AEROBIC EXERCISE TRAINING EFFECT ON *IN VIVO* AND *IN VITRO* VASCULAR ENDOTHELIAL INFLAMMATORY INDICES IN AFRICAN AMERICANS: IMPLICATIONS FOR HYPERTENSION AND CARDIOVASCULAR HEALTH

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ABSTRACT

African Americans have the highest prevalence of hypertension in the world which may emanate from their predisposition to heightened endothelial inflammation. The purpose of this study was to evaluate the *in vivo* influence of aerobic exercise training (AEXT) on the anti-inflammatory biomarker interleukin-10 (IL-10), the inflammatory biomarkers interleukin-6 (IL-6) and C-reactive protein (CRP), the endothelial activation marker CD62E+ endothelial microparticle (EMP), and the vasodilatory biomarker nitric oxide (NO) in an African American cohort. A secondary purpose was to conduct a complementary *in vitro* study on the influence of IL-10 and laminar shear stress (LSS) on African American endothelial cells. ***In Vivo* Methods:** The subjects were sedentary, putatively healthy, 45-71 y/o African American men and women. A pre-post study design was employed with baseline and post-intervention evaluations of office blood pressure, fasting blood sampling, and graded exercise testing. Subjects engaged in AEXT three times per week for six months at an intensity equivalent to 65% of their VO_{2max}. Plasma concentrations of IL-10 and IL-6 were determined using an enzyme-linked immunosorbent assay. Levels of nitric oxide metabolites (NOx) were determined using a modified Griess assay. Plasma samples for CRP were sent to Quest Diagnostics Inc. for analysis. Circulating CD62E+ EMPs were quantified using a flow cytometer. ***In Vitro* Methods:** Human umbilical vein endothelial cells (HUVEC) from an African American donor were cultured and exposed to four experimental conditions: *Static*, *Static with IL-10 Incubation*, *LSS at 20 dynes/cm^2*, and *LSS at 20 dynes/cm^2 with IL-10 Incubation*. Western blotting experiments were conducted to measure endothelial nitric oxide synthase (eNOS) protein expression and its phosphorylated form (p-eNOS) at

Serine 1177 in the cells in all four conditions. A modified Griess assay was used to measure NOx in the cell culture supernatant. ***In Vivo* Results:** There was a significant increase in NO (n=24; p=0.002), a significant decrease in IL-6 (n=32; p=0.04), a significant decrease in CRP (n=37; p=0.01), and a significant decrease in CD62E+ EMPs (n=28; p<0.001) following AEXT. IL-10 was increased following the AEXT intervention, however, it was not statistically significant (n=26; p=0.08). ***In Vitro* Results:** Protein expression levels of both eNOS and p-eNOS were significantly increased in the *LSS at 20 dynes/cm^2* and *LSS at 20 dynes/cm^2 with IL-10 Incubation* experimental conditions when compared to the *Static* experimental condition. NO concentration levels were significantly increased in the *LSS at 20 dynes/cm^2* and *LSS at 20 dynes/cm^2 with IL-10 Incubation* experimental conditions when compared to the *Static* experimental condition. **Conclusion:** Based on these results, AEXT may be a viable, non-pharmacologic method to improve vascular inflammation status and vasodilation, and thereby contribute to reduced hypertension and cardiovascular disease risk in African Americans.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

α-tubulin	Alpha tubulin
ACE	Angiotensin Converting Enzyme
ACSM	American College of Sports Medicine
AEXT	Aerobic Exercise Training
AHA	American Heart Association
ANG	Angiotensin
ANOVA	Analysis of Variance
ARB	Angiotensin Receptor Blocker
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Amendments
CRP	C-Reactive Protein
CV	Coefficient of Variation
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid

EMP	Endothelial Microparticle
eGFR	Estimated Glomerular Filtration Rate
eNOS	Endothelial Nitric Oxide Synthase
ET-1	Endothelin-1
FMD	Flow-mediated Dilation
HDL	High-density Lipoprotein
HUVEC	Human Umbilical Vein Endothelial Cell
IL-6	Interleukin-6
IL-10	Interleukin-10
LDL	Low-density lipoprotein
LSD	Least Significant Difference
LSS	Laminar Shear Stress
MDRD	Modification of Diet in Renal Disease
mRNA	Messenger Ribonucleic Acid
NADPH	Nicotinamide Adenosine Dinucleotide Phosphate
NF-κB	Nuclear Factor Kappa B
NIH	National Institutes of Health

NIST	National Institute of Standards and Technology
NO	Nitric Oxide
NOx	Nitric Oxide Metabolites (Nitrite/Nitrate)
ONOO-	Peroxynitrite
p-eNOS	Phosphorylated Endothelial Nitric Oxide Synthase
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate-Buffered Saline
PPP	Platelet-Poor Plasma
RIPA	Radioimmunoprecipitation Assay
ROS	Reactive Oxygen Species
SBP	Systolic Blood Pressure
SDS	Sodium Dodecyl Sulfate
SEM	Standard Error of the Mean
Ser	Serine
TNF-α	Tumor Necrosis Factor Alpha
VO_2	Oxygen Consumption
VO_{2max}	Maximal Oxygen Consumption

CHAPTER 1

INTRODUCTION

The preponderance of research on hypertension in ethnic populations supports the conclusion that African Americans have the highest prevalence of hypertension in the United States and in the world[1-5]. The earlier onset and increased severity of this pathology in African Americans leads to higher rates of morbidity and mortality when compared to other ethnic groups[5-12]. Hypertension has been linked to independent and interactive effects of multiple genetic and environmental factors[6]. One of these factors is inflammation of the endothelium, a pathological mechanism that can cause endothelial dysfunction, which is antecedent to hypertension[13-18].

The high incidence of hypertension in African Americans may be attributed to their predisposition to heightened systemic inflammation[11,19-22]. Long-term exposure of the endothelium to proinflammatory cytokines leads to increased inflammation and subsequent endothelial activation and dysfunction which support an environment favoring hypertension[23-27]. Recent evidence suggests that endothelial activation, characterized by increased inflammation, may be an early event in endothelial dysfunction and may be identified with the endothelial microparticle (EMP) inducible marker CD62E+[28-31]. It is hypothesized that the intravascular balance between pro- and anti-inflammation plays a crucial role as a determinant of endothelial health[32]. Research data have demonstrated a positive association between hypertension and proinflammatory markers including C-reactive protein (CRP) and interleukin-6 (IL-6)[13,15]. In contrast,

elevated circulating levels of the anti-inflammatory cytokine interleukin-10 (IL-10) have been associated with improved endothelial function[33-37]. Studies have also demonstrated that IL-10 is contributory in the up-regulation of endothelial nitric oxide synthase (eNOS) and subsequent bioavailability of nitric oxide (NO), a well-documented facilitator of vascular dilation that is critical for normal endothelial function[34,38].

Increased blood flow shear stress during aerobic exercise has been associated with favorable endothelial adaptations[39-62]. Aerobic exercise training (AEXT) may lead to the adaptive response of increasing plasma IL-10 and NO concentrations and decreasing plasma IL-6, CRP, and CD62E+ EMP concentrations in the African American population, thereby improving endothelial function through a reduction in vascular inflammation and endothelial activation. In addition, a high physiological level of laminar shear stress (LSS), used as an *in vitro* exercise mimetic, has been demonstrated to be important in protecting endothelial cells against inflammatory activation[19,56,63-69].

Despite the high prevalence of hypertension in African Americans, a paucity of research exists on the influence of AEXT on inflammation and endothelial health as a preventive measure to reduce the risk for cardiovascular disease (CVD) in this population. Therefore, the purpose of this study was to evaluate the *in vivo* influence of AEXT on the anti-inflammatory biomarker IL-10, the inflammatory biomarkers IL-6 and CRP, the endothelial activation marker CD62E+ EMP, and the vasodilatory biomarker NO in an African American cohort. Furthermore, a secondary purpose was to conduct a complementary *in vitro* study on the influence of IL-10 and LSS on African American endothelial cells.

The Specific Aims of this study were the following:

1. To measure the plasma levels of IL-10, NOx (nitrite/nitrate), IL-6, CRP, and CD62E+ EMPs in African American subjects prior and subsequent to six months of AEXT in order to potentially elucidate some of the etiological mechanisms of endothelial dysfunction as they relate to hypertension in this population.

   Hypothesis #1 was that plasma IL-10 and NOx levels would be increased and plasma levels of IL-6, CRP, and CD62E+ EMPs would be decreased in the African American subjects subsequent to six months of AEXT.
2. To measure phosphorylated eNOS (p-eNOS) at Serine 1177 relative to total eNOS protein expression and NOx (nitrite/nitrate) levels in African American endothelial cells in response to 24 hours of IL-10 incubation, high physiological levels of LSS, and a combination of IL-10 incubation with LSS. **Hypothesis #2** was that the greatest increase in p-eNOS relative to total eNOS protein expression and NOx levels in the African American endothelial cells would result from a combination of IL-10 incubation with LSS.

CHAPTER 2

REVIEW OF LITERATURE

Hypertension

The World Health Organization's *Global Brief on Hypertension* (2013) describes hypertension in the following statement:

> Hypertension, also known as high or raised blood pressure, is a condition in which the blood vessels have persistently raised pressure. The higher the pressure in blood vessels, the harder the heart has to work in order to pump blood. If left uncontrolled, hypertension can lead to a heart attack, an enlargement of the heart and eventually heart failure. Blood vessels may develop bulges (aneurysms) and weak spots due to high pressure, making them more likely to clog and burst. The pressure in the blood vessels can also cause blood to leak out into the brain. This can cause a stroke. Hypertension can also lead to kidney failure, blindness, rupture of blood vessels, and cognitive impairment[70].

African Americans and Hypertension

According to the Heart Disease and Stroke Statistical Update (2014) published by the American Heart Association (AHA), in conjunction with the Centers for Disease Control and Prevention (CDC), and the National Institutes of Health (NIH), African American adults have among the highest prevalence of hypertension (44%) in the world[1]. The prevalence of hypertension also remains highest among African Americans in the United States[2-5]. Hypertension is a major risk factor for cardiovascular disease (CVD) which is the leading cause of death in the United States[2,4,71]. In comparison with Caucasians in the United States, African Americans are at a greater risk for CVD-related morbidity and mortality including earlier onset, poorer control, increased target organ

damage, and more prevalent coexisting conditions including type 2 diabetes, left ventricular hypertrophy, heart failure, fatal and non-fatal stroke, coronary heart disease, coronary artery disease, and end-stage renal disease[5-12]. It is likely that multiple factors explain the excess of hypertension-related morbidity and mortality among African Americans[6]. Furthermore, hypertensive African Americans have less blood pressure-lowering responses to pharmacologic monotherapy and have lower rates of blood pressure control when compared to Caucasians[2,6]. Research data have also demonstrated that, even while engaging in antihypertensive therapy, African Americans have an increased prevalence of heart failure and an increased risk of sudden cardiac death when compared to Caucasians[72,73].

C-reactive protein (CRP), a non-specific marker of inflammation, has been positively associated with cardiovascular risk, and it has been demonstrated that African Americans have elevated levels of CRP when compared to Caucasians[8,10,74,75]. Ethnic differences have also been observed among African Americans when compared to Caucasians in relation to endothelial dysfunction, which has been positively associated with hypertension[13]. Multiple researchers have reported that African Americans have attenuated responses to vasodilators and decreased vascular function when compared to Caucasians[9,76-79]. Furthermore, studies conducted on human umbilical vein endothelial cells (HUVEC) have demonstrated that African American HUVECs have increased systemic inflammation, oxidative stress, and subsequent endothelial dysfunction when compared to Caucasian HUVECs[19-22].

Based on the disparity between African Americans and Caucasians regarding hypertension and subsequent CVD risk, research that may contribute to elucidating the

possible etiological mechanisms of increased hypertension and its possible relationship to detrimental physiological mechanisms among African Americans should be conducted. It is well documented in literature that oxidative stress and inflammation often occur simultaneously and have been linked to endothelial dysfunction, as well as hypertension[80-84]. A key message from the Seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (2003) was that hypertension is a significant CVD risk factor and that it is important to implement and maintain a treatment plan[71]. An increase in knowledge of the mechanisms relating to endothelial dysfunction and hypertension may be beneficial in developing preventive measures in reducing the CVD risk burden among African Americans.

Inflammation and Hypertension

Although the etiology of hypertension is not completely known, research data have demonstrated that inflammatory mechanisms play an important role in the pathophysiology of hypertension and CVD, and that vascular inflammation may be an important marker in terms of risk prediction[14]. Chronic low-grade inflammation has been identified as having an integral part in the pathogenesis of vascular disease and may play a significant role in the development and incidence of hypertension, either as a primary or secondary event[13,15-17]. It has been demonstrated that inflammation is associated in the initiation, progression, and clinical implications of cardiovascular disorders, including essential hypertension[18]. Additionally, a review article by Goldschmidt-Clermont et al. reported that genes relevant to mechanisms of inflammation are associated with hypertension[85].

Elevated inflammatory cytokines may lead to the development of endothelial dysfunction, impaired vasodilation, and subsequent hypertension[13,86,87]. Inflammation has been implicated in both endothelial dysfunction and the arterial stiffness that is associated with hypertension, with reduced nitric oxide (NO) bioavailability, as well as oxidative stress, demonstrated to be integral to this process[14,15]. The cooperative role of inflammation and oxidative stress in the pathogenesis of hypertension may be resultant of the inflammatory response subsequent to vascular oxidative stress[88]. It has been established that specific markers of inflammation are upregulated with various forms of CVD and correlate with vascular risk[14]. Bautista, as well as Boos and Lip, reported independent associations between hypertension and plasma levels of CRP and interleukin-6 (IL-6)[13,15]. Furthermore, elevated CRP levels have also been determined to be predictive of increased risk in the development of hypertension in both prehypertensive and normotensive patients[15,16].

Identifying markers of inflammation may lead to the development of new therapeutic targets that interfere favorably with these inflammatory mechanisms, and thus allow progress toward combating the complications resulting from CVD, specifically hypertension. There is evidence that pharmacologic treatments, such as statins, angiotensin converting enzyme (ACE) inhibitors, and angiotensin (ANG) II blockers, all of which have pleiotropic anti-inflammatory properties and are used in the management of hypertension, positively influence outcomes in patients by ameliorating the inflammatory state[15,89]. Although several therapeutic approaches targeting high blood pressure have demonstrated the efficacy of anti-inflammatory treatments on hypertension,

further research is needed to evaluate the reported anti-inflammatory effects in the sustained management of arterial hypertension[14,16].

Exercise Training and Hypertension

The role of non-pharmacologic interventions, including physical activity and exercise, have been emphasized in current treatment guidelines for hypertension[90]. According to the American Heart Association's scientific statement on alternative approaches to lowering blood pressure, there is strong evidence that exercise-based regimens, including aerobic exercise, may be effective in lowering blood pressure[91]. Physical activity and exercise effects on chronic conditions, including endothelial dysfunction, hypertension, and CVD, have been examined and demonstrated to primarily prevent and/or delay these chronic diseases[92]. Conversely, physical inactivity is considered to be a primary cause of these chronic diseases[39,92]. A rapidly advancing body of knowledge confirms an important and beneficial role for exercise in the prevention and treatment of CVD and its risk factors, including hypertension[39,40,90,93-100].

A meta-analysis by Cornelissen et al., including research studies in which participants engaged in at least four weeks of aerobic endurance training, revealed significant reductions in both resting and daytime ambulatory blood pressure[93]. The authors concluded that the aerobic endurance training decreased blood pressure through a reduction of vascular resistance and favorably affected concomitant CVD risk factors[93]. A more recent meta-analysis by Cornelissen et al., with similar criteria for study inclusion, revealed a significant reduction in daytime ambulatory blood pressure, but not nighttime ambulatory blood pressure, subsequent to aerobic endurance training[94]. A

study conducted on women with prehypertension demonstrated a significant reduction in systolic blood pressure following a 12-week diet and exercise training intervention[95]. Another investigation using an 8-week exercise intervention on sedentary, hypertensive subjects concluded that clinically significant decreases in blood pressure may be achieved with relatively modest increases in physical activity above sedentary levels[97]. Two independent reviews by Hagberg et al. on the effects of exercise training in patients with hypertension reported that exercise training decreased blood pressure in approximately 75% of the individuals with hypertension and reported a 10 mmHg reduction for both systolic and diastolic blood pressures in one review, and systolic and diastolic blood pressure reductions averaging approximately 11 and 8 mmHg, respectively, in the second review[90,96]. The authors also concluded that low-to-moderate intensity endurance training at ~40-70% maximal oxygen consumption (VO_{2max}) appears to be as, if not more, beneficial as higher intensity exercise training for reducing blood pressure in individuals with hypertension[90,96,98].

Cardiovascular exercise training has been demonstrated to be the most effective mode of exercise in the prevention and treatment of hypertension[98]. The most current position stand from the American College of Sports Medicine (ACSM) on exercise and hypertension recommends those with high blood pressure engage in primarily endurance physical activity at a moderate intensity for at least 30 minutes per day on most, preferably all, days of the week[99]. Although exercise has been associated with antihypertensive benefits, the optimal exercise dose (frequency, intensity, duration) required to lower blood pressure and maintain normotensive status remains unclear[97,100]. The mechanisms that have been linked to the antihypertensive benefits of exercise are

multi-factorial, and the optimal dosage may depend on the effects of exercise on independent risk markers of hypertension; therefore, the optimum exercise dose for the treatment of hypertension should be prescribed on an individual basis[100]. Research data have exposed that even modest increases in exercise intensity have hypotensive effects in sedentary, hypertensive patients, and that the volume of exercise required to reduce blood pressure may be relatively small[40,97]. It also has been reported that exercise training may be effective in reducing blood pressure without a change in body weight or body fat[98]. However, not all hypertensive patients respond favorably to exercise treatment. Differences in genetics and/or pathophysiology may be responsible for the inability or diminished ability of some to respond to exercise[99]. Further research is needed in order to identify the optimal training dose to improve the blood pressure-lowering capacities of exercise, particularly in certain ethnic groups such as African Americans[99].

Endothelium

The endothelium lines all blood vessels in the human body and is the basic structure which ensures the action of substances circulating in the bloodstream on the vascular wall[101,102]. It serves as a functional barrier between vessel wall and bloodstream, is metabolically active, and produces a variety of vasoactive mediators[103-105]. The endothelium is the endocrine organ essential for the physiological function of the vascular system, and it is effective in vascular tone and blood flow[101,102,104]. Impaired function of the endothelium has been demonstrated to be a factor in the development of vascular disease[101]. The endothelium modulates vascular tone by releasing several vasoactive substances including the vasodilator molecule NO and the vasoconstrictor protein endothelin[102-107].

Hypertension and Endothelial Dysfunction

When the endothelium is impaired, the balance between the release of vasodilation and vasoconstriction factors is typically disturbed[102-104,108-111]. Endothelial dysfunction is the term used for the resulting apparent decrease in endothelial-dependent relaxation[110,112]. It also may be characterized by a functional, yet reversible, alteration of endothelial cells resulting from impairment in NO availability and oxidative stress[113-120]. The decreased bioavailability of NO may be a result of the reduction in the activity of endothelial nitric oxide synthase (eNOS); however, the determining factor in endothelial dysfunction has been identified to be the increase in oxidative stress-producing superoxide radicals which combine with NO to form peroxynitrite (ONOO-)[112,119,121]. Furthermore, the superoxide radicals and the ONOO- activate the nuclear factor kappa B (NF-κB) transcription factor which controls the genes stimulating the expression of many proteins including some with vasoconstricting and inflammatory characteristics[112].

In hypertension, endothelial dysfunction has been identified at the level of both resistance and conduit arteries and, in patients with arterial hypertension, endothelial-dependent relaxation has been demonstrated to be impaired because of a reduced production and/or bioavailability of endothelial-derived NO[101,104,110,121,122]. This impairment of relaxation may account for the increased vascular tone that has been associated with hypertension[114]. Additionally, in the presence of hypertension, the endothelium becomes a source of vasoconstricting factors such as endothelin-1 (ET-1)[106]. Patients with essential hypertension have been characterized with endothelial dysfunction which has been evoked as a promoter of progressive vascular damage[113,119]. Endothelial

dysfunction has been implicated in both the development and pathophysiology of CVD, including essential hypertension[108,114,119].

