

THE INFLUENCE OF AEROBIC EXERCISE TRAINING ON BIOMARKERS OF ENDOTHELIAL  
ACTIVATION IN SEDENTARY AFRICAN AMERICANS

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## ABSTRACT

**Purpose:** Clinical, epidemiological and basic research evidence supports the inclusion of regular physical activity as a tool for the prevention of chronic disease and the enhancement of overall health. Cardiovascular disease (CVD), the number one cause of death in the United States, is more prevalent in African Americans when compared to other races. Extensive data suggests that increasing physical activity level, particularly with aerobic exercise training (AEXT), can improve modifiable risk factors (hypertension, obesity, dyslipidemia) for CVD. The common pathology for cardiovascular (CV) risk factors is atherosclerosis. Central to the complex pathology of atherosclerosis is the vascular endothelium. In recent years, autocrine and paracrine endothelial biomarkers that directly affect endothelial status (activated vs. inactivated) have been implicated in the pathogenesis of the development and progression of CVD and its precursors. Exercise interventions have been used to modify the concentrations of endothelial biomarkers in populations with varying disease states. The purpose of this study was to identify plasma and urinary biomarkers that are associated with aerobic capacity ( $VO_{2max}$ ) in a sedentary African American population and further determine the effect of 6-months of AEXT on the concentration and activity of the biomarkers. **Methods:** Participants were recruited from the Philadelphia, PA area. Twenty two pre-hypertensive African Americans (SBP  $122.15 \pm 10.33$ , DBP  $77.00 \pm 5.88$ ;  $52.27 \pm 6.25$  years of age) were included. Routine fasting blood samples were drawn to assess blood lipids and fasting blood glucose along with urinalysis to rule out kidney dysfunction or disease. Subjects had a physical examination and BP measured under standardized conditions. Exclusion criteria included smoking, a body mass index (BMI)  $> 40 \text{ kg/m}^2$ , alcohol intake of more than 3 drinks per day, diabetes (fasting glucose level  $> 126 \text{ mg/dl}$ ), total cholesterol  $> 240 \text{ mg/dl}$ , renal or CV disease. On a separate day, a sub-maximal graded exercise test with gas analysis was conducted to determine aerobic capacity.  $VO_{2max}$  was estimated from the baseline submaximal graded exercise test. Regression analysis was used to calculate  $VO_{2max}$ . Participants underwent 6 months of AEXT at a prescribed 3 sessions per week

for 40 minutes at 65%  $VO_{2max}$ . Plasma biomarkers of oxidative stress (8-isoprostane  $PGF_{2\alpha}$ ), cellular activation (VCAM-1), anti-oxidants (SOD), vascular tone (NO) and anti-thrombosis (2,3 dinor 6-keto Prostaglandin  $F_{1\alpha}$ ) were measured before and after AEXT by commercially available EIA and ELISA kits. CRP, a biomarker of systemic inflammation and predictor of CV events was assessed. **Results:** Estimated  $VO_{2max}$  values confirmed that the exercise group was untrained ( $VO_{2max}$ :  $25.31 \pm 3.91$  ml/kg/min). At baseline the most significant correlations observed were between  $VO_{2max}$  and CRP ( $r = -.50$ ,  $p = .01$ ) as well as CRP and 8-isoprostane  $PGF_{2\alpha}$  ( $r = .88$ ,  $p < .01$ ). Following AEXT, the association between  $VO_{2max}$  and CRP remained statistically significant ( $r = -.46$ ,  $p = .02$ ). Nitric oxide and VCAM-1 concentrations significantly differed following the AEXT intervention (NO: pre  $24.07 \pm 8.80$   $\mu\text{mol/L}$ , NO: post  $37.17 \pm 15.57$   $\mu\text{mol/L}$ ,  $p < .01$ ; VCAM-1: pre  $632.58 \pm 179.07$ , VCAM-1: post  $525.12 \pm 148.50$ ,  $p = .01$ ). **Conclusions:** Elevated basal plasma VCAM-1, CRP and 8- isoprostane  $PGF_{2\alpha}$  levels are evidence of endothelial activation and systemic inflammation. Pre-intervention findings provide evidence that having a higher  $VO_{2max}$  was strongly associated with decreased concentrations of CRP, a marker of systemic inflammation that is highly associated with risk for CVD. Post-intervention analysis suggests 6-months of AEXT is an appropriate intervention for elevating NO and decreasing VCAM-1 concentrations. This suggests there were cardioprotective modifications in the endothelial phenotype. The absence of significant change in SOD activity, 2,3 dinor 6-keto Prostaglandin  $F_{1\alpha}$  and CRP concentrations may suggest that AEXT is not a suitable mechanism to elicit improvements in all metabolic pathways that impact the state of the endothelium in previously sedentary African Americans.

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## ABBREVIATIONS

<b>Abbreviation</b>	<b>Word/Words</b>
2,3-d-6-keto	2,3 dinor 6-keto Prostaglandin F <sub>1α</sub>
8-iso-PGF <sub>2α</sub>	8-Isoprostane Prostaglandin F <sub>2α</sub>
AEXT	Aerobic Exercise Training
AHA	American Heart Association
ANOVA	Analysis of Variance
BIA	Bioelectrical Impedance
BMI	Body Mass Index
BP	Blood Pressure
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CRP	C-Reactive Protein
CV	Cardiovascular
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
ED	Endothelial Dysfunction
EIA	Enzyme Immunoassay
EKG	Electrocardiogram
ELISA	Enzyme Linked Immunosorbent Assay
eNOS	Endothelial Nitric Oxide Synthase
FMD	Flow Mediated Dilatation
FRS	Framingham risk score
GXT	Graded Exercise Test

H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
HDL	High Density Lipoprotein Cholesterol
HR	Heart Rate
HTN	Hypertension
HUVEC	Human Umbilical Vein Endothelial Cell
JNC7	Seventh Joint National Committee
LDL	Low Density Lipoprotein Cholesterol
NHANES	National Health and Nutrition Examination Survey
NO	Nitric Oxide
O <sub>2</sub> <sup>-</sup>	Superoxide
OH	Hydroxyl Radical
ONOO <sup>-</sup>	Peroxynitrite
PGI <sub>2</sub>	Prostacyclin
Pre-HTN	Pre-hypertension
ROS	Reactive Oxygen Species
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOD	Superoxide Dismutase
TXA <sub>2</sub>	Thromboxane A <sub>2</sub>
VCAM-1	Vascular Cell Adhesion Molecule – 1
VO <sub>2</sub>	Oxygen Consumption
VO <sub>2max</sub>	Maximal Oxygen Consumption

## CHAPTER 1

### REVIEW OF LITERATURE

#### Introduction

Clinical, epidemiological and basic research evidence supports the inclusion of regular physical activity as a tool for the prevention of chronic disease and the enhancement of overall health [1]. Maximal  $VO_{2max}$  is an independent risk factor for cardiovascular (CV) and all-cause mortality [2-3]. Extensive data suggests that increasing physical activity level, particularly with aerobic exercise training (AEXT), can improve modifiable risk factors (hypertension (HTN), obesity, dyslipidemia) for CV disease (CVD) [1,4-6]. CVD encompasses a number of diseases in which atherosclerosis is a common pathology. Atherosclerosis incorporates elements of the blood vessel wall along with circulating cells in its pathology with the central focus being the vascular endothelium. In recent years, biomarkers that are produced by the endothelium and/or that directly affect the function of the endothelium have been implicated in the development and progression of CVD and its antecedents [7-12].

Public health and medical authorities concur that reduced physical activity on the job and during leisure time increases the risk of CV events, as well as all-cause mortality. The National Health Interview Survey reported that only 23.2% of African Americans were engaging in regular physical activity [13-16]. CVD, the leading cause of death in the United States, is more prevalent in African Americans when compared to other races [11-13]. These racial/ethnic disparities in the prevalence of CVD may be reduced by promoting exercise programs specifically for African Americans. It is clear that aerobic exercise is cardioprotective and vasoprotective, though a detailed understanding of the cellular mechanisms responsible for this cardioprotection remains incomplete. Understanding the molecular basis for exercise-induced improvements in endothelial and cardiovascular health in African Americans will play an important role in developing optimal therapeutic exercise interventions.

#### Aerobic Exercise Capacity

Exercise capacity provides a wealth of clinically relevant diagnostic and prognostic information. Engaging in regular exercise lowers the risk of CVD and incidence of HTN [1-5, 18-

21]. Most recommendations suggest at least 30 minutes of moderate intensity physical activity on most (preferably all) days as a primary-prevention strategy for CVD in the general population [18-21]. Multiple studies report that after adjusting for age, peak exercise capacity ( $VO_{2max}$ ), was the strongest predictor of risk of death in healthy men and women and those with CVD. Risk for CV mortality associated with fitness was similar to that for cigarette smoking and elevated cholesterol levels [18,22-24]. These studies demonstrate that low aerobic capacity is a strong predictor of mortality and that an improvement in exercise capacity confers an increased likelihood of survival [15,20-22,25-28].

