THE EFFECTS OF COMBINED EXERCISE TRAINING ON NITRIC OXIDE AVAILABILITY AND ENDOTHELIAL HEALTH IN OVERWEIGHT TO OBESE, POSTMENOPAUSAL WOMEN

by

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by

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Dedication

Even though she is no longer with us, this thesis is dedicated to my mother, Debbie Wiechman (Cook). I believe that everything in life happens for a reason and were it not for her passing in 2015, I would never have had the courage to move 900 miles away from home to pursue a graduate degree. And without pursuing a degree at TCU, I would not have had the inspiration and encouragement to go into public health, let alone get accepted into Emory University's Master of Public Health program. Her academic background and our competitiveness continues to keep me motivated to further expand my education and enjoy every minute along the way.

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Chapter I: Introduction

Background

Obesity is a complex inflammatory disease that results from multiple environmental, biological, and behavioral factors. Characteristics of the disease often parallel with the development of metabolic syndrome, a condition that predicts cardiovascular disease risk (22). An accumulation of visceral abdominal fat causes diagnostic criteria of metabolic syndrome including insulin resistance, dyslipidemia, and hypertension (22). These factors cause injury to the inner walls of blood vessels that lead to the onset of cardiovascular disease through atherosclerotic plaque accumulation and insufficient vasodilation in response to sheer stress.

The endothelium is a thin layer of cells within the lumen of blood vessels that responds to chemical and physical stimuli to maintain vascular homeostasis (1,18). When exposed to sheer stress, such as elevated systolic blood pressure, a healthy endothelium dilates adequately to prevent further damage to the vessel. Nitric oxide is an important vasodilatory molecule released by the endothelial cells to diffuse into smooth muscle cells in arterial walls to relax myosin and dilate the blood vessel in response to sheer stress during exercise (20). Anti-inflammatory properties of nitric oxide inhibit the synthesis of adhesion molecules and cytokines that have chemotactic properties at the endothelium (7). Endothelial dysfunction occurs when the production and release of nitric oxide is impaired resulting in the endothelium being unable to adequately dilate, creating further inflammation and increasing cardiovascular disease risk (18). Exercise-induced nitric oxide release decreases consequent to aging, lack of consistent physical activity, and obesity (42,50).

Aging results in endothelial dysfunction, particularly within the heart and skeletal muscle arteries (42,50). Impairment in nitric oxide signaling is primarily caused by reduced endothelial nitric oxide synthase (eNOS) and increased superoxide production that causes eNOS uncoupling

(50). Markers of inflammation such as C-reactive protein (CRP) are positively correlated with body mass index (BMI) and are associated with decreased nitric oxide bioavailability and eNOS uncoupling (14,17). In addition, the significant decline in estrogen levels after menopause further increases the risk of cardiovascular disease in postmenopausal women (50). The increased risk of cardiovascular disease in postmenopausal women could also be a result of hypertension, dyslipidemia, weight gain, diabetes, and abdominal fat accumulation (50). When coupled with physical inactivity, these factors inhibit the bioavailability of nitric oxide which restrains vasodilatory responses (50). Chronic exercise training, however, improves nitric oxide production regardless of aging (42,50).

Acute exercise temporarily increases nitric oxide bioavailability in response to sheer stress to prevent further vascular damage during exercise (16,51). Increased eNOS phosphorylation throughout an exercise bout elevates nitric oxide bioavailability to enhance vasodilation (45). Vasodilation in response to increased sheer stress during exercise allows the endothelium to protect itself from potential damage. Studies show that exercise intensity influences the concentration of nitric oxide released (10,27,46). A positive correlation has been demonstrated between work output and nitric oxide levels when comparing moderate and vigorous intensities with aerobic and resistance exercise in untrained individuals (10,27). Chronic exposure to elevated nitric oxide levels from exercise training creates adaptations in higher resting and post-exercise nitric oxide concentration (10,20,46,50).

Resistance and aerobic exercise training have similar effects on improving endothelial health (10,20,46,48,50). Intense muscle contractions during resistance exercise elevates systolic blood pressure and induces sheer stress which improves nitric oxide synthesis by elevating eNOS levels (10). Similar increases in eNOS also occur after aerobic training (10,20,46,50). In response to exercise training, resting nitric oxide levels in postmenopausal women significantly

improve with reductions in resting blood pressure and heart rate (50). Changes in body composition and aerobic fitness in response to exercise also decrease CRP levels and hinders the production of other proinflammatory markers (6,24). Improvements in endothelial function due to chronic exercise training demonstrates the importance of consistent exercise in the postmenopausal population to improve and maintain cardiovascular function (50). Greater nitric oxide release as a result of chronic exercise training promotes vasodilation, decreases sheer stress, and improves endothelial health and function.

In addition to measuring nitric oxide concentration, arterial health can also be determined through flow mediated dilation (FMD). FMD is a noninvasive technique of measuring brachial artery dilation in response to blood flow occlusion that can be performed repeatedly without harm to the subject. When analyzing FMD, percent change is calculated from the average baseline diameter and maximum diameter after occlusion of blood flow is released. A greater positive percent change indicates a healthier blood vessel due to its ability to dilate efficiently in response to sheer stress. FMD has a biphasic response to acute exercise (11). Around 30 to 60 minutes after exercise, there is a decline in percent change from baseline artery diameter due to the vasodilatory responses from exercise still being present (11). After the initial 30 to 60 minutes pass, FMD may remain elevated up to 24 hours after exercise (11). As nitric oxide concentration increases in response to exercise training, FMD also improves as more nitric oxide is available to respond to induced sheer stress promoting greater vasodilation (19).

Exercise improves endothelial function through increases in nitric oxide bioavailability, suppression of CRP production, and resultant improvement in vasodilation measured via FMD. Aerobic and resistance training individually have similar effects on endothelial function measured through nitric oxide and flow-mediated dilation, regardless of aging and initial body composition. Obese, postmenopausal women, specifically, are at greater risk of developing

cardiovascular disease. Both excess fat accumulation and a decline in estrogen impede proper vasodilation in this population. With chronic exercise improving multiple mechanisms of endothelial function, exercise training decreases the risk of developing cardiovascular disease in obese, postmenopausal women. The extent to which combined aerobic and resistance exercise training improves endothelial function through nitric oxide concentration, CRP concentration, and flow-mediated dilation in obese, postmenopausal women, however, is an area of research that has not been well studied.

Purpose

The purpose of this study will be to examine the acute and chronic effects of combined resistance and aerobic exercise training on endothelial function in overweight to obese (BMI 26-47 $kg \bullet m^2$), postmenopausal (55-75 years) women. Endothelial function will be measured chemically as well as functionally by measuring nitrate/nitrite and CRP, and flow-mediated dilation analysis, respectively.

Hypotheses

Acute Response

1) Nitrate/nitrite concentration will increase after an acute exercise bout and will peak at two hours post exercise.
2) FMD will increase from before to two hours post exercise.
3) Acute exercise will not change CRP concentration.
4) Nitrate/nitrite, FMD, and CRP will not change in the resting control group.

Training Response

1) Pre exercise nitrate/nitrite concentration will increase in response to training.

2) Pre exercise FMD will significantly increase in response to training.
3) Pre exercise CRP concentration will significantly decrease in response to exercise training.
4) Nitrate/nitrite, FMD, and CRP will not change in the resting control group.

Project Significance

Cardiovascular disease is the primary cause of death in the United States. Development of cardiovascular disease begins with plaque formation on microscopic tears on the endothelium. Those microscopic tears can arise from inadequate vasodilation causing excess sheer stress. Overall endothelial function is a major determinant of cardiovascular health and is partially responsible for vascular homeostasis through appropriate responses to physical and chemical stimuli. Physical inactivity, increased visceral adiposity, and a decline in estrogen limiting nitric oxide bioavailability are among the major contributors of cardiovascular disease in postmenopausal women. Aerobic and resistance exercise training enhances nitric oxide bioavailability and lowers CRP levels regardless of age, initial body composition, and prior history of physical activity. Improvements in endothelial health as a result of chronic exercise may lead to a decrease in resting blood pressure and heart rate, and therefore sheer stress placed on the vascular system, all which directly improve vasodilation and prevent further progression of cardiovascular disease. Employing chronic exercise as a non-pharmacological approach to improve endothelial function can greatly benefit one's quality of life and overall cardiovascular health.

Chapter II: Literature Review

Introduction

Endothelial cells lining the lumen of blood vessels are responsive to chemical and physical stimuli in order to optimize blood flow to surrounding tissues. Nitric oxide, released in response to sheer stress from elevated systolic blood pressure, promotes vasodilation to protect the endothelium from further damage. Along with nitric oxide, there are other molecules and substances that can directly impact the bioavailability of nitric oxide, such as reactive oxygen species and proinflammatory cytokines including C-reactive protein (CRP). Reactive oxygen species and CRP increase in response to excess visceral fat, which is a defining characteristic of obesity. Without lifestyle interventions, cardiovascular disease progression in response to inadequate vasodilation can become fatal. Exercise helps hinder the production of proinflammatory cytokines and improves bioavailability of nitric oxide in order for the endothelium to adequately dilate in response to sheer stress.

Sympathetic Stimulation and Endothelial Function

Neural control of the cardiovascular system arises from central command in the cerebral cortex, the cardiovascular control center, and peripheral afferents including baroreceptors, chemoreceptors, and the hypothalamus (3). These mechanisms adapt vascular conductance to meet cardiac output demands. Central command uses the sympathetic and parasympathetic nervous systems to control heart rate, arterial blood pressure, and heart contractility (3). The sympathetic nervous system (SNS) is also responsible for regulating homeostatic mechanisms through stimulating sympathetic nerve fibers that innervate almost every organ system. Major sympathetic factors that temporarily regulate blood flow through vasodilation or vasoconstriction include nitric oxide, reactive oxygen species, and the renin-angiotensin system (4). Reactive

oxygen species and angiotensin II are vasoconstrictors that increase sympathetic activity which leads to elevated blood pressure. Oxidative stress from elevated concentrations of reactive oxygen species stimulates sympathetic outflow that enhances vasomotor tone, leading to elevated blood pressure. Angiotensin II increases arterial pressure by increasing total peripheral resistance in the arterioles and decreasing excretion of water and sodium by the kidneys. Obesity is associated with an affinity for sodium which increases SNS activity and impedes suppression of the renin-angiotensin system (38). In addition, higher levels of angiotensin II are associated with increases in oxidative stress and proinflammatory cytokines through activation of NADPH oxidase which also increases reactive oxygen species, giving the hormone a powerful vasoconstrictor property (12,41). Both reactive oxygen species and angiotensin II involved in the onset of cardiovascular disease through inhibition of nitric oxide production during periods of elevated sheer stress.

Cardiovascular disease, such as atherosclerosis, begins with injury on the endothelium. Injury allows plaque and other substances to accumulate, eventually leading to the hardening of arteries and restriction of blood flow. This progression of cardiovascular disease inhibits appropriate vasodilatory response in the endothelium. Endothelial dysfunction occurs when the cells are unable to successfully repair the endothelium to return to normal function. A main factor of endothelial dysfunction is decreased nitric oxide activity, preventing adequate vasodilation in response to sheer stress (30). Nitric oxide is a gas released from endothelial cells in response to sheer stress that diffuses into the smooth muscle cells (8). Once nitric oxide is inside the smooth muscle cells, it binds to the enzyme guanine cyclase to activate that enzyme. Activated guanine cyclase then cleaves two phosphate groups from guanine triphosphate, leading to the formation of cyclic guanine monophosphate which phosphorylates proteins such as myosin. The phosphorylation of myosin relaxes the muscle cell resulting in vasodilation. When

nitric oxide is unable to diffuse into the smooth muscle cells due to the buildup of plaque, fat, and cholesterol, the blood vessel cannot adequately dilate which may lead to further damage and the loosening of the plaque. Onset of cardiovascular disease can be prevented or delayed by maintaining adequate endothelial dysfunction to prevent further injury to the endothelium. Maintenance of endothelial function can occur through regular physical activity, regardless of age, by preventing the formation of reactive oxygen species that are responsible for cell damage (35,45).