It is well documented that endothelial dysfunction is implicated in hypertension and CVD[102-106,109,111,112,115-118,120,122-127]. Endothelial dysfunction in hypertensive patients has been associated with hypertensive target organ damage and may be predictive of future cardiovascular events[106,115,127,128]. It may also be used as a marker of an early change in those who develop hypertension, and the severity of endothelial dysfunction has been demonstrated to correlate with the development of CVD[109,112,121,128,129]. The fact that endothelial dysfunction may be used as a marker of future cardiovascular events emphasizes the importance of the clinical evaluation of endothelial function, as well as the effects of modulating the endothelium as a strategic and therapeutic target in the treatment of hypertension[101,108,109,114,121]. In the African American population, endothelial dysfunction has been identified as a key element for their excess CVD burden[130]. Therefore, elucidating and targeting mechanisms that improve the health of the endothelium may be especially beneficial in this population.

Inflammation and Endothelial Dysfunction

A link between inflammation and endothelial function has been well established in the literature, and inflammation has been implicated to be an important precursor to endothelial dysfunction[23,81,82,84,125,131-137]. At the site of an endothelial injury, inflammatory cells produce numerous proinflammatory factors which promote and amplify both local and systemic inflammation[134]. These inflammatory conditions have been associated with the loss of profound physiological functions of the endothelium[134].

One pivotal function of the endothelium altered by inflammation is the NO-mediated regulation of vessel tone and blood flow[23,134]. The endothelial-derived vasoactive modifications that have been identified as a result of inflammation include reduction in the activity of the relaxant NO, as well as enhancing the generation of the constricting factor ET-1, which impairs relaxation and contributes to endothelial dysfunction[84,137]. Additionally, inflammation has also been demonstrated to increase oxidative stress which contributes to endothelial dysfunction[82-84].

In a review article, Trepels et al. reported an association between inflammatory activation and endothelial dysfunction in healthy volunteers, patients at risk, and patients with established CVD[134]. MacKenzie concluded in another review article that inflammatory conditions are related to both the initiation and progression of vascular disease[137]. Furthermore, anti-inflammatory therapeutic interventions have been demonstrated to have a positive impact on endothelial function, as well as disease progression, thus providing an indirect line of evidence linking inflammation with endothelial dysfunction[134,138-143]. In a study conducted on ethnic differences in endothelial function, Marchesi et al. concluded that impaired endothelial vasoreactivity in apparently healthy African Americans, when compared to apparently healthy Caucasians, is related to their increased inflammatory state[136]. The tendency for African Americans to have heightened systemic vascular inflammation, which may contribute to endothelial dysfunction and their CVD-related morbidity and mortality, warrants further investigation into the potential underlying mechanisms.

Endothelial Cells and Activation

Endothelial cells line the internal lumen of the vasculature and are part of the complex system that regulates vasodilation and vasoconstriction, inflammation, and blood flow[114,131,144-146]. They play a key role in cardiovascular regulation by producing vasoactive mediators including NO and ET-1[107,114,145]. Endothelial cells from the umbilical vein of human umbilical cords have been established to be useful for culturing and in research as they are readily available, free from any pathological process, and physiologically more relevant than many established cell lines[146-148]. An example of a method of using endothelial cells to benefit research is the exposure of cultured endothelial cells to laminar shear stress (LSS) which has been demonstrated to result in the stimulation of NO production[149,150].

Inflammatory cytokines have the ability to activate endothelial cells and subsequently accelerate the inflammatory process[23-27]. Oxidative stress has also been demonstrated to contribute to endothelial activation[151]. Activation of endothelial cells has been associated with a loss of the biologic activity of endothelial-derived NO, an effect that can further accelerate the inflammatory process[23]. In addition, suppression of eNOS has been shown to promote endothelial cell activation[152].

Exercise Training and Endothelial Function

It has been well documented that exercise training in human and animal studies improves the health of the endothelium[39-62]. The most notable mechanism in this favorable alteration of endothelial function points to the augmentation of NO-dependent vasodilation of the endothelium in both conduit and resistance vessels[41,48,54]. Regular

physical activity increases blood flow and LSS, resulting in an increased expression of eNOS, as well as NO production and bioavailability[39,41-43,46,47,57]. This beneficial effect of exercise training on endothelial function may be mediated in various ways, including a more favorable oxidant/antioxidant balance, as well as stimulation of anti-inflammatory processes[39,43,44,54,57]. Research data demonstrate that short-term exercise training ameliorates vascular changes by increasing NO bioactivity, whereas long-term exercise training succeeds the short-term functional adaptation with NO-dependent structural changes leading to arterial remodeling[41,56,57].

Improvements in endothelial function have been observed in healthy individuals, hypertensive individuals, individuals with CVD, and those with other CVD risk factors in which endothelial dysfunction is antecedent[41,45-47,55]. The effects of exercise on traditional risk factors for CVD do not fully account for the magnitude of risk reduction[50,56]. Therefore, the direct beneficial effects on the vasculature and endothelial health provide a plausible contribution to the reduction in cardiovascular events associated with exercise training[50,54,56,61]. Furthermore, the protective effects of exercise on CVD risk have been demonstrated to be independent of traditional CVD risk factor modification[51].

Brachial artery flow-mediated dilation (FMD) has been the conventional method used to assess endothelial function and health in humans because of its high feasibility as a non-invasive, ultrasound testing modality. Its evaluation is thought to be an important index that may contribute to understanding the health status of the endothelium in subjects at risk for CVD[153,154]. In research studies that utilized FMD as a marker for endothelial health, favorable adaptations from forearm exercise have been demonstrated

to be restricted to the forearm vessels; however, lower body exercise training has been shown to induce a more generalized benefit[41]. Several studies, including both healthy individuals and those with CVD, reported a significant improvement in FMD subsequent to an exercise training program denoting a favorable adaptation of the endothelium[53,58,62]. The hemodynamic stimuli from exercise, also known as shear stress, exerts direct local effects on the vasculature contributing to improved endothelial function and providing the principal physiological stimulus for vascular remodeling[49,56,58-60].

A review article by Haram et al. reported that pharmacologic strategies used in the treatment of CVD incompletely repair endothelial dysfunction, whereas exercise training has been demonstrated to correct this dysfunction, primarily due to improved production and/or bioavailability of NO resulting in endothelial-dependent relaxation[46]. The ideal dose of exercise training for optimum improvement of endothelial function has not been established; however, moderate physical activity performed on a regular basis has been demonstrated to preserve endothelial function[41,43]. It has been reported that moderate-intensity exercise (~50% VO_{2max}) augments endothelial-dependent vasodilation through the increased production of NO, but high-intensity exercise may contribute to increased oxidative stress[43,45]. In addition, Thijssen et al. reported that exercise has a direct conditioning effect on vascular function, whereas inactivity has a direct deconditioning vascular effect[61]. Thus, exercise training, as it relates to positive adaptations in the health of the endothelium and its subsequent favorable effects on hypertension and CVD risk, should be considered as an important non-pharmacologic intervention in African Americans who have an increased risk for hypertension and CVD.

Inflammation and Endothelial Activation

Inflammation of the endothelium, a pathological mechanism that can cause endothelial dysfunction, has been identified as one factor that may contribute to the development of hypertension. Systemic inflammation involves many pathophysiological processes, and individual inflammatory markers, such as CRP and IL-6, have been used to gauge inflammation. It is thought that the balance between pro- and anti-inflammation plays a crucial role as a determinant of endothelial homeostasis and health[32]. Recent evidence suggests that endothelial activation, characterized by increased inflammation, is an early event in endothelial dysfunction and may be identified with the endothelial microparticle (EMP) that is sensitive to endothelial activation[155].

C-Reactive Protein

C-reactive protein (CRP) is an acute-phase reactant and nonspecific marker of inflammation, produced predominantly in the liver by hepatocytes in response to several cytokines including IL-6[13,15,156-158]. It has evolved as the most robust and reproducible marker of vascular inflammation and has been considered to be the prototypic downstream marker of inflammation[15]. Deposits of CRP have been found in the vascular wall of atherosclerotic plaques[15]. CRP stimulates the monocyte release of IL-6, as well as the expression of adhesion molecules by endothelial cells, further amplifying the inflammatory process[15,159].

Evidence has confirmed a consistent relationship between baseline CRP levels and the risk of future cardiovascular events in healthy subjects, as well as the prediction of future events in those who already exhibit CVD[15,156,157,159-164]. CRP and high blood

pressure, in combination, have an additional predictive value for cardiovascular outcomes since they contribute as independent determinants of cardiovascular risk[164]. Even mild elevations in CRP concentration have been demonstrated to predict cardiovascular events including myocardial infarction, stroke, and vascular death[165]. In addition, research data support an independent positive association between CRP and hypertension, and this association has been reported in healthy individuals, those with prehypertension, as well as those with hypertension[13,15,157,161,164-170]. Boos and Lip, as well as Schillaci and Pirro, reported elevated CRP levels to be predictive for the development of future hypertension in apparently normotensive individuals, and that prehypertensive individuals have higher CRP levels when compared with normotensive individuals[15,165]. The prognostic value of CRP has been demonstrated to be complementary to that of blood pressure values[165,171]. Furthermore, Virdis et al. reported a positive association between CRP and markers of arterial stiffness, thus suggesting a specific interaction between CRP and systolic blood pressure[171].

It has been demonstrated that CRP potently downregulates eNOS transcription in endothelial cells and destabilizes eNOS messenger ribonucleic acid (mRNA) resulting in decreases in both basal and stimulated NO release[15]. This reduction in available NO may be a critical step in the development of atherosclerosis, hypertension, and vascular events[15]. The decrease in eNOS mRNA protein expression demonstrated by CRP may lead to impaired endothelial-dependent relaxation, increased peripheral vascular resistance, large artery stiffness and subsequently hypertension[15,156,165]. In a review article, Bautista concluded that CRP concentration at levels known to predict adverse cardiovascular events significantly reduces eNOS mRNA stability, downregulates the

expression of eNOS, and attenuates NO release and bioactivity in endothelial cells[13]. Another review article by Trepels et al. reported that CRP downregulates eNOS expression and increases oxidative stress with a subsequent decrease in NO production and bioavailability[134].

Pharmacologic treatment, including statins and ANG II receptor blockers (ARBs), has been demonstrated in humans to reduce CRP and vascular inflammatory risk[156,158,160,164]. This decrease in inflammation may lead to subsequent reduction of hypertension and cardiovascular risk. Moreover, there is great variability in CRP levels among ethnicities, and it has been reported that African Americans have the highest levels of CRP[156]. This further emphasizes the importance of inflammation as a potential therapeutic target in the African American population.

Interleukin-6

Interleukin-6 (IL-6) is a pleiotropic cytokine produced by T-cells, macrophages, and endothelial cells under the influence of several mediums, including inflammatory cytokines such as tumor necrosis factor; and it has many diverse physiological roles, involving both proinflammatory responses and cyto-protective functions[13,15,172-174]. IL-6 is a powerful mediator of the hepatic acute phase response and stimulates the synthesis of acute-phase reaction proteins including CRP[13,15,173-176]. In two separate review articles, Bautista, as well as Boos and Lip, reported that IL-6 is known to prolong impaired endothelial-dependent relaxation, which may lead to increased peripheral vascular resistance and consequently hypertension[13,15].

It has been demonstrated that arterial hypertension is positively associated with the release of proinflammatory cytokines, including IL-6, which subsequently induces the synthesis of CRP[134]. Fernandez et al. reported that IL-6 concentration was significantly correlated with both systolic and diastolic blood pressure in apparently healthy subjects[176]. Most studies have reported positive associations between IL-6 and hypertension with the data linking IL-6 and hypertension as less convincing than that for CRP and hypertension[15,174,175,177-181]. Furthermore, Bautista et al. reported that IL-6 may be an independent risk factor for the development of hypertension in apparently healthy individuals[182]. IL-6 has been implicated in the pathogenesis of CVD and has been demonstrated to be positively associated with individuals exhibiting CVD[174,175,183]. In a review article, Fisman and Tenenbaum reported that large quantities of IL-6 have been found in human atherosclerotic plaques[174].

IL-6 has also been demonstrated to be related to endothelial dysfunction. In a study conducted by Naya et al., increased IL-6 levels predicted vascular resistance, used as a marker of endothelial dysfunction, in hypertensive subjects[180]. Another study by Weiss et al. concluded that IL-6 was related to arterial stiffness and other markers of endothelial dysfunction[184]. Bautista reported in a review article that a mild increase in inflammatory markers, including IL-6, results in significant endothelial dysfunction in resistant and conduit vessels by reducing the capacity of the endothelium to generate vasodilating factors, particularly NO[13]. In reviews conducted by Bautista, as well as Boos and Lip, an increase in IL-6 was demonstrated to result in decreased eNOS mRNA, decreased NO synthesis and bioavailability, an increase in NO breakdown and oxidative stress, and decreased vasodilation[13,15]. Based on the evidence that IL-6 is an

inflammatory marker linked to endothelial dysfunction, hypertension, and CVD, viable interventions that may decrease IL-6 levels should be important considerations for endothelial health in African Americans.

Endothelial Microparticles

Endothelial microparticles (EMP) are small vesicles released from the membrane of disturbed endothelial cells and may be distinguished from other classes of extracellular vesicles based on size, content, and mechanism of formation[28,155,185-188]. Endothelial cells release microparticles in response to activation by inflammatory cytokines, and these proinflammatory microparticles have been demonstrated to contribute to prolonged endothelial activation[187,189,190]. In a review article, Shantsila et al. reported that lines of evidence indicate that the shedding of microparticles from cells is a tightly regulated mechanism, and their role as biological messengers is supported by their differential and specific involvement in the pathophysiology of various cardiovascular disorders[28]. EMPs are considered to be biomarkers of vascular injury and proinflammatory conditions, and higher levels of EMPs have been demonstrated to be associated with vascular dysfunction and endothelial damage[28,188,191]. Because they contribute to both the consequence and pathology of conditions, EMPs are considered to be both markers and mediators of pathological conditions[188].

Evidence suggests that endothelial activation, characterized by increased inflammation, is an early event in endothelial dysfunction[155,187-190]. The activated endothelial cells that release plasma membrane submicron vesicles expressing CD62E (E-selectin) into the blood are known as CD62E+ EMPs[29]. CD62E+ is a member of the

selectin family of adhesion molecules expressed primarily on activated vascular endothelium and facilitates the rolling and adhesion of inflammatory cells[28,29]. A study by Lee et al. revealed that high levels of CD62E+ EMPs were associated with cardiovascular events in patients with stroke history, suggesting that systemic endothelial activation increases the risk for cardiovascular morbidities[29]. In a study by Jenkins et al., it was demonstrated that disturbed blood flow induced endothelial activation, as reflected by increases in local concentrations of CD62E+ EMPs[30]. Jimenez et al. conducted a study on endothelial cells by activating them with tumor necrosis factor alpha (TNF-α) to express surface adhesion molecules which participate in leukocyte and platelet recruitments, and demonstrated that the activated endothelial cells released CD62E+ EMPs[31]. The precise function of CD62E+ EMPs remains to be determined, but these EMPs are likely to be involved in the pathogenesis of CVD by promoting inflammation, vascular dysfunction, and coagulation[29]. Based on these findings, plasma levels of CD62E+ EMPs may be useful as a prognostic tool for predicting cardiovascular events.

Although microparticles have been detected in healthy people, the circulating levels of microparticles are significantly increased in pathological conditions such as hypertension and CVD[186-188,191]. EMPs appear to be very sensitive to hemodynamic changes in hypertension, and their numbers are increased even in mild hypertension and rise further in proportion to blood pressure elevation[28]. A review article conducted by Horstman et al. reported that EMPs are elevated in hypertension, and that EMPs are highest in severe hypertension with a significant positive correlation with both systolic and diastolic blood pressure[155]. Furthermore, in patients with CVD, it has been demonstrated that EMPs augment with increased endothelial dysfunction[186]. Therefore,

EMPs may be used as an important marker as their detection and quantification may be valuable tools to assess cardiovascular risk[185].

The endothelial dysfunction caused by circulating human microparticles seems to be mediated by particles of endothelial origin, and has been associated with a disabled production and release of NO from endothelial cells, but not with eNOS protein expression[185,187]. This unfavorable alteration in the endothelial NO transduction pathway has been demonstrated to result in impaired endothelial-dependent relaxation[29]. Horstman et al., as well as Boulanger et al., reported that experimental evidence suggests that plasma levels of EMPs are specific emerging markers of endothelial dysfunction and have a potential prognostic value for major adverse events in patients with CVD[155,186,187]. The expression of EMPs seems to reflect the degree of endothelial dysfunction; therefore, the detection and quantification of EMPs may be a potential valuable tool to assess cardiovascular risk even in asymptomatic patients[187]. Boulanger et al. concluded that the greater amount of EMPs detected, the less likely for arteries to normally vasodilate in response to blood flow[187]. The measurement of EMPs appears to be the most sensitive method for assessing blood pressure-induced effects on the endothelium, and subsequent risk of impending hypertensive vascular damage[155].

Pharmacologic treatments have demonstrated positive alterations in EMPs. It has been reported that statins impair the formation of EMPs, and antioxidants decrease the number of EMPs[187]. Interventions that target the reduction of EMPs and subsequently favorably alter the formation and/or concentration levels of EMPs should be considered based on the evidence.

Exercise Training and Inflammation

Aerobic exercise training (AEXT) has been demonstrated to improve the plasma inflammatory status, including CRP and IL-6, in certain populations; however, the effect of AEXT on levels of CD62E+ EMPs has not been previously investigated. In a review article on exercise training and CRP, Plaisance and Grandjean reported that the majority of studies conducted on a variety of populations suggest that long-term AEXT reduces CRP levels, and evidence from both cross-sectional and longitudinal studies suggests that exercise training independently lowers CRP as much or more than statins when compared in similar populations[192]. Furthermore, the authors reported that although CRP levels in those with medical conditions such as hypertension and CVD were higher at baseline, thereby demonstrating a higher reduction in CRP levels, this reduction reveals the robustness of CRP as a risk marker[192]. Independent review articles by Lavie et al., as well as Nicklas et al., concluded that the effects of AEXT on inflammatory markers, such as CRP and IL-6, remain unclear as some of the studies examined only report a significant reduction in inflammatory biomarkers with significant weight loss[193,194].

In a review conducted by Hopps et al., on studies including subjects with type 2 diabetes and engaged in a combination of AEXT and resistance training, significant reductions in plasma levels of CRP and IL-6 were reported[195]. In addition, Leung et al. concluded in a review article that both CRP and IL-6 were demonstrated to be significantly reduced subsequent to exercise training interventions[39]. Beavers et al. reviewed studies that included at least 20 subjects, had supervised AEXT programs, and in which there were no significant changes in body weight post-exercise training[196]. The authors reported that there were significant reductions in both CRP and IL-6 following

exercise training, and the reductions were greater in those with increased baseline inflammation[196]. In another review article by Kasapis and Thompson, the authors concluded that CRP significantly decreases subsequent to exercise training[197]. Downing and Balady reported in their review that there were significant reductions in IL-6 in patients with heart failure after participating in AEXT interventions[198]. In a review article involving studies conducted on coronary artery disease patients participating in AEXT interventions lasting at least two weeks, Swardfager et al. reported significant reductions in both CRP and IL-6 subsequent to exercise training[199].

A study conducted by Donges et al. on the effects of 10 weeks of aerobic exercise or resistance training on CRP and IL-6 demonstrated that, subsequent to the programs, both aerobic exercise and resistance training significantly decreased plasma levels of CRP; however, IL-6 was not significantly changed subsequent to either of the exercise training programs[200]. Another study conducted by Beckie et al. found that a 12-week cardiac rehabilitation AEXT program significantly reduced plasma levels of both CRP and IL-6 in women with coronary heart disease[201]. An additional study conducted by Goldhammer et al. on the effects of exercise training, including male and female coronary heart disease patients, demonstrated that both CRP and IL-6 plasma levels significantly decreased subsequent to 12 weeks of AEXT[202].