Recent data suggest that vigorous acute exercise (defined as 6 METs or more) could simultaneously increase the short-term risk of sudden death during and up to 30 minutes after vigorous exertion but offers protection from this risk in those who habitually engage in habitual exercise. Habitual vigorous exercise training attenuated the relative risk of sudden death that was associated with an isolated episode of vigorous exertion [25]. Considering the data available, there is a rational pathophysiological basis for recommending moderate-intensity but not vigorous-intensity as a therapeutic level of exercise [22, 24]. Available data suggest moderate intensity (40 to 70%  $VO_{2max}$  or 3 to 5.5 METs) training is associated with significant decreases in blood pressure (BP) [18, 24, 29]. Lee et al. reported that exercise intensity greater than 70%  $VO_{2max}$  did not show differing effects in BP but there was a difference found in hemostasis and platelet function. Moderate intensity exercise training suppresses platelet adhesion and aggregation with more pronounced effects in sedentary subjects when compared to physically active subjects [24, 30].

Exercise increases cardiac output, which in turn increases blood flow and vascular laminar shear stress. Shear stress can be defined as the frictional force acting on the endothelial cell surface as a result of blood flow [31-32]. Laminar shear stress is an important determinant of the function and structure of endothelial cells in vivo. Sustained high laminar shear stress upregulates gene and protein expression in endothelial cells, generating a protection against the atherosclerotic process [33-34]. Regular exercise can cause a reduction in oxidative stress, an increase in NO availability and improve the overall metabolic profile of an individual [35].

## The Endothelium

The endothelium consists of a monolayer of cells lining the lumen of blood vessels where it is constantly exposed to hemodynamic shear stress. The endothelium has multiple functions which include:

1. Maintenance of a selective permeability barrier between circulating blood and the underlying tissues;
2. Maintenance of vessel integrity and blood fluidity, prothrombotic, anticoagulant, antiplatelet and fibrinolytic activities;
3. Regulation of blood cell–vessel wall interactions;
4. Involvement in innate and adaptive immune responses;
5. Modulation of vascular smooth muscle tone (blood-flow distribution); and
6. Contribution to the maintenance of a quiescent, differentiated vascular smooth muscle phenotype [36].

These regulatory functions are carried out through the release of certain paracrine and autocrine factors including nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>), the expression of specific enzymes, and the surface expression of proteins such as cell adhesion molecules. Endothelial responses to stimuli can be acute such as the release of paracrine or autocrine factors, or prolonged, implying changes in gene expression. Fluid shear stress is an important determinant of the function and structure of endothelial cells in vivo. The physiological range of shear stress is 10-55 dynes/cm<sup>2</sup> [37]. The capacity of the endothelium to adapt its phenotype depending on the physiological conditions makes it very sensitive to pathological changes altering its structure and function [31-33].

### *Disruption of Endothelial Function*

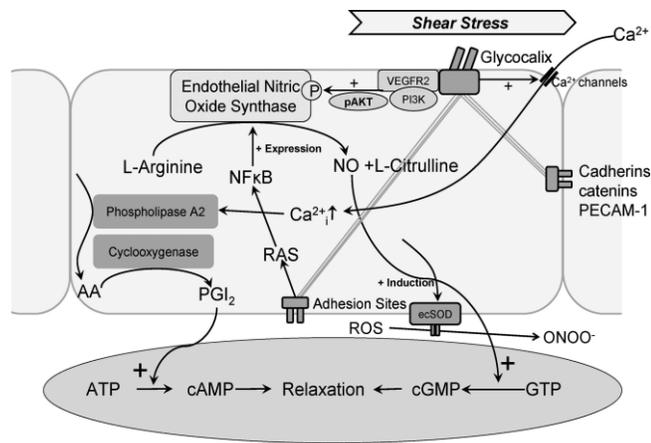
Endothelium-dependent vasodilation can be assessed in the coronary and peripheral circulations in humans. The primary surrogate marker for endothelium-dependent vasodilation in the plasma is nitric oxide NO. During endothelial cell activation, the loss of NO initiates a chronic inflammatory response of the arterial walls [31, 37]. Measurement of biomarkers including

specific adipokines, cellular adhesion molecules, and other pro-inflammatory markers are considered a good complement to the direct assessment of endothelial function (EF) and can be assessed years before a CV event [38]. Abnormalities in vascular endothelial and smooth muscle cell function contribute to atherosclerosis and other CVD risk factors. The link between endothelial dysfunction (ED) and CVD, smoking, HTN, diabetes, atherosclerosis and hyperlipidemia have all been consistently associated with impaired NO production and thus impaired endothelium-dependent vasodilation in animal and human studies [38-42].

Kalinowski and others report that the prevalence of endothelial impaired function disorders such as HTN and atherosclerosis is disproportionately higher in African Americans [43-45]. BP in the pre- and Stage-1 hypertensive ranges have been associated with oxidative damage to blood vessels and impaired endothelium-dependent vasodilation [40]. Age-related ED has been characterized in animals and humans, although the mechanisms involved are still under investigation [36-46].

#### *Exercise and the Endothelium*

Increased cardiac output during physical exertion results in intermittent increases in laminar shear stress. The majority of exercise effects on the vascular endothelium are mediated by the increased shear stress on the endothelial cells lining the blood vessels. As shown in Figure 1 below, shear stress causes deformation of the glycocalyx on the luminal side of the endothelial cell resulting in activation of calcium ion channels, phospholipase activity leading to calcium signaling, prostaglandin I<sub>2</sub> (PGI<sub>2</sub>) release, cAMP-mediated smooth muscle cell relaxation, and endothelial NO synthase (NOS) phosphorylation, resulting in increased NO and prostacyclin production [47]. Increased NO synthesis in response to shear stress induces superoxide dismutase (SOD) to inhibit the degradation of NO by superoxide anion (O<sub>2</sub><sup>-</sup>) by forming a less reactive ROS peroxynitrite (ONOO<sup>-</sup>) [48].



**Figure 1: Exercise Effects on the Vascular Endothelium.** Shear stress induced deformation of the glycocalyx can activate calcium channels to open which will increase phospholipase activity. PGI<sub>2</sub> and NO stimulate vasodilation, induce SOD to dismutate ROS to ONOO<sup>-</sup>. Akt indicates protein kinase B; PECAM-1, platelet endothelial cell adhesion molecule-1; Ras, small GTPase; ONOO<sup>-</sup>, peroxynitrite; and AA, Arachidonic acid. Davies & Helmke, 2010

The effects of exercise on the endothelium became a primary focus due to significant findings that disturbances in endothelial function are key in the development of CVD and predictive of future CV events and that exercise consistently improves EF [39,48]. Studies have evaluated the exercise training effects on endothelial function in various at-risk populations (hypertensives, diabetics and hypercholesterolemics) [48-52]. Higashi et al. investigated the effect of exercise training on endothelial function in hypertensive subjects in which the participants performed 12 weeks of aerobic exercise [48]. They suggested that the increase in shear stress from increased flow was responsible for changes in NO production and the subsequent improvement in endothelial function [48-50]. The effect of physical activity on age-related ED has also been assessed. Common findings indicate a progressive decline of endothelium-dependent vasodilation with aging and that regular physical activity could prevent age-induced endothelial dysfunction [39,48, 51].

### Oxidative Stress

Oxidative stress is defined as the pathological outcome of the overproduction of reactive oxygen species (ROS) that overwhelms the cellular antioxidant capacity. ROS are chemically-reactive molecules containing oxygen ions and peroxides. The process of ATP generation to

sustain the ongoing needs of living cells is accompanied by production of small amounts of ROS. Oxygen is a universal electron acceptor that allows aerobic organisms to use energy stored in carbohydrates, fats, and protein. It is widely accepted and experimentally proven that this catabolic process can generate oxygen free radicals and other ROS such as superoxide ( $O_2^-$ ), hydroxyl radical (OH), and hydrogen peroxide ( $H_2O_2$ ). Under normal physiological conditions, the majority of ROS are produced in the mitochondrial electron transport chain. These minute amounts of ROS are buffered by innate defenses which include SOD, catalase and glutathione. In certain disease states such as diabetes, HTN, and atherosclerosis, the innate defenses can become overwhelmed by ROS [13,39,52].

Lipid peroxidation occurs when ROS alter polyunsaturated fatty acids in the phospholipid membranes of cells. Modification of arachidonic acid, a constituent of the phospholipid bilayer, can result in the formation of biologically active compounds [36]. Oxidation of lipids, proteins and nucleic acids either systemically or locally, has been implicated in the pathogenesis of CVD risk factors including atherosclerosis and diabetes [36,41-42,52-54]. Additionally, stimulated neutrophils, endothelial and smooth muscle cells can release ROS (oxygen-derived free radicals and  $H_2O_2$ ). The interaction of  $O_2^-$  with NO leads to a decrease in NO expression and activity (decreased vasodilation) via the formation of ONOO- [37].

Individuals who do not exercise regularly reportedly produce greater levels of ROS following a bout of exercise than individuals who exercise regularly [20-21]. Studies examining the association of  $VO_{2max}$  on endothelium-dependent vasodilation and antioxidant capacity have concluded that a higher  $VO_{2max}$  is associated with better endothelium-dependent dilation and a higher basal concentration of antioxidants [47, 55].

