peripheral organs, primarily skeletal muscle and also adipose tissue.

The term ‘insulin resistance’ was first described among diabetic participants who required more insulin than expected to produce hypoglycemic attacks (Himsworth, 2013). More commonly, insulin resistance is used to describe the inability of tissue to be responsive to insulin for glucose uptake among diabetic populations as well as individuals who are at increased risk due physical inactivity. Insulin resistance is linked to physiological states including physical inactivity, but also, puberty, pregnancy, and advanced age. Pathological conditions where insulin resistance is thought to play a role

would be in the case of obesity, diabetes mellitus, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease (Scheen et al., 1994).

Insulin resistance is not a new physiological concept but the methods to adequately determine both insulin sensitivity and resistance are varied with their own advantages and disadvantages. The methods to determine the difference between individuals who are sensitive and insensitive to insulin administration following ingestion of glucose was first described in the 1930's (Himsworth, 2013). Since then numerous approaches have been developed including intravenous methods that bypass influences of the digestive system along with varied methods using the glucose ingested orally.

Hyperinsulinemic-euglycemic clamp

The gold standard method for measuring whole-body insulin sensitivity with the hyperinsulinemic-euglycemic clamp (HEC). (Muniyappa, Madan, & Quon, 2000; Muniyappa, Lee, Chen, & Quon, 2008). A known amount of insulin is infused, at a steady state that is above the fasting insulin level, so that a hyperinsulinemic state is reached. Glucose is then frequently monitored every 5 or 10 minutes and 20% dextrose is given intravenously, at a varying rate so that the blood glucose is "clamped" at a concentration in the normal range, so the blood is in a euglycemic state. Once the blood glucose concentration is in a steady-state and assuming the hepatic glucose production is suppressed, the glucose infusion rate during the HEC is equal to the disposal rate. The whole-body disposal of glucose during a steady-state conditions of greater than 30 min (after 1 hour of initiation of infusion) is used to obtain an estimate of insulin sensitivity. Whole body glucose disposal rate is typically normalized to fat-free mass or body weight for an estimate of insulin sensitivity. Insulin sensitivity index (S_I) is also derived using

this method. The S_I obtained from this method is defined as $SI_{Clamp} = M/(G \times \Delta I)$, where M is glucose disposal rate, G is steady-state blood glucose concentration and ΔI is the difference between fasting and steady-state plasma insulin concentrations. (Katz et al., 2000; Muniyappa et al., 2008).

There are a few but important assumptions and considerations when using this method. This method assumes that during the glucose clamp hepatic glucose production is completely suppressed. Radiolabeled glucose infusions can be used to determine hepatic versus muscle insulin sensitivity during the HEC procedure (Finegood, Bergman, & Vranic, 1987). Additionally, the rate of glucose disposal is determined using a specified insulin infusion and this infusion rate can differ between insulin resistant/sensitive individuals. This can lead to invalid conclusions when comparing different populations if a higher amount of insulin infusion is needed among insulin-resistant populations. Differing fasting glucose levels among participants can also be a limitation and some investigators use an isoglycemic level for the clamp instead of a pre-determined euglycemic clamp (Muniyappa et al., 2008).

Although the HEC procedure provides a direct measure of insulin sensitivity, the feasibility is limited because this method is time-consuming, requires specialized staff, is labor intensive, and costly. The HEC method is preferred due the direct measurement of metabolic insulin sensitivity, but this may not be feasible for all investigations nor is it a normal physiological stimulus such as a glucose load or oral meal.

Oral Glucose Tolerance Test

The oral glucose tolerance test is a less complex, inexpensive, and practical method for assessing glucose tolerance and insulin sensitivity. Following and overnight fast blood samples are taken for glucose and insulin analysis at 0, 30, 60, and 120 min following a standard oral glucose load (75g). After the glucose is ingested, the initial rise in plasma glucose stimulates the secretion of insulin from the β-cells, and with increased levels of insulin and glucose there it is assumed that endogenous glucose production is suppressed. Incremental area under the curve (AUC) can quantify the rise of plasma glucose and insulin relative to baseline. Stimulation of glucose disposal into peripheral tissues, primarily skeletal muscle, occurs with the rise in plasma glucose concentrations. Provided that endogenous glucose production is suppressed during the 60- to 120-min time, the decrease in plasma glucose concentration after 60-min is thought to mainly reflect skeletal muscle uptake (M. A. Abdul-Ghani, Matsuda, Balas, & DeFronzo, 2007).

Additionally, measuring impaired glucose tolerance at the 2-hour time point in an OGTT (140-199mg/dL) shows a greater risk of cardiovascular disease and future risk of developing type 2 diabetes. With more recent studies revealing the presence of microvascular complications (e.g. retinopathy and neuropathy) with impaired glucose tolerance (M. Abdul-Ghani, DeFronzo, & Jayyousi, 2016). To determine β-cell function and insulin sensitivity from the OGTT calculation can be done using the values obtained at the sampling times.

It is important to note that this method does not provide direct measures insulin sensitivity or insulin resistance. However, surrogate indexes have been developed that are strongly correlated with muscle insulin sensitivity measures from the HEC. Specific

indexes include the Matsuda index, Stumvoll index, Gutt index, Belfiore index and the oral glucose insulin sensitivity index. Data from a group of Mexican-American subjects with both normal glucose tolerance (n=100) and impaired glucose tolerance (n=55), reveal strong correlations (r=0.76-0.81) between OGTT-derived indices of muscle insulin resistance compared to the HEC (M. A. Abdul-Ghani et al., 2007). A study conducted among Japanese participants with a range of glucose tolerance (normal, n=40; impaired glucose tolerance, n=22; type 2 diabetes, n=15) found that insulin sensitivity measured from the HEC moderately correlated with indices from the OGTT for the Matsuda, Stumvoll and Gutt index (r=0.451, 0.641, 0.526, respectively) (Kanauchi, Tsujimoto, & Hashimoto, 2002). The Matsuda index was developed from a study of American adults with various degrees of glucose tolerance (normal, n=62; impaired, n=31; and type 2 diabetes, n=60). They found that this index was highly correlated with insulin sensitivity from the HEC (r=0.73), and this explains about 50% of the variance (Matsuda & DeFronzo, 1999). Lastly, a meta-analysis aimed to identify which surrogate marker of insulin sensitivity had the strongest correlation with the HEC reported strong correlations for many of the indices with the Matsuda Index correlation, r=0.67 and HOMA-IR, r=-0.60 (Otten, Ahren, & Olsson, 2014).

There is a general advantage of this low cost, normal physiological stimulus to provide surrogate measures of insulin sensitivity that correlate reasonably well with glucose clamp measures. Depending on the particular research question, population and feasibility, researchers may choose this approach. However, it is important to note that there have been reports of poor reproducibility resulting in within subject and day-to-day variability of this measure (Utzschneider et al., 2007).

Contraction-mediated Glucose Uptake

The primary source of glucose disposal in the body occurs at the skeletal muscle, and during exercise glucose delivery, transport and metabolism are all increased several fold (DeFronzo et al., 1981; Moghetti, Bacchi, Brangani, Dona, & Negri, 2016). Glucose transporter type 4 (GLUT4) is the major glucose transporter isoform in skeletal muscle that is both regulated by insulin and contractile activity (Jessen & Goodyear, 2005). Contraction-mediated glucose uptake involves a cascade of intracellular signaling that leads to the mobilization of GLUT4-containing vesicles from intracellular storage sites. Muscle contraction during exercise also alters the fuel status of the skeletal muscle cells, by reducing phosphocreatine and ATP concentrations. The increased ratio of AMP to ATP and creatine to phosphocreatine in turn activates 5' AMP-activate protein kinase (AMPK), playing a distinct role in the stimulation of glucose transport (Jessen & Goodyear, 2005).

Other important cellular mechanisms in contraction-mediated glucose uptake include calcium activated proteins, protein kinase C (PKC) and Ca/calmodulin-dependent protein kinase (CaMK), which act in parallel or in combination with the AMPK pathway (Santos, Ribeiro, Gaya, Appell, & Duarte, 2008). Additionally, distal signaling proteins atypical protein kinase C and AS160 are more potently regulated after exercise (Frosig & Richter, 2009). Other mediators of interest include reactive oxygen species (ROS), nitric oxide (NO) and bradykinin. These can also influence the signal for the translocation of GLUT4 to the cell membrane (Alvim, Cheuhen, Machado, Sousa, & Santos, 2015; Richter & Hargreaves, 2013). These cellular mechanisms are involved along with the integration of increased muscle blood flow and capillary recruitment during exercise to

keep interstitial glucose concertation during exercise high to be mobilized into the cell. Acute regulation of muscle glucose depends on GLUT4 translocation, and the expression of this gene is increased following exercise. With exercise training being a major stimulator of increased GLUT4 expression, this plays a pivotal role in the contribution of enhanced insulin action and glucose disposal.

It is important to recognize these metabolic and cellular effects of exercise. Acute alterations in the signaling pathway during exercise result in insulin-independent glucose uptake. With exercise training, there are also improvements in skeletal muscle insulin sensitivity. This would increase insulin-dependent glucose uptake and is thought to be an essential component for prevention and treatment of pre-diabetes and diabetes (Moghetti et al., 2016).

Post Exercise Insulin Sensitivity from OGTT

Insulin-independent glucose uptake during exercise is reversed around 2-3 hours post exercise, while muscle and whole-body insulin sensitivity is enhanced at 1-4 hours post exercise, with reports lasting for up to 48 hours (Cartee, 2015).

The OGTT method has been used in acute aerobic exercise studies as a measure of insulin sensitivity and glucose tolerance. An investigation by Brestoff and colleagues compared an endurance exercise protocol cycling at 75% of VO_{2peak} for 45 min and a sprint interval exercise protocol consisting of four 30 sec cycling sprints at 125%VO_{2peak} among 13 recreationally active men and women. They used fasting OGTT's 12-16 hours post exercise and compared that to a fasting no-exercise control OGTT and determined that the endurance exercise bout but not the sprint interval exercise increased insulin sensitivity (Matsuda index) compared to the control condition (Brestoff et al., 2009).

An investigation among 10 sedentary overweight/obese African American women used OGTTs to assess insulin sensitivity 1.5 hours following a 75-minute brisk walking treadmill exercise. They reported that insulin sensitivity (Matsuda index) was 18% higher in the exercise condition compared to control (Hasson, Freedson, & Braun, 2006). A study done among 10 young untrained obese males sought to investigate the relationship of glucose tolerance and inflammation effects of resistance training and aerobic training compared to a no exercise control. The OGTT was completed 24 hours post exercise and specifically looked at the insulin sensitivity results from the Matsuda index. They found no differences for both exercise conditions. These results were the same for the insulin and glucose AUC and HOMA. The aerobic exercise consisted of 60 min cycling at 70% VO_{2peak} (Mitchell, Phillips, Yellott, & Currie, 2011).

A well-designed study by Rynders and colleagues used the oral minimal model (OMM) and OGTT derived indices of insulin sensitivity post exercise among 18 obese males and females with prediabetes. They used the OMM as comparable 'gold-standard' to the clamp method (Cobelli et al., 2014). The OMM method included a 3-hr 75-g OGTT with frequent sampling of glucose and insulin at 5-min intervals during the first hour and every 10 min during the second and third hour. The exercise consisted isocaloric (~200kcal) bouts of moderate intensity exercise (~50% VO_{2peak}) and high intensity exercise (~80% VO_{2peak}), with a control no exercise condition. The glucose tolerance testing was done 1-hour post exercise and they found that insulin sensitivity was increased for both the OMM and OGTT indices of insulin sensitivity. However, there was high variability between indices under each condition. Therefore, they advised

caution when concluding insulin-sensitizing effects of exercise based on the OGTT method (Rynders et al., 2016).

Another comparison investigating insulin sensitizing effects of one bout of aerobic exercise compared the OGTT to the intravenous glucose tolerance test (IVGTT) among 10 young healthy active males. On the exercise condition days, they completed the test 20 min after exercise which consisted on 45 minutes of cycling at 65% VO_{2peak}. Interestingly, during the resting condition the Matsuda index correlated well with the IVGTT insulin sensitivity index (r=0.828), but not as well following exercise (r=0.547). The insulin sensitivity post exercise measured from the OGTT Matsuda index increased by 29%, but this was not statistically significant. The insulin sensitivity index from the IVGTT has been reported to increase significantly post exercise by 50% (Ortega, Hamouti, Fernandez-Elias, & Mora-Rodriguez, 2014).

King and colleagues conducted a time-course investigation using OGTTs immediately post exercise and 1, 3, 5 and 7 days following. The intervention consisted of 5 days of exercise for 45 min, cycling at 75% VO_{2peak} each session. In this study among moderately trained men and women they reported a marked impairment of insulin action post exercise with improved insulin action and glucose tolerance the day after exercise which persisted for 3 days, but not longer than 5 (King et al., 1995).

Measurement of Endothelial Function

The endothelial cells that line the interior surface of blood vessels are the interface for the circulating blood and the vessel wall play an important role in vascular homeostasis. This single layer of simple squamous cells makes up an organ system that plays an essential role in the regulation of vasomotor tone, inhibition of platelet

aggregation and thrombus formation (Lloyd-Jones & Bloch, 1996). Vasodilation occurs through a variety of pathways including prostacyclin, endothelial derived hyperpolarizing factor and nitric oxide (NO). Diseases such as diabetes and hypertension result in lower amount of NO release from the endothelial cells and an increased rate of endothelial cell apoptosis. Regenerated cells then replace the apoptotic cells. In this context they become dysfunctional, senescent, and not able to produce sufficient amounts of NO. This supports the inflammatory response leading to the production of atherosclerotic plaques. Reduced NO availability allows for and imbalance of vasomotor tone that amplifies the degree of endothelial dysfunction (Vanhoutte, Shimokawa, Feletou, & Tang, 2017).

Since the early 90's researchers have been able to study vascular physiology in systemic arteries, using high-resolution ultrasound imaging. This commonly used non-invasive method to determine vascular function is known as flow-mediated vasodilation (FMD) (Celermajer et al., 1992). FMD testing has been utilized as a surrogate marker of endothelial function in peripheral arteries and there is evidence to support its value as measure that can predict future cardiovascular disease events (Gokce et al., 2002; Neunteufl et al., 2000; Perticone et al., 2001). This method has been refined and utilized in a range of studies to investigate the physiological mechanism and clinical significance of FMD testing.

To help standardize this technique official guidelines have since been established. The guidelines put forth by Corretti et al., have been cited more than 2000 times since their technical report was published in 2002 (Corretti et al., 2002). The FMD test is simple in its method where an occlusion is placed on the arm resulting in reduced blood flow. This is done for 5 minutes using a suprasystolic occlusion. Following the release of

the blood pressure cuff used the brachial artery is imaged for 5-10 minutes post to detect the peak diameter change resulting from the shear stress induced vasodilation comparted to a baseline diameter image. The calculation of FMD is expressed as a percentage of vessel diameter change following reactive hyperemia in relation to the pre-occlusion baseline diameter (FMD% = Peak Diameter – Baseline Diameter/Baseline Diameter). Given that the baseline diameter is part of this equation it has been found that various diameter sizes among populations studied can result in a misinterpretation of the percentage of diameter change. To control for this statistical approach and to account for this variation guidelines have been put forth by Atkinson et al. and are now commonly used in the literature. They suggest computing a least squares regression slope for the relationship between the logarithmically transformed baseline diameter and peak diameter. If the upper confidence limit of the slope is less than then it would not be appropriate to use the simple ratio %FMD and allometric scaling should be implemented (Atkinson, Batterham, Thijssen, & Green, 2013).