These findings suggest an overall beneficial effect of AEXT on the inflammatory status as identified by plasma biomarkers in various populations. Although AEXT has been demonstrated to improve plasma levels of CRP and IL-6 in certain populations, the effect of AEXT on CD62E+ EMPs has not been elucidated. Furthermore, AEXT may lead to the adaptive response of decreasing plasma CRP and IL-6 concentrations, as well

as CD62E+ EMPs, in African Americans, thereby improving endothelial function by reducing inflammation. Few studies have investigated the effects of AEXT on inflammation and endothelial health in an effort to develop preventive measures to reduce hypertension and CVD among this high-risk population. Therefore, designing AEXT interventions that benefit African Americans may have a positive impact on their health.

Anti-Inflammation and Endothelial Health

As previously reported, there is a large body of evidence for the role of inflammation in endothelial dysfunction, hypertension, and CVD. Although there has been limited knowledge regarding the role of anti-inflammatory mechanisms, it has become an emerging topic as some of the mechanisms and plasma biomarkers involved in anti-inflammation and endothelial health have been elucidated.

Interleukin-10

Interleukin-10 (IL-10) is a pleiotropic cytokine secreted by activated macrophages, monocytes, and lymphocytes, and it has been demonstrated to have several anti-inflammatory characteristics including inhibition of NF-κB with subsequent suppression of cytokine production[134,203-208]. IL-10 potently inhibits production of proinflammatory cytokines, including IL-6 and TNF-α, and the inhibitory effects of IL-10 on TNF-α have been revealed to be crucial to its anti-inflammatory properties[207-209]. The principal routine function of IL-10 appears to be to physiologically limit and ultimately terminate inflammatory responses[206,208,210]. The STAT3 pathway is the signaling pathway that has been identified to be activated by the binding of IL-10 to the IL-10

receptor in order to generate the anti-inflammatory response, and it has also been identified to be essential for all known functions of IL-10[203,206,207].

IL-10 has been demonstrated to be capable of modulating pathways that may play an important role in the development, progression, and stability of atherosclerotic plaques[205]. Perez-Fernandez and Kaski reported, in a review comprising human and animal studies, that low levels of IL-10 lead to the development of extensive and unstable atherosclerotic lesions, thereby suggesting a potential protective role for IL-10 in atherosclerosis[204]. In a review conducted by Murray, it was reported that genetic studies using IL-10 deficient and IL-10 transgenic mice establish the unequivocal importance of IL-10 in controlling inflammation initiated and perpetuated by proinflammatory signals in both acute and chronic diseases[203]. Girndt and Kohler concluded in another review article that IL-10 was demonstrated to be protective against atherosclerotic disease in animal models[210].

In patients with coronary artery disease and evidence for an ongoing inflammation as indicated by elevated CRP levels, increased serum levels of IL-10 have been associated with improved systemic endothelial vasoreactivity, providing evidence for a crucial role of the balance between pro- and anti-inflammation as a major determinant of endothelial health[32]. A study conducted by Heeschen et al. demonstrated that IL-10 and CRP were inversely related, and that elevated IL-10 serum levels were associated with a more favorable prognosis in patients with acute coronary syndrome and elevated CRP levels[211]. Furthermore, recent evidence has indicated that IL-10 has a protective effect on hypertension through ameliorating endothelial function. IL-10 has been demonstrated to reduce oxidative stress, improve endothelial function, and lower

blood pressure in hypertensive mice[33]. In an additional study, Tinsley et al. reported that exogenous IL-10 normalized blood pressure and endothelial function in pregnancy-induced hypertensive rats[209].

IL-10 has been positively associated to endothelial function in various research studies, and the mechanisms by which IL-10 may improve endothelial health seem to point to a role for the balance of pro- and anti-inflammation[33-37,134]. A study by Kassan et al. revealed that IL-10 improved endothelial function by attenuating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, and this was positively associated with an increase in eNOS phosphorylation and improved endothelial-dependent relaxation in hypertensive mice[33]. Another study on mice conducted by Zemse et al. demonstrated that IL-10 restored the eNOS expression that had been reduced by TNF-α in both *in vivo* and *in vitro* experiments, and concluded that IL-10 may prevent impairment in endothelial-dependent relaxation caused by TNF-α by protecting eNOS expresssion[34]. Similarly, Giachini et al. demonstrated that IL-10 counteracted vascular constrictory responses to ET-1 following infusion of TNF-α in mice, which may be a mechanism for the vasculoprotective effects of a classic anti-inflammatory cytokine[35]. Gunnett et al. conducted a study using mice and demonstrated that IL-10 protected endothelial function by limiting increases in superoxide[36]. Another study utilizing mice provided evidence that endogenous IL-10 limited ANG II-mediated increases in IL-6, oxidative stress, and vascular dysfunction both *in vitro* and *in vivo* suggesting that at least some of the protective effects of IL-10 may occur within the vessel wall[37]. Cattaruzza et al. demonstrated that IL-10 increased eNOS expression in HUVECs that were pre-

incubated with TNF-α, and concluded that this increase in eNOS may be a mechanism in which IL-10 exerts its anti-inflammatory effects[38].

It is widely accepted that most drugs being used for primary and secondary preventions of CVD have anti-inflammatory properties[134]. In a review article, Trepels reported that studies have demonstrated a protective effect of anti-inflammatory drugs on endothelial function, suggesting that the detrimental influence of inflammation on endothelial integrity may be amenable to therapeutic interventions[134]. Typically, anti-inflammatory drugs target one proinflammatory mediator, whereas IL-10 has been demonstrated to target multiple proinflammatory mediators[203]. Based on this evidence, there may be significant benefits for using IL-10 to treat endothelial dysfunction, hypertension, and other inflammatory diseases[203].

Endothelial Nitric Oxide Synthase

Endothelial nitric oxide synthase (eNOS) is one of three isoforms of nitric oxide synthases that generates NO, an endothelial-derived relaxing factor that plays a crucial role in vascular tone and regulation, and also has anti-inflammatory actions[212-219]. The mechanism by which eNOS synthesizes NO is by catalyzing the oxidation of L-arginine to produce NO and L-citrulline[213,219,220]. Phosphorylation of eNOS at Serine 1177 by Akt has been demonstrated to be a critical requirement for eNOS activation[213,221,222]. The controlled regulation of eNOS affects the bioavailability of NO; hence, eNOS may be essential for endothelial function and cardiovascular health[217,219,221]. Superoxide can react with the NO released by eNOS, thereby generating ONOO- which in turn has been demonstrated to uncouple eNOS switching it from an anti-atherosclerotic NO-producing

enzyme to a dysfunctional enzyme that can produce superoxide and reaction oxygen species (ROS)[223-227]. Increased superoxide production by endothelial cells may have important consequences with respect to signaling[219,223].

The uncoupling of eNOS has been demonstrated to be one of the major underlying mechanisms of endothelial dysfunction which occurs in hypertension[217,228,229]. Impaired eNOS results in unfavorable alterations in the expression and bioactivity of eNOS and subsequent decreased NO bioavalability[216,225,230]. This altered expression of eNOS has been associated with endothelial dysfunction, as well as hypertension[212,214,216,219,220,227,229-231]. In a review article, Albrecht et al. reported that the inhibition of eNOS induced significant increases in blood pressure demonstrating that eNOS regulates vascular tone in humans[214]. Furthermore, the absence of eNOS activity in eNOS-deficient mice resulted in poor vasorelaxing activity, hypertension, and increased atherosclerotic lesions[214]. Other studies also have reported an association between dysfunctional eNOS and atherosclerosis[219,224,230].

It has been proposed that the modulation of eNOS may be a useful therapeutic strategy for endothelial function, hypertension, and CVD[232-234]. Optimizing eNOS function by enhancing the expression and activity of eNOS may reverse endothelial dysfunction and thus reduce hypertension and CVD[233,234]. Kietadisorn et al., as well as Roe and Ren, suggested that modulating eNOS by stabilizing its activity and reversing uncoupled eNOS may be a therapeutic approach to improve endothelial function[217,229]. Furthermore, Forstermann and Li proposed that preventing eNOS uncoupling and enhancing eNOS expression may be a viable therapeutic target[227]. Pharmacologic therapy, including statins, ACE inhibitors, and ARBs, have been demonstrated to

upregulate eNOS expression, increase NO, and have favorable effects on hypertension and CVD[215,231]. Furthermore, inhibition of eNOS activity using pharmacologic interventions has been demonstrated to play a potential role for eNOS in inflammatory diseases by increasing platelet aggregation[214]. Unfavorable alterations in eNOS may contribute to the inflammatory status that has been associated with hypertension and CVD, providing further rationale for interventions that may improve the bioactivity of eNOS.

Nitric Oxide

Nitric oxide (NO) is a gaseous, free radical, cellular signaling messenger released by the endothelium and generated from L-arginine by one of three nitric oxide synthases including eNOS[212,214,216,220,235-240]. It plays an important role in the protection against the onset and progression of endothelial dysfunction, hypertension, atherosclerosis, and CVD[214,235]. The cardioprotective roles of endothelial-derived NO include regulation of blood pressure and vascular tone, inhibition of platelet aggregation and leukocyte adhesion, as well as prevention of smooth muscle cell proliferation and migration[214,216,219,220,235-238,240-246]. Furthermore, NO has been demonstrated to exert an anti-inflammatory influence by protecting endothelial cells against inflammatory activation[216,245]. NO is considered to be the chief molecule in the regulation of endothelial homeostasis with vasodilation as its primary function[237,239,240,243-245,247]. Disturbances in NO bioavailability may lead to a loss of the cardioprotective actions and may increase disease progression[235].

A balanced release of vasoconstricting and vasorelaxing factors from the endothelium is characteristic of optimum physiological conditions[248,249]. The prototypic endothelial-dependent relaxing factor is NO which regulates endothelial-dependent vasodilation, and the prototypic endothelial-dependent vasoconstricting factor is ET-1, with ANG II and superoxide also contributing to constriction[242,248]. The vasorelaxing properties of NO antagonize the vasoconstrictive effects of ET-1, ANG II, and ROS, whereas increased oxidative stress mediates ET-1 and ANG II[249]. Conditions characterized by an impaired bioavailability of NO have been associated with enhanced synthesis of ET-1, and vice versa, thereby suggesting that these two factors have a reciprocal regulation[242]. An unfavorable alteration of the balance between vasorelaxing and vasoconstricting factors may play a decisive role in the development of hypertension, atherosclerosis, and CVD[248,249].

Vascular endothelial health depends on processes controlling the synthesis, bioavailability, and destruction of NO[250]. Diminished NO bioavailability and abnormalities in NO-dependent signaling have been demonstrated to be central factors of vascular disease[238]. NO has the capability of reducing the generation of ROS, but it also can react with superoxides resulting in the formation of ONOO-, which deteriorates endothelial function[249]. Endothelial dysfunction has been characterized by a reduction in the bioavailability of NO and subsequent impaired vasodilation, as well as an increase in the activity of vasoconstrictors including ET-1, ANG II, and ROS[251]. NO antagonizes the vasoconstrictive effects of ANG II by downregulating synthesis of ACE and ANG II receptors, whereas ANG II decreases NO bioavailability by promoting oxidative stress which contributes to endothelial dysfunction and hypertension[240,246,250,251].

Impaired endothelial-derived bioavailability of NO, in part due to excess vascular oxidative stress, impairs vasodilation and has been associated with endothelial dysfunction, hypertension, atherosclerosis, and CVD[216,219,220,226,234,237-244,248,250,252-257]. Hermann et al. demonstrated that the inhibition of NO is implicated in arterial stiffness and resulted in an increase in blood pressure, thereby suggesting that NO plays a crucial role in the regulation of blood pressure[253]. In a review article, Chowdhary and Townend reported that inhibiting NO production results in hypertension in both humans and animals[247]. Furthermore, it has been demonstrated that hypertensive patients have blunted vasodilatory responses to normal vasodilatory stimuli when infused with endothelial-dependent vasodilators[253,254].

Pharmacologic interventions, including exogenous NO, that enhance endothelial NO bioavailability have been demonstrated to restore vasodilation while reducing clinical events[254,257]. It has been demonstrated that ACE inhibitors augment endothelial-dependent vasodilation through an increase in NO bioavailability by an increase in NO production and a decrease in NO inactivation, and that ARBs have a favorable effect on endothelial function and the prevention of cardiovascular complications[142,143,236,255]. Other therapeutic agents that inhibit the synthesis and action of ANG II have also been demonstrated to increase the bioavailability of NO[246]. The balance between vasodilating substances such as NO and vasoconstricting substances has been implicated to be important for vascular function and endothelial health, and emphasizes the importance of therapeutic interventions that may enhance the bioavailability of NO.

Exercise Training and Anti-Inflammation

Research studies have examined the effect of exercise on circulating plasma levels of IL-10. In a review article on exercise training and anti-inflammation in heart failure patients, Batista et al. proposed that the anti-inflammatory effect induced by AEXT seems to primarily be mediated by IL-10[258]. Hopps et al. reported in a review article that combined aerobic and resistance exercise training in subjects with type 2 diabetes resulted in anti-inflammatory effects including a significant increase in IL-10[195]. The effect of eight weeks of AEXT on the plasma inflammatory status of post-myocardial infarction patients was examined by Ribeiro et al., and it was demonstrated that AEXT significantly increased IL-10 suggesting enhancement of anti-inflammation[259]. Furthermore, Goldhammer et al. demonstrated that a 12-week AEXT intervention in coronary artery disease patients was effective in increasing IL-10, leading to improvement in coronary risk status[202].

The effect of exercise training on expression of eNOS has been examined by researchers. In a review article on the effect of exercise on endothelial function in multiple animal studies, Leung et al. reported that exercise training mediates inflammation by increasing vascular eNOS expression, as well as eNOS phosphorylation at Serine 1177[39]. Furthermore, Hambrecht et al. conducted a study on the effect of four weeks of exercise on endothelial function in stable coronary artery disease patients and demonstrated that AEXT increased expression of both eNOS and phosphorylated eNOS on Serine 1177[260]. Two independent studies on mice conducted by Davis et al. and Fukai et al. demonstrated that eNOS expression was increased subsequent to AEXT[261,262].

Several studies have demonstrated an increase in NO production as a result of exercise training. In a review conducted by Green et al., it was reported that exercise training upregulates NO production which mediates exercise hyperemia and controls blood flow by modifying other physiological mechanisms[263]. Gomes et al. demonstrated that AEXT increased plasma levels of NO and decreased oxidative stress in patients with metabolic syndrome[264]. In another study conducted by Maeda et al., significant increases in NO and significant decreases in ET-1 were reported following eight weeks of exercise training in healthy, young humans[265]. Lewis et al. examined the effects of four weeks of cycle training on basal NO production in hypercholesterolemic patients, and a significant increase in endothelial-derived NO was demonstrated[266]. A recent study by Krause et al. reported that NO was increased in obese subjects following a 16-week moderate AEXT intervention[267].

Other research data have examined both plasma levels of NO and eNOS protein expression, and demonstrated favorable alterations. In a review article on multiple studies in humans and animals, Rush et al. reported that AEXT improves NO-dependent endothelial function through an increase in eNOS expression and bioavailability of NO, and this was reported in healthy individuals, as well as individuals with hypertension and CVD[250]. Green et al. conducted a review on multiple studies of exercise training in humans and animals and concluded that exercise training upregulates eNOS protein expression and phosphorylation, and that increases in NO with AEXT were demonstrated in human subjects with CVD, risk factors for CVD, and in whom antecedent endothelial dysfunction exists[41]. In another review by Kingwell, it was reported that exercise training increases NO bioavailability and eNOS protein expression in healthy individuals,

as well as individuals with CVD risk[268]. Furthermore, in a review by Higashi and Yoshizumi, it was demonstrated that eNOS protein expression was improved in healthy individuals and in hypertensive individuals, and that NO bioavailability was enhanced through an increase in NO production and a decrease in NO inactivation[47]. Zhou et al. examined the effects of exercise training on NO, eNOS protein expression, and phosphorylated eNOS protein expression in swine, and reported significant increases subsequent to exercise training[269]. An additional study conducted on rats by McAllister et al. reported significant increases in eNOS protein expression as well as NO-mediated dilation of conductant and resistance vessels[270].

The evidence for the favorable effect of exercise training on eNOS protein expression and NO bioavailability has been well-established; however, knowledge of the exercise training effect on IL-10 is somewhat limited. Specifically, for the African American population, in whom inflammation and subsequent endothelial dysfunction and hypertension may be more of a detriment when compared with other ethnic populations, research data, including the anti-inflammatory biomarkers, IL-10, eNOS and NO, are limited. Interventions that elucidate the possible favorable alterations of AEXT on mechanisms of anti-inflammation in African Americans would be advantageous for this population.

Shear Stress

Blood flow-induced shear stress is a hemodynamic force acting on the vessel wall and endothelial surface that is mechanotransduced into a biochemical signal, results in vascular changes, and affects endothelial cell phenotype and function[49,271-274]. Increased

cardiac output and blood flow during exercise modulate hemodynamic forces, and the shear stress generated by exercise has been attributed to the beneficial effects of exercise on vascular health[49,56]. Shear stress is computationally estimated using fluid dynamic models and is expressed in units of dynes/cm^2 which is frictional force per unit area[271,272,275]. Malek et al. reported that arterial level shear stress (>15 dynes/cm^2) induces endothelial quiescence and an atheroprotective gene expression profile, whereas low shear stress (<4 dynes/cm^2) stimulates an atherogenic phenotype[272]. Measurements have demonstrated that shear stress ranges from 1 to 6 dynes/cm^2 in the venous system and between 10 and 70 dyne/cm^2 in the arterial vascular network[272,276]. Shear stress actively influences vessel wall remodeling with chronic increases in blood flow and shear stress leading to expansion of the luminal radius, and decreases in blood flow and shear stress inducing a reduction in the luminal radius[56,272,275]. Endothelial cells are subjected to various forms and magnitudes of shear stress which produce differences in the resulting endothelial cell phenotype and function[49,275].

Laminar vs. Oscillatory Shear Stress

Laminar blood flow produces predominantly antegrade shear stress along the endothelial cell surface, and has been characterized by steady, undisturbed blood flow[49,272]. Although this type of flow is pulsatile, endothelial cells can well adapt because the flow is non-turbulent[272,277]. Cobblestone-shaped endothelial cells of random orientation are transformed into fusiform endothelial cells aligned in the direction of flow[272,278,279]. Endothelial cells exposed to LSS typically exhibit an anti-atherogenic phenotype[49,56,275]. The upregulation of eNOS protein expression and increased NO, as well as decreased ET-1, inflammatory activation, and oxidative stress, have been

demonstrated in response to physiological levels of LSS[49,56,149,150,271,272,275,277]. Maintenance of a physiologic, LSS is known to be crucial for normal vascular functioning, and is considered to be atheroprotective by promoting an anti-inflammatory, anti-oxidative, anti-proliferative, and anti-apoptotic environment[271,273,280,281].

Bifurcations in the arterial tree modify laminar blood flow and shear stress into areas of low shear stress and oscillatory flow patterns beyond the bifurcation[49,271,276,277]. These areas are prone to atherosclerotic lesions and plaques, and have negative effects on the endothelium resulting in endothelial dysfunction[49,271,274,276-278]. Endothelial cells that are exposed to conditions of low, oscillatory flow do not align in the direction of blood flow[272,277,278]. Oscillatory shear stress has been demonstrated to decrease eNOS protein expression, induce the expression of ET-1, and promote leukocyte adhesion, inflammation, and oxidative stress[49,271-273,275,277].