#### Vasoprotective Biomarkers

##### *Superoxide Dismutase*

One of the classes of enzymes with cytoprotective properties involved in the antioxidant defense system are the SOD's. SOD catalyzes the dismutation of  $O_2^-$  to  $O_2$  and  $H_2O_2$  [54]. Three forms of SOD exist (cytosolic, mitochondrial and extracellular). The effect of laminar shear stress on the expression of cytosolic SOD in cultured human aortic endothelial cells has been examined.

Inoue et al. found that laminar shear stress of 0.6 to 15 dyne/cm<sup>2</sup> increased cytosolic SOD mRNA, protein content and enzyme activity in a time- and dose-dependent manner in human aortic endothelial cells [33]. These findings indicate that increases in physiological levels of shear stress increase expression of cytoplasmic SOD in the endothelium.

### *Nitric Oxide*

In healthy individuals, the quiescent state of the vascular system is maintained by vasoactive compounds produced from endothelial cells, most notably NO. NO has a regulatory role in the vascular system, eliciting vasodilation and inhibiting platelet aggregation, leukocyte adhesion to the endothelium and vascular smooth muscle cell proliferation [56-60]. NO bioavailability is mediated by both constitutive and inducible forms of NOS [56]. When activated, NOS mobilizes, converting molecular oxygen and L-arginine to NO and L-citrulline (Figure 1) [56-57].

Shear stress is one of the most important mechanical regulators of endothelial NOS (eNOS) [56]. Laminar shear stresses induce phosphorylation of eNOS. An early biochemical marker of endothelial dysfunction is the loss of NO, initiating a chronic inflammatory response of the arterial walls. The most notable disruption in vascular homeostasis is an imbalance in the release of vasoconstrictor and vasodilator compounds. The bioavailability of NO is attenuated in many inflammatory conditions such as HTN, obesity, and atherosclerosis [57-58].

Loss of NO bioavailability involves decreases in vasodilation, increased adrenaline release and elevated tissue oxidative stress [9,61-64]. Significant increases in eNOS activity were observed when co-cultured cells (rat artery smooth muscle and bovine aortic endothelial cells) underwent shear stress at a rate of 15 dynes/cm<sup>2</sup> versus 0.5 dynes/cm<sup>2</sup> suggesting vessel function can be improved when blood flow through the vasculature is changed from pathologic to physiologic loads [65-67]. Low levels of laminar shear stress are thought to be involved in the development of CV pathologies. Hendrickson et al. reported that NO and PGI<sub>2</sub> are the best characterized endothelial-derived mediators in response to shear stress [65].

### *Prostacyclin*

Eicosanoids are 20-carbon molecules derived from arachidonic acid that exert a multitude of effects on the endothelium and other tissues. Arachidonic acid, a polyunsaturated fatty acid is freed from the lipid bilayer by phospholipases, primarily Phospholipase A<sub>2</sub>. Cyclooxygenase (COX) enzymes are ubiquitously expressed in mammalian blood vessel, kidney and stomach tissue. COX enzyme isoforms, (COX-1 or COX-2), and cell specific synthases convert the freed arachidonic acid to a biologically active compound which can be released into the circulation. The biologically active eicosanoid binds to receptors on the target cell plasma membrane to cause a wide range of biologic effects including platelet aggregation, lymphocyte aggregation and proliferation, bronchoconstriction, smooth muscle cell contraction, vasodilation or vasoconstriction. Stable metabolites of the eicosanoids can be measured in urine, plasma or tissue and are thought to represent whole-body synthesis of eicosanoids. The large family of eicosanoids can be more specifically classified as groups of prostaglandins, thromboxanes and lipoxigenases [68-71].

PGI<sub>2</sub>, a vasodilator and anti-platelet aggregator, is one of the best characterized prostaglandins influencing vascular tone. Vascular endothelial cells are the primary source of PGI<sub>2</sub>. The vasodilator activity of PGI<sub>2</sub> is determined by the expression of specific receptors on vascular smooth muscle cells. Receptors activated by PGI<sub>2</sub> are coupled to adenylyl cyclase. Vasodilation is achieved via increases in cyclic AMP levels (Figure 1). COX-2 is an inducible form of cyclooxygenase found in areas of inflammation as well as in the vascular endothelium which is subjected to hemodynamic shear stress. COX-2 is the primary precursor for prostacyclin synthase. PGI<sub>2</sub> is a very labile, biologically active compound with stable, inactive metabolites, 6-keto PGF<sub>1α</sub> and 2,3 dinor 6-keto PGF<sub>1α</sub> that can easily be assayed in plasma or urine, respectively. The expression of the gene encoding for PGI synthase in endothelial cells is augmented with age and by HTN [70-71]. PGI<sub>2</sub> is thought to have cytoprotective and cardioprotective properties.

## Markers of Inflammation and Cellular Activation

### *C-Reactive Protein*

C-reactive protein (CRP) has been validated as a marker of increased systemic inflammation and reported to have biological functions involved in decreasing production of eNOS leading to endothelial cell dysfunction and the progression of atherosclerosis [7-8,72-78], Cottone et al. revealed a significant relationship between lipid peroxidation, measured by 8-isoPGF<sub>2α</sub>, and risk for a CV event based on CRP risk categories [74-75]. In addition to inhibiting eNOS expression and bioactivity, CRP promotes tissue factor expression in monocytes and induces the expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) in human umbilical vein endothelial cells (HUVECS) and coronary artery endothelial cells [74,79]. Kawanami and colleagues found that CRP induced VCAM-1 mRNA expression and increased VCAM-1 promoter activity in vascular endothelial cells [79].

CRP adds to the predictive power of blood lipids (HDL and TC) to determine risk of future cardiac events. Significant independent associations exist between CRP and total cholesterol, triglycerides, systolic blood pressure and waist circumference. Prospective studies have shown that CRP levels of <1, 1 to 3, and >3 mg/L correspond to low, moderate and high risk groups for future CV events [7-8,74]. CRP is understood to be a mediator as well as a marker of atherothrombotic disease [72, 79]. LaMonte et al. report that CRP levels decrease across tertiles of fitness when African Americans, Native Americans and Caucasians were pooled. When racial/ethnic groups were examined separately, CRP did not decrease in the African American group as it did in the others [80]. Several studies have reported a reduction in CRP in response to diet and/or exercise intervention. Exercise interventions along with diet proved to be more effective in reducing CRP levels than those that used exercise alone in overweight/obese populations [80-82].

### *8-isoprostane PGF<sub>2α</sub>*

Oxidation of biomolecules including lipids, proteins and nucleic acids either systemically or locally, has been implicated in the pathogenesis of many diseases including atherosclerosis

[52,54,74,78, 83]. Oxidative stress-induced lipid peroxidation can lead to alterations in the biological properties of the membrane, such as the degree of fluidity and inactivation of membrane bound receptors or enzymes which may impair normal cellular function and increase membrane permeability [84]. Oxidative stress caused by ROS can be measured by primary or secondary end-products of lipid peroxidation [78]. 8-isoprostane  $\text{PGF}_{2\alpha}$  (8-iso $\text{PGF}_{2\alpha}$ ), an isoprostane produced by the oxidation of tissue phospholipids by ROS has emerged as an accurate surrogate marker for the assessment of oxidative stress in vivo and has been used extensively to quantify lipid peroxidation associated with CV risk factors [78, 83]. 8-iso $\text{PGF}_{2\alpha}$  formation can be induced by an overabundance of ROS or ROS generating molecules. Some ROS produce both NO and  $\text{O}_2^-$ , generating ONOO<sup>-</sup>. Under physiological conditions, ONOO<sup>-</sup> promotes the formation of 8-iso $\text{PGF}_{2\alpha}$ .

It has been suggested that measurement of 8-iso $\text{PGF}_{2\alpha}$  offers the best approach to an accurate quantification of oxidative stress in vivo than other available methods including TBARS and gaseous alkalines [83]. Fearheller et al. reported increased levels of 8-iso $\text{PGF}_{2\alpha}$  in response to a 6-month aerobic exercise intervention in an African American population [85]. Those results conflict with findings from others that report an exercise-induced reduction in 8-iso $\text{PGF}_{2\alpha}$  [86-87].

#### *Vascular Cell Adhesion Molecule-1*

Adhesion molecules enhance monocyte attachment to the endothelial wall allowing monocytes to migrate to the subendothelial space where they can develop into foam cells. Foam cells containing oxidized low density lipoproteins (LDL) contribute to the development and progression of atherosclerosis [76, 78, 88]. It has been well documented that adhesion molecules and CRP are potential predictors of atherosclerosis, coronary artery disease and ischemic CV events [63,72,74-75]. Upregulation of VCAM-1 in endothelial cells is induced by cytokines (i.e. TNF- $\alpha$  and IL-1). High intraluminal pressure (as associated with HTN) induces cytokine and adhesion molecule expression leading to monocyte adhesion. Kawanami and colleagues found that CRP induced VCAM-1 mRNA expression and increased VCAM-1 promoter activity in vascular endothelial cells [79]. VCAM-1 expression and activity is also responsive to alterations in shear stress which makes it potentially relevant to exercise [89-92].