Post Exercise Endothelial Function

A culmination of data demonstrates a biphasic response in FMD after an acute bout of exercise. Immediately post exercise FMD is decreased and improvements are typically seen about 1-24hr post exercise (Dawson et al., 2013). Utilizing the assessment of endothelial function acutely following exercise is beneficial to provide insight into the causes of longer-term adaptation. However, understanding of FMD responses in the acute exercise model has not been fully characterized and more specifically among an inactive obese population. To determine the interaction of endothelial function and a single bout of exercise, many methodological considerations need to be considered. Specifically, the

number of measurements are taken post exercise, use of a non-exercise condition to control for the diurnal variation in the assessment, and lastly if the measurement itself is reproducible in the acute exercise conditions. The optimal timing of measurement have yet to be determined, but it is clear that measuring more than once and for a longer duration post exercise should result in a clearer description of the characterization in endothelial function. Serial measurements have been found to not influence subsequent FMD outcomes, the reproducibility following exercise has been within an acceptable variation and the diurnal variation beyond the morning should be considered (Padilla et al., 2007).

There have been inconsistent findings in the literature as it pertains to the effects of acute exercise on endothelial function as measured by FMD. This could be in part to the methodological considerations above, but also due to population differences, individual variation and lack of investigations on a variety of populations. Single bouts of aerobic exercise have demonstrated an improved response, impaired and no effect.

A prolonged bout of aerobic exercise in the form of a marathon run in nonelite runners has been demonstrated to impair vascular function. A study of 15 male runners who completed the London marathon had FMDs completed before and within an hour finishing. They found there was a depression in femoral artery FMD, but no change in brachial artery FMD (Dawson et al., 2008). When comparing endurance-trained to sedentary adult males the effect of a single bout of high-intensity exercise has varying effects on FMD. Ten athletes and seven healthy sedentary control participants in this study. It is important to note that that ‘sedentary’ men were classified based on a VO2max value <55ml/kg/min and less than 1 hour or training per week. The high-

intensity aerobic exercise bout consisted of a 15-min warm-up running at 60-70% HRmax and then a 5 x 5 interval session with last 3 min of each 5-min bout at >90% HRmax and 2 min active recovery. FMD was measured 1-hour, 24-hours and 48-hours post exercise. The endurance-trained athletes had larger arterial diameters bus similar FMD results compared to the sedentary adults. Among the endurance athletes the 1-hour time point post exercise showed reduced FMD but that was normalized within 24 hours (Rognmo et al., 2008).

Clinical populations are also studied to examine the effects of acute exercise in endothelial function. A study involving 10 individuals with cardiovascular disease investigated the effects of moderate-intensity endurance exercise (MOD) and high-intensity interval exercise (HIE) on changes in FMD. The measurements were taken 30 min and 60 min post exercise. The MOD exercise involved 30 min of cycling at 55% power output peak and the HIE included ten 1-min bouts of cycling at 80% power output peak separated by 1-min active recover. They found that acute FMD 60 min after both exercise condition improved among patients with endothelial dysfunction and coronary artery disease (Currie, McKelvie, & Macdonald, 2012).

Among overweight individuals, activity status may alter the effect that acute exercise plays on FMD. Sixteen overweight inactive (n=8) and active (n=8) men performed three different intensity acute bouts of exercise and FMD was measured before and 1-hour post exercise. The exercise consisted of treadmill walking for 45 min at either low (25% VO_{2peak}), moderate (50% VO_{2peak}), or high (75% VO_{2peak}) intensity. Interestingly, they found that independent of intensity, the active males had a 24% increase in FMD following exercise and the inactive group had a 32% decrease (Harris,

Padilla, Hanlon, Rink, & Wallace, 2008b). The response to acute exercise among overweight postmenopausal women differs from normal weight premenopausal women. In a study of 13 overweight and 14 normal weight post- and premenopausal women found improved FMD post exercise in the overweight group and not in the normal weight group. The exercise in this study consisted of treadmill walking for 45 minutes at the heart rate at 60% of VO_{2max}, with the FMD conducted 1-hour post exercise (Harvey et al., 2005). Among obese populations the acute effects of exercise on endothelial function is less clear.

Obesity is associated with impaired FMD (Williams et al., 2005); however, the effect of acute exercise among obese individuals has been understudied. A study in young obese males found that an acute treadmill running for 45 min at a moderate intensity (80% HRmax) resulted in an increased FMD 1- and 2-hour post (Zhu et al., 2010). Acute changes in endothelial function among lean and obese adults were examined in an acute exercise study examining effects of exercise intensity (Hallmark et al., 2014). The participants in this study included 16 lean and 10 obese males and females who underwent a control no-excise condition and two exercise conditions consisting of 30 minutes of moderate- or high-intensity cycle exercise. The FMD was performed prior to the exercise and at each hour after exercise for 4 hours. An increase in FMD was only evident in the lean participants following the high-intensity exercise. FMD was unchanged among the obese individuals following both moderate and high intensity exercise.

Post Exercise Blood Pressure Control

Post exercise hypertension (PEH) is determined by comparing blood pressure (BP) values measured before and after an exercise session or by comparing the values during a control day to an exercise day (Cardoso et al., 2010). Measuring post exercise blood pressure changes is easily done with ambulatory blood pressure (ABP) monitors and they can be set to measure blood pressure at user specified intervals. One bout of exercise can lower blood pressure and be sustained for 13 hours (Kenney & Seals, 1993; Pescatello & Kulikowich, 2001). It is noteworthy that a 2mmHg reduction in systolic BP is associated with a 6% and 4% reduction in stroke and coronary heart disease mortality, respectively (Chobanian et al., 2003). This blood pressure lowering effect post exercise has been shown in hypertensive individuals as well as prehypertensive and normotensive, and provides a period during which the hemodynamic load is reduced (Forjaz et al., 2000). PEH can be attributed to the hemodynamic changes that occur in the vasculature following exercise and one of the main mechanisms is through a reduction in systemic vascular resistance. Other reports discuss that the reduction is, in part, due to a reduced cardiac output (Brito, Queiroz, & Forjaz, 2014).

There are multiple factors that can account for differences in the blood pressure response to exercise. These include, but are not limited to, the population studied, blood pressure status, medication use, age, gender, weight, exercise mode, type, duration, intensity etc. Although these variables mentioned can play a role in the magnitude of PEH following an acute bout of exercise, a recent meta-analysis reported that regardless of participant measurement features, and exercise characteristic, there was still a reduction in blood pressure in the hours following exercise (Carpio-Rivera et al., 2016).

There is a strong relationship between obesity and hypertension with evidence supporting the role of visceral obesity specifically as a key factor (Rahmouni, Correia, Haynes, & Mark, 2005; Sironi et al., 2004). The meta-analysis by Carpio-Rivera et al., reported that a lower BMI was associated with a greater reduction in systolic BP and that the implications of these findings are important because of the large proportion of obesity and hypertension in the population. However, their analyses only included a limited number of obese individuals, so those conclusions may not hold true and need to be investigated more (Carpio-Rivera et al., 2016). The participant characteristics included in PEH studies suggests a lack of information among obese individuals. High blood pressure is a major risk factor for cardiovascular disease and with over one-third of the US adult population being obese (Flegal, Carroll, Kit, & Ogden, 2012). Thus, having a better understanding of the effect of PEH among this population may have considerable clinical application.

Post exercise hypotension among 13 young healthy adults who underwent three exercise trials consisting of continuous steady state, aerobic interval and sprint interval exercise found that compared to the control they all had similar reductions in BP 1-hour post exercise. Sustained PEH for greater than 2 hours only occurred in the aerobic interval exercise group (Angadi, Bhammar, & Gaesser, 2015). Another study comparing aerobic interval exercise with submaximal constant-load exercise found no differences in PEH 1-hour post (Lacombe, Goodman, Spragg, Liu, & Thomas, 2011). The effect of high-intensity interval exercise on PEH has been reported in endurance trained men and women. They have also reported blood pressure lowing effects 1-hour post, but with no non-exercise control trial (Rossow et al., 2010; Scott et al., 2008). There is data to

support the role of intensity and its favorable effect on PEH (Kessler, Sisson, & Short, 2012; Wisloff et al., 2007), but still there is data lacking across all exercise types in the obese population.

Chapter 3

METHODS

Subjects were recruited through emails, flyers, and social media from the Phoenix area and at Arizona State University. All subjects were sedentary, obese (BMI 30-45 kg/m^2), non-smoking, adult males between the ages of 18 and 45 yr. All participants who responded to the recruitment flyer were provided an online pre-screening survey through Qualtrics Online Survey System to establish eligibility, which included questions from the physical activity readiness questionnaire (PAR-Q) (Appendix F). Online survey consent was obtained prior to advancing to questions. Participants responded to questions about gender, age, height, weight, and several "yes" or "no" questions related to smoking status, current exercise habits and weight loss or dieting efforts. If they self-reported a diagnosis of hypertriglyceridemia or hypercholesterolemia or answered "yes" to any of the PAR-Q questions they were excluded. Participants who were current smokers, diagnosed with diabetes or had a fasting blood glucose measurement above 125mg/dl on screening day, currently engaging in regular physical activity, or on a calorie-restricted diet were excluded. The Arizona State University Institutional Review Board approved the study.

Research Design

All subjects underwent 3 conditions in a randomized cross-over design to test the effect of muscle contraction frequency during moderate-vigorous exercise on glucose control, insulin sensitivity, endothelial function, and blood pressure. The conditions consisted of a non-exercise control day (CON) and two exercise conditions (45 RPM or

90 RPM). Conditions were completed in random order and separated by at least one week:

- Control: time-matched, no exercise
- 45 RPM: Cycling at 45 revolutions per minute at 65-75% of VO_{2max} for 45 minutes. Five-minute warm-up while increasing wattage to VO_2 intensity goal and a five-minute cool-down at 25-50 watts.
- 90 RPM: Cycling at 90 revolutions per minute at 65-75% of VO_{2max} for 45 minutes. Five-minute warm-up while increasing wattage to VO_2 intensity goal and a five-minute cool-down at 25-50 watts.

These cycling cadences of 45 and 90 RPM were chosen to simulate a double amount of muscle contractions during a same intensity for the same total exercise duration and energy expenditure. This could have been accomplished with other cadences (e.g., 35 vs. 70; 40 vs. 80; 50 vs. 100), but during pilot testing in the lab, a range closer to preferred cycling cadences that were not abnormally slow or fast was chosen. The selected range of 45-90 RPM does not result in substantial differences in motor unit recruitment patterns and amplitude of VO_2 slow component (Barstow et al., 1996).

A flow-diagram of the study design can be seen in Appendix A. Study duration for each participant was approximately 4 weeks to allow for a baseline assessment of physical activity and adequate temporal separation of conditions to avoid potential carry-over effects on outcome measures.

Sample size and participants

The sample size was estimated based on one-leg dynamic knee-extensor ergometer comparing insulin sensitivity and glucose control using a hyperinsulinemic-

euglycemic clamp in an exercised leg and non-exercised leg 4 hours after exercise (Wojtaszewski et al., 2000). The primary aim to detect area under the curve difference during the OGTT between control and exercise. Using G*Power 3.1 software (Faul, Erdfelder, Lang, & Buchner, 2007). We assumed 95% Power at 0.05% alpha level of significance (two-sided). Based on these parameters, a sample size of 4 would be required to detect significant differences between the exercise and control. Given that this reference study used a more robust measure of glucose tolerance and the goal of this study was to detect differences between exercise groups, a sample size of 10 participants was used.

Procedures

The procedures of this study included 7 visits to the laboratory:

Participant Screening (Visit 1)

The participants who met the eligibility criteria from the pre-screening survey were asked to visit the Healthy Lifestyles Research Center (HLRC) in the Arizona Biomedical Campus building on the ASU Downtown Campus. Upon arrival, they were provided with a copy of the consent form (Appendix B) to read, and all questions were answered. Study details were explained and written informed consent obtained. Participants also filled out a hard-copy and signed the PAR-Q (Appendix C), and were assigned a non-identifiable ID used on all saved documents and data. Those who consented had their height, weight, blood pressure, and blood glucose measured to ensure that they met our inclusion criteria. Blood glucose was measured using the fingerstick method to obtain fasting capillary blood glucose with a One Touch Ultra Glucose Meter to confirm fasting glucose was less than 126mg/dl. After this, subjects were offered a

snack (granola bar) and water and then performed a maximal exercise test on a cycle ergometer to determine maximal oxygen uptake (described later). Before leaving the laboratory, participants were provided an accelerometer (SenseWear Armband, Pittsburgh, USA) to wear for 5-7 days to confirm that they met the sedentary criteria for participation. Participants who agreed to take part in the study reported to the Healthy Lifestyles Research Center on the Downtown ASU campus for all subsequent visits. Participants were assigned to the three conditions (Control, 45 RPM, 90 RPM) in random order and all testing visits were scheduled 1 week apart.

Diet Control

To control for effects of the previous days diet on the modified-oral glucose tolerance test (OGTT), subjects were provided with pre-paid meal gift cards for each of the three days prior to the OGTT testing visit. On study visits 2, 4 and 6 (each day before the OGTT) participants reported to the lab and were provided specific instructions on what meals to purchase the day before the OGTT visits. This was done after discussing dietary preferences with the participant during the pre-screening visit. Pre-paid gift cards from chain restaurants (e.g., Chipotle, Subway) were purchased in advance. The participants were asked to provide the receipts or record of food eaten from the day prior to visit 3. On visit 4 and 6 each participant was instructed to eat the same foods/beverages from the day prior to visit 3. They were asked to refrain from consuming alcohol or caffeinated beverages on these days.