Nitric Oxide and Reactive Oxygen Species

Reactive oxygen species (ROS) are formed as a byproduct of metabolism of oxygen and are involved in cell signaling. Concentration of ROS can increase dramatically during times of stress and cause damage to cell structures, also called oxidative stress. ROS are related to endothelial dysfunction due to its reactive nature to chemical species that comprise oxygen. There are two main mechanisms through which ROS decrease nitric oxide bioavailability. First, superoxide interacts with nitric oxide to form peroxynitrite, a type of ROS that causes cell damage. Second, peroxynitrite and ROS reduce tetrahydrobiopterin (BH4), which is necessary for the formation of nitric oxide from endothelial nitric oxide synthase. Too much ROS production is maladaptive and causes cell proliferation, leading to cardiovascular disease and cancer (35). Free radicals oxidize low-density lipoprotein cholesterol (LDLC), allowing LDLC to become embedded in arterial walls leading to plaque formation and the onset of cardiovascular disease. Several causes of ROS, including diet-induced obesity, instigate increased oxidative stress which could lead to endothelial dysfunction. Hormone responses to exercise have the potential to facilitate ROS generation and create chronic benefits to training by decreasing the level of oxidative stress. Exercise training helps lower ROS production through hormone

responses and decreasing oxidative stress (45). Exercise training increases nitric oxide levels and ROS signaling in skeletal muscle arterioles to aid in maintaining the optimal level of ROS in order to promote vasodilation and prevent perfusion injury (42).

eNOS and iNOS

Endothelial nitric oxide synthase (eNOS) is the predominant isoform responsible for producing a majority of the nitric oxide in vascular tissue. When this isoform has reduced availability, nitric oxide signaling and release is impaired. Stimuli that inhibit eNOS expression include tumor necrosis factor-α, hypoxia, and inflammatory cytokines such as C-reactive protein. This dysfunction can occur from increased reactive oxygen species (ROS) which results from inactivity with aging and hypertension (31,42). Aerobic exercise restores reductions in nitric oxide that occur as a result of aging by enhancing the regulation of ROS and increasing the expression of eNOS (42). Having a negative impact on cardiovascular disease risk, eNOS uncoupling may be due to a high prevalence of low density lipoprotein which leads to endothelial dysfunction as well as hypercholesterolemia, diabetes, and hypertension (15). BH4 corrects eNOS dysfunction through multiple physiological pathways (15). Since BH4 is a powerful natural reducing agent, oxidation of BH4 could be a cause of eNOS dysfunction. Aging may also cause reductions in vascular BH4 availability associated with eNOS uncoupling (42). Through improvements in BH4 concentration, eNOS coupling increases which improves nitric oxide concentration and vasodilation. Chronic exercise may cause adaptations to increase resting eNOS expression.

Sindler et al. (42) determined a relationship between aging and exercise training on endothelial nitric oxide synthase was determined in mice through isolating soleus muscle. This approach used the muscle arterioles to measure bioavailability of nitric oxide. A fluorescent dye

was used to take pictures over a two-minute period of forced flow rate through the soleus muscle. Diameter of the vessel was recorded and analyzed to determine nitric oxide concentration and amount of vasodilation at the time the rats were euthanized. Older exercise trained mice had a significant increase ($p < 0.05$) in percent relaxation of flow-induced vasodilation of the arterioles when compared to sedentary older mice (45% and 20%, respectively). Neither age group, however, had an increase in soleus muscle mass in response to exercise training. This study demonstrates that eNOS availability increases in response to exercise training independent of age and amount of muscle mass.

Inducible nitric oxide synthase (iNOS) is expressed in response to increased inflammatory cytokines during exercise and produces large amounts of nitric oxide (40). Obesity is considered an inflammatory disease due to its proinflammatory effects at the cellular level, beginning with the accumulation of adipose tissue. iNOS expression is significantly elevated in obese persons compared to lean persons with a lower body mass index and less body fat ($p < 0.02$) (49). Chronic exercise decreases the size of adipose cells, so the volume of adipose tissue declines as amount of body fat lowers in response to training. Reducing the amount of adipose tissue results in less iNOS expression and eNOS uncoupling so that nitric oxide bioavailability can increase and improve vasodilation and endothelial health.

Silva et al. (40) demonstrated that inducing obesity in mice through a high-sugar diet caused excessive iNOS production, but exercise training decreased iNOS expression in comparison to their sedentary counterparts. The onset of obesity was associated with an inflammatory response and resultant activated signaling pathway that caused excessive iNOS expression. Since iNOS expression responds to inflammatory activation, nitric oxide has the potential to be overproduced which could reduce glucose uptake and increase cellular stress ($p < .001$). In addition, iNOS expression was also related to obesity-induced vascular dysfunction

after observing unusual aortic contractile responses in the obese mice. Exercise training facilitated the abnormal contractile responses evident by a 50% decrease in iNOS expression when comparing sedentary mice with a high sugar diet to trained mice with a high sugar diet ($p < .05$). Chronic exercise decreases adiposity which reduces iNOS expression and eNOS uncoupling, resulting in increased nitric oxide bioavailability.

C-Reactive Protein

C-reactive protein (CRP) is produced in response to inflammation. High levels of CRP in plasma can indicate inflammation from a variety conditions, including obesity. Because obesity is an inflammatory disease, CRP is positively correlated with body mass index (BMI) (17). As a result of increased oxidative stress from chronic inflammation, a decrease in nitric oxide bioavailability has been associated with higher CRP levels (14). The increased oxidative stress blunts vasodilatory responses that should be induced by nitric oxide which can further lead to atherosclerotic disease progression. A decline in inflammatory processes can occur as a result of chronic exercise (6,17,24).

Campbell et al. (6) conducted a 12-month aerobic exercise study comparing CRP and body composition in response to training in postmenopausal women (50-75 years). The exercise bouts consisted of walking at 60-75% of max heart rate for 45 minutes a day, five days a week. After 12 months of exercise training, the participants had a significant decrease in CRP (2.39 (1.85, 3.10) to 2.15 (1.66, 2.78); $p = 0.01$). Obese participants at baseline (BMI > 30 $kg \bullet m^2$ and waist circumference > 88 cm) had a greater reduction in CRP compared to those with a lower BMI and waist circumference ($p = 0.002$ and $p < 0.0001$, respectively). Body fat percentage also significantly decreased in response to training ($47.2 \pm 5.0\%$ at baseline, -1.5% change; $p < 0.0001$). Researchers suggested that as little as a 2% decrease in body fat could result in a

significant decrease in CRP, making the training effect partially dependent on fat loss due to proinflammatory markers that are released by adipose tissue (Campbell 2009).

Stewart et al. (44) examined the influence of 12 weeks of combined resistance and aerobic exercise training on CRP in young and old physically inactive adults. Training occurred three days a week, with resistance training consisting of two sets of eight exercises (leg extension, leg flexion, chest press, seated row, leg press, hip abduction, hip adduction, and lat pull down) and aerobic training including a 20-minute treadmill walk at 70-85% of heart rate reserve. BMI and body mass significantly decreased in response to training, as well as CRP concentrations ($p < 0.001$, $p < 0.01$, and $p < 0.01$, respectively). Phillips et al. (36) modeled their resistance training study after Stewart et al.'s resistance training protocol, also measuring CRP in response to training. In the 12-week study, obese (BMI 30-40 $kg \bullet m^2$), postmenopausal women (64.8 ± 2.4 years) exercised three days a week, completing three sets of 10 resistance exercises: chess press, latissimus dorsi pull down, chest fly, leg press, leg extension, and leg flexion. CRP significantly decreased by 33% in response to 12 weeks of resistance training ($p < 0.05$). Fat mass, however, did not significantly change in response to training (29.4 ± 4.3 to 29.0 ± 4.3 kg). Although previous studies report that is correlated with body fat, this study was the first to show that resistance training decreases CRP independent of changes in body fat. While weight loss and body fat loss may not be necessary to experience alterations in levels of CRP and other proinflammatory markers, aerobic and resistant exercise training has the ability to hinder the production of proinflammatory markers.

Flow-Mediated Dilation

Flow-mediated dilation (FMD) is a diagnostic tool used to determine responsiveness of the endothelium in the brachial artery to sheer stress from blood flow occlusion. Data are

recorded as a percent change from baseline diameter to peak diameter. Sheer stress caused from blood flow occlusion stimulates nitric oxide formation and release from the endothelium which leads to vasodilation to prevent further damage being placed on the blood vessel. At rest, a healthy FMD ranges from 4-18% (1,21,28,29,34,37,47). Risk for cardiovascular-related events increases when FMD is less than 2% due to the extensive variables that can negatively impact the ability of blood vessels to dilate in response to sheer stress, including age, gender, blood pressure, cholesterol, type 1 and type 2 diabetes, obesity, hormone levels, smoking, and prevalence of coronary artery disease (34). Those variables cause elevated levels of reactive oxygen species and superoxides that directly inhibit the production of nitric oxide, making vasodilation more arduous (32).

Endothelial function improves in response to exercise training as a result of structural remodeling after consistent periods of sheer stress and decreased adiposity (21,47). There is a biphasic response to FMD following an acute exercise bout (Dawson 2013). Within the first 30 minutes post exercise, there is a blunted response to FMD because immediately after exercise the endothelium is still dilated in response to exercise, so percent change from the already dilated baseline diameter to peak diameter is decreased (11). After the initial 30 to 60 minutes after exercise, there is a steady improvement in FMD over the next 24 hours before returning to baseline (11). Hallmark et al. (21) compared FMD responses to a moderate- and high-intensity bout between lean (22.6 ± 2.1 kg•m^2) and obese (36.4 ± 7.0 kg•m^2) individuals in a randomized-crossover study. The exercise bout consisted of a thirty-minute cycle ergometer ride at a moderate or high intensity. FMD was measured at baseline and hours one, two, and four after exercise completion. After the moderate-intensity exercise, FMD in the lean group was significantly increased above baseline at hours two and four ($p < 0.02$) while the obese group had no significant changes. The lean group had significant increases in FMD at all time points

compared to baseline in response to high-intensity exercise ($p < 0.001$) while FMD did not significantly change in the obese group. Lean subjects had significantly greater exercise responses at all time points compared to the obese subjects in both exercise conditions ($p < 0.05$). No differences were observed at baseline between the lean and obese subjects. This study supports that FMD may remain elevated at least two hours after completion of a moderate to high intensity exercise bout. Repeated exposure to sheer stress through exercise training may induce structural remodeling of the endothelium, further improving FMD in response to exercise training.

In a resistance and aerobic exercise training study, adult subjects completed a circuit training protocol of resistance exercises, cycle ergometry, and walking on a treadmill at 55-85% of max heart rate three days a week for eight weeks (19). FMD was measured at rest before and after training. The eight-week moderate- to high-intensity training protocol significantly increased FMD ($p < 0.0001$). This study further supports that a consistent exercise and combined resistance and aerobic training further improves FMD, inducing adaptations to the vasculature and overall endothelial function.

Exercise Protocol

Aerobic and resistance exercise induces similar exposure of sheer stress to the endothelium that elevates nitric oxide concentration in response to exercise. Exercise intensity may determine the extent to which nitric oxide elevates after exercise. Repeated exposure to sheer stress through consistent training creates adaptations to increase resting levels of nitric oxide as well as elevating their responses to exercise.

Acute Aerobic Exercise

Acute exercise protocols can vary from a single bout to a week-long intervention. Physical fitness has been correlated with resting nitric oxide bioavailability, suggesting that trained persons have a greater vasodilatory response during periods of sheer stress compared to untrained individuals. Jungersten et al. (27) related nitric oxide bioavailability at rest and with physical fitness level in an acute aerobic exercise study with healthy adults (20-39 years). Three different groups of either athletic or non-athletic participants performed varying exercise protocols. Group 1 was long-distance runner athletes that had blood collected at rest and immediately after a 135-minute (about 25 km) jog. Group 2 was a non-athletic control group and the participants completed a cycle ergometer max test, but blood was collected only at rest. Group 3 was also non-athletic that had blood collected at rest and immediately after and two-hours post a 35 km ride on a cycle ergometer. Nitrate in Group 1 athletes was significantly higher at rest compared to Group 2 (45 ± 2 and 34 ± 2 μM, respectively; $p < 0.01$). After exercise, nitrate in Group 1 significantly increased to 56 ± 3 μM ($p < 0.01$). In non-athletic Group 3, nitrate significantly increased from rest (32 ± 3 μM) to immediately after and two-hours after exercise (37 ± 4, $p < 0.05$ and 42 ± 6 μM, $p < 0.01$, respectively). Peak work rate from the max test in Group 2 was moderately significantly correlated with resting nitrate ($r = 0.53$, $p < 0.01$). No significance difference between groups post exercise nitrate was indicated. This study supported that greater physical fitness was related to resting nitric oxide bioavailability as well as a correlation existing between peak work rate and resting nitric oxide in a non-athletic population.