## CHAPTER 2

### PRELIMINARY STUDY

Eicosanoid Production Following One Bout of Exercise in Middle-Aged African American Pre- and

Stage-1 Hypertensives

#### Introduction

A preliminary study was conducted in order to establish plausibility for the current dissertation research study. The preliminary study explored the relationship between  $VO_{2max}$  and urinary metabolites of validated mediators of vascular tone and atherogenesis. There is limited data available on whether a relationship exists between cardiorespiratory fitness (measured by  $VO_{2max}$ ) and vasoactive eicosanoids.

#### Abstract

Endothelial dysfunction and a sedentary lifestyle may be involved in the development of HTN which is proliferative among middle-aged African Americans. Some signaling molecules derived from the oxidation of 20-carbon fatty acid molecules known as eicosanoids influence vascular tone. There is little known about the correlation of aerobic fitness and eicosanoid formation following exercise in middle-aged African American hypertensives. The purpose was to determine the relationship between  $VO_{2max}$  and eicosanoid formation after a bout of moderate intensity exercise in middle-aged African American hypertensives. Ten sedentary hypertensive African American adults underwent 50 min of aerobic exercise at 65%  $VO_{2max}$ . Urine was collected for 24-hr on two occasions; prior to testing and immediately following the bout of exercise. Urinary metabolites of prostacyclin (6-keto  $PGF_{1\alpha}$ ) and thromboxane (11-d $TXB_2$ ) were measured during the day and night periods by high performance liquid chromatography (HPLC). 6-keto  $PGF_{1\alpha}$  levels significantly increased ( $p=.04$ ) following the bout of exercise when compared to the control day. There was a significant relationship ( $r=.49$ ,  $p<.05$ ) between 6-keto  $PGF_{1\alpha}$  levels and  $VO_{2max}$  during the exercise day. Based on this preliminary study, there appears to be a relationship between  $VO_{2max}$  and exercise-induced 6-keto  $PGF_{1\alpha}$  production in middle-aged hypertensive African Americans. African Americans with lower  $VO_{2max}$  had lower 6-keto  $PGF_{1\alpha}$

formation. Impaired 6-keto PGF<sub>1α</sub> production in response to exercise may be suggestive of endothelial dysfunction.

### Introduction

HTN is a multi-factorial disease that has high prevalence in African Americans [13,43]. National surveys show that the majority of middle-aged, urban African Americans engage in little or no leisure-time physical activity [93]. This high prevalence of physical inactivity contributes to the disproportionate burden of obesity, HTN, diabetes, and coronary heart disease in African Americans [93-95].

A number of important causal factors for HTN have been identified. Research has implicated endothelial dysfunction as a factor involved in the pathogenesis of HTN and is evident in the early stages of the development of coronary atherosclerosis [13,44,69,78,83]. HTN is also more prevalent with advancing age. The prevalence of endothelial-impaired function disorders such as HTN is disproportionately higher in the African American population in contrast to Caucasians [13-14,45].

Eicosanoids are 20-carbon molecules derived from arachidonic acid, a polyunsaturated fatty acid. Cyclooxygenase 1 or 2 (COX-1 or COX-2) and cell specific synthases convert free arachidonic acid to a biologically active compound. The biologically active eicosanoid binds to receptors on the target cell plasma membrane to cause a wide range of biologic effects including platelet aggregation, lymphocyte aggregation and proliferation, bronchoconstriction, and vasodilation or vasoconstriction. Stable metabolites of the eicosanoids can be measured in urine, plasma or tissue and are thought to represent whole-body synthesis of eicosanoids [68,70].

Thromboxane (TXA<sub>2</sub>), a vasoconstrictor and platelet aggregator; and prostacyclin (PGI<sub>2</sub>), a vasodilator and anti-platelet aggregator, are the most well-characterized eicosanoids influencing vascular tone. TXA<sub>2</sub>, a very unstable compound produced by platelets, has thrombogenic properties and is cytotoxic. The stable metabolite of TXA<sub>2</sub> is 11-dehydro-Thromboxane B<sub>2</sub> (11-dTXB<sub>2</sub>). The stable metabolite of PGI<sub>2</sub> is 6-keto PGF<sub>1α</sub> [96].

Acute exercise enhances the generation of reactive oxygen species (ROS) which can lead to the oxidation of lipids, proteins and nucleic acids altering cellular function. The increase in

ROS after acute exercise promotes an acute phase of local inflammation that characteristically induces the release of inflammatory cytokines. These cytokines stimulate the release of arachidonic acid and ultimately an increase in PGI<sub>2</sub> production. Thus, the exercise-induced increase in PGI<sub>2</sub> is a normal consequence following exercise [97-98]. A recent study by Zoladz reported an attenuated PGI<sub>2</sub> release following exercise in coronary artery disease and hypertensive patients when compared to healthy controls [99]. There is contrasting evidence reported on exercise-induced TXA<sub>2</sub> production. Some research indicates an increase in TXA<sub>2</sub> following exercise when others have reported no change [96,99-102].

There is little, if anything, known about the acute exercise-induced responses of PGI<sub>2</sub> and TXA<sub>2</sub> in middle-aged African Americans. The purpose of the study was to assess the changes, if any, in PGI<sub>2</sub> and TXA<sub>2</sub> production following a single bout of aerobic exercise in middle-aged hypertensive African Americans.

#### Methods

Participants were recruited from the Baltimore, MD and Washington, DC area. Ten volunteers (5 male, 5 female; 58±2.3 yrs) completed the study. The participants were sedentary (regular aerobic exercise < 2 sessions/wk and < 20 min/session, sedentary occupation) African Americans who were categorized as having pre-HTN (pre-HTN) or Stage-1 HTN (systolic and diastolic BP 143±7/87±7 mmHg).

#### Screening

Subjects had a physical examination, routine fasting blood chemistries, and BP measured under standardized conditions. Inclusion criteria required participants to be pre or Stage-1 hypertensive according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7) guidelines [13] having an average systolic BP of 120-139 mmHg (pre-HTN), 140-159 mmHg (Stage-1) and a diastolic BP of 80-89 mmHg (pre-HTN), or 90-99 mmHg (Stage-1). No participants were taking anti-hypertensive medications. BP level was determined by the average of three casual BP readings from three separate days according to the standards established by the JNC 7 guidelines. Blood samples were drawn in the morning following a 12-hour fast and sent to Quest

Diagnostics for routine chemistries. In addition, blood lipids, serum creatinine and glucose were also measured. Blood lipids were assessed to rule out hyperlipidemia; serum creatinine was measured to rule out renal dysfunction; fasting glucose was measured to rule out diabetes. Individuals with abnormal blood chemistries were excluded from further participation in the study. Each participant then underwent a physical examination by the study physician. Exclusion criteria included smoking, a body mass index (BMI) > 35, alcohol intake of more than 3 drinks per day, diabetes (fasting glucose level > 126 mg/dl), total cholesterol >240 mg/dl, evidence of renal or CV disease.

The screening GXT was a maximal test to screen for signs and symptoms of coronary artery disease. During the test, the participants walked on a treadmill while their BP, heart rate and ECG response were monitored. The speed and/or degree of incline of the treadmill increased every 3 minutes until the participants could no longer continue or showed signs or symptoms of CV events. Participants having a negative screening graded exercise stress test (GXT) were excluded from further participation in the study. The study protocol was approved by the Institutional Review Board of the University of Maryland, College Park. Informed consent was obtained from each participant. A timeline of the protocol is provided in Figure 2 below.

Screening		Baseline					Testing							
Casual BP Screening Blood Draw Bruce Maximal TM test Modified GXT with Gas Analysis							24-Hr Urine	Diet Logs Returned					Exercise	24-Hr Urine
	Diet Log	Diet	Diet	Diet	Diet	Diet	Diet							
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6		

**Figure 2: Preliminary Study Timeline.**

*24-Hour Urine Collection*

Urine was collected for a 24-hour period one week prior to the exercise session (Baseline). Samples were collected in five time periods (00:00-08:00, 08:00-12:00, 12:00-16:00, 16:00-20:00 and 20:00-0:00). Urine for each time period was collected in a separate container. All urine collection containers were kept on ice in a cooler for the entire 24-hour period. Subjects

began urine collection after their first void in the morning and ended after their first void the following morning. Total urine volume was measured to the nearest 0.5 mL and an aliquot of the pooled 24 hour urine was sent to Quest Diagnostics for determination of creatinine concentration. Creatinine is a breakdown product of creatine phosphate in muscle. In the absence of kidney dysfunction, creatinine clearance is relatively constant and can be used as a means to compare other excreted metabolites. Aliquots from each time period were frozen at  $-80^{\circ}\text{C}$  until analysis. A second 24-hour urine collection was repeated immediately following the exercise session.

#### *Diet*

Three days prior to beginning baseline testing, subjects were instructed to maintain their usual diets and complete a 6-day dietary log. Each subject was given their dietary information recorded during the baseline period and instructed to repeat the 6-day diet leading to the exercise day.