Diet Control Visits 2, 4, 6:

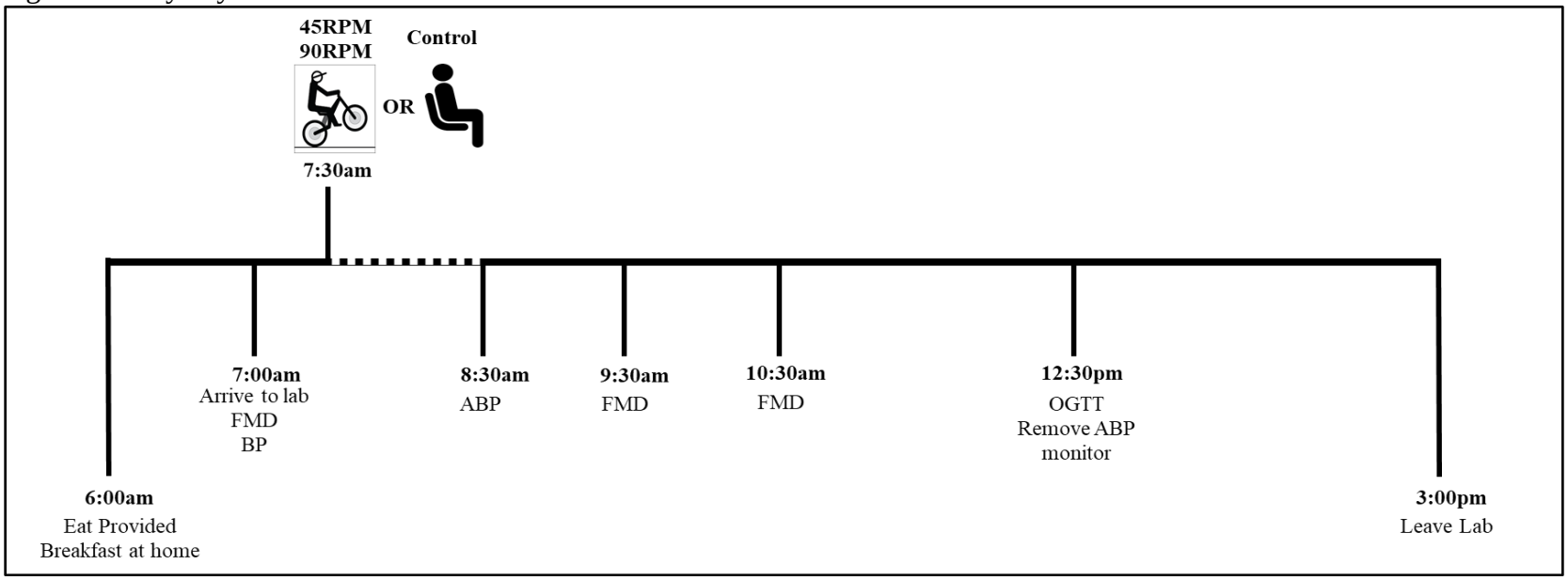
These visits were scheduled 1-3 days prior to testing visits (Control, 45 RPM, 90 RPM). Meal gift cards were given to the participants with diet instructions (reminder to

record what foods/beverages consumed, no alcohol, no caffeine) prior to the testing days. Participants were also provided with a light breakfast to consume the morning of their next visit between 6:00am and 6:30am. The breakfast meal consisted of a plain bagel, reduced-fat cream cheese, and low-fat chocolate milk (Nutrition facts; Appendix I). They were also given an accelerometer to wear prior to the next visit to confirm no engagement in physical activity prior to the testing visits and were reminded to not participate in any physical activity.

Exercise/Control Condition Visits 3, 5, 7:

These visits include the main testing days and were performed in random order separated by at least 1 week. The condition days started in the lab at 7am and the participants left at approximately 3pm (Figure 1). They are described below in the order of control visit and the two exercise condition visits.

Figure1: Study day timeline with conditions and assessments



FMD: flow-mediated dilation; BP: blood pressure; ABP: ambulatory blood pressure; OGTT: oral glucose tolerance test

Control Visit:

The participants reported for testing at the HLRC building at 7am and confirmed that they consumed the provided breakfast between 6:00am and 6:30am. Participants were asked to refrain from exercise for 78 hours prior to this visit, and to not consume caffeine, and dietary supplements for 48 hours prior to this visit. Participants had an ambulatory blood pressure (ABP) monitor placed on their right arm and this monitor was set to inflate and measure blood pressure every 15 minutes until they started their OGTT (ABP; described later). Participants rested for 15 minutes in the vascular assessment room and then had a brachial artery flow-mediated dilation ultrasound assessment completed (FMD; described later). This was repeated at 9:30am and 10:30am (approximately 1hr and 2hr post exercise). Participants waited comfortably in the lab between and after the flow-mediated dilation measurements for 4 hours prior to the oral glucose tolerance test (OGTT). Thereafter, subjects relaxed comfortably in reclining chairs while undergoing a modified-oral glucose tolerance test (OGTT; described in detail later). Following the placement of the intravenous catheter, body composition was measured (DEXA; described later).

Exercise Visits:

The participants reported for testing at the HLRC building at 7am and confirmed that they consumed the provided breakfast between 6:00am and 6:30am. Participants were asked to refrain from exercise for 78 hours prior to this visit, and to not consume caffeine, and dietary supplements for 48 hours prior to this visit. Participants had an ambulatory blood pressure monitor placed on their right arm and this monitor was set to inflate and measure blood pressure every 15 minutes until they start their OGTT

Participants rested for 15 minutes in our vascular assessment room and then had a FMD ultrasound assessment completed. The FMD assessment was repeated 1 hour and 2 hours following the exercise bout. The participants then completed their pre-assigned exercise condition of 45 RPM or 90 RPM exercise session on a cycle ergometer for 45 minutes at 65-70% of their VO_{2max} with a 5-minute warm-up and 5-minute cool-down at 20-50 watts. The participants were fitted with a mask connected to an expiratory hose and a metabolic measurement device (Parvo TrueMax 2400TM) to measure expired air and calculate oxygen consumption during the exercise session and a heart rate monitor. After the subjects completed the exercise session they remained in the lab for 4 hours prior to their OGTT. During the 4-hour break and between the flow-mediated dilation assessments, subjects remained fasted, but could read or work on a computer in our laboratory in a comfortable space.

Body Composition

Participants' heights and weights were measured on a standard scale (Seca274, Medical Measuring Systems, Chino, CA). Body composition was assessed via Dual-energy X-ray Absorptiometry (DEXA) (Lunar iDXA, GE Healthcare, Madison, WI). DEXA is considered a good tool to provide a rapid and accurate estimation of body mass, lean soft tissue, bone mineral and fat (Albanese, Diessel, & Genant, 2003; St-Onge et al., 2004). Participants assumed a supine position in the DEXA bed for 7-12 minutes while the DEXA arm passed over the entire body. A certified radiology technician completed all scans. The scan was completed on only one of the three condition days for body composition characteristic. Once the peripheral venous catheter was placed and secured

with clear tape for the OGTT, the first of two baseline blood draws was taken. The DEXA was then completed in the time between the two baseline collections.

Maximal Exercise Test

The details of the maximal exercise test were described to all participants including the graded exercise test portion and verification phase. The mask size for the Parvo was measured, head gear fitted for the mask to stay in place and Polar heart rate monitor was positioned on the chest. The mask was connected to an expiratory hose attached to the metabolic measurement device (Parvo TrueMax 2400TM) to continuously measure ventilation, respiratory gas exchange data, and heart rate. Participants were seated on a stationary cycle ergometer and resting data were collected for 2 minutes. Afterwards, they were prompted to cycle at their preferred cadence throughout the test. A 5-minute warm-up phase at 50 watts was completed, and then the test began by increasing 30 watts every minute until the participant could not continue or requested to stop. Then a cool down for 5-10 minutes was completed at 50 watts prior to proceeding with the verification phase. The verification phase was set at 100% of their watt max during the graded exercise test and each participant was encouraged to cycle until he could not continue. The verification phase has been described as an essential component for valid VO_{2max} testing, and this cardiorespiratory fitness protocol has been safely used in our lab among sedentary, overweight/obese adults (Poole & Jones, 2017; Sawyer, Tucker, Bhammar, & Gaesser, 2015). The highest oxygen uptake from either the ramp or verification phase was used as their max VO_2.

Ambulatory blood pressure monitor

Blood pressure was monitored by a SunTech Oscar2, Model 250 (Sun Tech Medical, Morrisville, North Carolina). This blood pressure monitor is designed to assess brachial blood pressure and estimate central blood pressure. This monitor was placed on the right arm of the participant upon arrival to the laboratory at ~7:00am. The monitor was removed during the exercise period, but then immediately replaced following the cool-down. The monitor was then only removed just prior to the start of the OGTT. The blood pressure monitor was set to record blood pressure every 15 minutes and participants were instructed to be still and not talk while the device was inflating. During the time period between exercise and OGTT, most of the measurements occurred while the participants were seated quietly. If they were walking or standing when a measurement started (e.g., walking to the restroom) they were instructed to stand quietly until the measurement was completed.

Brachial artery flow-mediated dilation

Brachial artery flow-mediated dilation was measured with a Terason uSmart 3300 dynamic depth resolution ultrasound machine (Terason Ultrasound, Burlington, MA) with a 15-4 MHz multi-frequency linear array probe. Procedures were conducted in accordance with published guidelines (Corretti et al., 2002; Thijssen, Black et al., 2011). Images obtained were saved to each participant's folder, and once all participants were done the videos were re-named in a separate folder with a random ID by the laboratory manager. The key was saved on the lab manager's computer and secured in a locked cabinet prior to analysis. Analysis was completed using a validated brachial artery edge-detection software (Diacom Blood Flow and FMD Encoder Analysis) to detect baseline

and peak diameter, blood flow velocity and the percent difference between baseline and peak diameter.

For this procedure participants laid quietly in a dimly lit room for 15 minutes on a vascular imaging table. The measurement was taken on the left arm (the arm without the ABP cuff). A baseline image of the brachial artery was first taken and recorded for 30-60 seconds. After the baseline image was completed an automatic blood pressure cuff placed on the upper forearm was inflated to 250mmHg for 5 minutes. At the 4-minute mark prior to cuff deflation the brachial artery was imaged again and for 3 minutes post occlusion. To minimize error between measurements the ultrasound settings were saved and the distance of the probe from the medial epicondyle of the humerus were marked and measured (Appendix H).

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) is a relatively inexpensive and practical technique for assessing insulin sensitivity. This method has been validated for indices of muscle insulin sensitivity index and hepatic insulin resistance against the gold standard euglycemic-hyperinsulinemic clamp (M. A. Abdul-Ghani et al., 2007). Participants had their OGTT performed at 12:00pm on visits 3, 5 and 7. This was approximately 4 hours after the exercise bout or at the same time of day on the control visit. All OGTT's were performed by a research nurse with extensive experience with this method. A small indwelling peripheral venous catheter was placed into a vein in the arm and then blood samples were collected at -10, 0, then at 30, 60, 90 and 120 min after the consumption of 75g of anhydrous orange flavored glucose solution (Glucola) for the measurement of plasma glucose and insulin concentrations. Approximately 5ml of blood was drawn at

each of the aforementioned time points during the OGTT. After each blood draw the catheter was flushed with 5mls of 0.9% saline to maintain patency. Participants could recline comfortably in recliner chairs and were allowed to watch videos or use a laptop for the duration of the OGTT. The blood was placed into labeled vacutainer tubes and processed according to type for plasma and serum collection. Aliquots of the plasma and serum were pipetted into labeled Eppendorf tubes and frozen at -80° C until analyzed.

Analytical Measures

Plasma glucose was analyzed using the *in vitro* hexokinase method (COBAS analyzer). Briefly, the enzyme hexokinase catalyzes the reaction between glucose and ATP to form glucose-6-phosphate and ADP. In the presence of nicotinamide adenine dinucleotide (NAD), glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide (NADH). NADH is directly proportional to glucose concentrations and measured spectrophotometrically at 340nm.

Serum insulin was measured by radioimmunoassay using a gamma counter. This process was completed over 2 days using a RIA kit (Millipore, Billerica, MA). On day one, the samples were processed in duplicate with an insulin tracer and insulin antibody followed by an overnight incubation. The following day involved a precipitation reaction and 20-minute incubation followed by the gamma counter. Radioimmunoassay uses fixed concentrations of labeled antigen incubated with a constant dilution of antiserum so that the concentration of antigen binding sites on the antibody is limited. This allows for a competition for unlabeled and labeled antigen with a constant number of binding sites on the antibody. When a mix of known amounts of radioisotope-tagged insulin and

antibodies is combined with a small portion of the samples, the insulin displaces some of the tagged insulin and the free-tagged insulin is then measured with the gamma isotope detector. The counts are fit to yield a normal curve to then result in the insulin values. For both glucose and insulin values, the duplicate coefficient of variance was reviewed and if they were out of range (>10%) samples were analyzed again.

Exercise Conditions

Participants were first fitted with a Parvo heart rate monitor and then set up with a mask and head gear connected to an expiratory hose attached to the Parvo metabolic measurement device. The participants were either pre-assigned to cycling at a cadence of either 45 or 90 RPM. The participants sat on the cycle ergometer for 2 minutes to collect resting data then they were prompted to start cycling for a 5-minute warm-up. The wattage during the warm-up was increased every 30 seconds while monitoring the VO_2, so that at the end of the warm-up period the participant was near or at 65-70% of their $VO_{2max.}$ The participants then sustained either 45 or 90 RPM for 45 minutes of continuous exercise. Cadence at either 45 or 90 RPM were monitored continually on the Parvo screen by the investigator, while the participants could view their cadence on the screen of the bike. If participants went below or above the assigned cadence, they were verbally instructed to increase or decrease their pedaling rate. The wattage was reduced or increased to maintain the intensity throughout the exercise session while monitoring 15-second VO_2 averages. At 5-minute intervals participants were asked their rating of perceived exertion (RPE). At the end of the exercise session a cool down at 25-50 watts for 5-minutes at their preferred cadence was completed.

Statistical Analysis

All statistical procedures were performed using SPSS (SPSS 23, Chicago IL, USA). Values were tested for normality and homogeneity. One-way analysis of variance was used to test for differences in baseline values between the three trials for fasting glucose, insulin, blood pressure and FMD%. Intra- and inter-class correlation coefficient were completed for a subset of FMD variables to determine intra-observer and between observer reliability. Linear mixed models were used to detect differences for areas under the curve (AUC) of insulin and glucose, indices of insulin sensitivity, hourly differences for ABP variables and FMD variables with both fixed and random effects explored. Covariates were included in the mixed models for baseline values, age and BMI. A p-value <0.05 was considered statistically significant.

Chapter 4

RESULTS

Sixteen adult males were enrolled in the study, and eleven completed the study. Two participants were excluded for being too active following the baseline evaluation. One participant was unable to start the study due to vein access and two dropped out following their first condition visit due to the time commitment. Descriptive characteristics of the eleven participants who completed the study are presented in Table 1.

Table 1. Participant characteristics

N	11
Age (yrs)	28 ± 5
Height (cm)	175.1 ± 6.1
Weight (kg)	105.6 ± 13.8
BMI (kg/m^2)	34.5 ± 4.0
Body Fat (%)	37.9 ± 4.4
Visceral Fat (g)	1588 ± 675
VO2max (ml/kg/min)	26.5 ± 5.9

Data presented represent Means ± SD

BMI = body mass index; VO_{2max} = maximal oxygen uptake.

To confirm that the exercise conditions were of similar intensity, measurements during the two exercise conditions from the Parvo Metabolic cart and electronically braked bike were recorded and compared. There were no differences in relative intensity, heart rate and work rate when averaged over the 45 min exercise bouts (Table 2).