A similar study by Franco et al. (16) compared plasma nitrite levels in semiprofessional football players (21.5 ± 2.88 years), older sedentary (65.7 ± 6.14 years), and younger sedentary (22.5 ± 3.45 years) males before and after a 10-minute cycle ergometer exercise bout at 80% of maximal heart rate. The athletes in this study had significantly higher baseline nitrite levels

compared to younger and older sedentary individuals (41.40 ± 6.06, 23.78 ± 5.74, and 22.17 ± 6.14 μM, respectively; $p < 0.001$). All groups had a significant increase in nitrite immediately post exercise with no differences between groups (athletes 41.40 ± 6.06 to 56.00 ± 9.90, younger sedentary 23.78 ± 5.74 to 44.73 ± 6.48, older sedentary 22.17 ± 6.14 to 45.88 ± 9.84 μM; $p < 0.05$). In response to sheer stress from acute exercise, nitric oxide increases to prevent further damage to the vascular endothelium. Trained individuals have a greater nitric oxide bioavailability at rest, supporting the importance of regular exercise to lower cardiovascular disease risk through the ability of the endothelium to adequately dilate during times of excess sheer stress.

Chronic Aerobic Exercise

Chronic exercise training creates adaptations in endothelial function as a result of repeated exposure to sheer stress during exercise. In 24-week chronic exercise study by Zaros et al. (50), postmenopausal women (50 ± 4 years) participated in a moderate-intensity aerobic exercise training using a cycle ergometer at 50% of their heart rate reserve for 60 minutes, three days a week. Nitric oxide concentration and blood pressure were measured before and after training. Nitrate and nitrite concentrations significantly increased in response to 24 weeks of training (10.0 ± 0.9 to 16.0 ± 2.0 μM, $p < .05$). In addition, systolic and diastolic blood pressure significantly decreased in response to training from stage 1 hypertension to pre-hypertension range (141 ± 10 to 123 ± 8 and 90 ± 5 to 80 ± 5 mmHg, respectively; $p < 0.05$). Weight and body mass index, however, did not significantly decrease in response to training. This study suggests that lighter intensity exercise can still lead to significant improvements in endothelial health and lower cardiovascular disease risk. Although significant improvements in endothelial function are not obtained in a short amount of time, lifestyle changes are still promoted with respect to overall cardiovascular health benefits (23,50).

Acute Resistance Exercise

During resistance exercise, systolic blood pressure increases in response to the excess stress being placed on the working muscles (10,20). This exposure to brief periods of hypertension induces sheer stress which creates a greater the amount of nitric oxide released to promote vasodilation. When comparing resistance exercise intensities, higher intensity exercise producing excess sheer stress may induce a greater nitric oxide production response to exercise compared to lower intensity exercise bouts. In a study conducted by Coelho-Junior et al. (10) in older men (67.1 ± 4.6 years), nitric oxide concentration was measured through immediate exercise recovery following either a low- or high-intensity acute resistance exercise bout. Both groups completed three sets of eight to 10 repetitions of nine resistance exercises: seated row, leg press, chest press, seated leg flexion, lateral raise, calf raise, bicep curl, triceps extension, and abdominal crunch. The low-intensity group at around 50% of one-repetition maximum (1RM) and the high-intensity group at around 70% of 1RM. Saliva samples were collected at rest, immediately after exercise, and at minutes 15, 30, and 60 after exercise completion. Nitrite levels were measured after the saliva was submerged in a Griess reagent, transferred into microplates, and then absorbency levels measured to quantify the amount of nitrite in the saliva sample. During recovery, nitric oxide reached was significantly elevated from baseline at all post-exercise time points in both groups with no difference between time points or groups ($p < 0.05$).

A similar acute resistance exercise study comparing low- and high-intensity exercise bouts in young males (27.8 ± 2.8 years) measured nitric oxide concentration over a 72-hour period recovery period in blood plasma (20). The exercise bout consisted of four exercises: squat, leg extension, latissimus dorsi pull, and chest press. Both groups did a total of 12 sets, but the high intensity group performed less repetitions at higher percentage of 1RM (three sets of two repetitions at 95% of 1RM, three sets of 4 repetitions at 90% of 1RM, three sets of six

repetitions at 85% of 1RM, three sets of 8 repetitions at 80%) while the low intensity group completed more repetitions at a lower percentage of 1RM (three sets of 20 repetitions at 35% 1RM, three sets of 23 repetitions at 30% 1RM, three sets of 26 repetitions at 25% 1RM, three sets of 30 repetitions at 20% 1RM). Blood was collected at rest, immediately after, and at hours six, 24, 48, and 72 post exercise. Plasma was then separated for conversion of nitrate to nitrite through a modified Griess reaction using vanadium chloride for the conversion. There were no significant increases in nitric oxide concentration in the low-intensity group (20). Nitric oxide concentration in the high-intensity group were significantly greater than pre-exercise concentration and the low-intensity group at hours six, 24, and 48 after exercise ($p < 0.05$). This study supports that greater sheer stress following a higher intensity resistance exercise bout creates a higher amount of nitric oxide which could lead to overall improved endothelial function through chronic exposure to similar exercise intensities.

Chronic Resistance Exercise

Chronic exposure to elevated sheer stress during exercise creates vascular adaptations through structural remodeling of the endothelium and increasing nitric oxide bioavailability at rest. Resistance exercise training induces similar improvements in nitric oxide concentration to that from aerobic exercise training. Tomeleri et al. (48) conducted a 12-week resistance exercise training study in pre- and hypertensive postmenopausal women (68.2 ± 5.7 years), measuring training responses in nitric oxide concentration and systolic and diastolic blood pressure. The participants trained twice a week, completing one set of 10-15 repetitions for eight resistance exercises: chest press, leg press, seated row, leg extension, preacher curl, leg flexion, triceps extension, and seated calf raise. After 12 weeks of training, nitric oxide significantly increased from pre- to post-training (5.4 ± 1.8 to 7.3 ± 1.7 μM, respectively; $p < 0.01$). Systolic and diastolic blood pressure also significantly decreased in response to training (142.2 ± 10.5 to

130.1 ± 9.7 and 79.5 ± 7.0 to 72.8 ± 4.3 mmHg, respectively; $p < 0.01$). A moderately significant negative correlation existed between systolic blood pressure and nitric oxide ($r = -0.63$, $p < 0.05$). Body fat percentage significantly decreased and skeletal muscle mass significantly increased in response to training as well (42.5 ± 5.5 to 40.7 ± 5.3% and 19.6 ± 2.9 to 20.9 ± 3.2 kg, respectively; $p < 0.01$). This study suggests that resistance training promotes improvements in endothelial function through increased nitric oxide concentration and decreased systolic and diastolic blood pressure.

Summary

Resistance and aerobic training can elicit similar salutary results in terms of nitric oxide production, vasodilation, and blood pressure (10,20,23,50). Light intensity chronic aerobic training may not have a high enough intensity to cause optimal endothelial adaptations, while too much endurance training can prevent maximum potential nitric oxide release through damaging over-training effects (23,46). Researchers tend to favor either aerobic or resistance exercise protocols, so overlaps of those forms of exercise are not commonly found. Since both forms of exercise individually result in overall improvements to cardiovascular health, combing resistance and aerobic training in sedentary, postmenopausal women may produce more significant endothelium improvements than simply targeting one form of exercise.

Chapter III: Methods

Study Design

For this study, a total of 50 sedentary, overweight to obese, postmenopausal women ages 55-75 years voluntarily participated in a 12-week intervention involving exercise training or health education sessions. A three-factor design was used in this study. The first factor was group with two levels: exercise (EX; n=22) and education (ED; n=18). Participants were randomly placed into either the EX or ED group after being approved to join the study. The second factor was the level of intervention: before the 12-week intervention (BT) and after the intervention (AT). The third factor was four acute exercise time points: pre-exercise (PRE), immediately post exercise (PO), one hour after exercise (1HR), and two hours after exercise (2HR) with identical time points for the ED group. Acute exercise data was collected BT and AT. FMD data was collected at the PRE and 2HR. Dependent variables are nitrate and nitrite and CRP concentrations from plasma aliquots, and percent dilation from FMD analysis. Nitrate and nitrite and CRP were measured at PRE, PO, 1HR, and 2HR, and FMD was analyzed at PRE and 2HR. All variables and time points were measured at BT and AT.

Participants

The population for this study included 50 postmenopausal, sedentary, overweight to obese women ages 55-75. Of those 52 women, 44 completed the 12-week intervention. Selection criteria for the participants included being postmenopausal surgically or naturally for at least two years, sedentary for at least six months (defined as not participating in more than one hour of mild-moderate physical activity per week for six months), and overweight to obese based on BMI (26-47 $kg \bullet m^2$). Exclusion criteria included: contraindications to the required exercise testing based on the ACSM Guidelines, chronic inflammatory or autoimmune disorders,

HIV/AIDS, acute or chronic infection, nervous system disorders, prior heart attack, chronic respiratory conditions, diabetes, previous stroke, kidney disease, blood disorders, tobacco use, and/or oral steroid use. Other exclusion criteria included regular use of over-the-counter non-steroidal anti-inflammatory medications, anti-coagulant use within 14 days, and/or surgery within the last three months.

Experimental Protocol

During the preliminary appointment, a potential participant was screened for health problems or medications that could potentially alter data collected. The participant also signed an informed consent that was approved by the Texas Christian University Institutional Review Board. Body composition measurements measured included height, weight, BMI, waist-to-hip ratio, and DEXA scan for bone density and lean and fat mass. After a participant was selected and randomly placed into either the education or exercise group, she participated in a three-session acclimation period to familiarize herself with the weight machines and to measure her eight-repetition maximum (8-RM) for each resistance training machine. Resistance exercises included leg press, chest press, seated row, leg extension, leg flexion, lateral pull down, leg abduction, and leg adduction. Further preliminary testing included a sub-maximal treadmill test to predict VO_2max by taking the participant to 80% of her calculated heart rate reserve (HRR = (.80 x (Age-predicted max HR – resting HR)) + resting HR) and extrapolating the data to her estimated VO_2max.

When the acclimation period and treadmill test was completed, each participant had an experimental trial day. Participants arrived to the lab well rested and after an overnight fast, withdrawn from medications for a predetermined amount of time. Prior to the experimental trial day, participants kept a three-day log of all food intake and any physical activity. If the

participant was in EX, she had first a resting blood sample and FMD taken prior completing an exercise session with two sets of 8-RM on all the weight machines followed by a treadmill walk for 25 minutes in a target heart rate range of 80% of heart rate reserve ±10bpm, followed by a two-minute cool down. Subsequent blood samples were collected at PO, 1HR, and 2HR. FMD was measured after completion of the 2HR blood draw. ED had blood drawn and FMD measured at similar time points and remained seated quietly during the entire experimental trial, with exceptions to go to the bathroom.

Once the experimental trial day was completed, the participant began the 12-week intervention period either exercising three days a week or attending education sessions twice a week. Exercising sessions included resistance and aerobic training similar to the experimental trial exercise bout. For resistance training, the first set was eight repetitions and the second set was up to 15 repetitions. Weights were increased as the participant is able to easily do more than 12 repetitions in the second set. After resistance training, the participant walked on the treadmill for a two-minute warm up, 25 minutes in the heart rate range, and a three-minute cool down. For the first two weeks the participant maintained 70% of heart rate reserve (HR), weeks three and four was increased to 75% HRR, and weeks five through study completion was at 80% HRR. The training protocol was selected to meet the American College of Sports Medicine recommendations for physical activity. Participants had to make up any missed exercise sessions at the end of their 12 weeks. Education sessions occurred twice a week and included a guest speaker lecturing about a health-related topic. Topics included yoga, stretching, end of life care, mindful meditation, stretching protocols, healthy diet, pharmacological information, and physiological changes associated with aging. An absence from an education session was made up at the end so that the participant attended 24 sessions total. At the end of the 12 weeks, the submaximal treadmill test, body composition measurements, and experimental trial were

performed on every participant. The participants were asked to repeat their BT three-day food log prior to the AT experimental trial.