In Vitro

The beneficial effects of exercise on vascular health have been attributed to the exercise-induced increase in mean shear stress, and this has been supported by data obtained from cell culture[19,56,63-69]. In a review article, Ando and Yamamoto reported that cultured endothelial cells subjected to LSS alter their morphology, function, and genetic expression[63]. Zhang and Friedman conducted a study on the effect of shear stress magnitude on porcine endothelial cells, and demonstrated that elevated shear stress has a predominantly anti-inflammatory and anti-oxidative atheroprotective effect on the endothelial cells[64]. In bovine aortic endothelial cells, Go et al. demonstrated that shear stress triggers activation of PI3k/Akt and increases eNOS expression leading to

production of NO[65]. Three independent studies conducted by Malek et al. on bovine aortic endothelial cells demonstrated that LSS increases eNOS protein expression and decreases ET-1[66-68].

The effects of shear stress on cultured human endothelial cells have also been reported. Brooks et al. conducted a study on cultured human aortic endothelial cells exposed to 24 hours of high levels of laminar flow (13 dynes/cm^2) or low levels of disturbed flow (<1 dyne/cm^2), and demonstrated that laminar flow represents changes that are atheroprotective, whereas disturbed flow induces a proatherogenic phenotype by upregulating proinflammatory, proapoptotic, and procoagulant molecules[69]. Brown and Feairheller examined the effects of high levels of shear stress on cultured African American HUVECs and reported significant improvements in eNOS protein expression and NO concentrations subsequent to high levels of LSS[19].

Subject-specific models of the human abdominal aorta were constructed from magnetic resonance angiograms of five healthy subjects, and computer simulations were performed under resting and exercise (50% increase in resting heart rate) pulsatile flow conditions in a study conducted by Tang et al[282]. The combination of magnetic resonance angiograms and computational fluid dynamics used in this investigation enabled a more complete characterization of the hemodynamic conditions that exist in the human abdominal aorta under resting and exercise conditions[282]. It was demonstrated that even relatively light levels of exercise were sufficient to improve local unfavorable hemodynamic conditions, such as low, oscillatory shear stress that were present at rest, and the authors concluded that exercise may lead to protection against the development or progression of atherosclerosis[282].

In Vivo

It is generally accepted that shear stress resulting from exercise is a principal physiological stimulus experienced by the endothelium in humans, and that exercise training has an impact on endothelial function[56]. In a review article, Newcomer reported that low-intensity exercise falls below a threshold for improvement in endothelial function, moderate-intensity exercise enhances endothelial function, and that at higher intensities, endothelial function may not necessarily be enhanced[56]. Effects on the vasculature and specific pathways associated with shear stress have been identified which provide a basis for the direct vascular conditioning effects of exercise[61].

The purpose of a study conducted by Tinken et al. was to demonstrate whether exercise-induced shear rate was responsible for improvements in endothelial function after eight weeks of bilateral handgrip training with shear rate restriction in one arm, and the results were improvements in FMD in the non-restricted arm and unaltered FMD in the restricted arm [58]. In another study by Tinken et al., FMD was examined in both brachial arteries of healthy young men before and after 30-minute interventions consisting of bilateral forearm heating, recumbent leg cycling, and bilateral handgrip exercise with a cuff placed on one arm to unilaterally manipulate the shear rate stimulus[59]. In the cuffed arm, antegrade shear rate was lower than in the noncuffed arm for all of the conditions, and the increase in FMD was abolished in the cuffed arm suggesting that differences in shear rate transduce differences in endothelial vasodilator function in humans[59]. Thijssen et al. conducted a study on different magnitudes of acute retrograde shear rate and their effect on endothelial function using an intervention that comprised 30 minutes of partial forearm occlusion with three distinct doses of retrograde

shear rate versus a control arm[60]. The result was a progressive dose-response decline of FMD in the cuffed arm and no changes in the noncuffed arm, suggesting that increases in retrograde shear rate induce a dose-dependent attenuation of endothelial function in humans[60].

Taken together, *in vitro* and *in vivo* data imply that the hemodynamic influences of shear stress on the vessel wall and endothelial surface combine to orchestrate a response to exercise and exercise training that regulates peripheral vascular resistance and results in vascular changes as well as changes in endothelial cell phenotype and function. These favorable alterations may contribute to the health of the endothelium and have a subsequent positive effect on CVD risk factors such as hypertension.

Conclusion

There are unique factors that have been linked to hypertension in different ethnic populations. Knowledge of anti-inflammatory mechanisms of endothelial activation and dysfunction is limited, especially in the African American population. More research is warranted that has the potential to elucidate anti-inflammatory mechanisms in the vascular wall of African Americans. Identifying these potential factors may contribute to our understanding of the possible etiological mechanisms of vascular inflammation and endothelial dysfunction in African Americans. This ultimately may translate to a healthier endothelium and a reduced risk for hypertension and CVD within this population who have a heightened level of systemic inflammation and the highest incidence of hypertension.

CHAPTER 3

RESEARCH DESIGN

In Vivo Study

The protocol for this study was approved by and conducted in accordance with Temple University's Institutional Review Board. This study employed a pre-post design following the completion of screening, dietary stabilization, and antihypertensive medication tapering. Sedentary, putatively healthy, middle-to-older age (40-71 y/o) African American men and women were recruited and underwent a series of screening tests to ensure they were free of disease and conditions that may confound interpretation of results. All qualified subjects completed a dietary stabilization period in order to control for the effects of interindividual variations in dietary intake. Subjects using antihypertensive monotherapy were appropriately tapered from their medication, and suspension of medication was continued for the duration of the study. This was done to avoid an aerobic exercise training (AEXT) by medication interactive effect. Following dietary stabilization and a minimum of two weeks after medication tapering, baseline testing was conducted. This included office blood pressure measurements, fasting blood sampling, and graded exercise testing. Upon completion of baseline testing, subjects engaged in a 6-month AEXT intervention under the direct supervision of appropriately trained laboratory personnel. At the conclusion of the 6-month intervention, subjects repeated all baseline tests. The post-AEXT testing was performed a minimum of 24 hours following the subject's final exercise session in order to control for the acute

effects of exercise on hemodynamic and biochemical variables. The *in vivo* study experimental design flowchart is depicted below. (Figure 3.1)

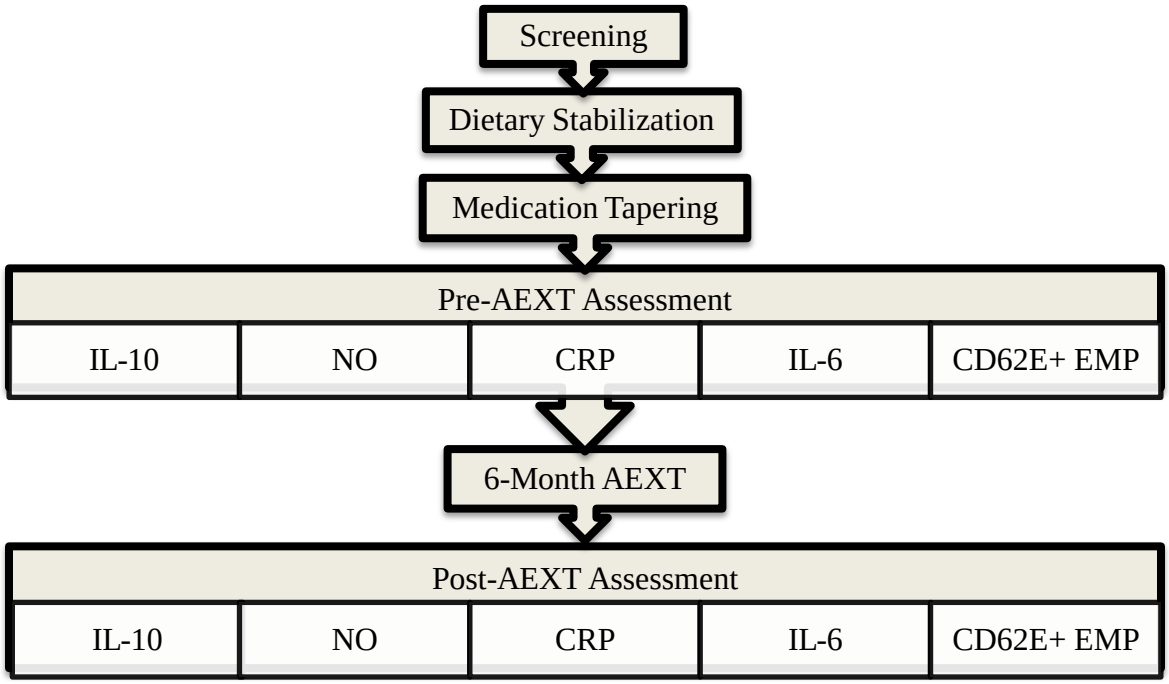

Figure 3.1. *In vivo* study experimental design flowchart.
IL-10 (Interleukin-10), NO (nitric oxide), CRP (C-reactive protein), IL-6 (Interleukin-6), EMP (endothelial microparticle)

Subjects

African American men and women within the city of Philadelphia and surrounding communities were recruited via mailed brochures and local newspaper advertisements. Prospective subjects were interviewed by laboratory personnel by telephone to assess eligibility. Subjects were between the ages of 40-71 years, sedentary (self-reported, regular aerobic exercisers $\leq$ 2 days per week), non-diabetic (fasting blood glucose < 126 mg/dL), non-smoking ($\geq$ 2 years), had a clinic resting blood pressure < 160/100 mmHg (i.e., not stage II hypertensive), and had no documented history of

cardiovascular disease (CVD), hypercholesterolemia (total cholesterol ≤ 240 mg/dL), renal disease, or pulmonary disease. Subjects on lipid-lowering medications, medications that affect cardiovascular or renal hemodynamics, or who were taking more than one antihypertensive medication were excluded from this study. Both premenopausal and postmenopausal (self-reported absence of menses) women were included in the study. All postmenopausal women were required to continue their hormone replacement therapy, either on or off, for the duration of the study. These inclusion criteria were used to create a more homogeneous group of middle-to-older age African Americans who were at low-to-moderate risk for CVD, but who were otherwise putatively healthy. Subjects who apparently met the inclusion criteria were scheduled for an orientation visit and mailed a medical history questionnaire. During the first laboratory orientation visit, subjects were given the explanation of the study, and medical history was reviewed to determine if there were any criteria that would exclude them from the study. Written informed consent (Appendix A) was given to the subjects following a complete explanation of the study.

Screening

Eligibility of all qualified subjects was ensured via completion of three screening visits prior to inclusion in the study. Screening visit one followed a 12-hour post-absorptive, single blood sampling to assess blood chemistries and a urinalysis to assess renal function. Any individual with a total cholesterol > 240 mg/dL or fasting blood glucose ≥ 126 mg/dL was excluded from this study. Estimated glomerular filtration rate (eGFR) was calculated using the four-variable modification of diet in renal disease

(MDRD) study equation specific to African Americans. Any participant who exhibited evidence of renal disease (eGFR < 60 ml min^{-1} per 1.73m^2) was excluded from the study.

Screening visits two and three required all qualified subjects to undergo a physician-administered physical examination and a cycle ergometer echocardiogram stress test to confirm that the subjects displayed no evidence of latent cardiovascular, pulmonary, or other chronic diseases. The echocardiogram stress test was conducted on a separate day following the completion of a successful physical exam. Subjects were required to have a < 2mV electrocardiogram (ECG) ST-segment depression and have no cardiovascular signs/symptoms at rest and at three different exercise workloads (25, 50, and 75 W) during the stress test.

Dietary Stabilization

In order to control for the confounding effects of variations in dietary intake, subjects who met the inclusion criteria after the screening visits underwent a dietary stabilization period for six consecutive weeks prior to testing. Subjects met with a Registered Dietician and were instructed on what to eat according to the American Heart Association (AHA) Dietary Guidelines for Healthy Adults, a diet formerly known as AHA Step 1 Diet[283]. All subjects were encouraged to remain on the diet for the duration of the study. Compliance to the prescribed eating plan was monitored by completion of a 3-day food log at the conclusion of dietary stabilization and bi-monthly thereafter.

Antihypertensive Medication Tapering

Subjects receiving antihypertensive monotherapy were tapered off their medication under the supervision of the study cardiologist. Those who needed

medication tapering were given a home blood pressure monitor in order to measure their blood pressure two times daily (morning and evening) which they logged and reported to lab personnel via telephone. If a participant's resting systolic or diastolic blood pressure was >159 or >99 mmHG, respectively, for three consecutive days, medication therapy was immediately resumed and the participant was excluded from further participation in the study. Medications were withdrawn for a minimum of two weeks before baseline testing.

Office Blood Pressure Measurements

Office blood pressure measurements were made on three separate visits by laboratory personnel in accordance with the guidelines from The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure[71]. Blood pressure was measured using a mercury sphygmomanometer subsequent to at least five minutes of quiet rest in a chair, with feet on the floor, and arm supported at heart level. An appropriate size cuff was determined by upper arm circumference. Blood pressure values were identified using the first and fifth phase of Korotkoff sounds. For each visit, blood pressure measurements were performed in triplicate, five minutes apart, and the mean of the three values was used as the blood pressure measurement for that visit.

Plasma Samples

Blood samples were collected in the morning following a 12-hour overnight fast. Blood was drawn into ethylenediaminetetraacetic acid (EDTA) contained vacutainer tubes, centrifuged at 2,000 g for 20 minutes at 4 ^{o}C, and the plasma was frozen at -80 ^{o}C

until the time of the assays. Levels of nitric oxide metabolites (NOx), including nitrite and nitrate, were measured using a modified Griess Assay. Reagents were obtained from Assay Designs (Ann Arbor, MI). Concentrations of Interleukin-10 (IL-10) and Interleukin-6 (IL-6) were determined using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Assays were conducted and analyzed according to manufacturer's protocol. Absorbance was recorded using a Spectra Max Microplate Reader (Molecular Devices, Sunnyvale, CA). The plate was read at 540 nm for NOx, at 490 nm with correction for optical imperfections at 650 nm for IL-10, and at 450 nm with correction for optical imperfections at 540 nm for IL-6. Intraassay and interassay coefficients of variation (CV) were 10.6% and 7.6% respectively for NOx, 5.5% and 11.9% respectively for IL-10, and 7.4% and 4.5% respectively for IL-6. Plasma samples for CRP were sent to Quest Diagnostics Inc. for analysis. Quest Diagnostics is a Clinical Laboratory Improvement Amendments (CLIA) certified lab pursuant to Section 353 of the Public Health Services Act 42 U.S.C. 263a.

Endothelial Microparticles Identification and Quantification

Circulating endothelial microparticles (EMP) were quantified using a venous blood sample obtained from the antecubital vein in the morning following a 12-hour overnight fast. Samples were collected in EDTA contained vacutainer tubes using a 21-gauge needle and were centrifuged at 2,000 g for 20 minutes at 4°C immediately after collection to separate plasma from whole blood. Plasma samples were stored at -80°C until measurement. On the day of analysis, two sequential centrifugation steps were used to reduce background signals contributed by plasma proteins and residual contaminating/unwanted cells, and to concentrate microparticles in order to improve the

signal-to-noise ratio during flow cytometric analysis. First, plasma samples were thawed and centrifuged at 1,500 g for 20 minutes at room temperature to obtain platelet-poor plasma (PPP). The top two-thirds volume of PPP were then transferred to a new tube and further centrifuged at 1,500 g for 20 minutes at room temperature to obtain cell free plasma. The supernatant was used for microparticle analysis. A volume of 100 µl supernatant was incubated with fluorochrome-labeled antibodies for 20 minutes at room temperature in the dark and then fixed by adding 93 µL of 10% formaldehyde. The mixture was protected from light and incubated while being gently mixed using a shaker for 20 minutes. The antibody CD62E-PE (15 µl per sample) was used to distinguish EMP subpopulations. All antibodies were obtained from BD Biosciences. After antibody incubation, samples were diluted with 500 ml of 0.22 µm double-filtered phosphate-buffered saline (PBS) before flow cytometric analysis. Two additional samples were also prepared to serve as negative controls and as a calibration. For the negative control tube, 733 µL of PBS was added to one tube. The calibrator sample was prepared using two drops of 0.9 µm standard precision National Institute of Standards and Technology (NIST) traceable polystyrene particle beads (Polysciences Inc, Warrington PA) and was added to the PBS according to the manufacturer's instructions. All samples were immediately analyzed by flow cytometry.

Samples and controls were analyzed using a BDLSRII flow cytometer (BD Biosciences, San Jose, CA) and BD FACSDIVA software (v 1.2.6; BD Biosciences). Forward scatter scale, side scatter scale, and each fluorescent channel were set in logarithmic scale. Events included in the set gate (< 1.0 µm) were identified in forward and side scatter intensity dot representation and plotted on 2-color fluorescence

histograms. CD62E+ events < 1.0 μm were defined as EMPs. Fluorescence minus one control and non-stained samples were used to discriminate true events from noise, and to increase the sensitivity for microparticle detection for each sample. The flow rate was set on medium on LSRII and all samples were run for 180 seconds. Using beads, medium flow rate was calculated; a mean sample volume of 101 μl per 180 seconds was processed. EMPs were expressed as events per μl plasma.

Exercise Testing

A sub-maximal graded exercise test was performed to determine subjects' cardiovascular fitness and to develop individualized exercise prescriptions for the AEXT intervention. A modified Bruce protocol sub-maximal treadmill graded exercise test was performed with continuous measurement of breath-by-breath gas sampling oxygen consumption (VO_2) using a calibrated metabolic cart (Vmax Encore, SensorMedics, Yorba Linda, CA). An ECG was continuously monitored, and the treadmill test was terminated when subjects reached 75-80% of their predicted heart rate reserve. A standard regression formula using data collected by indirect calorimetry (VO_2 averaged over each 60-second period) and ECG (minute heart rates) was used to predict maximal oxygen consumption (VO_{2max}), a measure of cardiovascular fitness, as recommended by the American College of Sports Medicine Guidelines for Exercise Testing and Prescription, 9th ed. 2013.

Aerobic Exercise Training Intervention

Subjects engaged in a 24-week AEXT intervention under direct supervision of lab personnel three times per week, beginning with 20 minutes of exercise per session at an

intensity equivalent to 50% of VO_{2max}. Training duration was increased five minutes each week until 40 minutes of exercise at 50% of VO_{2max} was reached. Upon reaching 40 minutes of exercise, training intensity was increased 5% each week until 65% of VO_{2max} was achieved. At week eight, subjects reached the desired exercise duration and intensity of 40 minutes at 65% of VO_{2max}, which they maintained as their exercise prescription for the remainder of the study. Exercise modes included treadmill walking/jogging, stair stepping, stationary cycling, rowing ergometry, arm ergometry, and elliptical cross-training. Subjects were instructed on the proper use of heart rate monitors in order to monitor exercise intensity. Study personnel recorded subjects' exercise mode, duration, and heart rates every 10 minutes in printed logs to ensure adherence to the prescribed exercise training program. At week 12, subjects completed a second sub-maximal treadmill exercise test as a basis for adjustment of their exercise prescription to account for changes in cardiovascular fitness. A gradual progression of training duration and intensity was used in order to avoid excessive fatigue and musculoskeletal complaints, thereby maximizing adherence.

Statistical Analyses

Data are expressed as mean ± the standard error of the mean (SEM). The distribution of all variables was examined using the Shapiro-Wilk test of normality. Pre-AEXT and Post-AEXT were compared using a paired samples Wilcoxon signed-rank test. Associations between the variables were identified using Spearman's Rho. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL).