Table 2: Exercise averages between conditions

	Average Work Rate (Watts)	Average VO_2 (L/min)	Average Heart Rate (BPM)
45RPM	97.9 ± 25.1	1.80 ± 0.34	147 ± 10
90RPM	82.3 ± 27.3	1.83 ± 0.37	151 ± 14
p-value	0.179	0.845	0.468

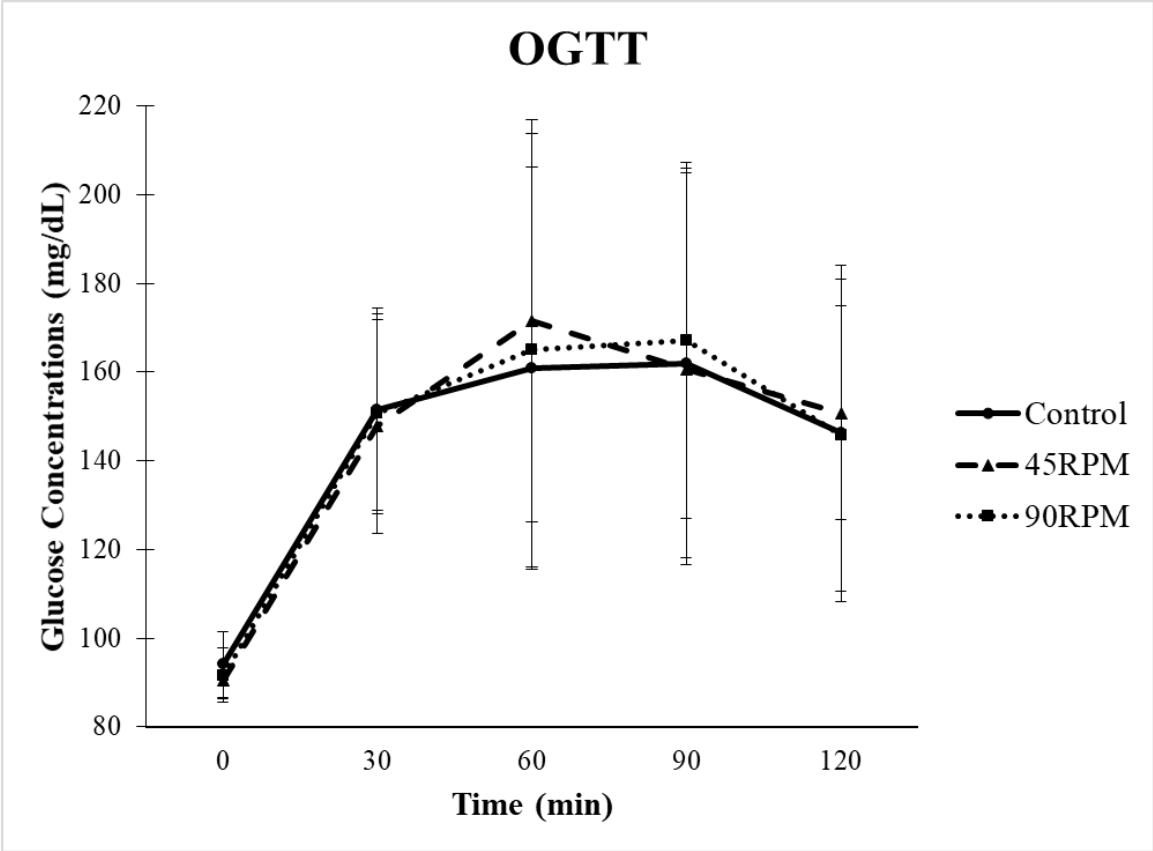
Data presented represent Means ± SD

RPM = revolution per minute; p-value = ANOVA between group

Oral Glucose Tolerance Tests

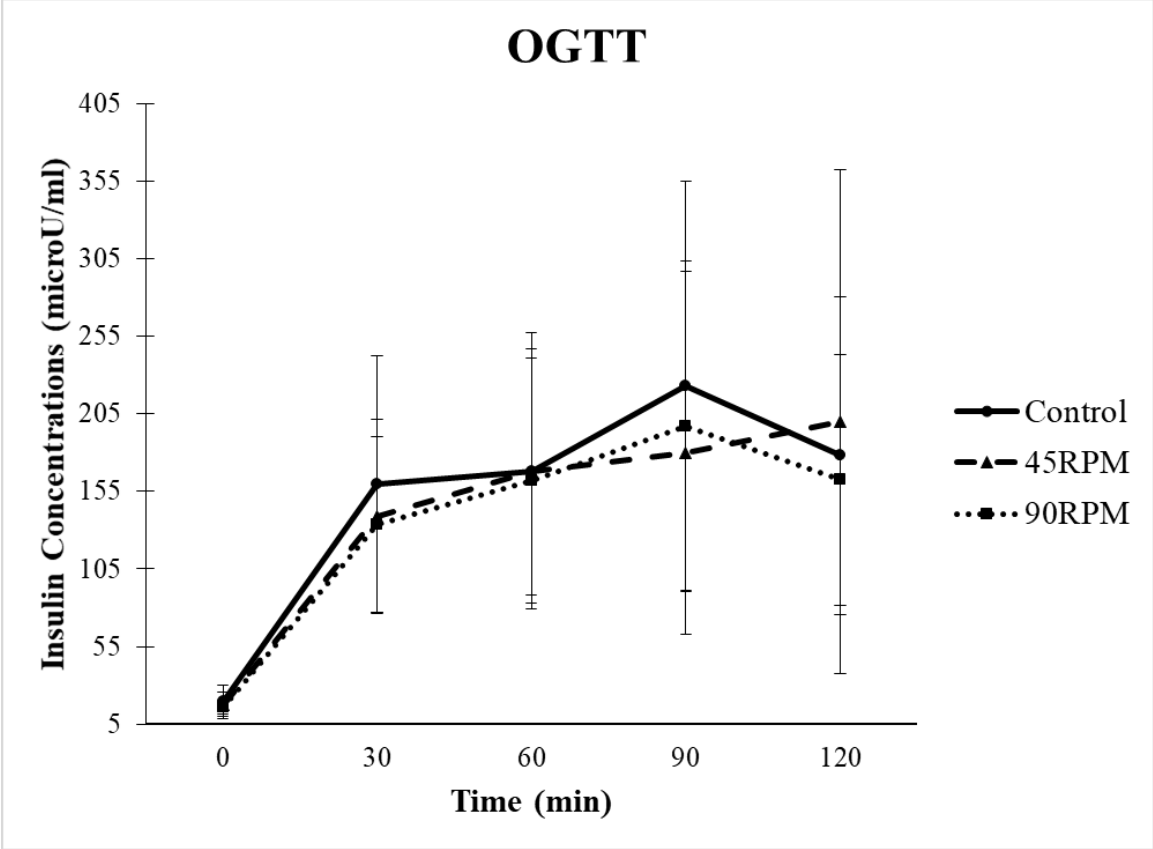
No significant differences for basal glucose and insulin were found between conditions. For each time point during the OGTT there were no significant differences found for the effects of exercise and no differences between exercise conditions (all $p>0.05$) (Figure 2 and 3). Similarly, there were no differences found for total area under the curve for both glucose and insulin ($p=.935$ and $p=0.562$, respectively) (Figure 4). Indices for beta cell function and insulin sensitivity calculated from the OGTT also revealed no significant differences (HOMA-IR, $p=0.570$; Insulinogenic Index, $p=0.445$; Matsuda Index, $p=0.095$) (Figure 4). Contrary to the hypothesis, increased muscle contraction frequency did not improve insulin sensitivity when measured 4 hours post exercise by an OGTT. Moreover, this 45 min moderate-vigorous exercise bout yielded a similar glucose and insulin rise as the no-exercise control day following standard glucose ingestion. Although the group averages displayed similar results across the conditions, there was individual variation among the participants. Specifically, the Matsuda Index which is an index of whole-body insulin sensitivity demonstrated considerable variation in responses to each exercise condition compared to the control day. Some individuals improved, had a worsened response or no change (Figure 5).

Figure 2: Mean glucose values from the oral glucose tolerance tests



Oral glucose tolerance test mean values of glucose concentrations at 0, 30, 60, 90 and 120 min for control condition and two exercise conditions. For both exercise conditions the OGTT was started ~4 hours post exercise and the control condition (no exercise) was time matched. Error bars represent SD. No significant differences between conditions.

Figure 3: Mean insulin values from the oral glucose tolerance tests



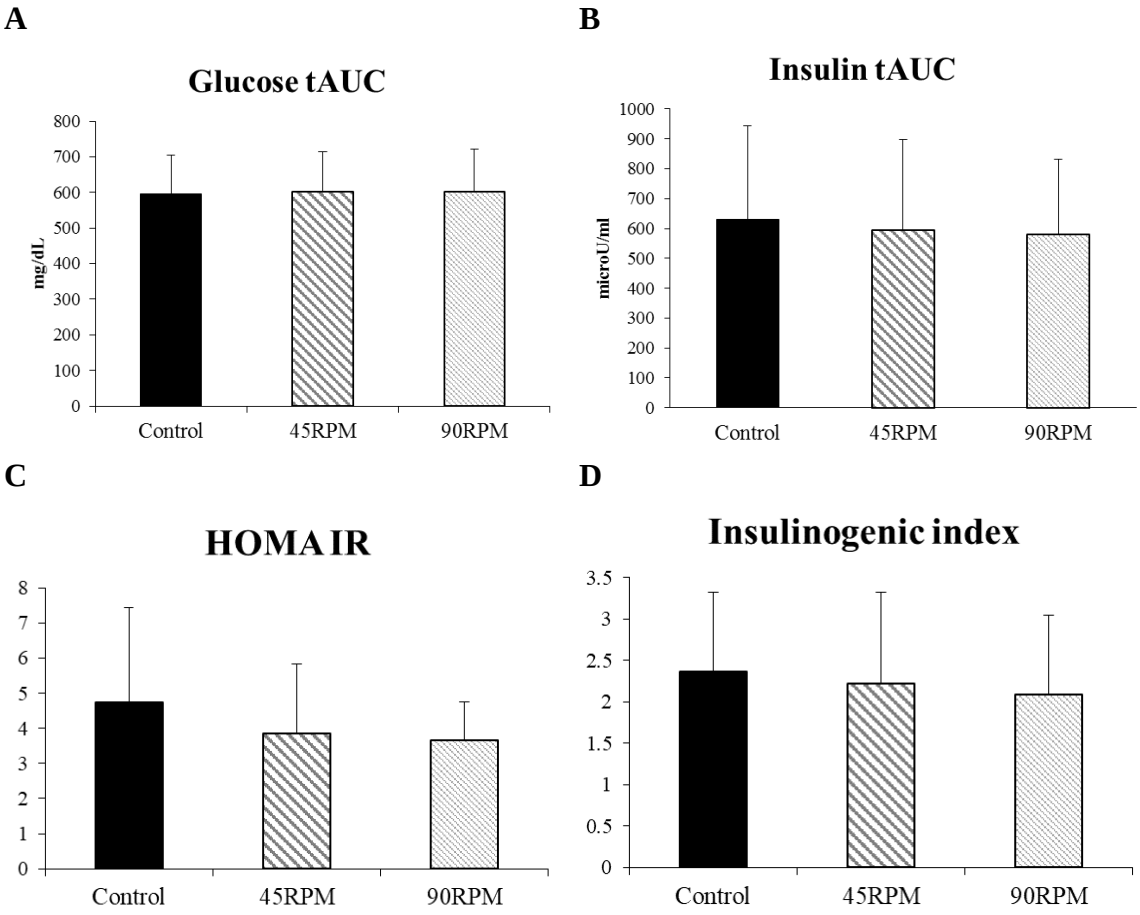
Oral glucose tolerance test mean values of insulin concentrations at 0, 30, 60, 90 and 120 min for control condition and two exercise conditions. For both exercise conditions the OGTT was started ~4 hours post exercise and the control condition (no exercise) was time matched. Error bars represent SD. No significant differences between conditions.

Table 3: Glucose and insulin OGTT averages

	Glucose (mg/dL)	Insulin (microU/ml)
Control		
Basal	94.1 ± 7.5	19.9 ± 9.8
30min	151.6 ± 22.8	159.9 ± 82.7
60min	160.9 ± 45.3	168.2 ± 89.0
90min	162.0 ± 43.9	223.2 ± 131.8
120min	146.2 ± 38.0	178.2 ± 102.6
45RPM		
Basal	90.5 ± 4.0	17.1 ± 8.5
30min	147.8 ± 24.1	138.7 ± 62.7
60min	171.6 ± 45.3	167.7 ± 79.3
90min	160.7 ± 44.1	179.8 ± 116.8
120min	150.9 ± 24.2	200.1 ± 162.2
90RPM		
Basal	91.7 ± 6.1	16.1 ± 4.4
30min	150.6 ± 22.5	133.8 ± 56.6
60min	165.0 ± 48.8	162.1 ± 79.0
90min	167.2 ± 40.1	196.9 ± 106.6
120min	145.8 ± 35.2	162.5 ± 80.8