Blood Analyses

Total blood drawn during each experimental trial day was around 220mL. EDTA tubes for plasma were centrifuged immediately upon blood draw for 10 minutes at temperature of 4°C and a speed of 2000 RCF x g. After the tubes were centrifuged, plasma was immediately aliquoted into 0.5mL cryotubes and frozen at -80°C for storage.

Nitric oxide was measured using the Cayman Chemical Nitrite/Nitrate Colorimetric Assay Kit (Item No. 780001). Frozen plasma aliquots at all time points were analyzed for nitrate and nitrite, the oxidized products of nitric oxide. The assay was run according to the provided directions, with the exception of the samples not being ultrafiltered. Samples were analyzed at an absorbance of 540 nm. C-Reactive Protein was measured using the Quantikine ELISA Human C-Reactive Protein/CRP Immunoassay Kit (Catalog No. DCRP00). Plasma samples were analyzed according to the provided directions at a 1:200 dilution, and samples were analyzed at an absorbance of 544 nm and subtracted from readings at 450 nm.

FMD Analyses

Resting blood pressure was measured on both treadmill test and experimental trial days. On experimental trial days, FMD was measured after PRE blood sample and immediately after 2HR blood sample. FMD was measured in a sound and temperature-controlled room with the participant in a supine position. An Acuson Sequoia 512 Ultrasound System (Mountain View, CA) with a 6L3transducer was used to obtain images of the brachial artery. The diameter of the brachial artery was measured along the anticubital space to obtain an image of the longitudinal

section of the artery. A blood pressure cuff was placed on the forearm and inflated to 200mmHg and remained inflated for five minutes. Video monitoring of the blood vessel began 30 seconds before the cuff was deflated and video continued to record for three minutes after deflation, taking three images per heart beat during that time period.

After the images were obtained, the software used for analysis of the diameter was Brachial Analyzer for Research (Medical Imaging Applications, LLC; Coralville, IA). Upon first opening the image file, a reasonable sized region of interest (ROI) was determined based on clarity of the artery walls throughout the period of measurement (Figure #). If possible, the same ROI was used for analysis throughout the baseline images and the reactive hyperemia images after brachial artery occlusion (Figure #). In the event that the same ROI could not be used for the entire time point analysis due to artery diameter disappearing or becoming unclear, the ROI would be moved to an area with more clear artery walls while staying as close to the original ROI from the baseline images (Figure #). Oftentimes the bright white line of the artery wall would disappear for a few frames. When this occurred, the artery diameter would be edited to be similar to the previous frames or the diameter would be placed along the usually-present faint white line (Figure #). If there were no clear artery borders due to the subject moving or technician error, the that frame would be removed from analysis. Baseline artery diameter and maximum artery diameter were used to calculate FMD, expressed as a percent change from the baseline diameter.

Anthropometric Measures Analyses

Height was measured twice to the nearest millimeter, without shoes, using a standiometer. Body weight was measured to the nearest 0.1 kilogram in light clothing and without shoes, and after subjects voided their bladder using a digital scale. Height and body

weight measurements were used to calculate BMI ($kg \bullet m^2$). The DEXA scan was done with a General Electric DEXA scanner to measure bone density, lean body mass, and fat body mass.

Statistical Analyses

The Shapiro-Wilk test was used to test for normality, and any non-normal data was log transformed. Mauchly's test for was used to test for sphericity and the Huynh-Feldt adjustment corrected degrees of freedom. A three-factor 2X2X4 repeated measures ANOVA was performed on nitrate/nitrite and CRP concentrations to test for significance within and between groups. The within factors included group (EX and ED), training (BT and AT), and time point (PRE, PO, 1HR, and 2HR). A three-factor 2X2X2 repeated measures ANOVA was performed on FMD to test for significance within and between groups. The within factors included group (EX and ED), training (BT and AT), and time point (PRE and 2HR). A two-factor 2X2 repeated measures ANOVA was performed on systolic and diastolic blood pressure, VO_2 max, leg press eight-repetition maximum, BMI, and android percent fat. The within factors included group (EX and ED) and training (BT and AT). For all ANOVAs, Bonferroni adjustments were used to determine significance, and significance was defined as $p < 0.05$. A Spearman Rho correlation was used to determine correlations between FMD, NO, CRP, BMI, SBP, and DBP at all time points, before and after training.

FMD Figures

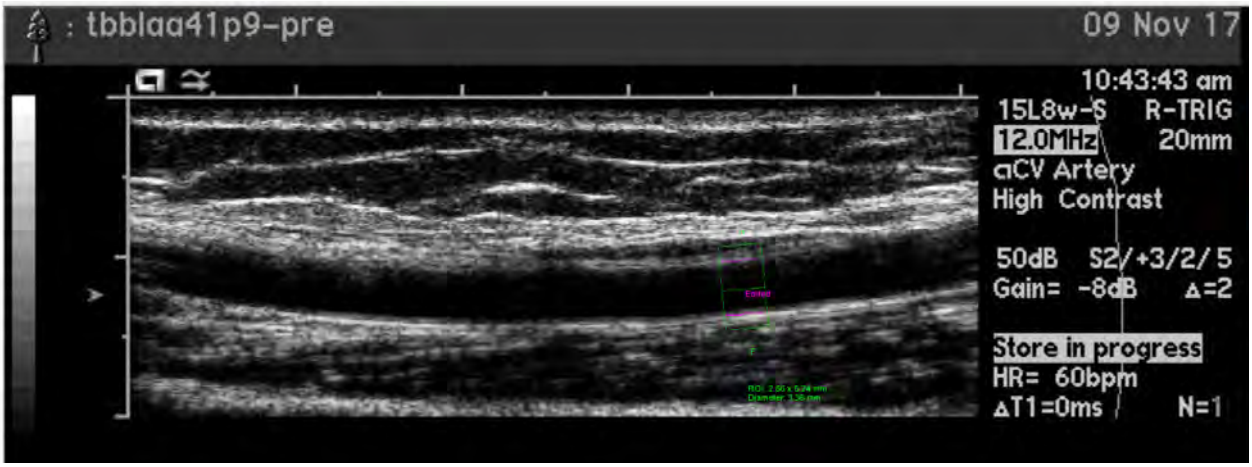

Figure 1: Subject A—baseline artery diameter

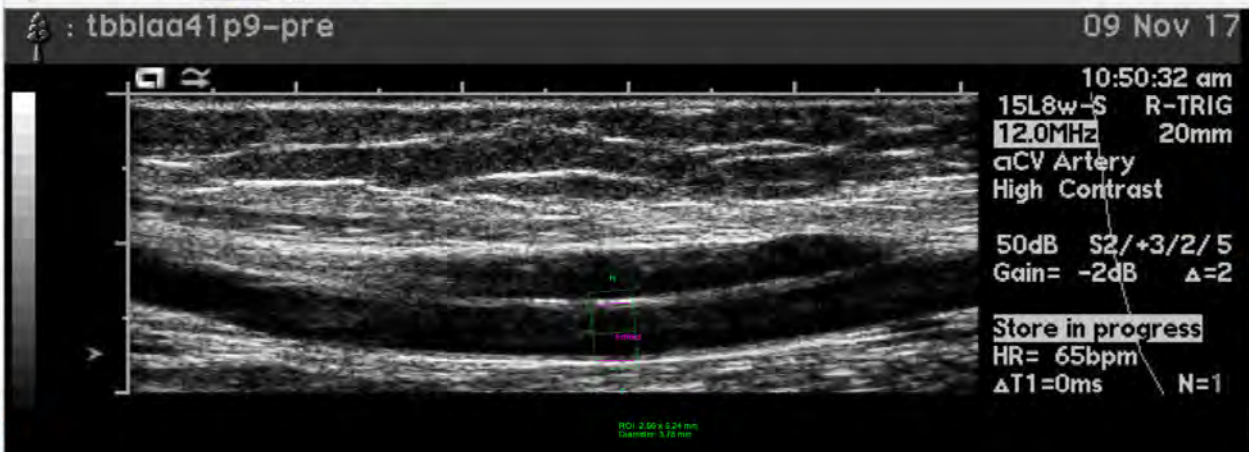

Figure 2: Subject A—peak artery diameter, ROI adjusted to an area with clearer arterial walls

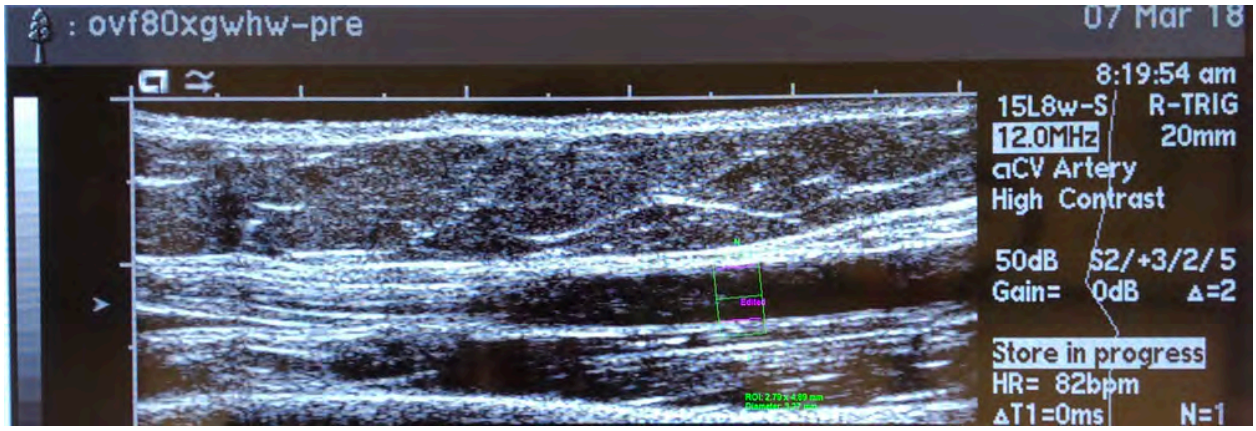

Figure 3: Subject B—baseline artery diameter

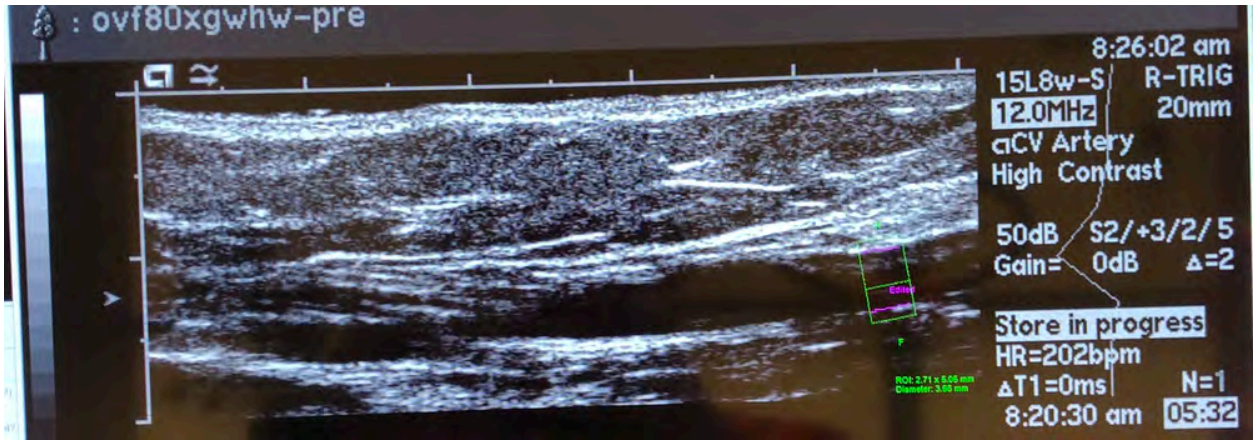

Figure 4: Subject B—reactive hyperemia diameter, ROI adjusted to an area with clearer arterial walls due to original ROI becoming unreasonably wide