#### *Graded Exercise Test to Measure Aerobic Capacity*

A second GXT was completed a week prior to the exercise session. All subjects completed a maximal treadmill exercise test to derive a valid prescription for the acute exercise session. When oxygen consumption ( $\text{VO}_2$ ) is added to a GXT it is possible to measure  $\text{VO}_{2\text{max}}$ , an index of CV fitness. Participants began at 70% of the peak heart rate achieved on the subject's screening exercise test and the treadmill grade was increased 2% every 2 minutes. BP, heart rate, and ECG were monitored during the test which was terminated when the subject could no longer continue.  $\text{VO}_2$  was measured continuously throughout this test using a mass spectrometer (Marquette), mixing chamber (Rayfield), turbine volume meter system (model VMM, Interface Associates), and customized validated metabolic software. Standard criteria were used to determine if  $\text{VO}_{2\text{max}}$  had been achieved. Subjective criteria included the subject's rating of their perceived exertion and their physical inability to continue exercise. The objective criteria that were monitored were a plateau in rise in heart rate and a respiratory exchange ratio of greater than 1.15. The respiratory exchange ratio is determined by dividing the volume of  $\text{CO}_2$  expired per minute by the volume of  $\text{O}_2$  inspired per minute.

### *Acute Exercise Session*

The goal of the exercise session was to accumulate 50 minutes of exercise. The exercise session began with a 10-minute warm-up consisting of walking and stretching exercises. The participants then walked on a treadmill for 30 minutes, followed by 5 minutes of rest, and then 20 additional minutes of treadmill walking or cycle ergometry for a total of 50 minutes of sub-maximal exercise. A heart rate monitor (Polar CIC, Inc) was used to ensure that each subject's exercise heart rate corresponded to 65% of their aerobic capacity that was measured during the  $VO_{2max}$  GXT. After completing the acute exercise session, participants began a 24-hour urine collection period in the same manner as during the baseline collection.

### *Measurement of 6-keto $PGF_{1\alpha}$ and 11-d $TX_{B2}$*

#### *Chemicals and Materials*

The prostaglandins (6-keto  $PGF_{1\alpha}$  and 11-d $TX_{B2}$ ) were purchased from Biomol (Plymouth meeting, PA, USA). Creatinine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ortho Phosphoric acid, HPLC water, and acetonitrile were purchased from Fisher Scientific (Waltham, MA, USA).

#### *Instrumentation*

The Jasco HPLC system consisted of a Jasco pumps (PU-980), a Jasco UV-VIS detector (UV-975) (Jasco Incorporated Easton, MD, USA) and a Rheodyne manual injector (Rheodyne LLC, Rohnert Park, CA, USA). Jasco-Borwin software (version 3.3.5) was used for data collection. The analysis was done on a Symmetry C18 4.6 x 250 mm column with 5  $\mu$ m particle size. (Waters Corporation, Milford, MA, USA).

#### *HPLC Method*

Acetonitrile was used as the organic phase. Phosphoric acid solution (pH=4) was used as the aqueous phase as it gave much better chromatographic results than acetic acid solution. An acidic solution (pH of 4) was necessary to maintain the eicosanoids in the neutral state. Creatinine, at pH =4 which is below its pKa value (4.83) will be in the protonated form. Creatinine in this form will be retained for a very short period of time on a non-polar stationary phase compared to the neutral form of 8-iso  $PGF_{2\alpha}$ , a long chain (C20) polyunsaturated fatty acid. The

flow rate and ratio of the aqueous and organic phase were selected to obtain a method with a practical run time and which resulted in good separation of all the compounds. All the chromatograms showed a negative drop at around 3.0 min. This is due to the change in the wavelength from 254 nm to 196 nm. The HPLC method employed an isocratic elution of 17mM phosphoric acid (solvent A) and acetonitrile (solvent B) in the ratio of 65:35. The analytes were separated at ambient temperature with an injection volume of 100  $\mu$ L and using a flow rate of 1.3 ml/min. The run time for the method was 16.5 min.

Urinary metabolites 6-keto PGF<sub>1 $\alpha$</sub>  and 11-dTXB<sub>2</sub> were dissolved in 1 ml methanol such that the stock mass concentration for all was 1mg/ml. 1000 mg of creatinine was dissolved in 100 ml HPLC water to obtain a stock having concentration of 10 mg/ml. Working standards for the analytes were prepared by serial dilution using 17mM phosphoric acid and acetonitrile in the ratio of 1:1 as the solvent. The solutions were stored at – 80°C when not in use. 75  $\mu$ l of the filtered urine samples was diluted to 500  $\mu$ l with a 1:1 mixture of 17mM phosphoric acid and acetonitrile before injecting into the HPLC system.

#### *Linearity, Accuracy, and Precision and Recovery*

The HPLC method was validated as per the FDA guidance for the industry: Bioanalytical method validation [107]. The linear range for 6-keto PGF<sub>1 $\alpha$</sub>  and 11-dTXB<sub>2</sub>, the metabolites of arachidonic acid, was established by injecting in triplicate standard solutions at mass concentrations ranging from their respective LOQ to 1000 ng. Both the compounds showed a good linear response with  $R^2 > 0.999$  in the range. Accuracy was determined by analyzing the compounds at three different mass concentration levels. Percent accuracy for the prostanoids was between 90.3 – 101.5%. Inter-assay and intra-assay precision were determined by injecting in triplicate. Intra-assay and inter-assay precision as indicated by the percent relative standard deviation (RSD) were <10%. The percent recovery for the prostanoids at the three concentration levels was within 95- 108%.

Instrument precision was determined by injecting a single mixture containing all the compounds six times and calculating the RSD values. RSD values were found to be less than

5% for all the analytes. The RSD values for all injections were <10% for intra-assay precision and all urine metabolites were normalized to ug of creatinine.

### *Statistical Analysis*

For statistical analyses, we redefined the time periods to AM (08:00-16:00) and PM (16:00-00:00) because of insufficient data collected between 00:00-08:00. Descriptive statistics were performed for subject characteristics and are presented as mean±SEM in Table 1. For each urinary metabolite (6-keto PGF<sub>1α</sub> and 11-dTXB<sub>2</sub>) a repeated measures ANOVA comparing the metabolite concentrations at the two time periods; before and after acute exercise bout. Repeated measures ANCOVA was used to covary for age, BMI and aerobic fitness. Separate simple linear regression analyses were performed with VO<sub>2max</sub> as the independent variable and each of the urinary metabolites as the dependent variables. Values are presented as mean±SEM. A value of P≤.05 was considered statistically significant. Statistical analysis was performed using STATVIEW (SAS Institute, Cary, NC).

Table 1. Baseline demographic and clinical characteristics

Male/Female	5/5
Age (years)	58±2.3
BMI (kg/m <sup>2</sup> )	30.5±1.1
VO <sub>2</sub> (mL/kg/min)	22.6±1.2
TC (mg/dl)	214.8±9.2
LDL-C (mg/dl)	131.8±10.1
VLDL-C (mg/dl)	27.9±4.0
HDL-C (mg/dl)	55.1±3.4
Casual Avg SBP (mmHg)	143±7
Casual Avg DBP (mmHg)	87±7

Values shown as Mean ± SEM

BMI indicates body mass index; VO<sub>2</sub>, aerobic capacity; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

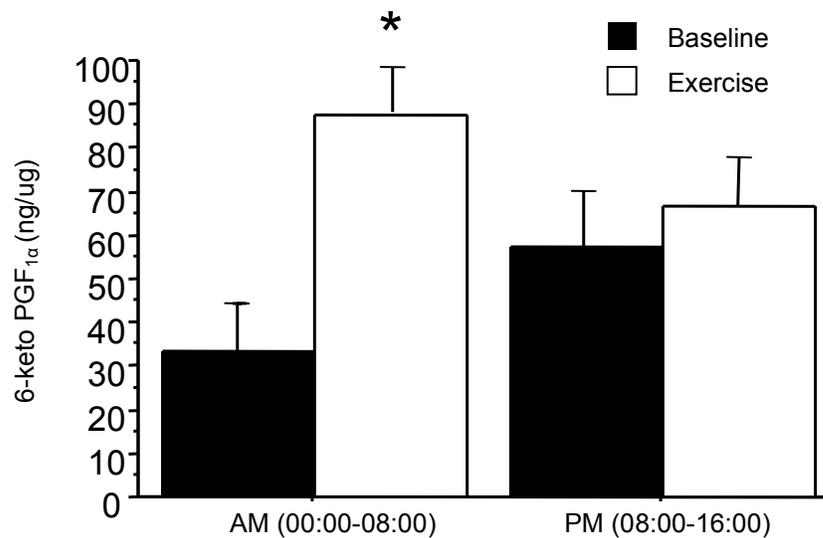
## Results

Subject characteristics are shown in Table 1. Ten African American men and women (5 male, 5 female), age  $58 \pm 2.3$  years, participated in the study.  $VO_{2max}$  determined from the GXT ranged from 17.4 – 29.3 ml/kg/min (mean  $22.6 \pm 1.2$  ml/kg/min), which would classify all participants as sedentary. The average LDL-C for the participants was  $131.8 \pm 10.1$ , which is borderline high according to the American Heart Association guidelines.