Data presented represent Means ± SD

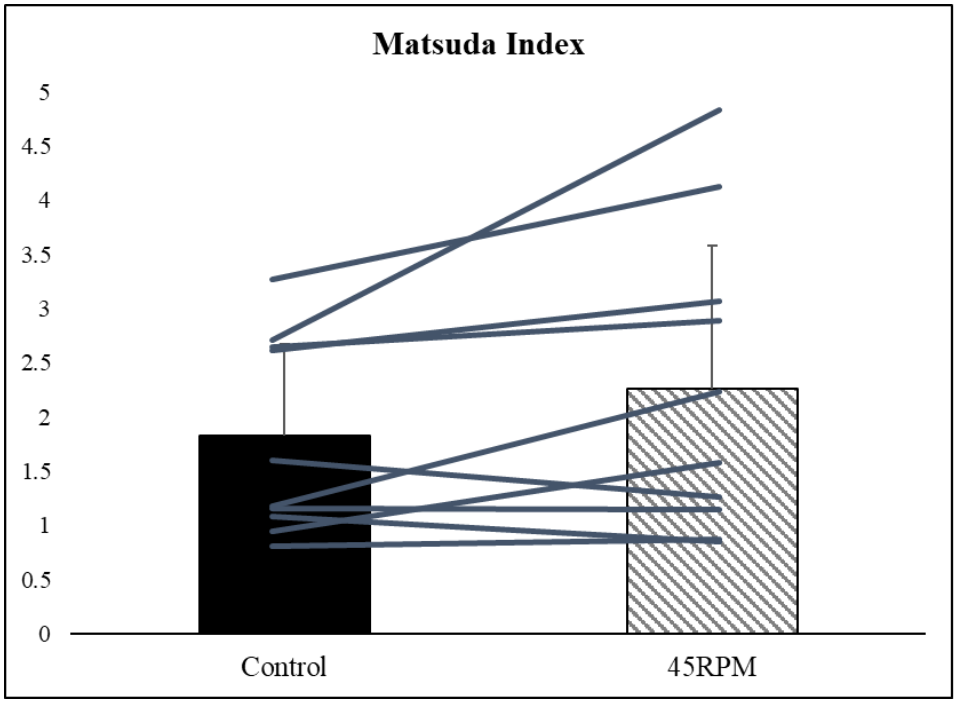
Figure 4: OGTT calculation means



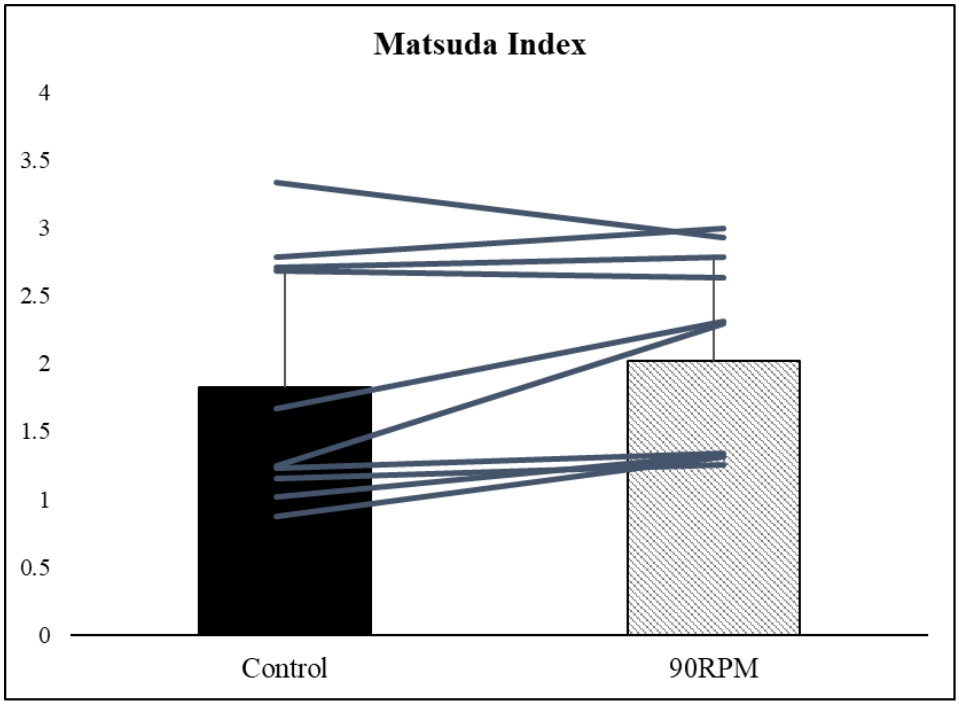
A. Glucose total area under the curve (tAUC) calculated from the oral glucose tolerance test for each condition. B. Insulin total area under the curve calculated from oral glucose tolerance test for each condition. C. Hemeostatic model assessment of insulin resistance (HOMA IR) calculated from the oral glucose tolerance test for each condition. D. Insulinogenic index calculated from the oral glucose tolerance test for each condition. No significant differences between conditions for all graphs. Error bars represent SD.

Figure 5: Mean Matsuda index with individual responses

A



B



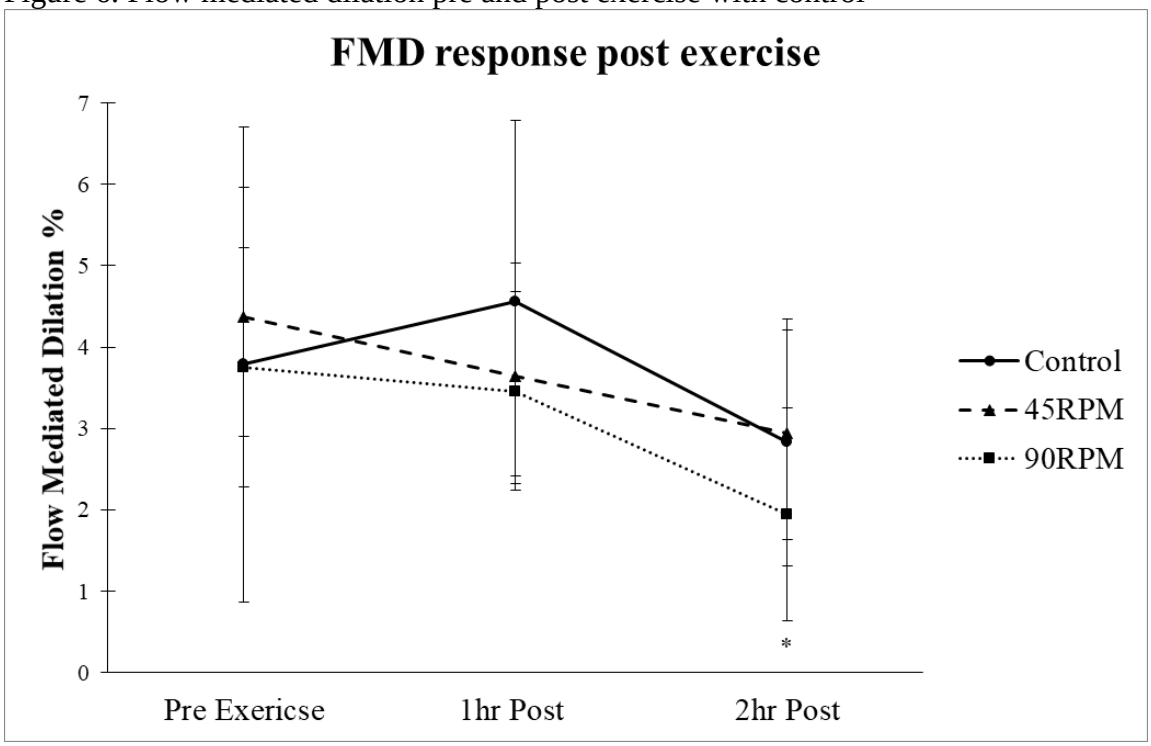
A. Control vs. 45RPM mean and individual Matsuda index values. B. Control vs. 90RPM mean and individual Matsuda index values. Individual variation between conditions with no significant differences found between conditions for the mean values. Error bars represent SD.

Endothelial Function

There were no significant differences across conditions for participants pre-exercise FMD. The FMD response post exercise with a time matched control day revealed no significant differences (Pre exercise, p=0.539; 1hr post, p=0.474; 2hr post, p=0.287) (Figure 6). Among the time points for the 90RPM condition there was a significant difference between the pre-exercise measurement and the 2-hr time point (p=0.021).

Adequate scaling for FMD was present as evidenced by the upper confidence limit of the regression slope of the relationship between the logarithmically transformed preocclusion baseline diameter and peak postocclusion diameter being >1 [$\beta \pm SE = 1.05$ plus minus 0.02; 95% confidence interval (CI) = 1.016-1.082] (Atkinson et al., 2013). Therefore, it was appropriate to use linear mixed models to detect condition by time interaction in FMD. The analysis was conducted using baseline diameter and shear as covariates in the model.

Figure 6. Flow mediated dilation pre and post exercise with control



Brachial artery flow-mediated dilation (FMD%) adjusted for baseline diameter and shear rate before and after 45RPM and 90RPM or time matched no exercise control condition. No significant differences between conditions. *indicates significant time difference between baseline and 2hr post for the 90RPM condition. Error bars represent SD.

Reliability testing for the analysis of FMD images was completed and revealed strong correlations for intra- and inter-user correlation coefficients (Table 4.)

Table 4: Intra- and Inter-class correlations for FMD variables

Inter-user ICC	**Correlation Coefficient**	**Intra-user ICC**	**Correlation Coefficient**
Base Diameter	0.997	Base Diameter	0.948
Peak Diameter	0.998	Peak Diameter	0.945
FMD %	0.877	FMD %	0.967

Table 5: FMD mean variables for each condition

	Control			45 RPM			90 RPM			
	Baseline	1hr Post	2hr Post	Baseline	1hr Post	2hr Post	Baseline	1hr Post	2hr Post	Condition x Time Interaction *P* Value
Preocclusion baseline diameter (mm)	4.72 ± 0.63	4.73 ± 0.59	4.83 ± 0.67	4.70 ± 0.71	4.74 ± 0.61	4.67 ± 0.52	4.79 ± 0.79	4.86 ± 0.68	4.87 ± 0.72	0.604
Postocclusion peak diameter (mm)	4.90 ± 0.63	4.94 ± 0.61	4.96 ± 0.63	4.90 ± 0.67	4.91 ± 0.59	4.81 ± 0.55	4.96 ± 0.77	5.02 ± 0.69	4.96 ± 0.69	0.582
Flow-mediate dilation (%)	3.79 ± 2.92	4.56 ± 2.23	2.83± 1.52	4.37 ± 1.60	3.64 ± 1.39	2.95 ± 1.26	3.75 ± 1.47	3.46 ± 1.22	1.95 ± 1.31	0.698
Average shear rate (1/s)	230.52 ± 82.03	235.94 ± 89.13	203.57 ± 63.18	278.34 ± 111.21	253.60 ± 105.01	231.21 ± 100.53	242.35 ± 93.90	247.02 ± 75.05	175.49 ± 71.80	0.668
Average velocity (cm/s)	27.11 ± 7.65	27.70 ± 8.68	24.06 ± 5.02	32.11 ± 9.05	29.61 ± 9.24	26.29 ± 8.92	28.46 ± 8.49	30.15 ± 8.14	25.93 ± 8.29	0.915

Data presented represents Means ± SD

Blood Pressure

Prior to exercise there were no significant differences in brachial or central blood pressure (BP). For brachial systolic blood pressure, there was a significant effect of exercise at 2- and 4-hours post exercise ($p<0.001$ and $p=0.004$, respectively), and for central systolic blood pressure a significant effect of exercise at 2-, 3- and 4-hours post exercise ($p<0.001$, $p=0.021$ and $p=0.004$, respectively). Both brachial and central diastolic BP increased slightly 1-hour post exercise, but was reduced at the 4-hour time point (Figures 6-9). The reduction in systolic blood pressure 2-hours post exercise was approximately 10mmHg, which remained lowed at the 4-hour time point for both 45 and 90RPM conditions. Similarly, central systolic BP was reduced post exercise and at the 2-hour time point. Central systolic BP was reduced approximately 7mmHg for both 45 and 90RPM conditions. At the 3-hour time point BP was reduced by 8mmHg and 9mmHg in the 45 and 90RPM condition, respectively. This lowering effect persisted to the 4th hour with a 5 and 7mmHg reduction in systolic blood pressure in the 45 and 90RPM conditions.

The other variables from the SunTech Oscar2 blood pressure monitor include mean arterial pressure (MAP), augmentation index normalized to 75 beats per min (AIX@75), central augmentation pressure (cAP) and central pulse pressure (cPP). Augmentation index is normalized to 75 beats per minute due to the variation in resting heart rate between conditions and between individuals. Since this variable is influenced by resting heart rate this is a standard index to use. There were not significant pre exercise differences for these variables ($p>0.05$), besides AIX@75. MAP initially increased significantly following the exercise bouts and subsequently lowered

significantly from 2-4 hours post exercise. The cPP was significantly lower at the 1- and 2-hour time point following both exercise conditions ($p<0.001$). Augmentation pressure was only significantly lowered at the 1-hour time point post exercise for both conditions ($p=0.041$). Whereas, augmentation index was significantly increased at the 1 and 2-hour time points post exercise ($p=0.020$ and $p=0.034$, respectively) (Table 6).

Table 6: Average ABP variables pre and post exercise with control

	SBP	DBP	cSBP	cDBP
Control				
Pre	139.5 ± 10.9	82.2 ± 9.7	124.7 ± 8.3	84.6 ± 10.4
1hr post	138.9 ± 12.8	77.8 ± 11.5	123.3 ± 11.3	78.9 ± 11.5
2hr post	139.0 ± 12.1	77.5 ± 9.4	124.0 ± 8.9	78.8 ± 9.5
3hr post	133.1 ± 12.0	77.6 ± 6.9	120.5 ± 10.2	79.2 ± 7.4
4hr post	138.0 ± 13.8	81.4 ± 10.5	124.5 ± 11.9	83.5 ± 11.0
45RPM				
Pre	141.4 ± 10.8	78.1 ± 9.8	124.5 ± 9.0	80.1 ± 10.5
1hr post	141.1 ± 11.3	82.7 ± 14.1*	125.0 ± 11.1	86.5 ± 13.4*
2hr post	131.1 ± 8.8*	76.4 ± 10.5	117.3 ± 9.0*	78.8 ± 11.3
3hr post	129.9 ± 12.5	75.5 ± 12.4	116.6 ± 11.6*	76.6 ± 12.4
4hr post	132.6 ± 12.5*	78.2 ± 11.0*	119.5 ± 12.1*	79.7 ± 19.6*
90RPM				
Pre	140.9 ± 10.2	78.9 ±9.0	125.5 ± 8.5	81.0 ± 9.8
1hr post	135.6 ± 9.8	83.2 ± 7.9*	120.3 ± 8.3	85.3 ± 9.2*
2hr post	131.2 ± 12.5*	78.0 ± 10.0	118.4 ± 10.5*	79.8 ± 10.0
3hr post	129.5 ± 13.5	75.4 ± 11.3	116.2 ± 12.5*	76.5 ± 11.6
4hr post	131.5 ± 10.1*	79.4 ± 9.7*	118.7 ± 9.9*	81.0 ± 9.5*

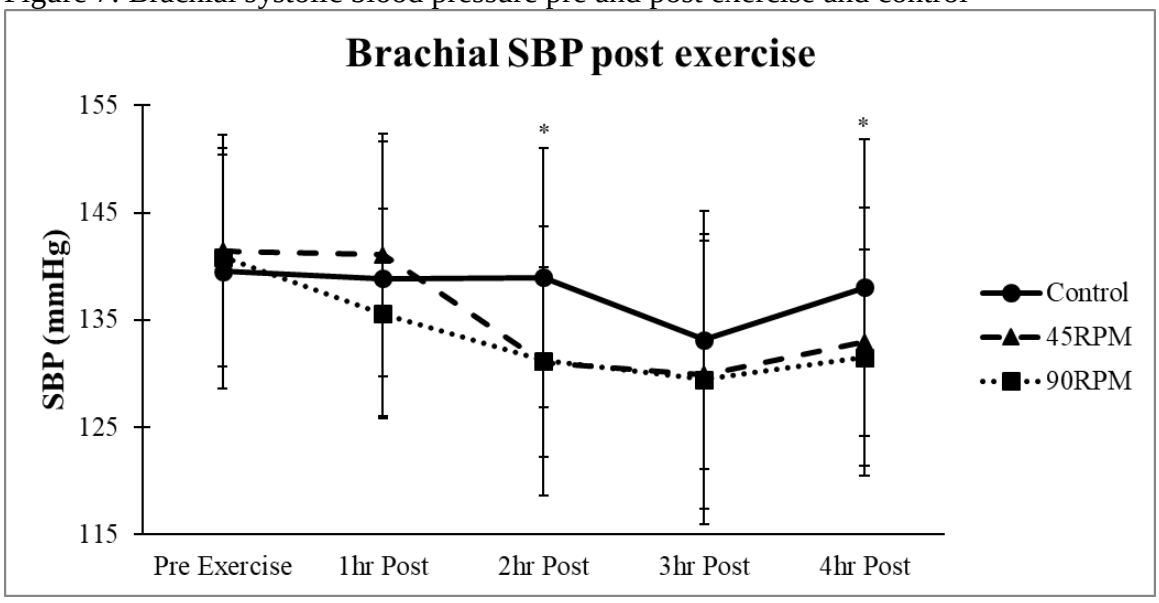
Data presented represent Means ± SD; *significant effect for exercise vs. contol $p<0.05$
SBP = systolic blood pressure; DBP = diastolic blood pressure;
cSPB = central SBP; cDBP = central DBP