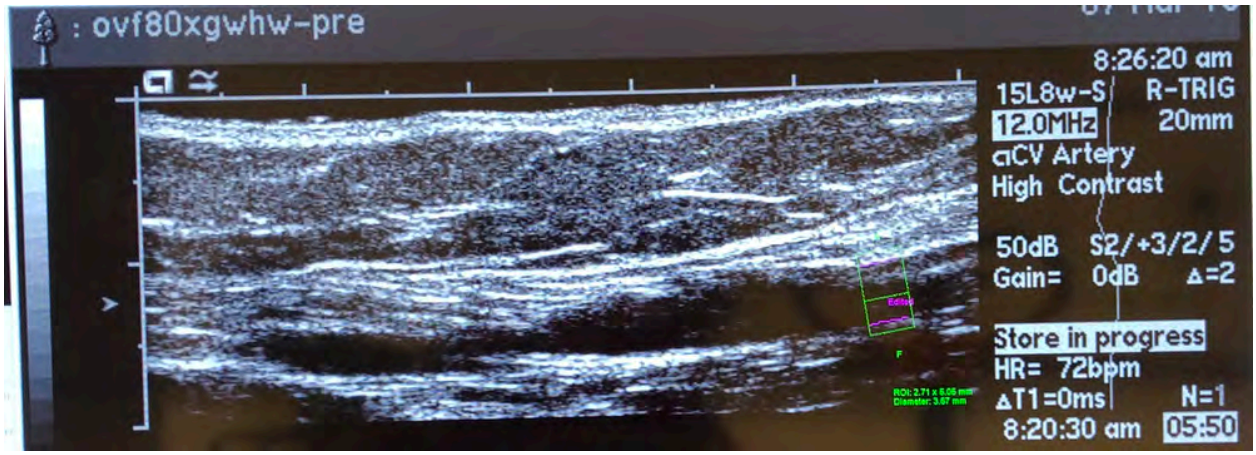

Figure 5: Subject B—reactive hyperemia diameter, bottom arterial wall not as illuminated so diameter placed in line with clearer arterial wall around the ROI.

Chapter IV: Results

At the start of the study, we had 48 total participants: 27 EX and 21 ED. By the end of the study, we had 22 EX and 19 ED. The five EX were dropped from the study due to lack of adherence to the training schedule or the inability to maintain the exercise intensity. Two ED were excluded after data collection had completed for altering their normal diet during the intervention. In addition, one ED was excluded after data analyses for having nitrate/nitrite and CRP concentrations well above normal values.

Age, Anthropometric Measures, and Blood Pressure

Age, anthropometric measures, and blood pressure (SBP and DBP) are reported in Table 1. Baseline measures did not differ between groups. BMI and android percent fat did not change in EX or ED after the 12-week intervention. There was a main effect of training where SBP decreased in both groups collapsed (Figure 1) ($p < 0.0001$). No changes were observed in DBP. BMI at BT and AT was correlated with resting CRP at BT and AT ($r = 0.350$, $p = 0.021$; $r = 0.352$, $p = 0.033$, respectively). DBP at BT and AT was correlated with resting CRP at BT and AT ($r = 0.305$, $p = 0.047$; $r = 0.422$, $p = 0.009$, respectively).

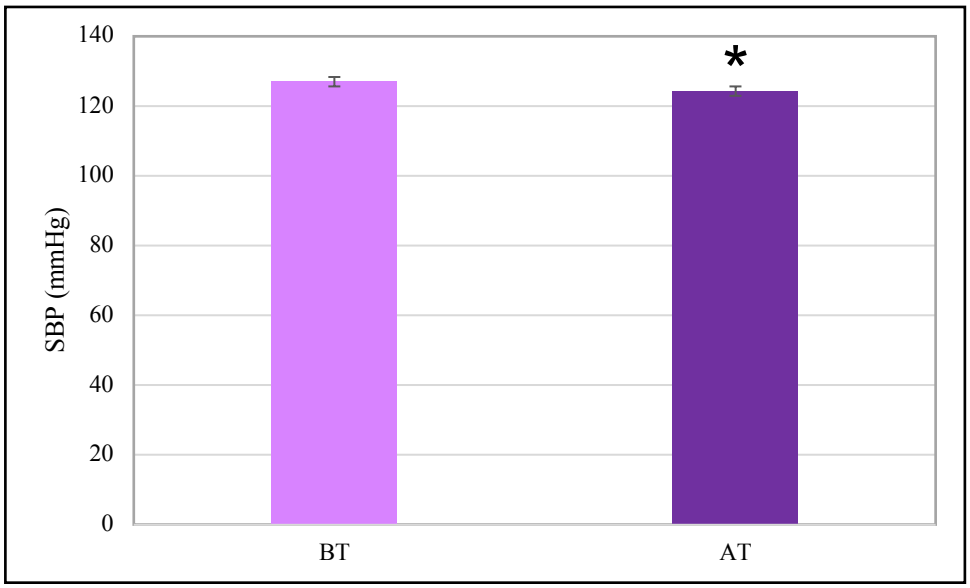

Figure 6: Main effect of the intervention on systolic blood pressure with the exercise (EX) and resting control (ED) groups collapsed. Main effect of the intervention.

* $p < 0.0001$, different from BT

Table 1

Age, anthropometric measures, and blood pressure (SBP and DBP) before (BT) and after (AT) the intervention in EX and ED. (Mean ± SE)

	EX (n = 22)		ED (n = 18)	
	BT	AT	BT	AT
Age (yr)	62.9 ± 5.1		65.9 ± 5.1	
BMI ($kg \cdot m^2$)	32.9 ± 1.00	33.0 ± 0.98	32.7 ± 1.11	33.2 ± 1.08
SBP (mmHg)	127.3 ± 2.2	123.6 ± 2.2*	125.8 ± 2.5	124.0 ± 2.4*
DBP (mmHg)	78.1 ± 1.5	77.5 ± 1.5	72.8 ± 1.6	74.4 ± 1.6
Android Fat (%)	54.5 ± 1.50	53.2 ± 1.42	55.2 ± 1.7	54.9 ± 1.6

* $p < 0.0001$, main effect different from BT

Aerobic Fitness and Strength Measures

Aerobic fitness and muscular strength significantly improved in response to the employed training protocol. VO_2 max significantly increased in EX while ED had no changes in response to the intervention (Figure 7) ($p = 0.009$). The exercise intervention also significantly increased leg press 8-repetition maximum in EX with no changes in ED (Figure 8) ($p < 0.0001$).

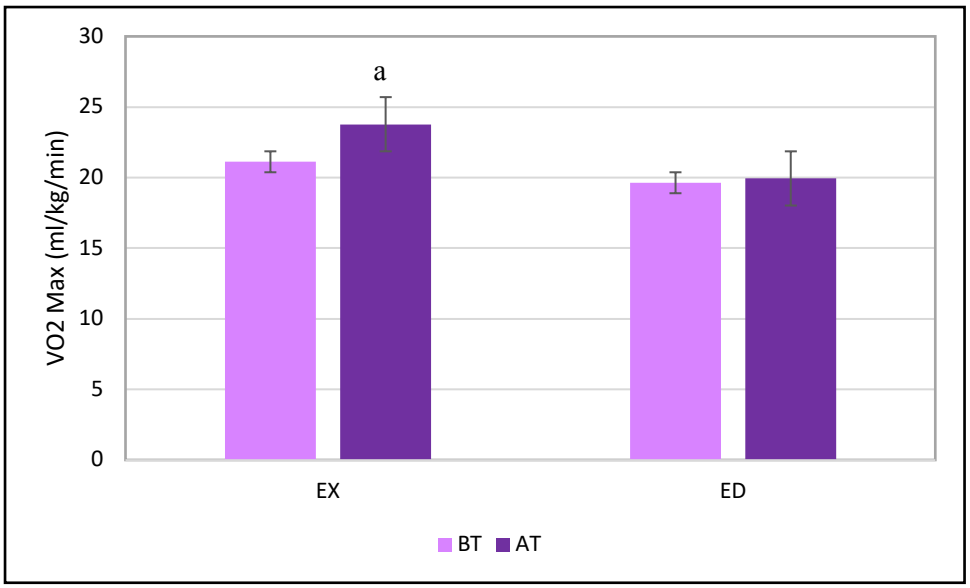

Figure 7: Relative VO_2 max (ml/kg/min) before (BT) and after (AT) the intervention in the exercise (EX) and education control (ED) groups.

[a] $p = 0.009$, different from BT, group by training interaction

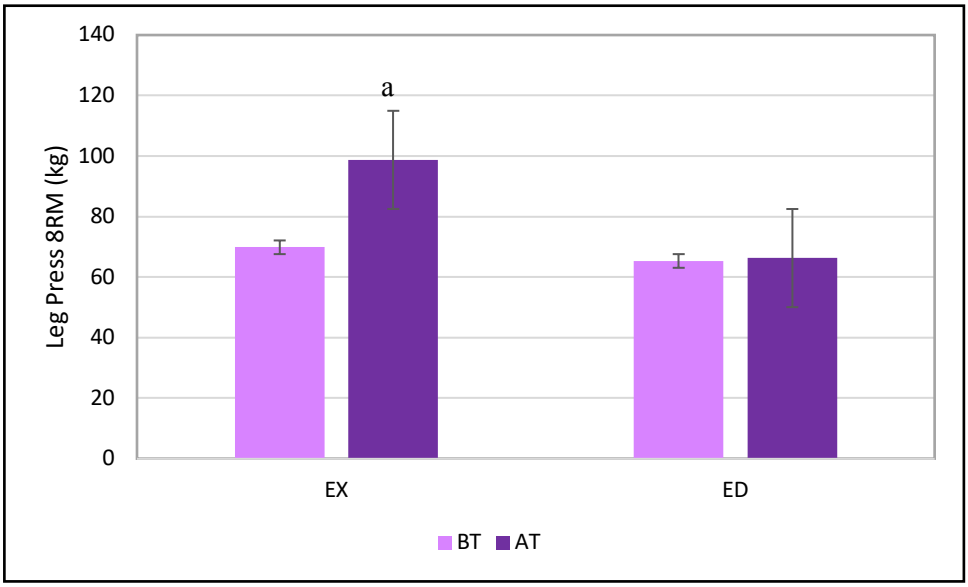

Figure 8: Leg press 8-repetition maximum (kg) before (BT) and after (AT) the intervention in the exercise (EX) and education control (ED) groups.

[a] $p < 0.0001$, different from BT, group by training interaction

Nitrate + Nitrite

Analyses include only those subjects with data from all eight time points (four at BT and four at AT). Two EX and one ED were missing at least one time point. In addition, one ED had clearly erroneous values from BT to AT. Nitrate and nitrite (NO) were similar between groups at baseline. NO concentrations at each time point in each group are listed in Table 3. There was a time point by group interaction with BT and AT collapsed (Figure 9, Table 2) ($p = 0.004$). In response to acute exercise, NO had a biphasic response such that NO concentration increased immediately after exercise, returned to near-baseline levels at one-hour post exercise, then increased again at the two-hours post exercise time point ($p < 0.0001$, $p = 0.001$, and $p = 0.009$, respectively). NO did not change diurnally in ED. There was no effect of the intervention observed in EX or ED. No correlations existed between NO and BMI, SBP, DBP, FMD, or CRP.