In Vitro Study

Human umbilical vein endothelial cells (HUVEC) from an African American donor were cultured. The experiment consisted of the following four experimental conditions: *Static, Static with IL-10 Incubation, Laminar Shear Stress (LSS) at 20 dynes/cm^2*, and *LSS at 20 dynes/cm^2 with IL-10 Incubation*. After the LSS, cell lysates and cell culture media were collected from the four experimental conditions. Western blotting was used to measure endothelial nitric oxide synthase (eNOS) protein expression and its phosphorylated form (p-eNOS) at Serine 1177 in the cells. A modified Griess assay was used to measure NOx levels in the cell culture supernatant. The *in vitro* study experimental design flowchart is depicted below. (Figure 3.2)

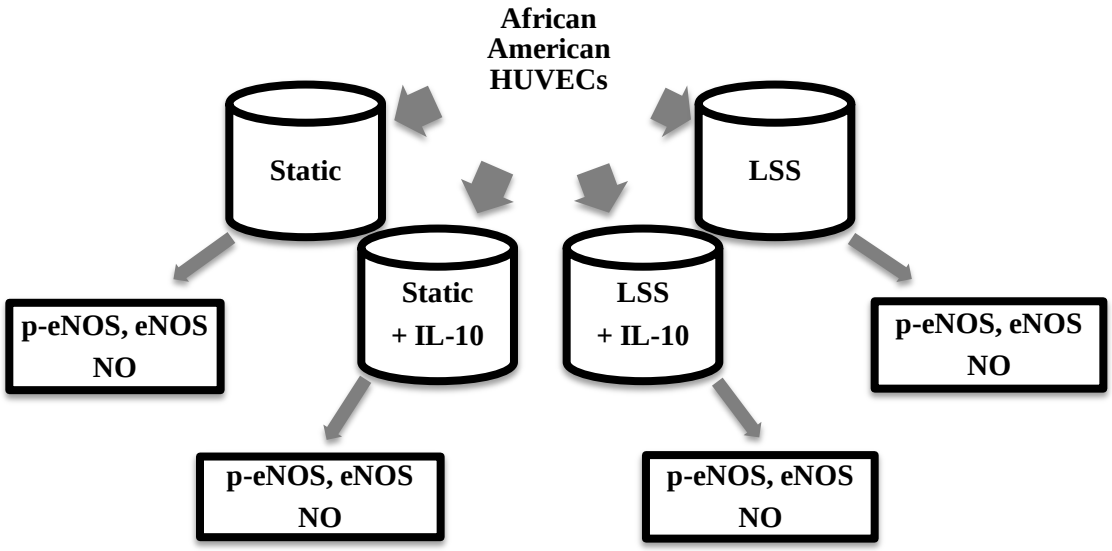

Figure 3.2. *In vitro* study experimental design flowchart.
HUVECs (human umbilical vein endothelial cells), IL-10 (interleukin-10), LSS (laminar shear stress), p-eNOS (phosphorylated endothelial nitric oxide synthase), eNOS (endothelial nitric oxide synthase), NO (nitric oxide)

Cell Culture

HUVECs from an African American donor were obtained from Lonza (Walkersville, MD) and preserved in liquid nitrogen until time of culture. Experiments were conducted with HUVECs between passages 4-7. HUVECs were cultured in M199, phenol red-free media and supplemented with 20% fetal bovine serum and endothelial cell growth supplement from bovine neural tissue in 10% gelatin coated dishes. It has been demonstrated that the addition of phenol red to media could potentially interfere with nitrate and nitrite measurements, and that measurement of NOx is most reliable when levels are quantified from cells that have been cultured in phenol red-free media[284,285]. Cells were maintained at 37 ^{o}C, 97% humidity, and 5% CO_2 in tissue culture dishes and were examined daily for confluency and morphology observation. LSS was applied when cells reached 95-100% confluency. The culture dishes were exposed for a period of 24 hours to the four experimental conditions. Recombinant human IL-10 was reconstituted and diluted to a final concentration of 2.0 ng/mL in the cell media. This dose was used for the incubation of cells in the tissue culture dishes that were included in the IL-10 experimental conditions.

Laminar Shear Stress

Unidirectional LSS was used as a model for aerobic exercise-induced vascular shear stress. Confluent HUVECs grown in 100-mm tissue culture dishes were exposed for a period of 24 hours to LSS at an exercise flow of 20 dynes/cm^2 using a rotating Teflon cone (cone and plate viscometer, 0.5 degree cone angle). Shear stress experiments were conducted in a cell incubator at 37 ^{o}C, 97% humidity, and 5% CO_2.

Immediately following LSS application, both the static and LSS culture dishes were harvested for protein analysis. Radio-Immunoprecipitation Assay (RIPA) Buffer made with 150 mM NaCl, 10 mM Tris-Cl (pH 7.5), 0.1% sodium dodecyl sulphate (SDS), 1.0% Triton X-100, 1.0% Deoxycholate, 5 mM EDTA, and 500 mL water, as well as protease inhibitor and phosphatase inhibitor, were used to enable cell lysis and to stabilize the protein solution in order to measure protein concentration in the cell lysate. Phenylmethylsulfonyl fluoride protease inhibitor was freshly added to eliminate interference. Briefly, cells were washed twice with cold Dulbecco's PBS. A 300 µL volume of the RIPA cocktail was added and a rubber scraper was used to harvest adherent cells. Cells were collected and centrifuged at 16,000 g for 15 minutes at 4 ^{o}C. Cells were stored at -80 ^{o}C until time of assay. A Bradford protein assay using Bio-Rad protein reagent was conducted to measure the protein concentration. A 5X SDS solution was made with 10% SDS, 10 mM dithiothreitol, 20% glycerol, 0.2 M Tris-HCl (pH 6.8), and 0.05% bromophenolblue. The protein-SDS samples were boiled for 3 minutes at 95 ^{o}C, and the sample was immediately frozen at -80 ^{o}C until use.

Western Blotting

Levels of eNOS, p-eNOS, and alpha tubulin (α-tubulin) were analyzed by Western blotting with mouse monoclonal anti-eNOS (BD Biosciences, 610296), mouse monoclonal anti-p-eNOS; s1177 (BD Biosciences, 612392), and mouse monoclonal anti-α-tubulin (Sigma-Aldrich, T9026) antibodies, respectively. The anti-α-tubulin antibody was used as the internal control. Proteins were separated by SDS-Polyacrylamide gel electrophoresis (PAGE) on 10% gels and electrotransferred to polyvinylidene difluroide immobilion transfer membranes (Millipore). Membranes were incubated overnight with

a primary antibody at 4 ^{o}C. After washing and incubating with a secondary antibody conjugated with horseradish peroxidase, total protein was detected by chemiluminescence. Band densitometry analyses were completed using ImageJ software (National Institutes of Health, Bethesda, MD).

Nitric Oxide

Cell culture supernatant was collected from all four experiment conditions and stored at -80 ^{o}C until the time of the assay. On the day of assay, all samples were centrifuged to remove particulates at 16,000 g for 20 minutes at 4 ^{o}C and then ultrafiltered through a 10,000 molecular weight cut-off filter (Sigma-Aldrich) by micro-centrifuge at 14,000 g for 20 minutes at 4 ^{o}C. Concentrations of NOx (nitrite/nitrate) in the cell culture supernatant were determined using an assay based on the enzymatic conversion of nitrate to nitrite by nitrate reductase, followed by colorimetric detection of nitrite as an azo dye product of the Griess reaction (R & D Systems, Minneapolis, MN). The intra-assay CV value was 1.8%.

Statistical Analyses

Data are expressed as mean ± the standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by post-hoc testing with Fisher's least significant difference (LSD) were used to assess differences across the four experimental conditions. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL).

CHAPTER 4

RESULTS

In Vivo Study

Among the 42 subjects who completed the 6-month aerobic exercise training (AEXT) intervention, the data used in the statistical analysis for each primary outcome variable were interleukin-10 (IL-10; n=26), nitric oxide (NO; n=24), C-reactive protein (CRP; n=37), interleukin-6 (IL-6; n=32), and CD62E+ endothelial microparticle (CD62E+ EMP; n=28). The differences in each variable's sample size are related to issues with subject scheduling, acquiring blood samples, or assay procedure.

Clinical Laboratory Values of Subjects

The study group consisted of 42 African American men (n=6; 14.3%) and women (n=36; 85.7%). The mean age of the group was 52.7 ± 1.0 years. The clinical laboratory values of the subjects measured prior and subsequent to the AEXT intervention are presented in Table 4.1. The 6-month AEXT intervention significantly increased maximal oxygen consumption (VO_{2max}) and significantly decreased body mass index (BMI), fasting plasma triglycerides, and fasting blood glucose. Total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) were not significantly changed following the AEXT intervention.

Table 4.1: *Clinical laboratory values of subjects pre- and post-AEXT.*

Variable	Subject Number	Pre-AEXT	Post-AEXT	Percent Change
BMI (kg/m^2)	n=42	31.4 ± 0.9	30.6 ± 0.8*	-2.5%
VO_{2max} (mL/kg/min)	n=41	25.9 ± 0.9	28.2 ± 1.1**	8.9%
SBP (mm Hg)	n=41	124.2 ± 1.9	123.6 ± 2.2	-0.5%
DBP (mm Hg)	n=41	78.7 ± 1.1	78.9 ± 1.2	0.3%
Total Cholesterol (mg/dL)	n=36	192.1 ± 4.3	191.4 ± 5.1	-0.4%
LDL Cholesterol (mg/dL)	n=36	108.7 ± 3.6	111.9 ± 4.3	2.9%
HDL Cholesterol (mg/dL)	n=36	66.8 ± 3.3	65.6 ± 3.4	-1.8%
Triglycerides (mg/dL)	n=36	83.0 ± 5.7	70.1 ± 3.3**	-15.5%
Fasting Glucose (mg/dL)	n=34	95.1 ± 1.7	88.5 ± 1.8**	-6.9%

Subject number represents usable sample for variables.
Values are expressed as mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
****Denotes significant difference; p < 0.05***
*****Denotes significant differences; p < 0.01***

Vascular Health Biomarkers of Subjects

The vascular biomarkers: IL-10, NO, IL-6, CRP, and CD62E+ EMP, considered indices of vascular health, were measured prior and subsequent to the AEXT intervention and are presented in Figures 4.1 through 4.5. The 6-month AEXT intervention elicited statistically significant changes in NO, IL-6, CRP, and CD62E+ EMPs. There was a significant 56.5% increase in NO (23.7 ± 1.8 vs. 37.1 ± 3.2; p=0.002), a significant 12% decrease in IL-6 (5.0 ± 0.2 vs. 4.4 ± 0.4; p=0.04), a significant 15.2% decrease in CRP

(3.3 ± 0.5 vs. 2.8 ± 0.5; p=0.01), and a significant 47.3% decrease in CD62E+ EMPs (42.5 ± 5.3 vs. 22.4 ± 3.8; p<0.001) following AEXT. IL-10 was increased by 4.9% (0.81 ± 0.06 vs. 0.85 ± 0.06; p=0.08) following the AEXT intervention, however the increase was not statistically significant.

Correlations Among the Changes in Variables

Spearman's Rho using change values calculated for each of the vascular health biomarkers revealed that there were no significant associations among the changes pre-post-AEXT for IL-10, NO, IL-6, CRP, or CD62E+ EMP.

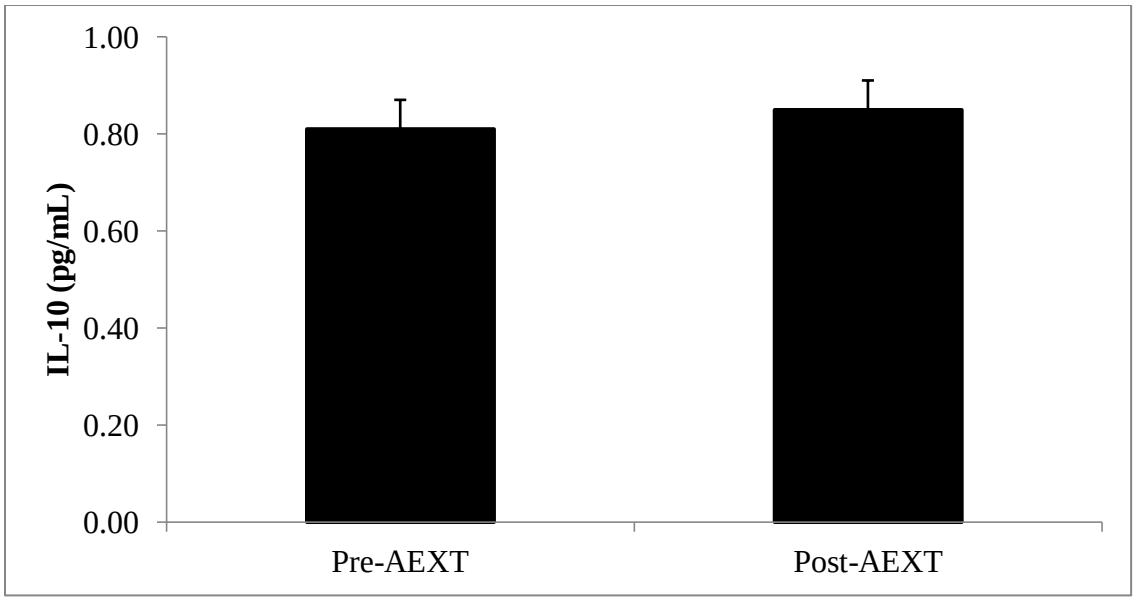

Figure 4.1. Interleukin-10 (IL-10) pre- and post-AEXT.
Bars are expressed as mean ± SEM.

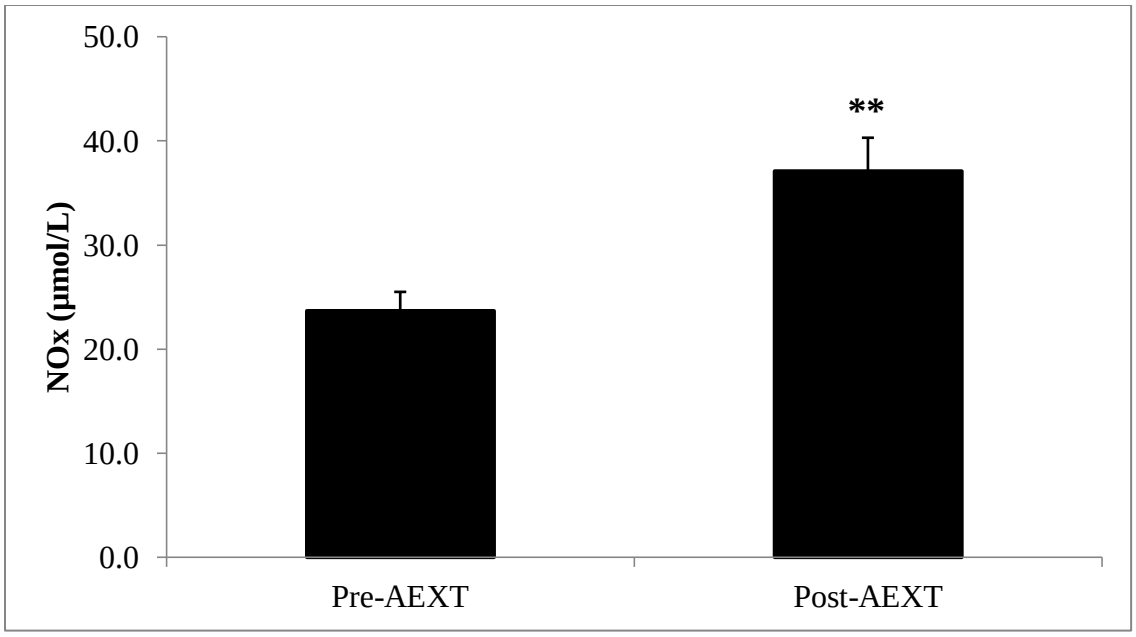

Figure 4.2. Nitric oxide (NOx) pre- and post-AEXT.
Bars are expressed as mean ± SEM. **Denotes significant difference; $p < 0.01$.

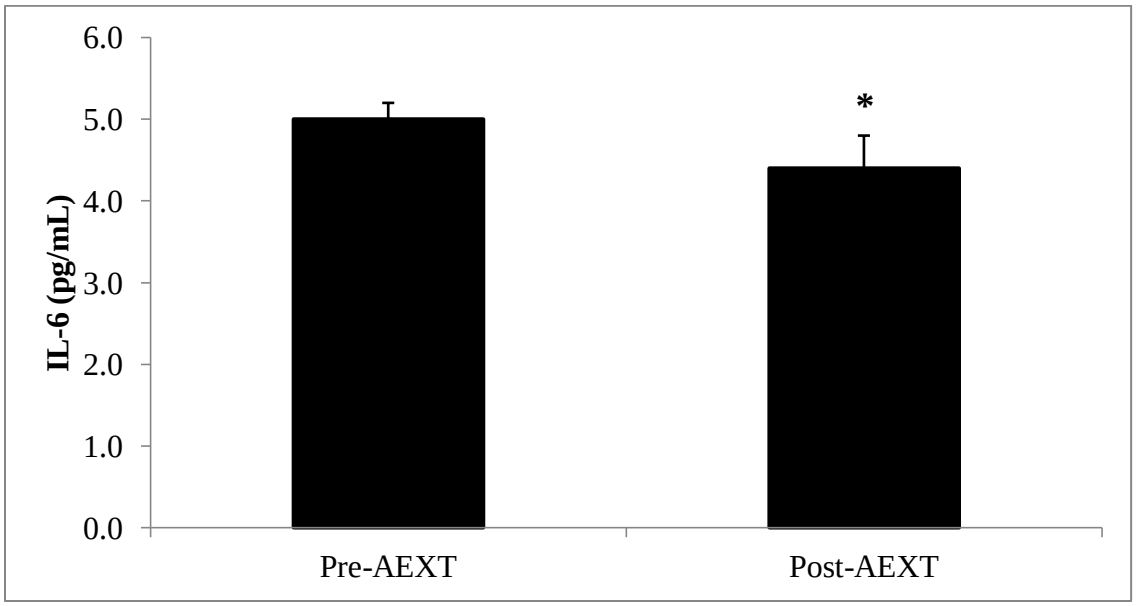

Figure 4.3. Interleukin-6 (IL-6) pre- and post-AEXT.
Bars are expressed as mean ± SEM. *Denotes significant difference; $p < 0.05$.

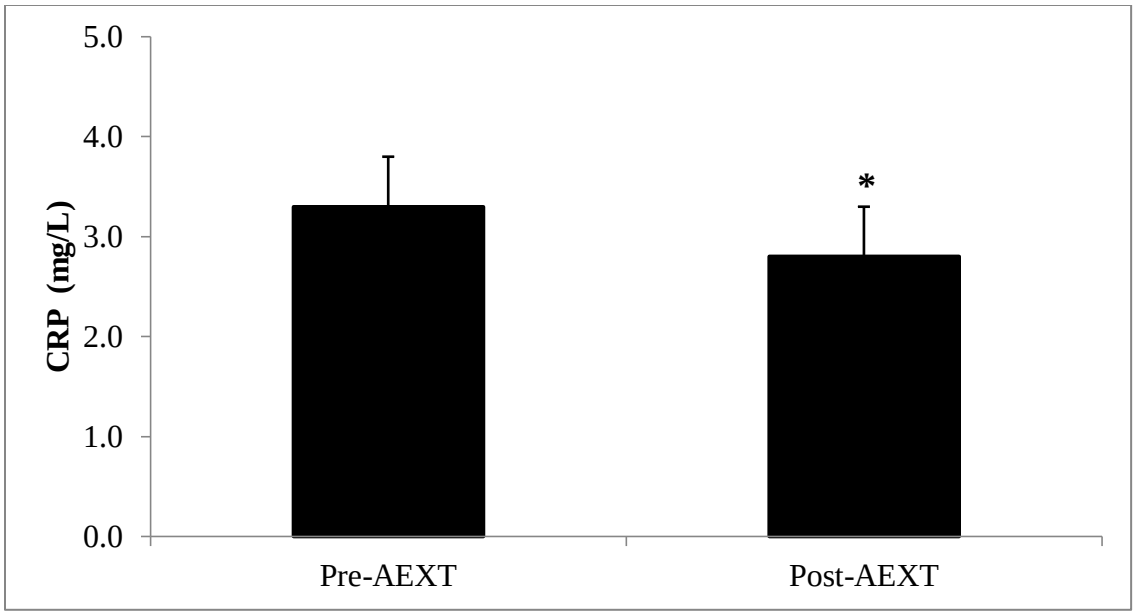

Figure 4.4. C-reactive protein (CRP) pre- and post-AEXT.
Bars are expressed as mean ± SEM. *Denotes significant difference; $p < 0.05$.