Table 7: Average ABP variables pre and post exercise with control

	MAP	AIX@75	cAP	cPP
Control				
Pre	101.3 ± 9.2	19.9 ± 10.7	8.1 ± 5.0	40 ± 6.8
1hr post	98.2 ± 10.8	15.4 ± 11.6	8.8 ± 4.9	44.7 ± 7.5
2hr post	98.0 ± 8.5	11.2 ± 11.2	7.7 ± 4.8	45.0 ± 8.9
3hr post	96.1 ± 7.2	13.8 ± 15.9	8.8 ± 6.9	41.3 ± 8.6
4hr post	100.3 ± 104	11.7 ± 18.9	7.1 ± 6.4	40.6 ± 7.6
45RPM				
Pre	99.1 ± 9.6	12.3 ± 8.6	8.0 ± 5.5	44.4 ± 8.0
1hr post	102.1 ± 12.8*	24.5 ± 11.4*	6.7 ± 5.8*	38.6 ± 6.2*
2hr post	94.6 ± 8.3*	15.7 ± 16.4*	6.9 ± 5.7	38.5 ± 10.1*
3hr post	93.7 ± 11.5*	11.5 ± 12.3	7.4 ± 4.7	40.3 ± 7.9
4hr post	96.7 ± 10.2*	11.4 ± 18.3	7.3 ± 7.0	39.7 ± 8.3
90RPM				
Pre	99.5 ± 9.0	20.1 ± 5.4	10.3 ± 5.2	43.8 ± 5.4
1hr post	101.2 ± 8.8*	23.1 ± 10.2*	6.3 ± 5.3*	36.5 ± 9.1*
2hr post	95.6 ± 10.2*	20.7 ± 12.8*	9.0 ± 6.6	38.6 ± 6.1*
3hr post	93.4 ± 11.4*	16.1 ± 13.8	8.5 ± 6.4	39.6 ± 7.1
4hr post	96.7 ± 9.4*	15.8 ± 17.9	7.8 ± 6.2	37.7 ± 5.1

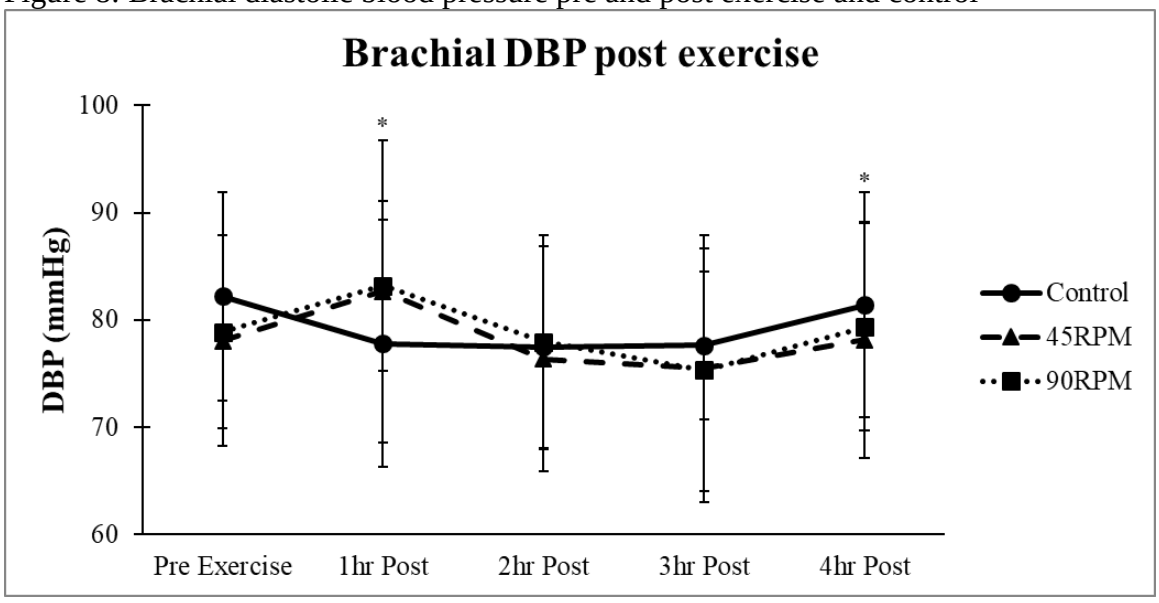
Data presented represent Means ± SD; *significant effect for exercise vs. control $p<0.05$
MAP = mean arterial pressure; AIX@75 = augmentation intex at 75 beats per minute;
cAP = central augmentation pressure; cPP = central pulse pressure

Figure 7: Brachial systolic blood pressure pre and post exercise and control



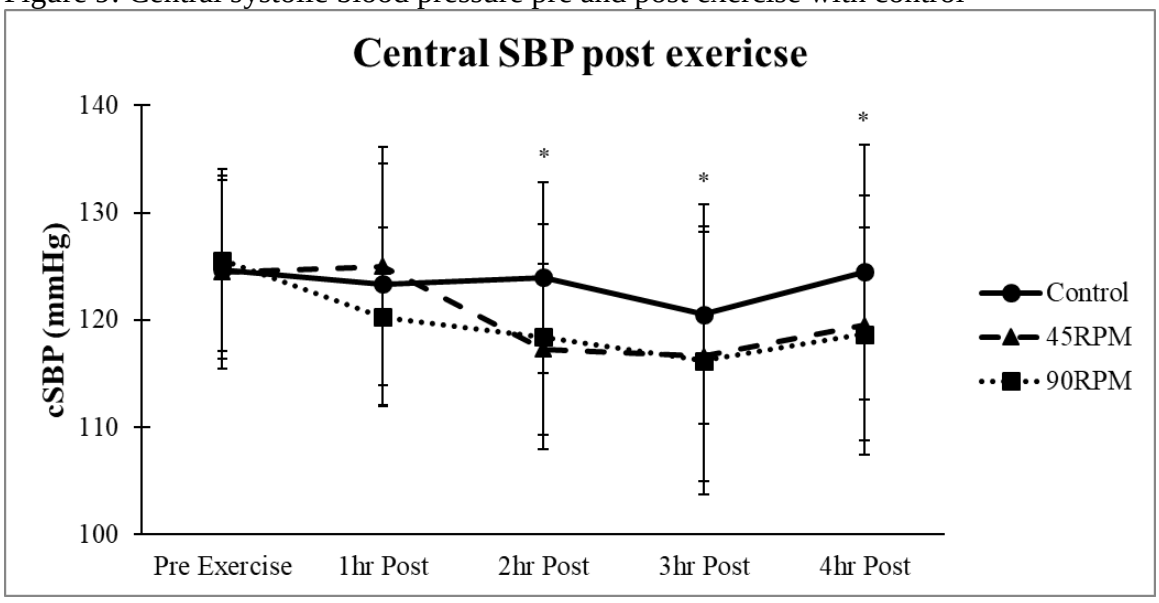
Brachial systolic blood pressure (SBP) post exercise with time matched control. Data presented represent Means ± SD *significant difference between control and exercise conditions $p<0.05$

Figure 8: Brachial diastolic blood pressure pre and post exercise and control



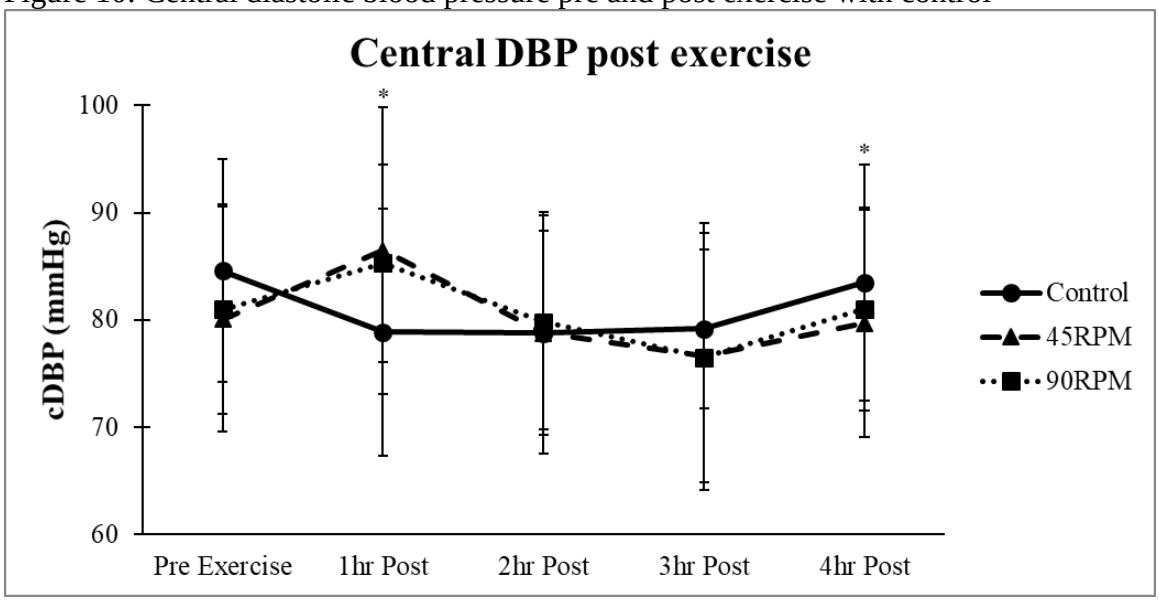
Brachial diastolic blood pressure (DBP) post exercise with time matched control. Data presented represent Means ± SD *significant difference between control and exercise conditions $p<0.05$

Figure 9: Central systolic blood pressure pre and post exercise with control



Central systolic blood pressure (cSBP) post exercise with time matched control. Data presented represent Means ± SD *significant difference between control and exercise conditions $p<0.05$

Figure 10: Central diastolic blood pressure pre and post exercise with control



Central diastolic blood pressure (cDBP) post exercise with time matched control. Data presented represent Means ± SD *significant difference between control and exercise conditions $p<0.05$

Chapter 5

DISCUSSION

I hypothesized that the increased muscle contraction frequency during an acute bout of moderate-vigorous intensity aerobic exercise would result in a greater improvement in insulin sensitivity, and this was not confirmed. The main findings from this study do not confirm any primary hypotheses. Moreover, I hypothesized that both exercise conditions would result in improved insulin sensitivity over a non-exercise control day and the results show no differences among the groups or compared to the control conditions. Exploratory analyses were included into this design to examine the effects of an acute bout of exercise in an obese population on endothelial function and post exercise hypotension. There were no significant effects of exercise on endothelial function as measured by brachial artery at FMD 1- and 2-hours post exercise compared to the non-exercise control condition. In the 90RPM group alone there was a significant attenuation in FMD at the 2-hour post exercise time point. I hypothesized that there would be a post exercise blood pressure lowering effect of exercise and this hypothesis was supported as both 45 and 90RPM conditions demonstrated significant PEH.

Post Exercise Glucose Control and Insulin Sensitivity

Contrary to the findings here, other acute aerobic exercise studies have found significant improvements in insulin sensitivity (Brestoff et al., 2009; Hasson et al., 2006; Howlett, Mathews, Garnham, & Sakamoto, 2008; Mikines, Sonne, Farrell, Tronier, & Galbo, 1988; Newsom et al., 2013; Ortega, Fernandez-Elias, Hamouti, Pallares, & Mora-Rodriguez, 2015; Rose, Howlett, King, & Hargreaves, 2001). These studies demonstrate improvements in insulin sensitivity, and were comprised of various post exercise time

points ranging from immediately post exercise to 48 hours post exercise. They also studied various populations including healthy men and women, exercise trained men, and one investigation among healthy obese males and females. Newsom and colleagues used an obese healthy population, to determine the insulin sensitizing effects of low and moderate acute aerobic exercise compared to a no exercise control. Insulin sensitivity, measured using a hyperinsulinemic euglycemic clamp (HEC) showed a 35% significant increase following the low intensity (50% VO_{2peak}) condition and a 20% nonsignificant increase following the moderate intensity (65%VO_{2peak}) condition compared to control (Newsom et al., 2013). This measurement was done the morning following the exercise bout, which was approximately 13 hours post exercise. To control for activity and diet they provided standardized meals prior to the condition days and they were admitted to the research unit and remained there until the following morning for the HEC. It is important to note that the low intensity condition resulted in 15 minutes of additional exercise to maintain 350kcal of expenditure of each exercise condition. The longer duration of exercise may have played a role in the greater improvements seen in the low intensity condition.

Investigations using the OGTT method to assess insulin sensitivity changes following an acute bout of exercise has revealed improvements compared to control conditions the following day (12-24hr post) (Brestoff et al., 2009; Rose et al., 2001). Brestoff and colleagues found a significant improvement in insulin sensitivity the day following an acute bout of endurance cycling exercise at 75% VO_{2max} compared to control. By contrast, a high-intensity (125%VO_{2max}) sprint interval exercise bout did not result in significant improvements. Their study population included healthy males and

females, which may differ from an obese population when evaluating the effects of acute endurance exercise on insulin sensitivity. They reported dietary control via 3-day food diaries, but with no standardized meals provided prior to the condition days. The improvements in insulin sensitivity may not be due to exercise intensity in this study, but exercise duration may have played a role. The endurance exercise condition consisted of 3 bouts of 15-minute intervals totaling 45 min, while the sprint interval condition consisted of 5 30-second intervals interspersed with 4 5-min rest periods. By contrast, in this current investigation the OGTT was done on the same day and the exercise conditions consisted of 45 minutes of continuous exercise.

Another study that found significant improvements the next day was conducted using exercise trained men and they used the OGTT method with a double tracer of deuterated glucose (Rose et al., 2001). The exercise consisted of a 60-minute cycling bout at 70% $VO_{2peak,}$ and they found a 24% increase in whole body glucose disposal which they attribute to the greater glucose disposal in the previously active muscle. Again, these results are limited to healthy males that are trained athletes. Insulin sensitivity changes following aerobic exercise the same day among a sedentary population from the OGTT method has varied results.