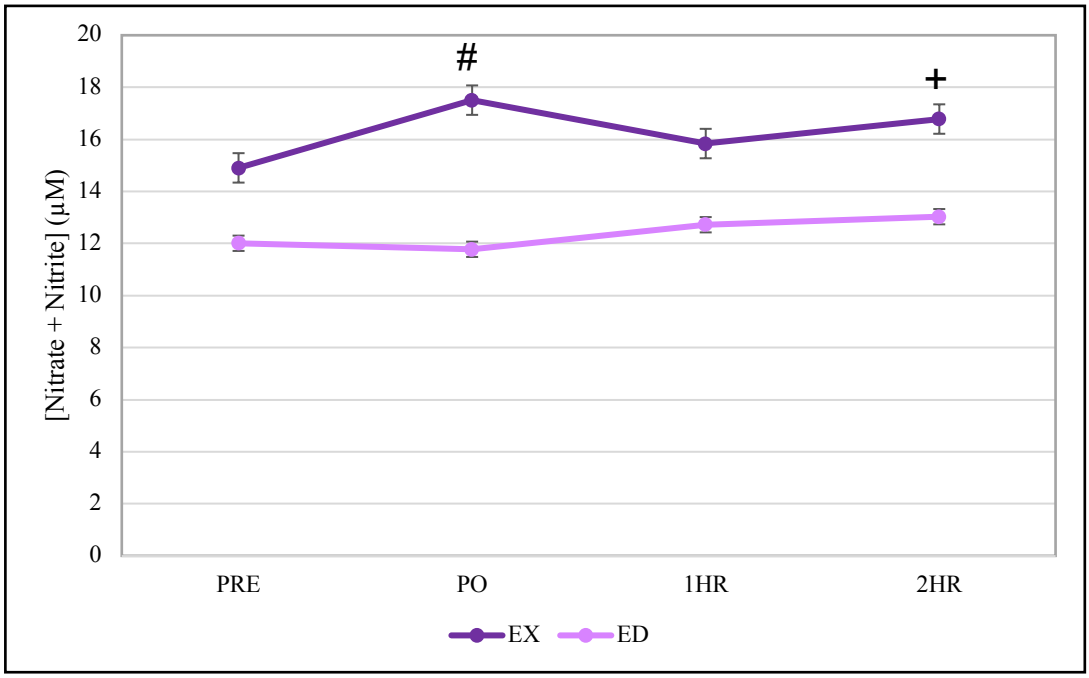

Figure 9: Nitrate + Nitrite at PRE-, PO-, 1HR, and 2HR-post exercise (EX) or quiet sitting (ED) with before (BT) and after (AT) the intervention combined.

+ $p = 0.009$, different from PRE within group
# $p < 0.0001$, different from PRE, and $p = 0.001$, different from 1HR within group

Table 2

Nitrate + nitrite concentrations (μM) at four time points (PRE, PO, 1HR, 2HR) with before (BT) and after (AT) the intervention combined in EX and ED. (Mean ± SE)

	EX (n = 20)	ED (n = 18)
PRE	14.9 ± 1.03	12.0 ± 1.14
PO	17.5 ± 0.98#	11.8 ± 1.10
1HR	15.8 ± 0.87	12.7 ± 0.98
2HR	16.8 ± 0.97+	13.0 ± 1.09

+ $p = 0.009$, different from PRE within group
# $p < 0.0001$, different from PRE, and $p = 0.001$, different from 1HR within group

Table 3

Nitrate + Nitrite Concentrations (μM) in EX and ED at four time points (PRE, PO, 1HR, 2HR) before (BT) and after (AT) the intervention. (Mean ± SE)

		EX (n = 20)	ED (n = 16)
BT	PRE	13.7 ± 1.00	11.3 ± 1.11
	PO	16.6 ± 0.94	11.2 ± 1.05
	1HR	14.9 ± 0.95	12.6 ± 1.06
	2HR	16.6 ± 1.14	12.5 ± 1.27
AT	PRE	16.1 ± 1.34	12.7 ± 1.50
	PO	18.4 ± 1.27	12.4 ± 1.42
	1HR	16.8 ± 1.12	12.9 ± 1.25
	2HR	17.0 ± 1.17	13.6 ± 1.31

Flow-Mediated Dilation

FMD analyses included those subjects with data at all time points. Two EX were missing BT FMD data, so they were not included in analyses. FMD was similar between EX and ED at baseline. A group by time point interaction revealed that a bout of moderate-high intensity resistance and aerobic exercise improved percent FMD at 2HR with BT and AT collapsed (p = 0.04) (Figure 10, Table 4). Additionally, 2HR percent FMD was greater in EX compared to ED with BT and AT collapsed (Figure 10) (p = 0.04). There was no diurnal effect observed for FMD in ED. There was no effect of the intervention on FMD observed in EX or ED. Baseline brachial artery diameter did not change in EX or ED between BT and AT (Table 6). Reactive hyperemia (RH) diameter, or peak diameter, also did change. No correlations existed between FMD and SBP, DBP, BMI, NO, or CRP at any time points.

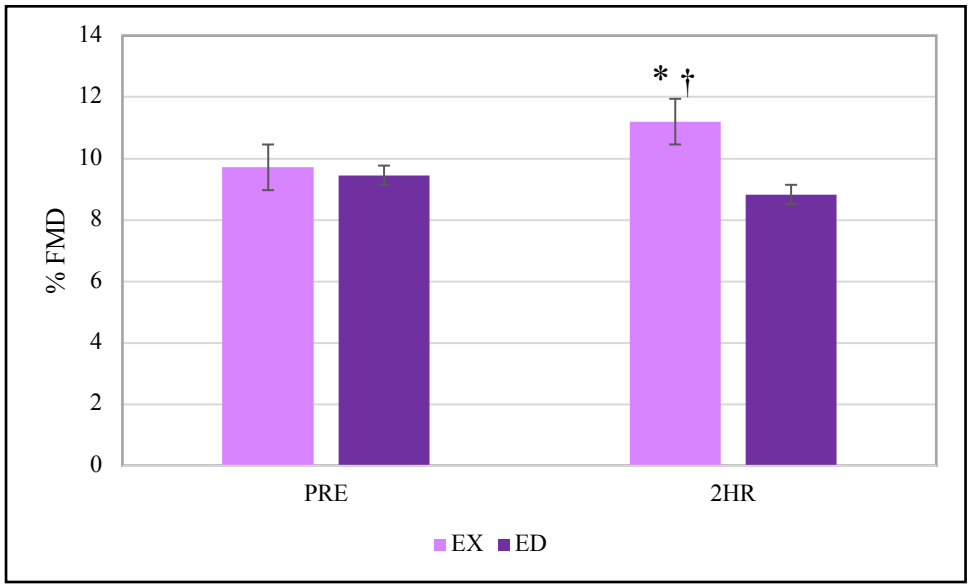

Figure 10: Percent FMD at PRE- and 2HR-post exercise (EX) or quiet sitting (ED) with before (BT) and after (AT) the intervention collapsed. Group by time point interaction.

* $p = 0.04$, different from PRE within group
† $p = 0.04$, different from ED

Table 4

Percent FMD at PRE- and 2HR-post exercise or quiet sitting with training (before and after intervention) collapsed. (Mean ± SE)

	EX (n = 20)	ED (n = 18)
PRE	9.7 ± 0.48	9.5 ± 0.51
2HR	11.2 ± 0.5*†	8.8 ± 0.53

* $p = 0.036$, different from PRE within group
† $p = 0.003$, different from ED

Table 5

FMD percent change from baseline brachial artery diameter to peak diameter after blood flow occlusion to the hand at two time points (PRE and 2HR) before (BT) and after (AT) the intervention in EX and ED. (Mean ± SE)

		EX (n = 20)	ED (n = 18)
BT	PRE	9.5 ± 0.70	9.9 ± 0.71
	2HR	11.3 ± 0.72	9.5 ± 0.76
AT	PRE	9.9 ± 0.71	9.0 ± 0.75
	2HR	11.1 ± 0.69	8.1 ± 0.73

Table 6

Brachial artery diameter (mm) at baseline and at peak diameter during reactive hyperemia (RH) after blood flow occlusion to the hand, at two time points (PRE and 2HR), before (BT) and after (AT) the intervention in EX and ED. (Mean ± SE)

			EX (n = 20)	ED (n = 18)
BT	PRE	Baseline	3.7 ± 0.12	3.7 ± 0.12
		RH	4.1 ± 0.12	4.1 ± 0.12
	2HR	Baseline	3.8 ± 0.13	3.7 ± 0.13
		RH	4.3 ± 0.13	4.0 ± 0.14
AT	PRE	Baseline	3.7 ± 0.12	3.7 ± 0.12
		RH	4.1 ± 0.12	4.0 ± 0.13
	2HR	Baseline	3.6 ± 0.11	3.7 ± 0.11
		RH	4.0 ± 0.11	4.0 ± 0.11

C-Reactive Protein

CRP analyses included those subjects with data from every time point. Two EX and 1 ED were missing data from at least one time point. Because CRP is a clinical marker of acute infection and inflammation, we had to exclude two EX and two ED due to values above 10 mg/L which generally is indicative of an acute event. CRP was similar between groups at baseline. A group by time point interaction existed with BT and AT collapsed (Figure 11, Table 7) (p = 0.036). Acute exercise increased CRP by PO regardless of training status (p = 0.005). There was no effect of the intervention on CRP in EX or ED. Baseline CRP (BT PRE) was positively correlated with BMI at BT (r = 0.350, p = 0.021). CRP at BT PRE was also positively correlated with BT DBP (r = 0.305, p = 0.047). At AT, CRP was positively correlated with AT DBP and AT BMI (r = 0.422, p = 0.009; r = 0.352, p = 0.033, respectively).

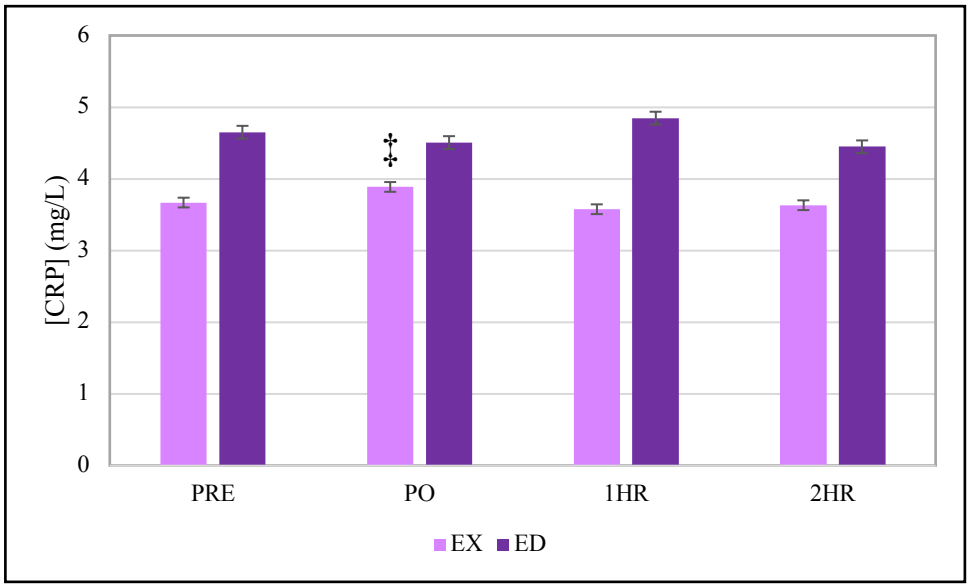

Figure 11: CRP concentrations (mg/L) comparing EX and ED at four time points (PRE, PO, 1HR, 2HR) with the before (BT) and after (AT) intervention values collapsed. Group by time point interaction.

‡ $p = 0.005$, different from 2HR within group

Table 7

CRP concentrations (mg/L) in EX and ED at four time points (PRE, PO, 1HR, 2HR) with the before (BT) and after (AT) intervention values collapsed. (Mean ± SE)

	EX (n = 18)	ED (n = 16)
PRE	3.7 ± 0.61	4.7 ± 0.65
PO	3.9 ± 0.57‡	4.5 ± 0.61
1HR	3.6 ± 0.59	4.9 ± 0.63
2HR	3.7 ± 0.55	4.5 ± 0.58

‡ $p = 0.005$, different from 2HR within group

Table 8

CRP concentrations (mg/L) in EX and ED at four time points (PRE, PO, 1HR, 2HR) with before (BT) and after (AT)the intervention. (Mean ± SE)

		EX (n = 18)	ED (n = 16)
BT	PRE	4.0 ± 0.66	4.7 ± 0.69
	PO	4.2 ± 0.58	4.4 ± 0.62
	1HR	3.9 ± 0.60	4.8 ± 0.64
	2HR	3.8 ± 0.52	4.1 ± 0.55
AT	PRE	3.4 ± 0.59	4.6 ± 0.63
	PO	3.7 ± 0.59	4.7 ± 0.63
	1HR	3.4 ± 0.64	4.9 ± 0.67
	2HR	3.6 ± 0.63	4.8 ± 0.66

Chapter V: Discussion

The acute exercise protocol employed in this study significantly increased FMD, nitrate/nitrite, and CRP; however, the intervention had no effects in either EX or ED on these variables. Systolic blood pressure significantly decreased in response to the intervention with both groups collapsed. The main effect of training that revealed a significant reduction in SBP is difficult to explain. When examining the data, we see the mean SBP in EX decreased from 127.3 ± 2.2 to 123.6 ± 2.2 mmHg, which is close to a 4mmHg decline. Comparing that to the almost 2 mmHg decrease in ED from 125.8 ± 2.5 to 124.0 ± 2.4 mmHg, we could attribute the significant reduction in SBP was partially driven by the decrease in EX given that SBP in EX dropped twice as much as ED. Although ED was consistently reminded not to change their diet or physical activity level, it is possible that participants may have altered their lifestyle without our knowledge. In addition, acclimation and familiarization to the study procedures on the data collection days, or "white coat syndrome", may have falsely increased the blood pressure measured during the BT experimental trial. Although no training effect was observed in nitrate/nitrite, FMD, or CRP, evidence of vasodilatory responses to acute exercise were supported based off increased nitric oxide bioavailability and enhanced FMD response after a single exercise bout.