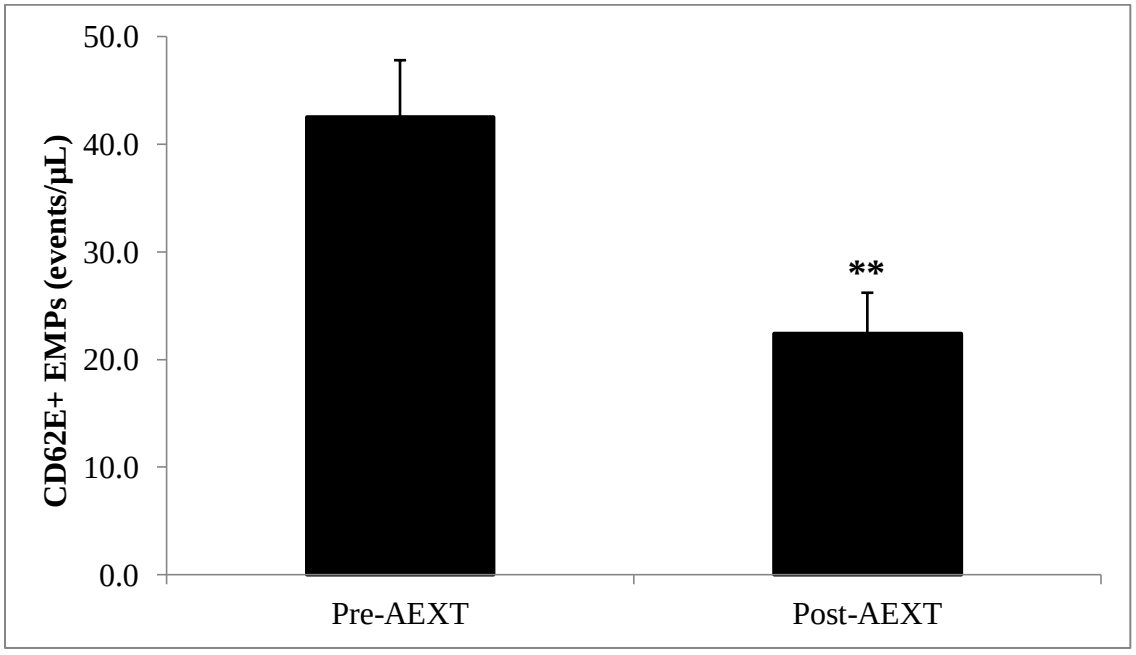

Figure 4.5. CD62E+ endothelial microparticles (EMPs) pre- and post-AEXT.
Bars are expressed as mean ± SEM. **Denotes significant difference; $p < 0.01$.

In Vitro Study

Human umbilical vein endothelial cells (HUVEC) were cultured and divided among four experimental conditions including the following: *Static*, *Static with IL-10 Incubation, Laminar Shear Stress (LSS) at 20 dynes/cm^2*, and *LSS at 20 dynes/cm^2 with IL-10 Incubation.* The cells were grown until they reached 95-100% confluency. As expected, HUVECs exposed to the static conditions were polygonal and randomly aligned, and HUVECs exposed to LSS conditions were elongated and aligned in the direction of flow. Phase contrast images of HUVECs exposed to the four experimental conditions are presented in Figure 4.6.

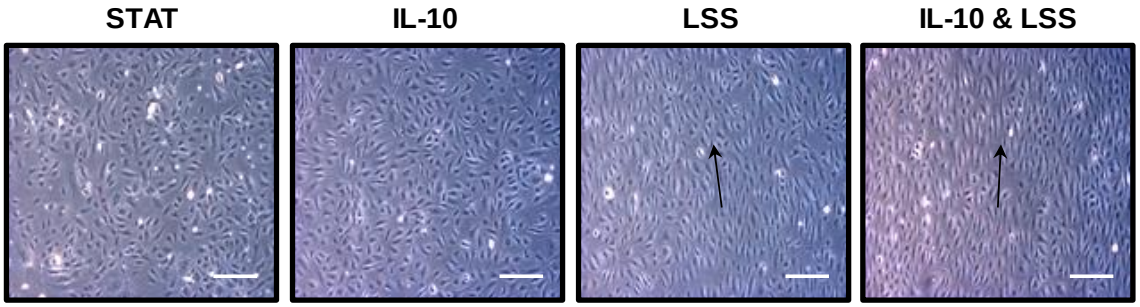

Figure 4.6. Phase-contrast images of HUVECs exposed to the four experimental conditions: STAT, *Static;* IL-10, *Static with Interleukin-10 (IL-10) Incubation;* LSS, *Laminar shear stress (LSS) at 20 dynes/cm^2*; IL-10 & LSS, *LSS at 20 dynes/cm^2 with IL-10 Incubation.* Arrows indicate the direction of shear stress. Scale bars indicate 100 µm.

Western Blotting

Three independent western blotting experiments were conducted to measure endothelial nitric oxide synthase (eNOS) protein expression and its phosphorylated form (p-eNOS) at Serine 1177 in the cells in all four experimental conditions, and alpha tubulin (α-tubulin) was used as the internal control. Levels of eNOS, p-eNOS, and

α-tubulin were analyzed with anti-eNOS, anti-p-eNOS (s1177), and anti-α-tubulin, respectively. Band densitometry analyses were used to depict the results as shown in the bar graphs. The results are presented in Figures 4.7 through 4.11.

Protein expression levels of both eNOS and p-eNOS were significantly increased in the *LSS at 20 dynes/cm^2* and *LSS at 20 dynes/cm^2 with IL-10 Incubation* experimental conditions when compared to the *Static* experimental condition (Figures 4.7, 4.8, 4.9). There were no significant differences in the *Static with IL-10 Incubation* compared to the *Static* experimental condition or *the LSS at 20 dynes/cm^2 with IL-10 Incubation* compared to the *LSS at 20 dynes/cm^2* experimental condition. In addition, there were no statistically significant differences in the protein expression of p-eNOS relative to eNOS protein expression among any of the four experimental conditions (Figures 4.10, 4.11).

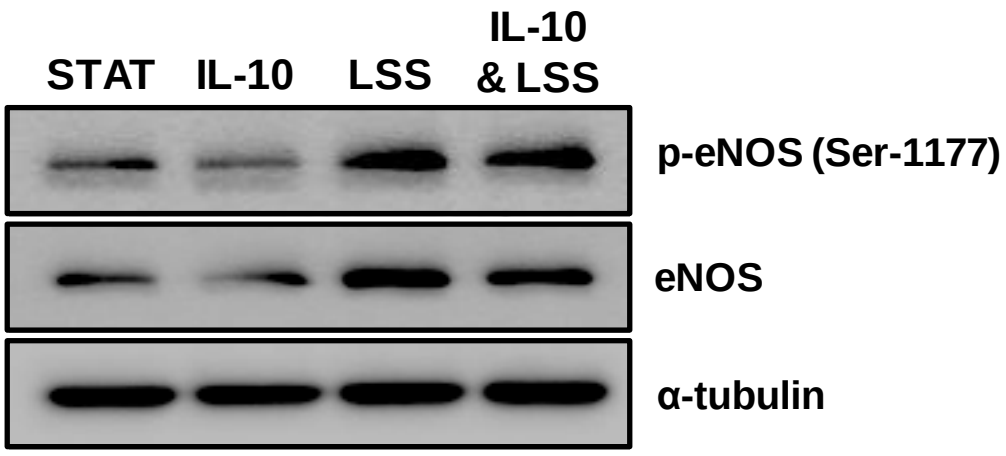

Figure 4.7. Western blotting results of phosphorylated endothelial nitric oxide synthase (p-eNOS) at Serine 1177 (Ser-1177) and endothelial nitric oxide synthase (eNOS) normalized to alpha tubulin (α-tubulin) for the four experimental conditions. STAT, *Static;* IL-10, *Static with Interleukin-10 (IL-10) Incubation;* LSS, *Laminar shear stress (LSS) at 20 dynes/cm^2*; IL-10 & LSS, *LSS at 20 dynes/cm^2 with IL-10 Incubation*.

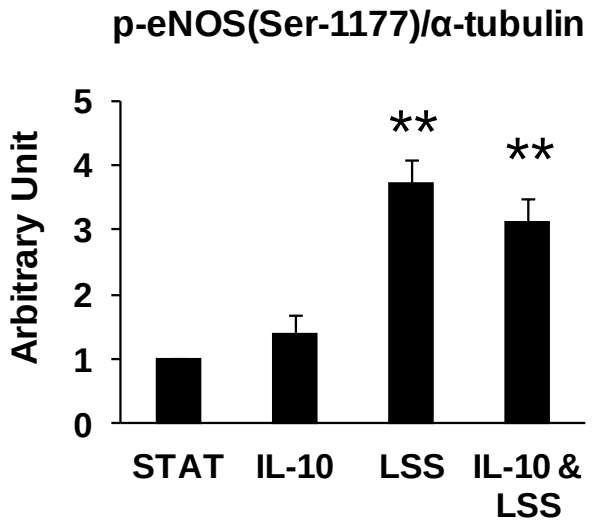

Figure 4.8. The bar graph depicts the results from densitometry analyses from western blotting experiments of phosphorylated endothelial nitric oxide synthase (p-eNOS) at Serine 1177 (Ser-1177) normalized to alpha tubulin (α-tubulin) for the four experimental conditions. STAT, *Static;* IL-10, *Static with Interleukin-10 (IL-10) Incubation;* LSS, *Laminar shear stress (LSS) at 20 dynes/cm*^{2}; IL-10 & LSS, *LSS at 20 dynes/cm*^{2} *with IL-10 Incubation.* Bars are expressed as mean ± SEM. **Denotes significant differences from STAT; $p < 0.01$.

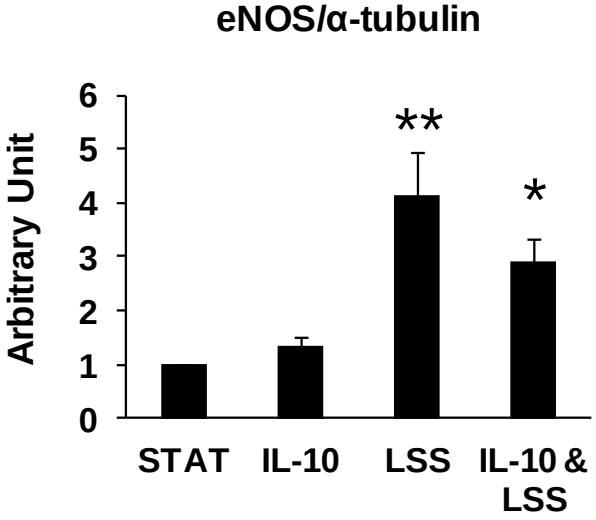

Figure 4.9. The bar graph depicts the results from densitometry analyses from western blotting experiments of endothelial nitric oxide synthase (eNOS) normalized to alpha tubulin (α-tubulin) for the four experimental conditions. STAT, *Static;* IL-10, *Static with Interleukin-10 (IL-10) Incubation;* LSS, *Laminar shear stress (LSS) at 20 dynes/cm*^{2}; IL-10 & LSS, *LSS at 20 dynes/cm*^{2} *with IL-10 Incubation.* Bars are expressed as mean ± SEM. *Denotes significant difference from STAT; $p < 0.05$. **Denotes significant difference from STAT; $p < 0.01$.

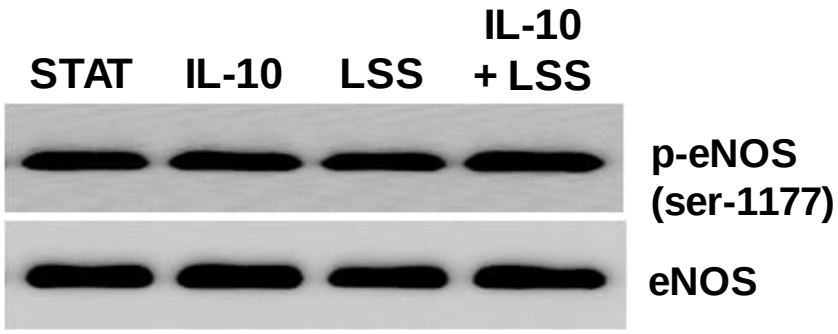

Figure 4.10. Western blotting results of phosphorylated endothelial nitric oxide synthase (p-eNOS) at Serine 1177 (Ser-1177) normalized to endothelial nitric oxide synthase (eNOS) for the four experimental conditions. STAT, *Static;* IL-10, *Static with Interleukin-10 (IL-10) Incubation;* LSS, *Laminar shear stress (LSS) at 20 dynes/cm^2*; IL-10 & LSS, *LSS at 20 dynes/cm^2 with IL-10 Incubation.*

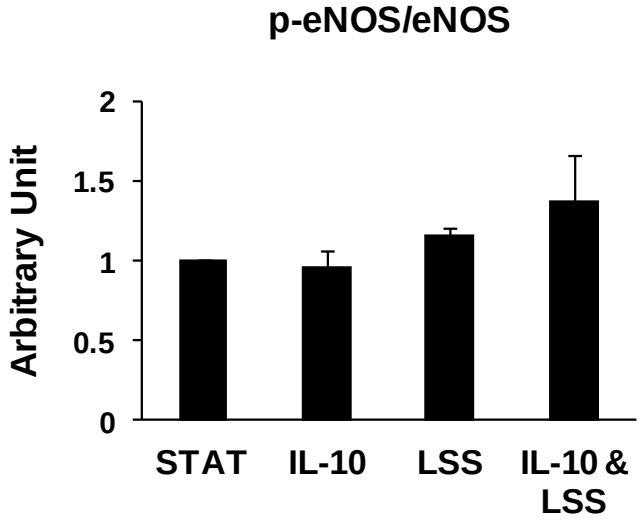

Figure 4.11. The bar graph depicts the results from densitometry analyses from western blotting experiments of phosphorylated endothelial nitric oxide synthase (p-eNOS) normalized to endothelial nitric oxide synthase (eNOS) for the four experimental conditions. STAT, *Static;* IL-10, *Static with Interleukin-10 (IL-10) Incubation;* LSS, *Laminar shear stress (LSS) at 20 dynes/cm^2*; IL-10 & LSS, *LSS at 20 dynes/cm^2 with IL-10 Incubation.* Bars are expressed as mean ± SEM.

Nitric Oxide Assay

Cell culture supernatant was collected from the four experimental conditions and included three independent samples for each condition. Total concentration of nitric oxide (NOx), including nitrite and nitrate, was measured in the cell culture supernatant, and concentrations were determined using an assay based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The results are presented in Figure 4.12. NOx concentration levels were significantly increased in the *LSS at 20 dynes/cm^2* and *LSS at 20 dynes/cm^2 with IL-10 Incubation* experimental conditions when compared to the *Static* experimental condition. There were no significant differences in the *Static with IL-10 Incubation* compared to the *Static* experimental condition or *the LSS at 20 dynes/cm^2 with IL-10 Incubation* compared to the *LSS at 20 dynes/cm^2* experimental condition.

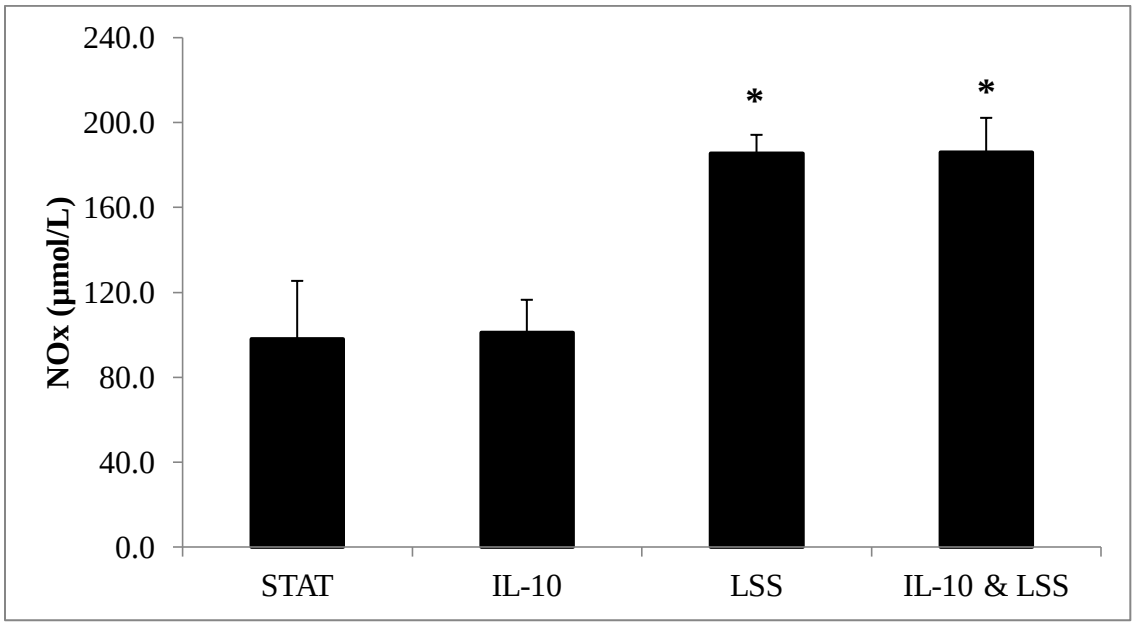

Figure 4.12. Total nitric oxide (NOx) concentrations from cell culture supernatant exposed to the four experimental conditions: STAT, *Static;* IL-10, *Static with Interleukin-10 (IL-10) Incubation;* LSS, *Laminar shear stress (LSS) at 20 dynes/cm^2*; IL-10 & LSS, *LSS at 20 dynes/cm^2 with IL-10 Incubation.* Bars are expressed as mean ± SEM. *Denotes significant differences from STAT; $p < 0.05$.

CHAPTER 5

DISCUSSION

In Vivo Study

The primary findings of the *in vivo* study demonstrated that six months of aerobic exercise training (AEXT) elicited significant positive improvements in the inflammatory biomarkers interleukin-6 (IL-6), C-reactive protein (CRP), and CD62E+ endothelial microparticle (EMP), as well as the vasodilatory biomarker nitric oxide (NO), in a cohort of middle-to-older age African Americans. Other research studies that measured inflammatory and vasodilatory biomarkers have demonstrated similar results, but this is the first study, confirmed by an extensive review of the literature, that measured all of these complementary biomarkers prior and subsequent to AEXT in an exclusively African American population.

C-Reactive Protein

Elevated plasma levels of CRP have been determined to be predictive of increased risk for the development of hypertension[15,16]. Furthermore, CRP levels have been positively associated with cardiovascular risk, and have been demonstrated to be elevated in African Americans when compared to Caucasians[8,10,74,75]. In review articles comprising multiple studies, independent associations between hypertension and plasma levels of CRP have been reported[13,15]. Pharmacologic treatment with anti-inflammatory properties has been demonstrated to reduce CRP in humans[156,158,160,164]. Therefore,

targeting inflammation as an intervention may decrease the risk of hypertension and subsequent cardiovascular disease (CVD) in the African American population.

Improvements in plasma levels of CRP following AEXT have been well documented in previous research. It has been demonstrated that exercise training significantly reduces CRP in a variety of populations including apparently healthy individuals, subjects with type 2 diabetes, and CVD patients[39,192,195-197,199-202]. This previous research data suggest that AEXT has a beneficial effect on plasma levels of CRP and inflammatory status. The present study provides some evidence that AEXT may also be beneficial for improving inflammatory status in African Americans, as indicated by significantly decreased CRP levels in this African American cohort.

Interleukin-6

Independent associations between hypertension and plasma levels of IL-6 have also been reported in review articles comprising multiple studies[13,15]. Arterial hypertension has been positively associated with the release of IL-6 which subsequently induces CRP synthesis[134]. IL-6 may be an independent risk factor for the development of hypertension in apparently healthy individuals[182]. It has also been reported that IL-6 is known to protract impaired endothelial-dependent relaxation, which may lead to increased peripheral vascular resistance and consequently hypertension[13,15]. Furthermore, IL-6 has been demonstrated to be positively related to vascular resistance and endothelial dysfunction[13,15,180,184]. For the African American population, interventions that decrease plasma IL-6 levels may be an important target for endothelial health in order to lessen their risk for hypertension and CVD.