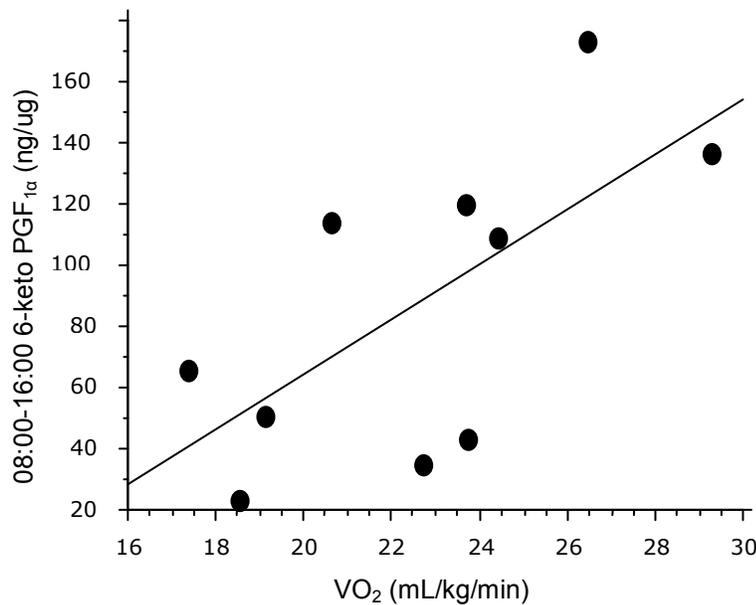
The urinary metabolites were examined at two time periods: 08:00-16:00 (AM) and 16:00-00:00 (PM) by ANOVA (Figure 3). Baseline levels of 6-keto  $PGF_{1\alpha}$  and 11-dTXB<sub>2</sub> were  $33.5 \pm 10.9$  ng/ug and  $50.8 \pm 20.4$  ng/ug in the AM, respectively. The 6-keto  $PGF_{1\alpha}$  levels increased by more than two-fold from  $33.5 \pm 10.9$  ng/ug at baseline to  $77.8 \pm 14.1$  ng/ug ( $p < .05$ ) during the AM collection period following the acute exercise session. Based on ANOVA, there was a significant relationship ( $p = .04$ ) between 6-keto  $PGF_{1\alpha}$  levels and  $VO_{2max}$  during the AM collection period following exercise (Figure 4). ANCOVA identified  $VO_{2max}$  as a significant predictor of 6-keto  $PGF_{1\alpha}$  following the exercise bout. ANCOVA with  $VO_{2max}$  as the sole covariate, followed by Fisher's LSD, improved the effect of the acute exercise bout ( $p = .03$ ).

Correlational analysis conducted using the baseline values revealed a significant positive relationship ( $r = .97$ ,  $p = .04$  AM collection;  $r = .99$ ,  $p = .01$  PM collection) between LDL-C and 11-dTXB<sub>2</sub>. This relationship was not observed between LDL-C and 6-keto  $PGF_{1\alpha}$ . Analysis also revealed a significant negative correlation between 6-keto  $PGF_{1\alpha}$  levels and age ( $r = -.92$ ,  $p = .003$ ).

The urinary 11-dTXB<sub>2</sub> concentrations were decreased at both collection times; 51% AM and 35% PM relative to baseline levels. However this trend, though reproducible and consistent throughout the study did not meet the test of significance as defined by  $p \leq 0.05$  following the acute exercise session.



**Figure 3. Urinary 6-keto PGF<sub>1α</sub> before and after exercise bout.** Urinary 6-keto PGF<sub>1α</sub> levels increased 33.5±11ng/ug to 77.8±14ng/ug during the AM period after the bout of exercise when compared to baseline. Levels returned to near baseline during the PM collection following the exercise bout.  
\* p<.05



**Figure 4. Urinary 6-keto PGF<sub>1α</sub> correlates with VO<sub>2max</sub>.** Urinary 6-keto PGF<sub>1α</sub> collected in the AM following a moderate intensity exercise bout was found to significantly correlate with VO<sub>2max</sub> in sedentary African American pre- and Stage-1 hypertensives (r = .67, p < .05).

## Discussion

Increases in vascular shear stress and mobilization and activation of cytokines and immune cells occur as a result of exercise. This leads to arachidonic acid liberation from the plasma membrane. COX-1 or COX-2 and cell specific synthases convert the free arachidonic acid to a biologically active compound with cytoprotective properties.

Platelet activation increases during exercise. It has been suggested that metabolic activity in the arachidonic acid cascade may be increased equally at various exercise intensity levels, but that the synthesis of PGI<sub>2</sub> and TXA<sub>2</sub> may be more heavily influenced by the exercise intensity level [96]. Alternatively, the conversion of arachidonic acid to eicosanoids other than PGI<sub>2</sub> and TXA<sub>2</sub> may be affected by exercise, which may be responsible for the observed decrease in 11-dTXB<sub>2</sub> following the acute exercise bout. The increase in PGI<sub>2</sub> generation could be compensating for the prothrombotic environment induced by exercise. Prostacyclin production is initiated by an influx of calcium into the cytoplasm resulting from the emptying of intracellular stores, which occurs only in the initial minutes of cellular activation [104]. This could explain why the increase in the excretion of 6-keto PGF<sub>1α</sub> occurred in the morning following the exercise bout and not later in the day.

Rodrigo et al. reported that blood antioxidant activity was lower in hypertensives when compared to normotensive controls [84]. Acute exercise enhances the generation of ROS, which can lead to the oxidation of lipids, proteins and nucleic acids leading to altered cellular function. ROS increases the expression of antioxidant enzymes, but this is attenuated in hypertensives [105-106]. It has been documented that in a hypertensive population, plasma levels of 6-keto PGF<sub>1α</sub> were significantly lower than that of healthy controls [104]. In the current study, individuals who were more aerobically fit (assessed by VO<sub>2max</sub>) produced higher concentrations of urinary 6-keto PGF<sub>1α</sub> following an acute bout of exercise. This may suggest that there is greater antioxidant activity in those individuals who are more aerobically fit than those who are less aerobically fit.

Investigators have observed plasma levels of 6-keto PGF<sub>1α</sub> increased following a low intensity (30-50% VO<sub>2max</sub>) bout of exercise, which suggests that exercise at lower intensities may

stimulate a greater production of 6-keto PGF<sub>1α</sub> [96-97,99]. This trend was seen in the present study population with a 1.5-fold to 2-fold increase in 6-keto PGF<sub>1α</sub>. Todd et al. showed that 6-keto PGF<sub>1α</sub> levels decreased incrementally as exercise intensity increased to near maximal levels [96]. In the present study, moderate intensity aerobic exercise increased urinary levels of 6-keto PGF<sub>1α</sub> following exercise in the AM urine sample. This is consistent with findings by Okahara et al. that have documented an increase in 6-keto PGF<sub>1α</sub> production as a result of increased vascular shear stress and cytokine release [32]. The 6-keto PGF<sub>1α</sub> levels during the PM sample were not different than the baseline day.

Tokunga et al. demonstrated an age-related decline in PGI<sub>2</sub> synthesis which is consistent with our findings. Reduced PGI<sub>2</sub> production, as a result of aging or selective COX-2 inhibition, has been proposed as a mechanism involved in the atherogenic process, myocardial infarctions and stroke [107-108]. Nicholson et al. reported that forearm vasodilation responses to PGI<sub>2</sub> were significantly impaired in an older group when compared to a younger group matched for BMI and sex. They further suggested age-related differences in PGI<sub>2</sub>-mediated vasodilation are likely attributed to a reduced contribution of the endothelium-derived relaxing factor, nitric oxide (NO) [108]. In a race comparison study, healthy African Americans demonstrated blunted NO-mediated vasodilation when compared to a group of healthy Caucasians supporting evidence of race-related defects in endothelial function [45].

The highly reactive oxygen radical, superoxide anion (O<sub>2</sub><sup>-</sup>) has a high affinity for NO, which increases the likelihood of the inactivation of NO and the formation of peroxynitrite (ONOO<sup>-</sup>), a potent oxidant [109-110]. Kalinowski et al. reported racial differences in the steady-state NO/ O<sub>2</sub><sup>-</sup>/ONOO<sup>-</sup> balance in endothelial cells from African Americans. Basal levels are reportedly closer to the redox states characteristic for endothelium-impaired function disorders [13]. In the current study, LDL-C was highly correlated with the urinary metabolite 11-dTXB<sub>2</sub> at baseline. LDL-C has been reported to increase the generation of O<sub>2</sub><sup>-</sup>, along with NO. Weisser et al. showed that LDL-C increases thromboxane synthesis in a dose-dependent manner [111]. Kuklinska et al. reported a significant negative correlation between LDL-C and PGI<sub>2</sub> but we did not observe such a finding [69].

Decreases of 11-dTXB<sub>2</sub> following the exercise session could be due to arachidonic acid being used as a precursor for prostacyclin to compensate for the prothrombotic state. TXA<sub>2</sub> synthase may be at the same expression level but competing PGI synthase may be in higher concentrations. Todd et al. suggested that exercise intensities below 70% of VO<sub>2max</sub> may not be sufficient to stimulate significant alterations in TXA<sub>2</sub> activity [96]. There has been some conflicting evidence in regards to how exercise influences TXA<sub>2</sub> concentrations following exercise. Aerobically trained individuals seem to have an enhanced antioxidant system [110], which could explain the lack of significant alterations in TXA<sub>2</sub> activity at the low exercise intensity.