Acute Endothelial Response

These results suggest that in response to sheer stress from the moderate-to-high intensity resistance and aerobic exercise bout, eNOS phosphorylation increased leading to elevated nitric oxide bioavailability and enhanced vasodilation (45). Vasodilation occurs during exercise to protect the endothelium lining the arterial walls from damage. Greater cardiac output and blood redistribution to the working muscles during exercise to increase oxygen and nutrient delivery

elicits widespread responses to sheer stress, which supports elevated nitric oxide concentrations continuing after exercise completion (20). Although our subjects had a biphasic nitric oxide response to exercise, the elevated concentrations at 2HR compared to PRE supports that nitric oxide remains elevated at two hours post-exercise. Peak nitric oxide concentrations, however, occurred at PO at BT and AT, rejecting our hypothesis that nitrate/nitrite concentration would peak at 2HR. Our data are supported by results reported by Coelho-Junior et al. (10) and Guzel et al. (20) who reported that nitric oxide significantly increased post a resistance exercise bout. Coelho-Junior et al. (10) reported that nitric oxide was elevated in postmenopausal women up to one hour following three sets of eight to 10 repetitions on nine whole-body resistance exercises at 50% and 70% of participants' one-repetition maximum. Nitric oxide was measured immediately post-exercise and at minutes 15, 30, and 60 post-exercise. Significant increases in nitric oxide concentration from baseline occurred at all time points with groups; however, although the high intensity group had higher nitric oxide concentrations at immediately-post, 15, and 30 minutes post-exercise and a decrease at 60 minutes post exercise, there was no significant differences between groups at any time point ($p < 0.05$). Guzel et al. (20) reported that NO was elevated at up to 48 hours following 12 total sets of two to eight repetitions on four whole-body resistance exercises at 80-95% of the participants' one-repetition maximum while participants completing the same number of sets with 20 to 30 repetitions at 20-35% of participants' one-repetition maximum had no significant increases in nitric oxide ($p < 0.05$). Between the studies by Coelho-Junior et al. (10) and Guzel et al. (20), we could infer that nitric oxide decreases at one-hour post exercise in postmenopausal women and elevates later in the recovery period. Since neither study measured nitric oxide at the two-hour time point, however, we are unable to provide validation of the biphasic response in nitric oxide following our study protocol.

Similar to nitric oxide responses, FMD significantly increased from PRE to 2HR with BT and AT collapsed. During blood flow occlusion, tissue hypoxia and the accumulation of vasodilator substances such as adenosine and carbon dioxide increase sympathetic activity leading to greater vasodilation when blood flow is restored. As discussed previously, nitric oxide levels may remain elevated up to 48 hours post exercise (20). When residual concentrations of nitric oxide and other vasodilator metabolites are elevated exercise after exercise completion, increased vasodilation occurs during FMD because higher nitric oxide bioavailability promotes greater smooth muscle relaxation. Our results are consistent with previous studies that reported significant increases in FMD after exercise from two hours to up to 24-hours post moderate-to-high intensity aerobic exercise (11,21). The increase in FMD at 2HR following our exercise protocol accepts our hypothesis that FMD would increase in response to exercise. Although no correlations existed between FMD and nitric oxide at any time points, the significant increases in FMD and nitric oxide at 2HR supports the conclusion that endothelial function was enhanced in response to our acute exercise protocol to two hours post-exercise.

Endothelial Function Adaptations

The 12-week combined resistance and aerobic exercise training protocol did not elicit chronic adaptations to endothelial function measured via nitric oxide concentration and FMD, rejecting our hypotheses that stated nitrate/nitrite concentration and FMD would increase in response to training. Nitrate/nitrite concentrations measured in our study population were consistent with previous exercise training studies in a similar age population using the same assay (13,26,43,50). Sponton et al. (43) reported no significant changes in nitric oxide in males and females (50.8 ± 0.6 years) despite improvements in aerobic capacity and resting heart rate after eight weeks treadmill training at 100% LT. In a previous study, Sponton et al. (43) reported previously that they found increases in nitric oxide concentration in obese, postmenopausal

women in response to a similar training protocol, however, with a training duration of six months. This suggests that the duration of our 12-week training protocol may not have been of long enough duration to measure significant increases in nitric oxide concentration. Zaros et al. (50) performed a six-month moderate-intensity aerobic exercise training study in hypertensive, postmenopausal women (50 ± 4 years). In response to training, nitrate and nitrite concentrations significantly increased along with a significant decrease in systolic and diastolic blood pressure. Even though Zaros et al. (50) employed a comparatively lighter exercise intensity at 50% of heart rate reserve, the duration of the program may have had a greater impact on nitric oxide concentration than the intensity since their protocol was twice as long as the protocol we employed. Esposti et al. (13) also conducted an eight-week moderate-intensity aerobic training study in postmenopausal women that resulted in a non-significant increase in nitric oxide concentration. Systolic and diastolic blood pressure, however, significantly decreased. Prior to that study, the researchers also performed a 24-week training study that resulted in a significant increase in nitric oxide concentration, also suggesting that volume rather than intensity of exercise is more important in benefiting endothelial function.

The absence of significant increase in nitrate and nitrite could be attributed to the length of our training protocol considering the previously mentioned studies suggested that duration rather than intensity may be a more important factor in influencing nitric oxide adaptations. Holding intensity constant throughout the aerobic training may have also been a limiting factor in nitric oxide improvements. Izadi et al. (25) reported significant decreases in nitric oxide concentration following a six-week aerobic high intensity interval training program in hypertensive adults (61.7 ± 5.8 years). Lack of improvements in body composition may also have prevented significant improvements in nitric oxide availability. BMI and android percent fat remained unchanged in both groups after the intervention, stating that body composition

remained relatively unchanged. Since android fat did not decrease in response to training, then CRP produced in response to inflammation from excess visceral tissue may not have declined which would inhibit nitric oxide production. Choi et al. (9) reported a significant positive correlation between BMI and percent body fat with resting nitric oxide concentration to BMI and percent body fat. Higher nitric oxide in obese persons could be produced by inducible nitric oxide synthase (iNOS) which is expressed in response to inflammation and can produce large quantities of nitric oxide (40). Reactive oxygen species are also increased in response to inflammation, so when these free radicals such as superoxide reacts with nitric oxide, it is reduced to peroxynitrate which is attributed to the process of atherosclerosis (40). Production of iNOS within vascular smooth muscle cells following exposure to proinflammatory cytokines is also a major cause of hypertension. So even though nitric oxide concentrations may be elevated in obese persons, the conditions by which it is produced does not promote enhanced endothelial function. In addition, the amount of circulating free radicals may not have decreased without a loss of visceral adipose tissue, leading to continued eNOS uncoupling and inability to produce nitric oxide during a period of elevated sheer stress. Since higher levels of angiotensin II are associated with increases in oxidative stress and proinflammatory cytokines through NADPH oxidase activation that increases reactive oxygen species, the absence of reduction in oxidative stress would inhibit a decrease in the vasoconstricting properties of angiotensin II (12,41). Angiotensin II concentration may also not have decreased due to the potential lack of decrease in visceral adipose tissue. Higher activity of angiotensin converting enzyme, the enzyme that forms angiotensin II from angiotensin I in adipose tissue, can cause increased renin-angiotensin system activity for extended periods of time (38). Increased levels of sodium retention in obesity causes increased activity of the sympathetic nervous system which impedes suppression of the renin-

angiotensin system (38). The reduction in systolic blood pressure and improvements in aerobic fitness, however, still provide basis for cardiovascular-related benefits of our exercise protocol.

Although FMD increased two hours after exercise completion similar to nitrate and nitrite, the 12-week intervention did not improve resting FMD. At all time points, the participants were within normal values (4-18% dilation) of FMD with no participants at a higher risk of cardiovascular related events based on FMD-related criteria (< 2% dilation) (11,21,28,29,34,37,47). Improvements in FMD as a result of training occur from structural remodeling after consistent periods of sheer stress during exercise and decreased adiposity (21,47). Structural remodeling can be determined through FMD by comparing baseline brachial artery diameters before and after an intervention. If structural remodeling occurs, the artery diameter would increase as an adaptation to consistent vasodilatory responses to sheer stress from exercise. In our subjects, brachial artery diameter did not significantly change after the intervention, rejecting this method of determining structural remodeling of the endothelium in the absence of improvements in FMD. Tinken et al. (47) conducted an eight-week aerobic training study in young, active males with FMD measured at weeks 0, 2, 4, 6, and 8. FMD significantly increased from week 0 at weeks 2 and 4, but had no significant improvements compared to baseline after week 4. Brachial artery diameter did not significantly change at any of the time points throughout the study. These findings were proposed to be a result of functional vascular endothelial adaptations occurring before actual arterial remodeling in response to exercise training. Because we only measured FMD before and after the 12-week intervention, we do not know if FMD improved in the first few weeks of the exercise training protocol before returning to similar baseline levels after 12 weeks. Since systolic blood pressure improved in response to training, structural remodeling of the endothelium may have occurred, allowing the arterial wall to normalize during the repeated bouts of elevated sheer stress. In addition, the

absence of significant body composition changes may also have prevented improvements in FMD. If the amount of visceral adipose tissue did not decrease, then circulating inflammatory markers and free radicals would still be preventing adequate nitric oxide bioavailability which would inhibit an improved FMD response to training. Technician error may also have occurred during FMD measurement and analysis which would alter results. The ultrasound probe was placed in the same spot on the arm at PRE and 2HR within each experimental trial, but the location between trials may have changed slightly which could impact a potential training response. Both training time points (i.e. BT PRE and 2HR) were analyzed blinded by one technician in one sitting, however, since all analyses took place over the course of five months, within subject trial interpretation of the arterial borders may have occurred.

Inflammatory Response

Within our study population, most of the participants were classified as being at high risk for cardiovascular disease based on having a CRP concentration greater than 3 ng/L. Because obesity is an inflammatory disease, CRP is positively correlated with BMI (17). This was confirmed in our population with a moderate, significant correlation between BMI and CRP at BT and AT PRE ($r = 0.350$, $p = 0.021$; $r = 0.352$, $p = 0.033$, respectively). In response to exercise, we hypothesized that acute exercise would not change CRP concentration, and CRP would decrease in response to exercise training. Due to the lack of significance between time points within EX, we can accept our hypothesis that CRP would not change in response to acute exercise. There was a group by time point interaction, however, in EX with BT and AT collapsed such that PO was greater than 2HR ($p = 0.005$). In response to a moderate-intensity exercise bout, CRP may increase as a result of exercise-induced muscle injury stimulating IL-6 secretion that induces CRP synthesis and release into the blood immediately post-exercise (2). This

mechanism may be responsible for significant elevations in CRP in response to our protocol immediately post-exercise.