Significant reductions in IL-6 subsequent to AEXT interventions have also been demonstrated in various populations including apparently healthy individuals, subjects with type 2 diabetes, and CVD patients[39,195,196,198,199,201,202]. The present study provides further evidence that AEXT may attenuate plasma IL-6 concentrations in the African American population. The significant decrease in plasma IL-6 levels demonstrated in this study may translate to a more favorable inflammatory status, as well as to the health of the endothelium, within this population.

CD62E+ Endothelial Microparticles

Levels of EMPs have been demonstrated to be associated with vascular dysfunction and endothelial damage, and EMPs have been considered to be biomarkers of proinflammatory conditions, vascular injury, and endothelial dysfunction[28,155,186-188,191]. Circulating levels of EMPs have been reported to be significantly increased in individuals with hypertension and CVD when compared to healthy subjects[186-188,191]. Endothelial activation, characterized by increased inflammation, has been identified as an early event in endothelial dysfunction, and CD62E+ EMPs have been identified as markers of inflammatory endothelial cell activation[28-31,155,187]. Therefore, the detection and quantification of EMPs may be a valuable tool in the early detection of cardiovascular risk.

The review of literature revealed that the effect of AEXT on CD62E+ EMPs has not been previously investigated in any population. Lee et al. demonstrated that high levels of CD62E+ EMPs were associated with cardiovascular events in patients with a history of stroke, suggesting that systemic endothelial activation may be associated with

increased risk for cardiovascular morbidities[29]. The present study provides some of the first evidence that AEXT may attenuate endothelial activation in the African American population which may have clinical importance given the recent findings reported by Lee et al.

Interleukin-10

Interleukin-10 (IL-10) potently inhibits proinflammatory cytokines such as IL-6 and tumor necrosis factor alpha (TNF-α), and the primary function of IL-10 seems to be to limit and ultimately terminate inflammatory responses[206-210]. In CVD patients with elevated plasma CRP levels, IL-10 has been associated with improved vasoreactivity and a more favorable prognosis, providing some evidence for the importance of IL-10 in endothelial health[32,211]. In multiple studies conducted on mice, IL-10 has been positively associated with endothelial function through various mechanisms that point to a role for the balance of pro- and anti-inflammation[33-37]. IL-10 has also been demonstrated to lower blood pressure by ameliorating endothelial dysfunction in rats and mice[33,209]. Pharmacologic treatment with anti-inflammatory properties has been reported to protect endothelial function, suggesting the detrimental influence of inflammation on endothelial integrity is amenable to therapeutic interventions[134].

Most studies that have measured serum or plasma IL-10 concentrations have found detectable levels of IL-10 in a diseased population versus healthy subjects. Blay et al. measured IL-10 in 153 subjects with non-Hodgkin's lymphoma and compared them to a control group of 60 healthy subjects[286]. The researchers found that IL-10 was not detectable in any of the healthy subjects; however, it was detectable in about half of the

diseased subjects[286]. In the present study, the fact that IL-10 was able to be detected, even at relatively low levels in this putatively healthy African American cohort, may be interpreted as a result of the increased disposition in this population to chronic low-grade inflammation.

Several studies have previously examined the effect of AEXT on circulating levels of IL-10 in subjects with type 2 diabetes and patients with CVD, and reported a significant increase in IL-10 subsequent to an AEXT intervention[195,202,258,259]. In the present study, the effect of AEXT on circulating levels of IL-10 was investigated for the first time in an exclusively African American cohort, according to a review of the literature. The results of this study demonstrated that IL-10 was able to be detected at low levels in this African American cohort, and there was a tendency for IL-10 to be increased subsequent to AEXT, although statistical significance was not achieved.

The significant improvements in IL-10 that were reported in previous research as a result of AEXT included subjects with type 2 diabetes or CVD, and inflammation has been demonstrated to be manifested in those disease conditions. An interpretation based on those findings may be that a critical basal level of inflammation needs to be present in order for IL-10 to be necessary to exert its anti-inflammatory properties. A review conducted by Batista et al. on the role of TNF-α and IL-10 on the anti-inflammatory effect of AEXT included heart failure patients who exhibited elevated baseline levels of TNF-α[258]. The authors concluded that the anti-inflammatory effect induced by AEXT seems to be primarily mediated by IL-10[258]. Based on the results of the present study, more research is warranted that may include an African American cohort with higher known levels of vascular inflammation.

Nitric Oxide

NO is released by the endothelium and has an important role in the protection against the onset and progression of endothelial function, hypertension, and CVD[214,235]. NO is the prototypic endothelial-dependent relaxing factor with its primary function to regulate vasodilation, and vascular endothelial health has been demonstrated to be contingent on processes controlling the synthesis, bioavailability, and destruction of NO[242,248,250]. The balance between vasodilation and vasoconstriction has been implicated to be important for endothelial health; therefore, therapeutic interventions that may enhance the bioavailability of NO should be considered.

Research studies have demonstrated an increase in NO bioavailability as a result of AEXT interventions. Increased plasma levels of NO subsequent to exercise training have been reported in healthy individuals, in obese subjects, in patients with metabolic syndrome, and hypercholesterolemic subjects[264-267]. Furthermore, endothelial nitric oxide synthase (eNOS) and NO have been reported to significantly increase following an AEXT intervention in healthy subjects and in individuals with endothelial dysfunction, hypertension, CVD, and CVD risk factors[41,47,250,268]. The present study provides additional evidence that AEXT may also be beneficial for improving the bioavailability of NO in the African American population.

Blood Pressure

In the present study, there were no significant changes in mean resting blood pressure subsequent to the AEXT intervention. The mean resting blood pressure of the subjects in this study would categorize the subjects as having prehypertension. These

findings are in agreement with another study that measured resting blood pressure following AEXT in prehypertensive individuals in which blood pressure did not significantly change in most cases[287]. An additional study that measured resting blood pressure in normotensive and prehypertensive subjects before and after AEXT demonstrated no significant difference in resting blood pressure in the normotensive subjects, but found a significant decrease in the prehypertensive subjects[95]. Conversely, in two independent reviews by Hagberg et al. comprising hypertensive subjects, blood pressure significantly decreased in 75% of the subjects subsequent to AEXT[90,96]. Based on the evidence from the literature, it seems that decreases in blood pressure as a result of AEXT have been demonstrated to be more pronounced in subjects with hypertension when compared to prehypertensive or normotensive subjects.

Despite the fact that mean blood pressure did not change significantly in the present study, the vasodilatory and inflammatory biomarkers measured in this study related to endothelial health, hypertension, and CVD improved considerably. These positive changes in the vasodilatory and inflammatory biomarkers following AEXT demonstrated in this study may indicate considerable improvement in CVD risk for the African American population. A substantial portion of the CVD risk reduction associated with exercise training cannot be entirely explained by changes in conventional CVD risk factors[50,56,61]. It has been suggested that direct beneficial effects of exercise on the vessel wall may account for some of the remaining risk factor reduction[50,54,56,61]. Therefore, the pronounced benefits on CVD risk reduction resulting from AEXT may go beyond simple blood pressure reduction in an African American population as elucidated by the results of the present study.

Limitations

Several limitations must be noted when interpreting these study findings. First, the sample size is small, but this was due to the exclusion of diabetics, smokers, participants with CVD or other chronic diseases, and those on medications that affect cardiovascular or renal hemodynamics, on lipid lowering medications, or who were on more than one antihypertensive medication. This was done to create a more homogenous group and to ensure lack of confounding variables that may influence vasodilatory or inflammatory biomarker levels. It should be noted that, even with a relatively small sample size, significant changes were observed in four of the five primary outcome measures subsequent to AEXT. Second, because of the observational nature of the study design, mechanisms underlying exercise training-induced changes in vasodilatory or inflammatory status cannot be inferred. Third, there are presently no standardized methods for the measurement of microparticles. Processing and analyzing techniques differ between investigators, thus comparisons across studies for EMPs should be done cautiously. Fourth, no control group was included in the study design, thus it is difficult to ascertain whether the observed changes were exclusively due to AEXT and not the result of an unidentified confounding factor. Finally, the sample population was predominately female, thus the findings may have limited generalizability to African American males.

In Vitro Study

The primary findings of the *in vitro* study conducted on human umbilical vein endothelial cells (HUVEC) demonstrated that there were significant increases in

phosphorylated endothelial nitric oxide synthase (p-eNOS), endothelial nitric oxide synthase (eNOS), and nitric oxide (NO) in the *Laminar Shear Stress (LSS)* and *LSS with IL-10 Incubation* experimental conditions when compared to the *Static* experimental condition. There were no significant differences in p-eNOS, eNOS, or NO in the *Static with IL-10 Incubation* experimental condition when compared to the *Static* experimental condition. Furthermore, there were no significant differences between the *LSS* and *LSS with IL-10 Incubation* experimental conditions. These findings suggest that IL-10 had little effect in the present study.

Research studies conducted on HUVECs have demonstrated that African American HUVECs have increased systemic inflammation, oxidative stress, and subsequent endothelial dysfunction when compared to Caucasian HUVECs[19-22]. It has been well documented in literature that oxidative stress and inflammation often occur simultaneously and have been linked to endothelial dysfunction and hypertension[80-84]. The cooperative role of inflammation and oxidative stress in the pathogenesis of hypertension may be resultant of the inflammatory response subsequent to oxidative stress[88]. Elucidating the mechanisms relating to inflammation, endothelial dysfunction, and hypertension may be beneficial in developing preventive measures in reducing the CVD risk burden among the African American population.

It has previously been reported that African American endothelial cells had significantly greater levels of IL-6 protein expression and produced greater amounts of IL-6 in response to TNF-α, an inflammatory cytokine[20]. In addition it has been demonstrated that, compared to Caucasian endothelial cells, African American endothelial cells had significantly greater protein expression of nicotinamide adenine

dinucleotide phosphate (NADPH) oxidase, the principal source of reactive oxygen species (ROS) in endothelial cells[21]. The findings from research studies suggest a heightened inflammatory and oxidative stress status in African American endothelial cells. Therefore, an intervention that can diminish this condition before endothelial dysfunction develops to the point where it manifests clinically may be very important.

A pivotal function of the endothelium altered by inflammation is NO-mediated regulation of vessel tone and blood flow, and this modification includes the reduction in the bioavailability of NO which impairs relaxation and contributes to endothelial dysfunction[23,84,134,137]. Research studies have demonstrated that NO exerts an anti-inflammatory influence by protecting endothelial cells against inflammatory activation[216,245]. In a study conducted on ethnic differences in endothelial function, Marchesi et al. concluded that apparently healthy African Americans have impaired endothelial vasoreactivity when compared to apparently healthy Caucasians, and this disparity may be related to the increased inflammatory state demonstrated in African Americans[136]. This further emphasizes the importance of interventions for the African American population that target the bioavailability of NO which has been demonstrated to be critically important for vasodilation and subsequent endothelial health.

Research data obtained from cell culture have demonstrated the beneficial effects of exercise on vascular health which have been attributed to the increased exercise-induced shear stress[19,56,63-69]. In studies conducted on animal aortic endothelial cells, eNOS protein expression and NO were reported to be increased subsequent to the exposure of the cells to LSS[65-68]. The effect of LSS on cultured African American HUVECs has previously been reported, and significant improvements were demonstrated

in eNOS protein expression, as well as NO concentrations subsequent to high levels of LSS[19]. The results of the present study indicate a similar outcome with significant increases in eNOS and p-eNOS protein expression, as well as concentrations of NO, subsequent to LSS.

In the present study, the effect of IL-10 on the protein expression of p-eNOS relative to eNOS, as well as NO concentrations in the cell culture supernatant, were also examined. It was hypothesized that IL-10 would provide an additive beneficial effect on p-eNOS relative to eNOS, as well as NO concentrations. The *Static with IL-10 Incubation* and the *LSS with IL-10 Incubation* experimental conditions did not significantly increase the protein expression of p-eNOS relative to eNOS, nor did it increase NO concentrations, when compared to the respective *Static* and *LSS* experimental conditions as was originally hypothesized.

Other studies have examined the effect of IL-10 on eNOS protein expression and concentrations of NO. In a study conducted on mice, Zemse et al. reported that IL-10 exerted its anti-inflammatory influence by inhibiting the *in vivo* and *in vitro* adverse effects of TNF-α on the endothelium of murine aorta by restoring the eNOS protein expression that was reduced by TNF-α[34]. Furthermore, IL-10 without the presence of TNF- α had no effect on eNOS expression in the study[34]. An additional study was conducted on HUVECs pre-incubated with TNF-α, and it was demonstrated that eNOS protein expression and NO concentration were increased in the cells subsequent to incubation with IL-10[38]. Based on the results of the study, Cattaruzza et al. concluded that in the presence of inflammatory conditions, increased eNOS protein expression was mediated by the anti-inflammatory effect of IL-10[38]. An important commonality in both

of these studies that should be noted is that IL-10 exerted its anti-inflammatory influence by increasing eNOS protein expression and NO in the presence of TNF-α, an inflammatory cytokine. These findings complement previous *in vivo* studies demonstrating that IL-10 exerted its anti-inflammatory effects in subjects with diseases in which higher levels of inflammation are manifested.

In the present study, the protein expression of p-eNOS relative to eNOS, as well as NO concentration, were measured in HUVECs that were incubated with IL-10, however, the cultured cells were not previously exposed to any inflammatory medium such as TNF-α. A future direction for an in *vitro study* may be to pre-incubate the cultured cells with TNF-α and subsequently measure eNOS protein expression and NO concentration under the same four experimental conditions as the present study.

Conclusion

The results of this study are novel because plasma levels of CRP, IL-6, CD62E+ EMPs, IL-10, and NO have not previously been measured together prior and subsequent to an AEXT intervention in an African American sample population. The primary findings of this study revealed favorable alterations in the inflammatory and vasodilatory biomarkers measured subsequent to AEXT. Furthermore, in African American HUVECs, p-eNOS and eNOS protein expression, as well as NO concentrations, were demonstrated to be significantly increased as a result of LSS. Therefore, AEXT may be a viable, non-pharmacologic method to improve vascular inflammation status and vasodilation, and thereby contribute to reduced hypertension and CVD risk in African Americans.

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APPENDIX A

INSTITUTIONAL INFORMED CONSENT

Project Title: **Genetics of In Vivo and In Vitro Endothelial Function in African Americans**

IRB Protocol #: 10831

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1. PURPOSE OF THE STUDY

African Americans have hypertension more often than any other population in the United States. Most of the time, African Americans get hypertension at an earlier age and it causes more damage. Changes that happen to the blood vessels (the hollow tubes that carry blood through the body) may help to explain how a person gets high blood pressure. It is also known that a person's genetic make-up can play a role in getting hypertension. In most people, exercise can help to make these damaged blood vessels better, but a person's genetic make-up may affect how well exercise works for them.

You are being asked to join this study because you are between 40-75 years old and have a blood pressure between 120/80 and 159/99.

This is a research study and the purpose of the study is to understand how aerobic exercise and genes affect your blood pressure and blood vessels. Examples of aerobic exercise are fast walking, bicycling, and stair stepping.

2. DESCRIPTION OF THE PROJECT

If you qualify for the study, you will be enrolled for a total of 9-10 months. This includes a screening process, a diet, exercising, and testing before and after the exercise program. You will be one of many people participating in this study at Temple University.

This is not a weight loss study. In fact, the investigators want you to keep your body weight about the same during the study so that they can only look at the effects of exercise on your blood pressure and blood vessels. If you are a woman and taking hormone replacement medication for menopause, then you will continue your usual medication as prescribed by your doctor. A table showing the visits you will make and the amount of time needed for each visit is shown on the last page of this consent form.

Screening

You will have two or three separate screening visits to Dr. Brown's laboratory in the Department of Kinesiology at Temple University.

The first screening visit will take place in the morning after you have not eaten for 12 hours. Once you arrive to the laboratory, the staff will review with you what you will be doing on this first visit. First, you will give a urine sample for testing and then you will have your weight, height, and blood pressure measured. To give the urine sample, the staff will give you the appropriate items depending on if you are a man or a woman which you will take to the restroom next to the laboratory. You will collect some of your urine in the plastic container and return to the laboratory. The staff will then take the container of urine and dispose of the urine collection containers. Next, you will sit quietly for 15 minutes and then your blood pressure will be measured. You will then have a blood sample taken from your arm by staff trained in the procedure. The staff member will tighten a band around your upper arm, wipe your arm with alcohol and then insert a small needle in a vein in your arm. Three tubes of blood will be filled.

One tube will be used to measure chemicals in your blood like glucose and salt in order get information about your health. The second tube of blood will be used to measure cholesterol and fat levels and the third tube will be for getting your DNA (Genetic material). The total amount of blood that will be taken is about 1½ tablespoons. The total time for this visit is approximately 1 hour.

It is possible that some of your DNA will also be frozen for future studies. However, this can only be done if you sign a separate consent form indicating that the investigators can store a sample of your DNA for future use. If you decline to give consent for storage and future use of you samples, this will not affect your participation in the study.

If you are not using any medicine to lower your blood pressure, and your blood pressure is between 120/80 and 159/99, then you will qualify for the next screening visit. If you are using only one medicine to lower your blood pressure, then your blood pressure must be less than 130/85 in order to slowly stop your medication. If it is higher than this, then you will not be allowed to participate in the study. The study physician, Dr. Crabbe, will watch over the stopping of your medicine. Before your medication is slowly tapered, you will visit Dr. Brown's laboratory to get a small blood pressure machine and to go over the plan for stopping your medicine. During the time that your medication is being stopped, you must check your blood pressure every day and keep a log of the blood pressure values. You will also be given information telling you how to safely stop your medication. During the time that your medication is being stopped, you will begin an American Heart Association diet (see below). If your systolic blood pressure (top number) goes to 160 mmHg or your diastolic blood pressure (lower number) goes to 100 mmHg, then you will immediately contact the investigators. If you must restart your blood pressure medication you cannot take part in this study. If this happens, a letter will be sent to your personal physician explaining that you should start your usual treatment for your blood pressure. Four weeks after your blood pressure medication has been stopped, you will visit the laboratory in the morning for a second screening visit. During this visit, you will have your blood pressure measured. If your systolic blood pressure is between 120 and 159 and your diastolic blood pressure is between 80 and 99, while you are not taking blood pressure medication, then you will qualify for the next phase of the screening.

During the second screening visit you will have a physical examination by Dr. Crabbe, an ECG (a way for the doctors to look at how your heart functions to see if it is healthy) and have your blood pressure measured after 15 min of seated quiet rest. In order to have this test, a technician will apply small sticky pads to the skin of your upper body. At the location where the sticky pads are placed, your skin will be rubbed with an alcohol pad. Next, you will have an exercise test to see if you have any signs of heart disease. This test will be performed so that the investigators can be sure that the exercise program will be safe for your heart. During the exercise test, you will ride a bicycle and have pictures of your heart taken by echocardiography, sometimes called cardiac ultrasound. Echocardiography is one of the most commonly used tests for heart disease. It is non-invasive and involves placing a small wand on your chest. It uses sound waves to take pictures of the heart. The test will take place at the Cardiovascular

Center in Temple University Hospital. The test will begin easy and the pedaling will get harder every three minutes. The total time for the bicycle is approximately 8-12 minutes. You can ask the technician to stop the test at any time if you become uncomfortable. During this exercise test, your blood pressure and heart will be monitored. At certain times during the test, a technician will ask you to point to a chart to indicate how difficult the exercise is feeling. A physician will be present during the test. You understand that, if the test shows that you might have heart disease you will be excluded from the study at this point and you will be asked to be seen by your personal doctor or arrangements will be made for you to be seen by a doctor at Temple University Hospital. The total amount of time for this visit is 1 hour.

Baseline testing

Diet Program: After the second screening visit, you will go to a dietary class once per week for 6 weeks to learn how to eat an American Heart Association (AHA) Diet. This diet is called a "Step 1" diet because it is the first step in eating foods that are healthy for your heart. At each diet class, your weight and blood pressure will be measured. If the diet is causing you to lose weight, you will be asked to increase your intake of healthy foods slightly. The staff will help you figure out ways to do this. The amount of salt in your diet will be measured at the end of the 1 month period by providing another urine sample.