A study among overweight/obese sedentary women used the OGTT method to assess insulin sensitivity changes the same day following an aerobic bout of exercise. They had these women walk at a brisk pace for 75 minutes and had an OGTT completed 90 minutes post exercise. Insulin sensitivity was 18% higher following the exercise compared to the control (Hasson et al., 2006). A later study from this same group found no significant improvements in a group of overweight/obese sedentary adults following

the same brisk walking protocol (Hasson, Granados, Chipkin, Freedson, & Braun, 2010). This investigation also looked at changes in insulin sensitivity the next day, but they used the HEC method. Both investigations controlled their participants' diets by providing standardized meals and they completed the same brisk walking protocol. The difference in findings may be due to the more robust method used in the latter investigation (HEC vs. OGTT).

Other studies have also found no changes in insulin sensitivity following an acute bout of aerobic exercise using this clamp method (Devlin, Barlow, & Horton, 1989; Hasson et al., 2010; Howlett et al., 2008). Similar to this study design, these investigators chose a time point within 2-4 hours post exercise recovery when exercised muscle groups are likely to be maximally glycogen-depleted and have enhanced insulin-stimulated glucose uptake. Among a group of healthy men and women cycling at 70% of VO2max for 15-minute bouts interspersed with 5-minute rest periods until exhaustion, insulin-mediated glucose utilization was not increased during the post exercise recovery period (Devlin et al., 1989). Another cycling study using the HEC found similar results 3-hours post exercise. They examined 7 untrained men who exercised at 60% VO2 peak for 60 minutes and found in the post exercise recovery period no significant changes in insulin sensitivity during post exercise recovery. However, a t-test conducted compared to the control condition revealed a trend for increased insulin sensitivity (Howlett et al., 2008). Although these null findings were among healthy normal weight participants the results are similar to the findings in the current study, demonstrating no significant improvements in insulin sensitivity following an acute aerobic exercise intervention.

It is important to note that interpretation of results from the OGTT method may be subject to individual day-to-day variability. Possible mechanisms include changes in gastrointestinal absorption and motility, variations in response to incretin hormones that stimulate insulin secretion in response to a glucose ingestion, differences in recent glucose exposure and changes in physical activity. A study conducted to investigate day-to-day variability in beta cell function indices derived from an OGTT reported some measures with high within-subject variability (Utzschneider et al., 2007). They recruited 13 adults with normal glucose tolerance, 10 with impaired glucose tolerance and 14 with type to diabetes to all undergo 2 OGTTs separated by ~7 days. The beta cell function measures included insulinogenic index, incremental AUC, integrated insulin secretion response from 0-120 min and a mathematical model. The insulinogenic index demonstrated the highest within-subject variability (CV 57.1%), with the CV's of the other measures ranging from ~17-30%. They also included measures of insulin sensitivity with their CV's ranging from ~7-24% and these CV's did not differ by glucose tolerance category. Although the insulin sensitivity indices were not the primary outcome of this paper, they did report that formulas utilized from the OGTTs for insulin sensitivity all showed reasonable variability. In the current investigation, all participants were provided with pre-paid meal gift cards to consume the same lunch and dinner food/beverage prior to each condition visit and they consumed the same light breakfast meal on the morning prior to arriving at the lab.

Variability of insulin sensitivity indices from the OGTT following an acute exercise bout has also been recognized. In an acute exercise study comparing moderate- and high-intensity aerobic exercise on acute changes in insulin sensitivity markers

Rynders et al., compared OGTT derived insulin sensitivity measures to more robust values derived from the oral minimal model method (Rynders et al., 2016). They found that OGTT-derived values for insulin sensitivity underpredicted the change following the moderate- and high-intensity exercise bouts by 35% and 75% respectively. Among my participants, there was considerable individual variation, where some individuals had improvements in insulin sensitivity while others showed no change or a reduction resulting in mean changes that were no different than the control condition.

Post Exercise Endothelial Function

It has been reported extensively that exercise training contributes to a significant increase in brachial artery flow-mediated dilation (Early et al., 2017). The acute post exercise effects have been shown to enhance (Currie et al., 2012; Hallmark et al., 2014; Harris et al., 2008b; Harvey et al., 2005; Padilla, Harris, Fly, Rink, & Wallace, 2006; Zhu et al., 2010), impair (Jones, Green, George, & Atkinson, 2010; McGowan et al., 2006; Rognmo et al., 2008; Silvestro et al., 2002), or result in no change (Hallmark et al., 2014; Harris et al., 2008a; Jones et al., 2010; Rognmo et al., 2008) in endothelial function. Data on the acute effects of aerobic exercise on endothelial function among obese populations are lacking. This current investigation indicated that among this cohort of inactive obese males that a 45-minute bout of moderate-vigorous aerobic exercise does not acutely improve brachial artery flow-mediated dilation. There is evidence among young healthy adults to suggest that FMD in the brachial artery does not represent a systemic index of endothelial function. Investigations by Thijssen and colleagues found no correlations between brachial artery FMD and in both superficial femoral artery and popliteal artery FMD (r=0.09 and r=0.05, respectively) (Thijssen, Rowley, Padilla, Simmons, Laughlin,

Whyte, Cable, & Green, 2011b). Given that our exercise mode involved cycling exercise, it could be a possibility that measuring the arm as a surrogate measure of systemic endothelial function was not the most appropriate measure for acute changes. We cannot speculate what the FMD in the femoral or popliteal artery would have revealed, but this should be investigated further in this population.

An investigation by Hallmark et al. compared the effect of high- and moderate-intensity exercise on FMD in both healthy obese and lean adults (Hallmark et al., 2014). They found that lean individuals have a significant improvement in FMD following the high-intensity exercise bout at 1-, 2-, and 4-hours post exercise and for the obese participants there was a trend from increased FMD at the 2-, and 4- hour time point following the moderate-intensity exercise. In contrast to our investigation, not only did we not see any trend for improvement in FMD following exercise but there was a significant decrease in FMD in the 90RPM condition at the 2-hr time point when directly compared to the pre-exercise value. This may be reflective of heterogeneity in the response of FMD following exercise in the obese population. Although improvements in brachial artery FMD were not observed, improvements in endothelial function systemically cannot be determined. Future studies should investigate vascular beds in the legs and arm to see if there are improvements in FMD post exercise.

Post Exercise Blood Pressure

Post exercise hypotension has been well established in the literature (Carpio-Rivera et al., 2016; Cornelissen & Fagard, 2005). Post exercise hypotension following aerobic exercise has been reported to persist for 2-4 hours under laboratory conditions (Kenney & Seals, 1993), with significant reductions among both normotensive and

hypertensive individuals (Brito et al., 2014). The PEH response reported among obese populations is lacking. In a study of overweight healthy men, it was reported that PEH was not significantly correlated with body mass index (BMI) (Hamer & Boutcher, 2006). A recent meta-analysis reported a significant correlation between BMI and PEH, where a lower BMI was associated with a greater reduction in SBP (Carpio-Rivera et al., 2016); however, the correlation coefficient was weak (r=0.26). Their regression line for reduction in SBP and BMI indicates that there would be no PEH response among individuals with a BMI above 30. In a different meta-analysis that depicts the physiological characteristics influencing PEH such as changes in peripheral vascular resistance and cardiac output, the articles included were primarily among normal weight or overweight participants (Brito et al., 2014). Only one study had obese participants, and this study was among middle-aged women with and without type 2 diabetes and normal resting blood pressure (Figueroa, Baynard, Fernhall, Carhart, & Kanaley, 2007). They found a significant reduction in SBP after a 20-minute walk at 65% of VO_{2peak}. This reduction persisted for only 10-20 minutes following the walk and returned to baseline 30 minutes post. This demonstrates a lack of published data on the effects of exercise on BP in the obese population.

The primary findings from our blood pressure data demonstrated that both central and brachial blood pressures were lowered following exercise. For brachial SBP the reduction was significantly lower at the 2- and 4-hour time point post exercise. The central SBP was lower at 2-, 3-, and 4-hour time point post exercise. This reveals that there is a pronounced PEH effect of a moderate-vigorous aerobic exercise bout. The males from our study did have a range of normal to prehypertensive blood pressure at

rest. This effect may not have been found if these males all had normal blood pressure values at baseline.

A recent investigation that included obese males and females with metabolic syndrome compared blood pressure responses following high-intensity interval exercise (HIIE) or moderate continuous exercise (CE) (Morales-Palomo, Ramirez-Jimenez, Ortega, Pallares, & Mora-Rodriguez, 2017). The interval exercise consisted of 5 x 4-min intervals at 90% HR_{peak} and the continuous exercise was an isocaloric bout at 90% HR_{peak} for around 70 minutes. They found that HIIE produced a larger and significant reduction in SBP in both the hypertensive (-20 vs. -5 mmHg) and normotensive group (-8 vs -3mmHg) compared to the CE. PEH was tested 45 minutes after exercise, so persistent effects of PEH beyond this time point are unknown among this group. In this current study blood pressure was measured for a much longer time period, which demonstrated that PEH in obese males is reduced 2-4 hours post exercise.

Investigators have identified that as many as 25% of individuals with elevated blood pressure have minimal antihypertensive benefits from endurance training (Hagberg, Park, & Brown, 2000). However, identifying individuals who demonstrate PEH following an acute bout of aerobic exercise may predict their response to chronic training. Liu and colleagues demonstrated in a group of hypertensive men and women that their magnitude of change in systolic blood pressure after acute exercise was significantly correlated with the magnitude of change after chronic training ($r=0.89$, $p<0.01$) (Liu, Goodman, Nolan, Lacombe, & Thomas, 2012). Individuals who do not see blood pressure reductions following an acute bout of exercise can be identified and alternative forms of exercise prescribed to explore their effects of PEH prior to chronic

exercise prescription. The participants in this study all had reduction in central and brachial systolic blood pressure in the post exercise period. This would provide rationale for exercise training in the form moderate-vigorous intensity aerobic cycling in this population for blood pressure reductions. Future studies among obese participants should also explore PEH following acute aerobic exercise for a 24-hour period using an ambulatory blood pressure monitor outside of the laboratory setting.

Strengths and Limitations

With all investigations, there were many strengths of the research design, but also some limitations. The randomized cross-over design was a strength, because we could compare each condition to a non-exercise control day to control for possible diurnal variation in outcome measures. Participants in this study were provided with meal gift cards and asked to consume the same lunch and dinner prior to the testing days and were provided the exact same breakfast meal the morning of testing. During the exercise conditions, intensity and power output were constantly monitored and adjusted to ensure that the exercise intensity was the same during both conditions and the only variation was the pedal rate.

One major limitation of this investigation was that I used the OGTT as the primary outcome to assess insulin sensitivity. Although OGTT derived measures of insulin sensitivity have been validated against the gold standard HEC, there is less evidence to show its reproducibility following an acute bout of exercise. Other methods to assess insulin sensitivity are costlier, but may have revealed differences among the exercise condition or exercise compared to control. The OGTT does provide a more natural physiological stimulus of glucose intake that must be digested compared to

methods that bypass the gastrointestinal system. However, we were not interested in this question, but rather the changes in glucose uptake following an acute bout of exercise that differed in muscle contraction frequency. An alternative may have been to use the oral minimal model method that utilizes the oral glucose ingestion method, but has more frequent sampling time points. The measurement of endothelial function at the brachial artery was also a limitation in this study where our mode of exercise was stationary cycling, and we may have missed changes to endothelial function in the lower extremities post exercise. The interpretation of the finding from this study is limited to relatively healthy, inactive, obese male adults.

Conclusions

In conclusion, the results show that contraction frequency during endurance exercise does not alter insulin sensitivity post exercise among obese males. An acute aerobic exercise bout does not improve glucose control or insulin sensitivity when compared to the absence of exercise. Previous studies have reported that duration of exercise plays an important role in glucose control and insulin sensitivity, which may be attributable to the number of muscle contractions performed during the exercise bouts. However, when controlling for muscle contraction frequency by altering the cycling cadence no effect was found. Similarly, this aerobic exercise stimulus does not enhance endothelial function, and there were no differences when muscle contraction frequency was altered during cycling. The non-effect on FMD following an acute bout of exercise in an obese population adds to the limited data known about the effects on vascular function following acute exercise. An important finding of this investigation revealed a significant and persistent blood pressure lowering effect of this exercise intervention.

There is limited data with regards to PEH among obese individuals and we demonstrate that an acute bout of aerobic exercise can elicit a significant reduction in central and brachial systolic blood pressure. The magnitude of systolic blood pressure lowering is clinically relevant and provides insight that obesity per se does not prevent PEH. The determination of muscle contraction frequency during aerobic exercise and its physiological effects have yet to be determined among the obese population, which permit further investigation.

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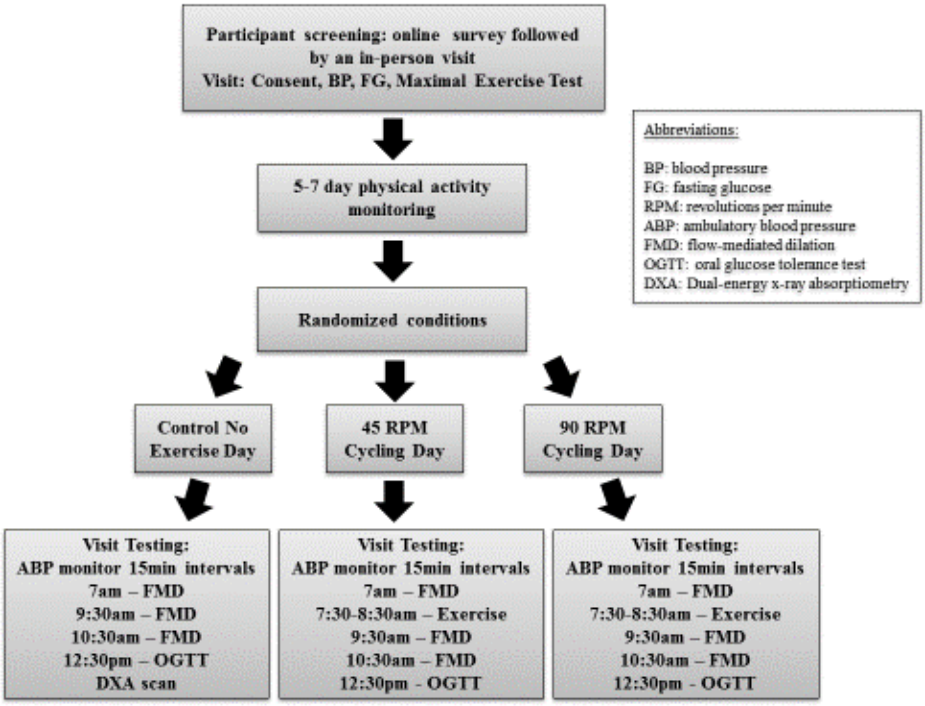
APPENDIX A

STUDY FLOW DIAGRAM


Participant screening: online survey followed by an in-person visit
Visit: Consent, BP, FG, Maximal Exercise Test
5-7 day physical activity monitoring
Randomized conditions
Control No Exercise Day
45 RPM Cycling Day
90 RPM Cycling Day
Visit Testing:
ABP monitor 15min intervals
7am – FMD
9:30am – FMD
10:30am – FMD
12:30pm – OGTT
DXA scan
Visit Testing:
ABP monitor 15min intervals
7am – FMD
7:30-8:30am – Exercise
9:30am – FMD
10:30am – FMD
12:30pm - OGTT
Visit Testing:
ABP monitor 15min intervals
7am – FMD
7:30-8:30am – Exercise
9:30am – FMD
10:30am – FMD
12:30pm - OGTT
Abbreviations:
BP: blood pressure
FG: fasting glucose
RPM: revolutions per minute
ABP: ambulatory blood pressure
FMD: flow-mediated dilation
OGTT: oral glucose tolerance test
DXA: Dual-energy x-ray absorptiometry

APPENDIX B

INFORMED CONSENT

CONSENT FORM

Effects of acute exercise bouts on blood glucose control and insulin sensitivity in obese sedentary males

INTRODUCTION

The purpose of this form is 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, Siddhartha Angadi, PhD, (faculty), and Catherine Jarrett and Wesley Tucker, doctoral students, in the Physical Activity, Nutrition and Wellness Program in the School of Nutrition and Health Promotion, have requested your participation in a research study.