The 12-week intervention did not decrease resting CRP concentrations, thus rejecting our hypothesis that CRP would decrease in response to exercise training. Although prior research supported the inclusion of resistance training with aerobic training to decrease CRP concentrations in postmenopausal women, we were not able to further confirm those results (36,44). In a 12-month moderate-intensity aerobic training study in middle-aged participants conducted by Campbell et al. (5), CRP concentration did not significantly decrease. Researchers attributed the absence of changes in CRP to the lack of significant weight loss in the exercise group. In our participants, BMI and android percent fat did not change in response to training. With a lack of decrease in visceral adipose tissue, chronic inflammation is still present causing increased oxidative stress. As a result of increased oxidative stress, a blunted vasodilatory response occurs from decreased nitric oxide bioavailability that has been associated with higher CRP concentrations (14). CRP was also significantly correlated with DBP in our study population at BT and AT ($r = 0.305$, $p = 0.047$; $r = 0.422$, $p = 0.009$, respectively). DBP is a measure of arterial relaxation between heart beats, so a higher DBP is indicative of inadequate vasodilation. The positive correlation between CRP and DBP further demonstrates the inability of adequate arterial relaxation to occur in the presence of elevated inflammatory markers. CRP inhibits the production of nitric oxide which blunts vasodilatory responses, and this is supported in our study with the positive correlation between DBP and CRP (39). Since CRP did not decrease in response to training, we can relate the lack of increases in nitric oxide concentration and FMD as well as the positive correlation with DBP to the absence of decreases in CRP inhibiting vasodilation.

Conclusion

In our study measuring the effects of combined exercise training on endothelial function in overweight to obese, postmenopausal women, acute exercise increased nitric oxide concentration, FMD, and CRP but these variables did not improve in response to 12 weeks of training. Lack of improvements in those variables could be attributed to the absence of changes in BMI and android percent fat, the duration of the training program not being long enough to induce significant changes in endothelial function, or the exercise intensity may not have been high enough to promote structural remodeling of the endothelium. Systolic blood pressure, however, did improve in response to training which suggests that there was some enhancement in endothelial function independent of improvements in FMD and nitric oxide availability.

This study did have a few limitations. Although our subjects were healthy and free of symptoms to indicate acute infection on experimental trial days, some reported general allergy symptoms which may have altered nitric oxide and CRP concentrations. Potential participants with uncontrolled arthritis were excluded from entering the study, but it is possible that our participants had mild arthritis that was unreported. University holiday closings lengthened overall training duration in most participants, but all participants completed at least 36 training sessions with a minimum of nine consecutive training sessions in three weeks prior to the AT experimental trial. Experimental trial days were also delayed for some participants due to scheduling difficulties of the participant and student researchers. Participants may have adjusted their diet or increased their usual level of activity outside of training or education sessions without our knowledge over the course of the intervention. We attempted to minimize these confounding factors by reminding the participants that they were not to change their lifestyles.

In conclusion, acute exercise enhanced vasodilation by increasing nitric oxide availability which promoted improved FMD two hours after exercise completion. Although no chronic

improvements were measured in nitric oxide, FMD, and CRP after training, the improvement in systolic blood pressure, aerobic fitness, and muscular strength favors the ACSM-recommended guidelines for combined resistance and aerobic exercise training on improving overall fitness. Maximal oxygen consumption is the gold standard measurement of aerobic fitness and is inversely related to all-cause mortality (33). By significantly improving VO_2 max, EX decreased their overall risk of mortality. Future studies could extend the protocol up to six to twelve months to determine if exercise training duration is a more important factor in improving endothelial function than exercise training intensity with a combined resistance and aerobic training protocol. In addition, data collection could take place every two to four weeks to measure progressive improvements in response to the training protocol. Results of this study provide insight that endothelial function is enhanced in response to acute exercise in the overweight to obese, postmenopausal population, and continual exercise may further decrease cardiovascular disease risk factors by lowering systolic and diastolic blood pressure, improving vasodilation in response to sheer stress, and decreasing circulating inflammatory markers.

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ABSTRACT

THE EFFECTS OF COMBINED EXERCISE TRAINING ON NITRIC OXIDE AVAILABILITY AND ENDOTHELIAL HEALTH IN OVERWEIGHT TO OBESE, POSTMENOPAUSAL WOMEN

by Carmen Anastasia Cook, M.S., 2019
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Thesis Advisor: Dr. Melody Phillips, Associate Professor of Kinesiology

PURPOSE: To determine the influence of acute and chronic combined aerobic and resistance exercise training on endothelial function in overweight-obese, postmenopausal women. METHODS: Overweight-obese (BMI 27-46 $kg \bullet m^2$), postmenopausal women (55-75 years) were randomized into either exercise (EX, n = 22) or education control (ED, n = 18) groups. Blood was collected before (PRE), immediately after (PO) and at 1- (1HR) and 2-hours (2HR) after exercise for EX or at similar time points for ED for nitrate/nitrite (NO) and CRP analysis. FMD was performed at PRE and 2HR. Testing was repeated after 12-weeks of training or education sessions. RESULTS: With training collapsed, acute exercise caused increases in NO at PO and 2HR, FMD at 2HR, and CRP at PO. No diurnal or training effects were measured in either group. CONCLUSION: Acute exercise enhanced endothelial function up to 2 hours post exercise but did not elicit chronic improvements in endothelial function.

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Education

Bachelor of Science, Exercise Science, Berry College, Rome, GA. December 2016
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Research Experience

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A 12-week resistance and aerobic training study in obese, postmenopausal women measuring inflammatory markers of cardiovascular disease. Analyses included assays for inflammatory markers on plasma and flow-mediated dilation.

Texas Christian University, 2017

Measuring the effect of prior aerobic exercise on endothelial dysfunction after consumption of a high-sugar meal in post-menopausal women. Analyses included flow-mediated dilation.

Publications and Abstracts

Bailey S, Glockener A, Kreutzer A, Garrity E, Adams L, Cook C, Mitchell J, Adams-Huet B, Cheek C, Oliver J, Phillips M, Shah M. Effect of Prior Aerobic Exercise on High-Sugar Meal Induced Endothelial Dysfunction in Postmenopausal Women. International Journal of Exercise Science: Conference Proceedings: 2018;2(10):article 76. Poster presentation, March 2018 Texas American College of Sports Medicine, Austin, TX.

Glockener A, Bailey S, Kreutzer A, Garrity E, Adams L, Cook C, Shah M, Adams-Huet B, Cheek C, Jada S, Mitchell J. The Effects of Prior Aerobic Exercise on Lipid and Lipoprotein Responses Following a High-Carbohydrate Meal in Postmenopausal Women. International Journal of Exercise Science: Conference Proceedings: 2018;2(10):article 80. Poster presentation, March 2018 Texas American College of Sports Medicine, Austin, TX.

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Cardenas, Maria A.; Levitt, Michael M.; Richie, Bryan; Lu, Shaohan; Erickson, Elise; Cook, Carmen; Haynes, Jay; Kreutzer, Andreas; Mitchell, Joel B.; and Phillips, Melody D. (2018) "Exercise-Induced Th17 Lymphocyte Response and Their Relationship to Cardiovascular Disease Risk Factors in Obese, Post-Menopausal Women," *International Journal of Exercise Science: Conference Proceedings*: Vol. 2 : Iss. 10 , Article 13.

Cook, Carmen A.; Kreutzer, Andreas; Levitt, Michael M.; Cardenas, Maria A.; Teagle, Gail; Thames, Kirby; Cheek, Dennis J.; and Phillips, Melody D. (2019) "The Effects of Combined Exercise Training on Flow-Mediated Dilation and C-Reactive Protein in Overweight, Postmenopausal Women," *International Journal of Exercise Science: Conference Proceedings*: Vol. 2 : Iss. 11 , Article 79.

Presentations

Texas American College of Sports Medicine, Annual Conference, Austin, TX, February 2018. Grant award presentation on the effect of combined exercise training on endothelial function in obese, postmenopausal women.

Texas American College of Sports Medicine, Annual Conference, Fort Worth, TX, February 2019. Poster presentation titled "The Effects of Combined Exercise Training on Flow-Mediated Dilation and C-Reactive Protein in Overweight, Postmenopausal Women."

Experimental Biology, Annual Conference, Orlando, FL, April 2019. Poster presentation titled "The Effects of Combined Aerobic and Resistance Exercise Training on Flow-Mediated Dilation in Overweight, Postmenopausal Women."

Teaching Experience

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Lectured an undergraduate exercise physiology lab section. Assisted in grading and leading review sessions in anatomical kinesiology. Helped run in-class labs in exercise assessment and prescription.

Research Grants

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Harris College of Nursing and Health Science Student Research Grant. Funded $500. Awarded March 2018. Principal investigator: Dr. Melody D. Phillips

Harris College of Nursing and Health Science Student Research Grant. Funded $500. Awarded October 2018. Principal investigator: Dr. Melody Phillips

Student Research and Development Award from Texas American College of Sports Medicine. Application denied. February 2019. Principal investigator: Dr. Melody D. Phillips

Professional Affiliations

American College of Sports Medicine, 2017-Present
Texas Chapter of American College of Sports Medicine, 2017-Present

Work Experience

Atlanta Swim Academy, Marietta, GA

Lifeguard, August 2013-December 2015. Responsibilities included lifeguarding pool parties and cleaning the pool deck.

Water Safety Instructor, November 2013-December 2015. Responsibilities included teaching swim lessons for children ages 6 months to 15 years old and to adults, cleaning the pool deck, providing parents with regular skills updates, and substituting in for instructors when needed.

Berry College, Rome, GA

Lifeguard, August 2013-May 2015. Responsibilities included checking pool chemicals, keeping pool deck clean, and lifeguarding various classes and free-swim.

Berry College Elementary and Middle School Front Office Assistant, August 2013-May 2015. Responsibilities included answering and making phone calls, making copies, designing the school year book, caring for sick and injured students, and other miscellaneous school and office tasks.

Advance Rehabilitation, Rome, GA

Physical Therapy Technician, November 2015-December 2016. Responsibilities included taking patients through exercises under the supervision of a physical therapist, preparing ice and hot packs as needed, cleaning the clinic, laundry, product inventory, and chart organization.

Office Assistant, January 2017-August 2017. Responsibilities included verifying insurance benefits, preparing new patient charts, filing and organizing charts, scheduling patients, collecting payments, and doing the deposit weekly.

Texas Christian University, Fort Worth, TX

Graduate Assistant, August 2017-Present. Responsibilities include running research studies, working closely with study participants, running various assays, maintaining lab equipment, keeping track of inventory in the lab, writing grants, and other teaching assistant responsibilities.

Escape the Room, Fort Worth, TX

Clue Master, May 2018-July 2018. Responsibilities included greeting customers, providing guidance as needed during their time in the room, resetting the room, and the facility clean.

Assistant Manager, July 2018-November 2018. Responsibilities include maintaining the front desk, answering phone, scheduling bookings, advising potential customers, opening and closing the facility when needed, scheduling employees, and ensuring customer satisfaction.

Volunteer Experience

Mt. Bethel United Methodist Church, Marietta, GA

Sunday School Assistant, August 2009-May 2013. I assisted the Sunday School teachers with lessons and projects every Sunday with second through fifth grade students.

Church Orchestra, August 2011-June 2015. I would play French Horn in church once a month with the church orchestra and attend weekly practices.

East Cobb Library, Marietta, GA

Student library volunteer, May 2012-August 2012. I would volunteer at the library three times a week for four hours each day. Responsibilities would include shelving books, organizing the children's books area, distributing books to other county branches, and checking in returned books.

Berry College, Rome, GA

First Year Service Projects, September 2013. In our orientation groups, we were assigned various community projects in which to participate. We picked up trash along roads in various areas of Rome, GA.

Year of Service with Bundles of Joy, August 2014-May 2015. After applying and being accepted into the Year of Service program at Berry College, our group had to spend the school year working with our organization of choice, Bundles of Joy, Inc. We spent the year making blankets for infants in the NICU and distributed the blankets to hospitals in Rome, GA at the end of the year.

Event Committees

Relay for Life, January 2016-April 2016. I was a part of the publicity committee when putting together the annual Relay for Life fundraiser event at

Berry College. We decorated campus with signs, balloons, and sidewalk chalk as well as decorating the inside of main buildings on campus. On the night of the event, we helped with set up, ensured that events flowed smoothly, and cleaned up after the event.

Marthapalooza, August 2016-October 2016. I was a part of the volunteer committee for Berry College's annual Marthapalooza event. As a part of the volunteer committee, leading up to the event I recruited volunteers for the event, coordinated volunteer schedules, and collected T-shirt sizes. On the night of the event, I checked in volunteers upon arrival, distributed T-shirts, and did damage control as needed.

Brass Ensemble, August 2013-December 2016. After attending weekly rehearsals throughout the semester, the brass ensemble would have four to five performances around the community at various events and churches.