Submaximal VO_2 test: VO_2 stands for the amount of oxygen that your body uses when you are resting or doing physical work. Before the test begins, you will have your resting metabolic rate (A measure of how many calories your body burns) measured during 20 minutes of quiet rest while lying down on a table. VO_2 will be measured continuously during the 20 minutes by placing a hard plastic covering around your head for 20 minutes. You will just relax and breathe normally. After 20 minutes of quiet breathing, you will be prepared for the exercise test. The investigators need to measure your VO_2 during exercise in order to plan your exercise program. During this test, you will walk on a treadmill and wear a clip on your nose and have a tube connected to a mouthpiece so that the air you breathe out during the test will go into a machine that will measure oxygen and carbon dioxide. This test will start at a medium walking peed and the hill of the treadmill will get steeper and the walking speed will get a little faster every 3 minutes. Your blood pressure, heart rate, and your heart tracing (ECG) will be monitored before, during, and after the treadmill test. The test will be stopped when you reach 75% of your maximal exercise capacity. You will have this test three times, once before starting the exercise program and after 3 and 6 months of being in the exercise training program. The total amount of time that you will be on the treadmill is 8-12 minutes. The total amount of time for the visit is about 1 hour.

Ambulatory Blood Pressure Monitoring and Urine collection: Ambulatory blood pressure is the blood pressure in your body as you go about your regular day. On a separate day, you will begin a 24-hour blood pressure monitoring and urine collection period. This will happen on a day in which you have a normal schedule. You will visit Dr. Brown's laboratory in the morning between 7:00 AM and 9:00 AM. Laboratory staff will give you all of the materials required to complete the 24-hour period. The urine collection

period will begin immediately. You will be fitted with a blood pressure monitor that will measure your blood pressure during the next 24 hours. The blood pressure monitor is a small electronic device that can go under your clothes. The monitor is connected to a blood pressure cuff that goes around your upper arm just like when you have your blood pressure measured. The blood pressure monitor will measure your blood pressure every 30 minutes during your waking hours and every 60 minutes during your sleeping hours. You will have the monitor for 24 hours so this means that you will have it when you go home and even when you go to bed. You will be asked to not exercise before or during the day of blood pressure monitoring. This means that you will not do any exercise or other physical activities that you would not regularly do. If you are walking about at the time of a blood pressure measurement, then you will stop if it is safe and pause until the measurement is completed. For example, if you are walking across the street and the machine begins to measure your blood pressure, you should continue across the street and then find a place to stop for a few minutes. You will be given a log book so that youcan write down what you are doing each time that your blood pressure is measured. You will be instructed to not remove the monitor except for bathing purposes, after which you will put the blood pressure monitor and cuff back on. Staff will show you how to take off and put on the blood pressure monitor and cuff. You will also be given the materials in order save all of your urine during the 24-hour period. 24-hours from the start of the blood pressure monitoring period you will give your last urine sample and remove the blood pressure cuff and turn off the monitor. This will end the 24-hour period. You will do have this test two times, once before and once after 6 the month exercise program.

Body composition and blood drawing: On the same day as the 24-hour ambulatory blood pressure monitoring and urine collection period, you will have your body composition (the amount of fat muscle and bone) measured. This measurement will tell the investigators what percentage of your body is fat. The instrument that measures your body composition is called bioelectrical impedance (BIA). The machine will cause a very small electrical current to go through your body for 2-3 seconds. It is one of the most common ways to measure your body composition. People who join a gym to workout often have this done at the gym before they start their exercise program. To do this test, you will lie on a table on your back with your left foot exposed. You will have to take off your left shoe and sock or remove any stockings. A technician will place two sticky pads on your left foot and two sticky pads on your left hand. The day before this test, you will be told to not exercise, drink alcohol, or eat food that is more salty than what you eat in your regular diet. This will help the investigators and you to get the most accurate information.

After your body composition is measured you will have blood samples taken so that the investigators can measure how you body changes with exercise training. This will be done twice during the study; once before and once after the exercise training. The blood will be taken the same way a described above in the screening visit. A needle will be placed in your arm vein and 6 tubes of blood will be obtained. These blood samples will be used to measure chemicals in your blood that help the investigators to know more about your blood vessels and blood pressure. Approximately 1 ounce (2½ tablespoonfuls) of blood will be taken. You will have your body composition measured two times, once

before and once after the month exercise program. You will have your blood taken three times, once before, mid-way through, and at the end of the 6 month exercise program.

Blood Vessel Function Testing: The blood vessels are the small hollow tubes that carry blood through your body. They are called arteries and veins. This test will be done at the Cardiology Section at Temple University Hospital after an overnight fast (12 hours) so that the investigators can measure how well the blood vessels in your arm work. The investigators use an ultrasound machine to take pictures of a blood vessel in your arm. If you are right-handed, the test will be done on your left arm. If you are left-handed then the test will be done on your right arm. You will be asked to not eat or drink food or liquid that has caffeine, alcohol, or pain medicines like aspirin, Advil, or Motrin, and not take any decongestants, cold or allergy medicines for the whole day before the study. You will lie down comfortably on a table. Following 20 minutes of quiet rest on the table, a blood sample (about 1½ tablespoons) will be taken. First, the doctor will put a gel (Similar to Vaseline) on your arm. The doctor will place a small device called a wand on your skin near your elbow and hold it still for several minutes while pictures are being taken.

Next, the same measurement will be made, but this time, it will happen after 5 minutes of stopping the blood flow going into your arm. To do this, the doctor will put a cuff around your arm. The cuff is just like the cuff that is put on your arm to measure your blood pressure. Just like when your blood pressure is measured, the cuff is pumped up until the blood stops going into your arm. This test is the same except that the cuff will stay pumped up for 5 minutes. Your hand may begin to feel "numb and tingly" similar to the feeling when your hand or foot falls asleep. When the air is let out of the cuff, the measurements with the ultrasound machine will be made for three minutes. During this time you will continue to lie down on the table in a comfortable position.

After a 10-15 minute rest period, the same test will be done again but this time it will be done after small amount of a substance called a nitroglycerine tablet is placed under your tongue. Nitroglycerine is a substance that causes your blood vessels to relax. It is most often used when people have chest pain due to heart disease. Nitroglycerine can also lower your blood pressure for a short time. Very rarely, it causes a mild headache that last for 5-10 minutes.

During the same visit, two blood vessels in your neck (carotid arteries) will be measured to find out the thickness of the blood vessel walls. The thickness of the blood vessel walls in your neck is sometimes related to the risk for cardiovascular disease. This test will be done using the same ultrasound machine that was used to measure the blood vessel in your arm. The doctor will place a small amount of gel on each side of your neck and then place a small wand on the skin. Pictures will be taken for 3-5 minutes. The total time for this visit to measure arm and neck blood vessels is approximately 1 ½ hours. You will have this test done two times during the study; once before and once after the exercise training.

On a separate day, you will visit Dr. Brown's laboratory in the Department of Kinesiology at Temple University to have your blood vessels measured using a different kind of machine. For this test, you will also lie down comfortably on a table after not

eating for 12 hours. You should not eat foods or liquids that have caffeine or alcohol in them and you will be told not to take an pain relievers, decongestants, cold or allergy medicines for the whole day before the test. Measurements will be made after 20 minutes of quiet rest. During the rest time, the investigators will comfortably support your arm in an armrest and put a blood pressure cuff on your upper arm. A second smaller blood pressure cuff will be put around your wrist. Next, a very thin hollow rubber band filled with mercury, called a strain gauge will be placed around your forearm. The test will begin when the investigators pump up the cuff around your wrist. Your hand will start to fell numb. The cuff around your upper arm will then be pumped up only a little bit every 15 seconds. During this time, blood pressure will be measured in your other arm. After these measurements and a 15-minute rest period, the investigators will again do the test but this time it will be after 5 minutes of having the cuff inflated just like what was done in the other test. This is when the cuff on your arm is pumped up very high for 5 minutes. After the 5 minutes, the air is let out of the cuff and the measurements will begin again and last for 3 minutes. This entire visit will last approximately 1 hour. You will have this test done two times during the study; once before and once after the exercise training.

Exercise Training Program

After completing the Baseline Testing described above, you will begin an aerobic exercise training program for 6 months. Aerobic exercise is physical exercise that uses large muscles like the legs and is continuous meaning is done for 20 minutes or more. It is not exercise like lifting weights. Aerobic exercise is the kind of exercise that doctors say will help to lower blood pressure, lower cholesterol levels, and lower the chances of getting diabetes. Examples of aerobic exercise are fast walking and bicycling. You will visit the exercise facility in the Department of Kinesiology at Temple University 3 times per week. Study personnel will supervise all exercise sessions. You will learn how to measure your heart rate and to use heart rate monitors so that you will know how hard you are exercising. At your first exercise session, you will exercise for 15-20 minutes at the lowest level of difficulty. As you get in better shape, the amount of exercise you do will increase gradually until you are exercising for 40 minutes of moderate intensity exercise every session. The investigators do not want you to exercise as hard as you can because they know that lower levels of exercise are most healthy. They call this level of exercise "moderate intensity". You will be able to choose from different exercise machines. Exercise sessions will last between 40 and 60 minutes.

Final Testing

After you finish the 6 month exercise program, you will have everything re-tested in the same order as the testing that occurred during Baseline Testing. In addition, you will have the treadmill exercise test to measure your fitness level after the exercise training program. These final tests will happen 36-48 hours after one of your regular exercise sessions.

The total number of times that you will be stuck with a needle during the entire study is 4 (once during screening, once during baseline testing, one mid-way through the exercise program, and once during final testing). The total amount of blood

that will be taken from your arm during the entire study is about 12 tablespoons over the 9-10 month period that you participate in the study.

Possible risks related to participation in this research study

The following risks, although low, are related to your participation in this research study.

Exercise testing: During the study, there are times when you will do a treadmill test that requires you to exercise as hard as you can. These tests are called maximal exercise tests. This is not the same as the exercise training in which you exercise 3 times a week. The risk of a maximal exercise test is that out of 10,000 tests, someone has a medical problem. In 1 out of every 70,000 exercise tests, a person will die from heart problems. In medical terms, doctors call this a rare event. The investigators will make sure it is as safe as possible for you to do this test because you will already have had tests including blood tests and a physical examination that will help the doctor to find out whether you are healthy enough to perform maximal exercise. Also, a doctor will be present when you do the test.

Giving blood: The research staff will take your blood in exactly the same way as when you have your blood taken at the doctor's office. There is a small risk of bruising and rarely infection. These risks will be lowered by using sterile procedures and by having trained research staff take all blood samples. There is also some pain associated with needle sticks and sometimes, people have been known to faint during needle sticks and blood drawing. We will take your blood while you are lying down which helps to prevent fainting.

Stopping your blood pressure medicine: The risks are that your blood pressure could increase to unsafe levels (greater than 180/120). Unsafe levels of blood pressure can lead to headache, stroke, chest pain, heart attack and damage organs such as the kidneys and heart. These types of very high blood pressure emergencies are rare. Many doctors that treat high blood pressure feel that it is a good idea to reduce medicine once a year to see if the amount of medicine can be lowered. The investigators will only talk to you about stopping your medication if your blood pressure is not higher than 130/85 while you are taking your medicine. Your risk will be reduced because during this time you will also be changing your diet which may help to lower your blood pressure. In addition, the study doctor will check you as you begin to slowly stop your medicine. In order to help the study doctor make sure it is safe for you to stop your blood pressure medicine, the investigators will give you a blood pressure monitor to take home. The investigators will show you how to measure your blood pressure during the day. You will keep a log of your blood pressure numbers and report it to the investigators. If your blood pressure increases to more than 160/100, then the investigators will tell you to resume your medicine.

Measuring your body composition: There are no known risks of having the amount of fat measured in your body. There are no needles and no pain. Sticky pads are placed on your foot and hand. The test takes about 5 minutes.

Measuring Blood Vessel Function: The blood vessels are the small hollow tubes that carry the blood in your body. The risk of these tests is the minor discomfort you will feel when the blood pressure cuff is pumped up because it will cause the blood to stop going into your arm and hand and this will happen for 5 minutes. There are no procedures to lower the chances of having this discomfort. This discomfort is the same as when your foot falls asleep. There are no known risks of having ultrasound. During part of the test, a small nitroglycerine tablet will be placed under your tongue. Nitroglycerine can sometimes lower your blood pressure and sometimes cause a headache for 5-10 minutes. Your blood pressure will be prevented from going lower because you will be lying on a table. A Cardiologist will be performing the test and will monitor you during the entire visit.

Measuring your ambulatory blood pressure: You will be wearing a small device that will measure your blood pressure during a regular day. When the blood pressure monitor pumps up the cuff, it is possible to hear the sound of the pump when you are in a quiet place. About 2 out of 100 people say that they have woken up during the night. These people also say that they are light sleepers. At night, the machine will measure your blood pressure 1 time every hour. There are no procedures to lower the chances that the blood pressure machine might wake you while you are sleeping. The investigators will show you ways that might help so that this does not happen.

Exercise training: The risk of exercise training is that it is possible to have a medical problem usually related to your heart. Out of every 375,000 hours of exercise training there are 2 times in which a person has a medical problem. This is the same as 1 medical problem for every 1.7 million miles of walking. These risks will be lowered because you will have a physical examination and an exercise test to make sure it is safe for you to train. There will also be trained staff that knows how to handle medical problems if it happens during an exercise training session.

Genetic Testing: As part of the study, the investigators will be analyzing your DNA to see if it gives them information about how your blood vessels work and how your blood vessels and blood pressure are affected by exercise. DNA is the material in your body that is passed on from parent to child and from generation to generation. The investigators will get your DNA during one of the times that they take your blood at the start of the study. The risks of having your blood taken have already been described above. The risk of genetics testing is finding out that you have a gene that shows that you may have a higher risk for getting a disease in the future. These risks are low because the places in your DNA that the investigators are looking at do not tell them if you will or will not get cardiovascular disease in the future.

Since there may be unknown risks to pregnant women and their unborn child, if you are nursing, pregnant, or planning to become pregnant, you will not be allowed to participate in this research study.

You confirm to the best of your knowledge that you are not pregnant and if you become pregnant during the course of this study, you must notify your physician and the investigators immediately.

Possible benefits of participating in this study

It is well known that African Americans suffer more from high blood pressure (hypertension) compared to other populations in the United States. There are direct benefits to you as a result of your participation in this study. Some of these benefits are greater than those you would have from usual medical testing. For example, 24-hour ambulatory blood pressure monitoring, dietary counseling, exercise testing, cardiac ultrasound, and supervised exercise training are not usual medical practice procedures. You will benefit from the medical and cardiovascular testing, measurement of your cholesterol and glucose. Most experts think that exercise is usually good for your overall health. The benefits of aerobic exercise training on risk factors for cardiovascular disease are well known. When blood pressure is lowered, it lowers your chances of getting heart disease and having a stroke. Even when blood pressure is not lowered with exercise training, healthy changes in body composition, cholesterol, and glucose and insulin almost always happen. You will also benefit from the diet. This diet is the first step to a low fat/low salt diet that is healthy for your heart. The benefits of a lower fat and salt diet are also well known. It is the investigator's hope that the exercise becomes an enjoyable experience and that you will enjoy exercising with others who share many of the same health and fitness goals as you do. The benefits of dietary counseling and exercise training have been shown in large studies involving many participants. Whether these benefits will occur in you cannot be guaranteed.

Alternative Treatments

Alternative treatments to aerobic exercise training are very limited. Of course, under your physician's direction, there is the option of increasing your medications to control your blood pressure. This may be the case even if exercise does lower your blood pressure. However, blood pressure medicine cannot do all of the things that aerobic exercise can. All of the side effects of aerobic exercise training in terms of health are beneficial. There are other treatments that do not use medication. Lowering the amount of salt in your diet and reducing your body weight if you are overweight may help to lower your blood pressure too. As with exercise, these treatments may not be effective for every person, and, each person may respond differently to them. You should always ask your doctor before you start any of these ways to help treat your blood pressure. You also have the choice to not participate in this study.

Confidentiality Statement

All documents and information about to this study will be kept confidential in accordance with federal, state, and local laws and regulations. You understand that medical records and data generated by the study may be reviewed by Temple University's Institutional Review Board, the Office for Human Research Protections, and the National Institutes of Health to assure proper conduct of the study and compliance with federal regulations. You understand that the results of this study may be published. If results are published, you will not be identified by name.

Voluntary Participation Statement

You understand that participation in this study is entirely voluntary, and that refusal to participate will involve no penalty or loss of benefits to you. You may discontinue your participation at any time without penalty or loss of benefits.

Compensation Statement

You understand that you will receive $150 if you complete this study and attend at least 90% of the exercise training sessions. You understand that you will receive $50 if you complete the baseline testing, an additional $50 if you complete the exercise training with at least 90% attendance, and an additional $50 if you complete the final testing. You will receive compensation for your participation in the form of cash at the end of the study. If you do not complete the entire study you will receive partial compensation for those parts of the study you do complete.

Institutional Contact

If you have questions about your rights as a research participant, you may contact the Institutional Review Board Coordinator at (215) 707-3390

If you have questions about research-related injuries, you may contact the Principal Investigator, Dr. Michael Brown, in the Department of Kinesiology at (215) 204-5218.

Standard Injury Statement

You understand that if you sustain an injury as a result of participation in this study, the physician's fees and medical expenses that result will be billed to your insurance company or you in the usual manner. You understand that financial compensation for such injuries is not available. You understand that you have not waived any legal rights that you would otherwise have as a participant in an investigational study.

Costs Statement

You understand that any doctor's fees, medical tests, or other tests associated with this study will be provided at no cost to you. You understand that you are responsible for transportation to the study site and parking.

Termination Statement

The investigators have the right to terminate your participation without regard to your consent. This could occur if you cannot make your appointments, miss more than 10% of your exercise sessions, or experience a change in your medical condition during the course of the study.

Statement of Significant New Findings

You will be informed in a timely manner of any new information regarding this study that may have an effect on your willingness to participate, continue your participation, or after your participation that may have an effect on your future medical care. You may be asked to sign a revised informed consent that contains this new information.

Final Statement and Signature

This study has been explained to me, I have read the consent form and I agree to participate. I have been given a copy of this consent form.

_____	_____
Participant's signature	Date
_____	_____
Principal Investigator's signature	Date
_____	_____
Witness' signature	Date

Timeline	Visit	Procedure	Required
Month 1	*Orientation Visit*	**1.** Review medical history questionnaire, informed consent **2.** Blood pressure taken.	1 hour time
Month 1	*Before Screening Visit 1*	12 hour overnight fast evening before screening visit 1	12 hours intake monitoring
Month 1	*Screening Visit 1*	**1.** Blood and urine sample drawn **2.** Blood pressure taken.	1 hour time
Month 1	*Screening Visit 2*	Physical exam and exercise stress echo test	1 ½ hours time
Month 2	*Dietary Stabilization Period*	**1.** Learn and maintain AHA diet. **2**. Complete food records. **3**. Meet 1 session/week for 6 weeks	**1.** Monitor and maintain dietary intake. **2.** Attend 2 dietary sessions a week for 4 wks.
Month 2	*Before Baseline Testing*	12 hour overnight fast evening before first visit	12 hours intake monitoring
Month 2	*Baseline Testing*	**1.** Blood samples **2**. Body composition tested. **3.** Blood pressure taken. **4.** Blood vessel function tests **5.** 24 hour urine and BP collection. **6.** Submaximal treadmill test to measure fitness level	*Several visits:* **1.** 1 ½ hours: Collection of blood, urine, blood pressure. **2.** Body comp. taken and take home supplies for 24 hour collection. **3.** After 24 hour collection, drop off supplies and samples. **4.** 1 ½ hours for blood vessel function testing
Months 3-8	*Exercise Training*	Supervised exercise training sessions:	3 sessions a week for 6 months
Month 9	*Before Final Testing*	12 hour overnight fast evening before first visit of final testing.	12 hours intake monitoring
Month 9	*Final Testing*	Repeat Baseline Testing	Same as baseline testing