STUDY PURPOSE

Our primary objective is to determine whether increased muscle contractions by cycling at a faster cadence on stationary bike at a moderate to vigorous intensity can improve blood sugar control as measured by an Oral Glucose Tolerance Test (OGTT).

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 two moderate to vigorous exercise conditions on blood sugar control.

You are being asked to participate in this study because you are considered obese by current public health guidelines, (BMI>30 kg/m^2), male, 18 - 45 years of age, in good health, and capable of performing vigorous physical activity. You must also be classified as sedentary which means you cannot currently be engaged in any regular structured exercise program.

As a study participant you will have 7 visits to the Healthy Lifestyles Laboratory on the Downtown Phoenix campus of ASU. You will be asked to visit for consent and screening, gift card pick-up and meal instruction days, aerobic fitness testing, 2 supervised exercise sessions, 3 Oral Glucose Tolerance Test days.

Testing:

There will be 3 days of OGTT testing in the laboratory with our research nurse (Ginger Hook RN, CDE) and this will take place over a 3 week period. These testing days will be completed in a random order and 2 of the days will include an acute bout of aerobic exercise on a stationary bike. At the end of the consenting visit you will be asked to perform an aerobic fitness test on a stationary bike to appropriately prescribe the intensity of exercise.

All tests will be listed in chronological order with detailed descriptions following the list of visits.

The descriptions for visits 3, 5 and 7 include either exercise or no exercise, a 4 hour wait in the lab and OGTT. These three visits will be given to you in a random order.

Visit 1 (Consent & Screening; 1.5 Hour):

Your first visit will involve coming to the test site where the study will be explained to you, we will answer any questions you may have and you will be asked to sign this consent. When the consent is signed you will go through the following screening tests to confirm that you are eligible for the study:

1. Fill out and sign a questionnaire called the PAR-Q to acknowledge that you are suitable to perform exercise. Based on the results of this form, you may need to obtain permission from a physician to participate in this study.
2. Measurement of you blood sugar, blood pressure, height and weight to ensure that you meet the criteria of participation.
3. You will be asked self-reported health questions and smoking status for eligibility.
4. You will be asked to undergo a maximal exercise test on a stationary bike.

5. You will be asked to wear a small movement sensor for one week to measure your physical activity.
6. You will be asked to fill out a Three-day Food Record.

Visit 2 (Diet Control; 10 Minutes):

You will be provided with gift cards to purchase meals and beverages (e.g. Starbucks, Subway) at no cost to you to consume the day prior to visit 3. The items you choose will be decided based on your preferences, calorie needs and appropriate distribution of protein, carbohydrate and fat. The diet you consume on this day will be replicated on the two other days prior to your following OGTT test days. You will also be provided with a light breakfast consisting of a bagel, cream cheese and a low-fat plain or flavored milk to consume the morning prior to visit 3. If you have any allergies or dietary restrictions regarding the light breakfast we will modify to your liking. The breakfast will be the exact same the morning of visits 3, 5 and 7.

Visit 3 (Testing and Control OGTT; 8 Hours):

You will consume your provided breakfast at home between 6:00am and 6:30am. You will arrive in the lab before 8am and comfortably wait 4 hours prior to testing. You will have body composition measurements taken (DEXA). Then you will undergo an OGTT.

Visit 4 (Diet Control; 10 min):

You will be instructed to eat the same meals/beverages you ate prior to visit 3. Pre-paid gift cards and breakfast will be provided. You will be provided with an accelerometer.

Visit 5 (Exercise session and OGTT; 8 Hours):

You will consume your provided breakfast at home between 6:00am and 6:30am. You will be assigned to one of two exercise conditions and 4 hours following the exercise have an OGTT done.

Visit 6 (Diet Control; 10 min):

You will be instructed to eat the same meals/beverages you ate prior to visit 3. Pre-paid gift cards and breakfast will be provided. You will be provided with an accelerometer.

Visit 7 (Exercise session and OGTT; 8 Hours):

You will consume your provided breakfast at home between 6:00am and 6:30am. You will be assigned to one of two exercise conditions and 4 hours following the exercise have an OGTT done.

Details of all measurements and tests:

Measurement of Physical Activity and Dietary Intake (visit 1):
We will be measuring your movement and dietary intake for the purposes of this study by using a small movement sensor called an accelerometer and having you fill out a three-day food record. We will have you wear the accelerometer for a week before you start the study and prior to any testing day. This device is worn on your non-dominant arm during the day. The device is very light, easy to wear and easy to conceal. Also, to assess your dietary intake we will have you fill out a three-day food record. You will record everything you eat and drink for 2 weekdays and 1 weekend day during the week that you are required to keep a three-day food record and during the first visit we will conduct a usual dietary intake interview.

Maximal Exercise Test (visit 1):
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. You will sit quietly on the stationary bicycle for 2 minutes then you will be asked to pedal at light resistance for 5 minutes for the warm-up phase. After the warm-up phase the resistance will increase continuously every minute until you cannot continue. We will encourage you to push yourself as hard as you feel comfortable. 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. The whole test will take about 30 minutes.

Oral Glucose Tolerance Test (OGTT) (visit 3, 5, 7):

- You will need to arrive to the laboratory approximately 2 hours after consuming the light breakfast provided.
- You will be asked to not drink any **caffeine, alcohol or dietary supplements** for 48 hours prior to arrival at the laboratory for this visit.
- You will be asked not to perform any exercise 72 hours prior.

The OGTT will take start between 11:00am and noon depending on the timing of arrival to the lab. An OGTT allows us to assess blood sugar control and insulin sensitivity following the exercise or no exercise conditions. A small plastic catheter will be placed into a vein in your forearm. You will drink a sugary orange drink containing 75 grams of carbohydrate. Blood samples will be collected from the catheter before the drink and every 30 minutes after the drink for 2 hours. There will be a total of 6 blood draws during this test. We will draw approximately 40ml (8tsp) of blood to measure blood markers before and after the intervention. All OGTTs and blood draws will be performed by a highly trained nurse and certified phlebotomist. This test takes about 3 hours. All OGTT's will be performed 4 hours following either arrival to the laboratory on the no exercise visit or 4 hour following the exercise bout.

Dual-Energy X-ray Absorptiometry (DEXA) (visit 3):
Your body composition (amounts of fat and lean tissue) will be determined by using a bone density measurement machine, called a Dual-energy X-ray Absorptiometry (DEXA). You will be asked to lie face up, on a padded table for about 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 regular clothing (no metal allowed). A certified X-ray technician will complete all DEXA scans.
This test takes about 15 minutes.

Exercise visits (visit 5 and 7):
You will be asked to complete 2 different exercise sessions on separate weeks. These training sessions will both be continuous moderate-vigorous intensity exercise sessions completed on a stationary bike. The difference between the two sessions will be how fast you are cycling on the bike, 45 or 90 revolutions per minute. Each exercise session will last a total of 45 minutes including a 5 minute warm-up and cool-down. The intensity will be based on 65-70% of your VO_{2max}. During the exercise session you will be wearing a mask attached to a long tube to collect the air you breathe out and a heart rate monitor.

Meals provided (visit 2, 4, 6):
After reviewing your three day food recalls we will be providing you will gift cards to restaurants to eat the days prior to OGTT testing days. You will eat the same diet for each of those three days. You will also be provided with a light breakfast to consume at home in the morning prior to the testing visits. These visits will take approximately 10 minutes.

Total time commitment for completion of this study is approximately 26 hours.

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 (0.001% event rate). 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.

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

The OGTT involves a needle puncture in your forearm and hence may lead to some discomfort and bruising as well as a slight risk of infection with catheter use and multiple draws over time. These procedures will be carried out by experienced medical staff who will properly clean the insertion site and use standard sterile technique during the procedures. 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.

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

BENEFITS

Although there may be no direct benefits to you, you will be provided information on your test results and the study results if you would like us to contact you when the study is complete.

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. You will be paid $150 (cash or gift card) for the completion of the study. Partial payment will be made if unable to complete all visits for the study **following visit 1**.

Prorated payment will be divided by either hours or complete visits days:

Visit days: For each day (150$/7visits) 21$

Hours: In the case of multiple visits with an uncompleted visit at withdraw we will prorate compensation based on hours. For each hour (150$/26 hours) 5.75$

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; glenn.gaesser@asu.edu.

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_____

APPENDIX C

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

2015 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it *does not limit your current ability* to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

01-01-2015

APPENDIX D

RECRUITMENT FLYER

ARIZONA STATE UNIVERSITY

Healthy Sedentary Men 18 – 45 years old and over a certain body weight are needed for a study investigating the effects of different exercise protocols glucose control

Compensation: $150

This study is designed to determine the effects of different aerobic exercise protocols on glucose control and insulin sensitivity. This study includes 7 visits to the Healthy Lifestyles Research Center on the Arizona State University Downtown Campus in Phoenix. Time commitment: 26 hours over the course of 3-4 weeks. **Your participation throughout the study is completely voluntary.**

Eligible men must be nonsmokers, in good health, have no restrictions for participating in vigorous intensity physical activity, and must not be taking any medications for blood pressure, cholesterol, diabetes or a heart condition. **If your weight is greater than the number indicated for your height, you may qualify for this study:**

Height	5'3"	5'4"	5'5"	5'6"	5'7"	5'8"	5'9"	5'10"	5'11"	6'0"	6'1"	6'2"	6'3"	6'4"	6'5"
Weight (pounds)	170	175	180	190	195	200	205	210	215	225	230	235	245	250	255

Please contact:
Catherine Jarrett (602 827-2492; Catherine.Jarrett@asu.edu)

Catherine Jarrett 602-827-2492 catherine.jarrett@asu.edu

ASU IRB IRB # STUDY00003717 | Approval Period 1/20/2016 – 1/19/2018

APPENDIX E

SCREENING QUESTIONS

RPM

Screening Questions

1. Age: Males must be between 18 and 45. Age: _____
2. BMI: >30. Height: _____ Weight: _____ BMI: _____
3. Blood Pressure: <160/100 mmHg Blood Pressure:_____
4. Fasting Blood Glucose: <126 mg/dl Blood Glucose: _____
5. Medications: Heart, blood pressure, diabetes, thyroid.
6. History: personal history of heart attack, angina, stroke, fainting during exercise, hypercholesterolemia, hypertriglyceridemia.
7. Current exercise routine: must be relatively sedentary; Willing to keep same routine outside of study.
8. Diet: Must be willing to keep current diet and make no changes throughout the course of the study
9. Schedule: must be able to do 3 fasting visits early in morning during the week.
10. Timeline: Approximately 3-4 weeks depending on lab and participant schedule.

APPENDIX F

QUALTRICS SCREENING QUESTIONS

RPM Pre-Screening Questions Sheet

Short consent for participation in the pre-screening process

1.) The following pre-screening questions will allow us to establish your eligibility for participation in our study (a brief description of the study is displayed in the flyer). Participation in the pre-screening process is completely voluntary and you may end the process at any time. This survey should only take a few minutes to complete.

Do you consent to participate in the pre-screening survey?

Screening Questions

1. How old are you?
2. How tall are you in feet and inches? (Example: 6 foot 2 inches).
3. What is your body weight in pounds? (Example: 200 pounds).
4. Do you smoke?
5. Have you ever been told by your doctor that you have high triglycerides or cholesterol?
6. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
7. Do you feel pain in your chest when you do physical activity?
8. In the past month, have you had chest pain when you were not doing physical activity?
9. Do you lose your balance because of dizziness or do you ever lose consciousness?
10. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
11. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
12. Do you know of any other reason why you should not do physical activity?
13. Are you currently training for a specific athletic event, such as an endurance race or marathon?
14. Do you currently perform regular vigorous exercise, including resistance exercise or weight training?
15. Do you currently perform moderate-intensity physical activity (such as brisk walking for health) more than 1-2 hours per week?
16. Are you currently dieting or trying to lose weight?
17. Have you lost more than 15 pounds in the last 60 days?
18. Do nurses or phlebotomists ever have major difficulty finding a vein and drawing blood from your arm?
19. Have you previously fainted or had any adverse reactions to blood collection?
20. What is the preferred method for us to contact you?
21. Please include your name, email address and phone number below so we can contact you about participation in this study if you are eligible:

APPENDIX G

SUBJECT RECORDING SHEET

Date:

Participant ID:	Age:	Height(cm):	Weight(kg):	BMI:
Date of Birth:				
Resting Blood pressure:				
Finger Stick:				
Handedness:				

Consent form	☐	Arm band	☐	Food Record	☐	PAR-Q	☐	Food Gift Cards	☐

Max Test Results:

Estimated prescription
65-70%VO2max

Control OGTT Date:

RPM Date:

Warm up: Wattage adjustment notes:

RPM Date:

Warm up: Wattage adjustment notes:

Notes:

APPENDIX H

BRACHIAL ARTERY FLOW-MEDIATED DILATION NOTES

Brachial Artery Ultrasound Subject Settings

Study _____ Subject ID _____ Date _____ Time _____

Arm Used	
Cuff Size	
Distance from medial epicondyle of humerus (measure from distal side of probe)	
Frequency	
Depth (cm)	
Focal Zone setting (how many and location i.e: @)	
Compression	
Noise Rejection	
Gain (# of ticks right or left of midline)	
Zoomed? how much of the visible artery pre-zoom)	
SV Size	
Steering Angle	-15
Correction Angle	60
Baseline	
Angle of Probe (acute/obtuse with table closest to sonographer)	

Time Gain Compensation (sliders)
Insert a line where each is moved
If not moved no line is needed

_____
_____
_____
_____
_____
_____
_____
_____

Additional notes on subject and settings:

APPENDIX I

BREAKFAST NUTRITION INFORMATION

Item	kcal	Fat(g)	Sat Fat (g)	Carb (g)	Sugar (g)	Fiber (g)	Pro (g)
Chocolate milk (335ml)	300	4	2.5	49	44	2	20
Bagel (95g)	260	1	0	52	4	2	8
Cream cheese (30g)	70	5	3.5	3	2	0	2
Total	630	10	6	104	50	4	30