Increased risk and incidence of disease are associated with low aerobic capacity [95]. In the present study, there was a relationship between aerobic capacity as assessed by VO<sub>2max</sub> and exercise-induced PGI<sub>2</sub> production in pre- and Stage-1 middle-aged hypertensive African Americans. African American subjects with lower VO<sub>2max</sub> had lower PGI<sub>2</sub> formation. Impaired PGI<sub>2</sub> production in response to exercise may be suggestive of endothelial dysfunction. When racial comparisons are made, the endothelium of African Americans is closer to the state characteristic for endothelium-impaired functional disorders than Caucasians [13]. This may explain, in part, the predisposition of African Americans to complications associated with CV disease.

The middle-aged participants were carefully screened to provide us with a homogenous population of healthy participants. The strict inclusion criteria lead to the small sample size which is a limiting factor of this study. The lack of significance found for the urinary metabolite 11-dTXB<sub>2</sub> following the acute exercise bout could be due to the small sample size and/or the intensity level of the exercise session.

### *Conclusion*

The preliminary study examined the best-characterized eicosanoids affecting endothelial function in a population of sedentary, middle-aged pre- and Stage-1 hypertensive African Americans who reside in an urban area. Results of the preliminary study show that those with a lower VO<sub>2max</sub> had a diminished capacity to produce PGI<sub>2</sub>, the cardio-protective eicosanoid produced by the vascular endothelium, after a single bout of exercise. The findings from this

preliminary study incited the examination of other possible biomarkers affecting the endothelium that also correlate to  $VO_{2max}$ . Further, we concluded that it would be beneficial to examine the effects of long-term aerobic exercise training on endothelial function in sedentary African Americans.

### Summary

The findings from the preliminary study revealed a significant relationship between  $VO_{2max}$  and the urinary metabolite of prostacyclin, a molecule with vasoprotective properties. In an effort to further explore the relationship between  $VO_{2max}$  and vasoactive substances, in addition to prostacyclin, end products of NO, SOD, CRP, 8-isoPGF<sub>2 $\alpha$</sub> , and VCAM-1 were measured. These 6 substances are well-characterized and are susceptible to alteration through exercise training or by shear stress (in vivo). There is a great deal of evidence that supports the use of aerobic exercise training to improve vascular function in various populations. The present study is novel and important because it attempts to identify endothelial biomarkers that have regulatory roles in the maintenance of endothelial health or endothelial activation that are correlated to changes in  $VO_{2max}$ . This study provides insight into which vascular biomarkers are more closely associated with  $VO_{2max}$  in African Americans. The prevalence of CVD in the African American population has been well documented. There are differences in CVD risk factor burden, differential clustering of risk factors as well as socioeconomic statuses between races. Albert and Ridker report that approximately 40% of people who eventually develop CVD have none or only one of the traditional CVD risk factors which may suggest a necessity for alternative biomarkers to enhance current screening mechanisms [28]. Identification of these biomarkers may provide insight for future research investigating surrogate biomarkers to enhance traditional risk factor assessment in African Americans specifically.

Intervention strategies such as aerobic exercise training have been shown to improve endothelial function in healthy populations and those with CV risk. There are little data evaluating this relationship in African Americans. Biomarkers of vascular health have been identified and validated. Not only can they be easily obtained with limited invasiveness, but can be processed in a cost-effective manner. To my knowledge, there has not been a human study that has

examined exercise effects on NO, SOD, CRP, VCAM-1, 8-isoPGF<sub>2α</sub> and PGI<sub>2</sub> in a African American population.

#### Statement of Purpose

In recent years, biomarkers that are produced by the endothelium and/or that directly affect endothelial health have been implicated in the pathogenesis of the development and progression of CVD and its precursors. The purpose of this study was to identify biomarkers that are associated with aerobic capacity (VO<sub>2max</sub>) in an at-risk population and determine the effect of AEXT on the levels of activity of NO, SOD, CRP, VCAM-1, 8-isoPGF<sub>2α</sub> and PGI<sub>2</sub> in a middle-aged African American population.

#### Specific Aims

1. Determine the relationship of NO, SOD, CRP, VCAM-1, 8-isoPGF<sub>2α</sub> and PGI<sub>2</sub> to basal VO<sub>2max</sub> in a group of sedentary African Americans.
2. Determine if 6 months of AEXT will modify the concentrations or enzymatic activity of NO, SOD, CRP, VCAM-1, 8-isoPGF<sub>2α</sub> and PGI<sub>2</sub> in relation to an increase in VO<sub>2max</sub>.

#### Hypotheses

It is hypothesized that at baseline, individuals with a low VO<sub>2max</sub> will have higher concentrations of biomarkers of endothelial activation and inflammation (CRP, VCAM-1, 8-isoPGF<sub>2α</sub>) than those with higher VO<sub>2max</sub>. It is also hypothesized that the concentrations of the cardio-protective NO and PGI<sub>2</sub>, and antioxidant activity of SOD, will have a positive correlation with VO<sub>2max</sub>.

Following the 6-month AEXT, it is hypothesized that the biomarkers that were found to be significantly associated with VO<sub>2max</sub> at baseline will remain significantly associated. It is also hypothesized that the participants that have the greatest increase in VO<sub>2max</sub> will have the greatest improvement in endothelial biomarkers. These changes will reflect alterations in favor of endothelial inactivation.

## CHAPTER 3

### METHODS

#### Recruitment and Participants

Participants from the Philadelphia, PA (N=22; 20 women, 2 men) area were recruited via printed advertisements and pre-screened through telephone interview to determine eligibility to participate in the study. Eligible participants met the following criteria: middle-aged (40-70 years), sedentary (regular aerobic exercise  $\leq$  2 sessions/wk and  $<$  20 min/session, sedentary occupation) African Americans with a body mass index (BMI) of  $<40$  kg/m<sup>2</sup> who are categorized as having pre-HTN (pre-HTN) or Stage-1 HTN by the JNC 7 guidelines (pre-HTN: systolic 120-139 mmHg, diastolic 80-89 mmHg; Stage-1 HTN: systolic 140-159 mmHg, diastolic 90-99 mmHg) [12].

Middle-aged participants will be the focus of this study based on the finding from the NHANES study in 2000 [13]. The rationale being that the disparity in the prevalence of HTN in African Americans when compared to other racial groups is the greatest once they reach middle-age, beginning at the age of 40, including both men and women. After 75 years, there is little difference in the prevalence of HTN between races [13].

The rationale for including participants with a sedentary lifestyle is that having a sedentary lifestyle is one of the modifiable risk factors for CV disease. Physical inactivity is not only a predictor of CV mortality but also total mortality in middle-aged men and women. Evidence has shown that regular exercise can increase aerobic capacity and improve vascular health. Exercise can also lead to beneficial alterations in BP [18-23,30,35,48]. Having subjects who are not currently exercising will allow for the observation of any alterations in BP.

The rationale for excluding individuals with BMI  $>$  40 is that excessive weight would interfere with an individual's ability to exercise. Obesity has been reported to lead to HTN by activating the rennin-angiotensin-aldosterone system, increasing sympathetic activity, promoting insulin and leptin resistance, increasing pro-coagulating activity, promoting endothelial dysfunction and increasing renal sodium absorption, ultimately causing volume expansion [113].

Individuals with pre-and Stage-1 HTN were selected to participate in the study. BPs in the pre- and Stage-1 hypertensive ranges has been associated with having oxidative damage to

blood vessels and endothelial dysfunction. Participants need to have stable BP without medication. Persons having sustained BPs  $\geq$  160/100 mmHg are typically prescribed a combination of drugs to control their BPs [12].

The participants were free of diseases that would cause contraindications to exercise or where exercise would exacerbate contraindications including CV, renal or lung disease, diabetes, or any condition that impedes performing aerobic exercise. Any post-menopausal women meeting the above inclusion criteria and who were on hormone replacement therapy (HRT) continued while participants not on HRT refrained from taking any HRT during the length of the study. Hormone therapy has been reported to have anti-oxidative properties that could potentially decrease oxidative stress [114-115]. Additionally, participants refrained from taking any over the counter antioxidant therapy throughout the study. All study participants signed an informed consent (Appendix A). The study protocol was approved by the Institutional Review Board of Temple University, Philadelphia, PA.

Individuals who preliminarily met the inclusion criteria were asked to attend an orientation visit where the study protocol, risks and benefits were explained. The orientation visit provided an opportunity for individuals to have any questions or concerns addressed. Those individuals who remained interested in participating were scheduled to return to the HyMAP laboratory for screening visit #1.

## Screening

### *Screening Visit #1*

Subjects arrived at the HyMAP laboratory after a 12-hour fast to provide blood and urine samples. Samples were sent to Quest Diagnostics for routine chemistries. Upon evaluation of test results, individuals were excluded from the study if they had serum creatinine  $>1.5$  mg/dl, total cholesterol  $<240$  mg/dL, or fasting blood glucose levels  $> 126$  mg/dL.

Creatinine, an end-product of muscle metabolism, is excreted solely by the kidneys. Its production rate is proportional to the patient's muscle mass and is, therefore, relatively stable over time. In adults, the normal range for plasma creatinine is 0.8 to 1.4 mg/dL. In men, 0.8 to 1.3 mg/dL and 0.6 to 1.0 mg/dL in women. The rationale for screening for creatinine clearance is

that a creatinine level of 1.4 may represent normal renal function in a muscular man but may represent markedly decreased function in a less muscular man. This is not a concern considering solely untrained persons are participating. Creatinine excretion can be used to estimate the glomerular filtration rate because creatinine is freely filtered at the glomerulus and is not reabsorbed [116].

Individuals with diabetes were not included because of the consequences of the disease affect the vasculature. The macrovascular manifestations include atherosclerosis and medial artery calcification. The microvascular consequences (retinopathy and nephropathy) are major causes of blindness and end-stage renal failure [117]. Diabetes is associated with an increased prevalence of atherosclerotic vascular disease and CV mortality. In diabetic patients, medial calcification appears to be a strong independent predictor of CV mortality [118]. Clinical studies have found that endothelium-dependent vasodilation is abnormal in patients with type 1 or type 2 diabetes. Experimental evidence supports the notion that hyperglycemia decreases endothelium-derived NO [119]. Hyperglycemia induces a series of cellular events that increase the production of reactive oxygen species (such as superoxide anion) that inactivate NO to form peroxynitrite [118-120]. Eliminating diabetes as a confounding factor eliminated a potential contributing pathway that could have led to endothelial dysfunction and increased production of ROS.

Hyperlipidemia is one of the primary risk factors for CVD. Persons with hyperlipidemia are frequently prescribed lipid-lowering medications. A person having high levels of blood lipids is highly susceptible to endothelial dysfunction and accelerated progression of atherosclerosis [121-122].

#### *Screening Visit #2*

A. Eligible participants had a CV examination by the study cardiologist in order to rule out any CV, pulmonary or any other disease that would have prohibited vigorous exercise. Evidence of HTN-related end organ damage also met criteria for exclusion from the study.

B. A sub-maximal bicycle stress echocardiogram test was administered by the study cardiologist to screen for signs and symptoms of coronary artery disease. The resistance of the cycle ergometer increased every 3 minutes according to the Bruce protocol. The test concluded

when the participants completed the 3 stages, could no longer continue, showed signs or symptoms of CV events or had ST-segment depression of  $> 2$  mV. Participants having a negative screening echocardiogram stress test were excluded from further participation in the study. Additional study exclusion criteria included smoking, alcohol intake of more than 3 drinks per day, taking more than one BP-lowering medication, any cholesterol-lowering or anti-inflammatory medication.

#### Dietary Stabilization

Participants were required to meet with the study registered dietician (RD) once a week for 6 consecutive weeks. In these sessions, the RD informed the participants about the AHA Dietary Guidelines for Healthy American Adults, formerly the AHA “Step 1” diet. Salt intake was to be limited to 3-4 grams per day. Diet logs were collected during diet class, and every 8 weeks during the exercise training period. Logs were analyzed using FoodWorks software, version 11 (Long Valley, NJ, USA) to monitor adherence to the DASH diet.

#### Casual Blood Pressure Measurement

Casual BP was measured in all participants on three separate days according to the JNC 7 guidelines. Casual BP refers to a BP measurement taken in a physician's office or clinic setting using one of several available auscultatory techniques. The guidelines state that the participant should be seated quietly for at least 5 minutes in a chair, with feet on the floor and arm supported at heart level [12]. Measurements were made in triplicate, at least two minutes apart with an appropriate sized cuff. The average of the three separately recorded BP values over three office visits were used in data analyses.

#### 24-hour Urine Collection

Urine was collected for a 24-hour period. Participants were given a cooler that contained ice packs and two collection containers-- one for morning and one for the evening collections. An aliquot of total (AM and PM combined) urine was used to measure urinary 2,3-dinor-6-keto PGF<sub>2α</sub>. Urine samples were stored in -20°C until analysis. 24-hour urine was collected once before and once after the 6-month exercise program.

### Graded Exercise Test

All subjects completed a modified Bruce sub-maximal treadmill graded exercise test (GXT). Oxygen consumption (breath by breath gas analysis) was calculated along with the GXT to estimate  $VO_{2max}$ , an index of CV fitness. The purpose of this test was to determine the prescribed exercise intensity for each individual for the exercise training sessions. According to the guidelines set forth by the American College of Sports Medicine, treadmill speed and/or grade increased every 3 minutes [123]. The test concluded when the participants reached between 75-80% of their age-predicted max heart rate or felt as if they could no longer continue. BP, heart rate, and ECG were monitored throughout the test. Oxygen consumption ( $VO_{2max}$ ) was measured continuously throughout the test with the Viasys  $VO_2$  system (VMax Encore, Sensormedics Software, Yorba Linda, CA). Each subject's rating of their perceived exertion served as subjective criteria. Regression analysis was utilized to calculate estimated  $VO_{2max}$ . A maximal graded exercise test could cause undue stress to persons unaccustomed to exercise. According to Marsh and others, the protocol followed is appropriate for the estimation of maximal  $VO_{2max}$  in a middle-aged, untrained population [124-127]. The GXT was conducted once before beginning the exercise program and again at 6 months.

### Body Composition

Prior to test day subjects were to refrain from exercise, alcohol or excessive salt intake as these can alter water balance. Body composition was measured by bioelectrical impedance (BIA). BIA was measured after 20 minutes of lying still to ensure body water was equilibrated throughout the body. Briefly, two electrodes were placed on the right hand and foot of the subject. The BIA machine sends an electrical current between the sets of electrodes. Percent lean body mass, fat mass, intracellular water, extracellular water and total body water are calculated by ImpediMed DF50 software (Version 2.0.1, Australia, 2005). These tests will be conducted before and after 6-months of exercise training.

### Metabolic Blood Draw

Participants arrived at the HyMAP laboratory following a 12-hour fast. Blood was drawn into vacutainer tubes containing heparin sulfate, EDTA and EDTA with BHT additive. Blood was

spun at 2000 G for 20 minutes. Plasma aliquots were frozen at -80°C until assay. Metabolic blood draws took place before exercise training and after 6 months of exercise training.

#### Exercise Intervention

Studies that have assessed the effects of exercise as a mechanism to restore endothelial function have strengthened decisions to employ exercise training as an important therapeutic treatment [22,24,32-34,48,49]. Therefore, participants were prescribed 3 doses of supervised moderate intensity aerobic exercise per week. Work rate was determined from the predicted  $VO_{2max}$  derived from the sub-maximal GXT. The intensity for the first dose of exercise was 50% of the predicted  $VO_{2max}$ . The duration of the first 3 sessions was 20 minutes. Participants gradually increased time and duration until exercise intensity of 65% and duration of 40 minutes is met. Weeks 1-5 increased to an intensity of 50%  $VO_{2max}$  and had incremental increases of 5 minutes each week until participants reached 40 minutes. During weeks 6-8, exercise intensity increased in 5% increments until 65%  $VO_{2max}$  was reached. Study personnel supervise all exercise sessions. Each participant had their body weight measured weekly to make certain body weight was maintained. Participants were encouraged to maintain their normal caloric intake while adhering to the diet. Although a small amount of weight loss is common with increased energy expenditure, excessive weight loss was discouraged due to the risk of dehydration and electrolyte imbalances as well as common side effects of fatigue, dizziness, irritability and headaches [128].

BP was measured and resting heart rate recorded before and after each bout of exercise. If BP prior to exercise is above 160/90 mmHg, participants were asked to sit quietly for a few minutes before having BP measured again. If the BP is lower, participants were allowed to exercise. If not, participants were not allowed to exercise that day. Following the bout of exercise and cool down/stretch, participants had their BP measured and heart rate recorded. Participants were allowed to leave when BP and heart rate were similar to those before the exercise session (BP 4 mmHg, HR 10 bpm).

## Enzyme Immunoassay and Enzyme-linked Immunosorbent Assay

In order to preserve data integrity, quality control and quality assurance protocols were followed when handling samples and performing assays. All endothelium-derived parameters were measured by commercially available enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA). Standard curves were constructed using appropriated concentrations for each factor. When necessary, extraction and purification were implemented to avoid interference with other components in serum. All plasma samples were stored at -80°C until analysis and urine was stored at -20°C. Absorbance for all assays was measured using a SpectraMax Microplate Reader (Molecular Devices, Sunnyvale, California, USA).

Risk for a CV event was assessed by measuring hsCRP and the adhesion molecule VCAM-1. Blood samples to determine hsCRP levels were sent to Quest Diagnostics, Inc for analysis.

### *Vascular Cell Adhesion Molecule-1*

A commercially available solid phase sandwich enzyme linked immuno-sorbent assay kit (Abcam, Cambridge, MA) was used to detect VCAM-1 levels in plasma. Plasma samples were diluted 1:20 in sample buffer (.09% sodium azide) prior to assay. A monoclonal antibody specific for VCAM-1 coated each well. There were two incubation periods, the first involves the VCAM-1 antigen and monoclonal antibody (specific for VCAM-1) being simultaneously incubated. The plate was then washed and incubated with the enzyme streptavidin peroxidase. After a second wash to remove any unbound enzyme, a substrate solution (TMB solution) was added to act on the bound enzyme, inducing a colored reaction product. The intensity of the colored reaction is directly proportional to the concentration of VCAM-1 in the samples. Inter- and intra-assay coefficients of variation from preliminary trials were 6.1% and 7.2%, respectively.

### *Nitric Oxide*

All reagents used were obtained from Assay Designs (Ann Arbor, Mich., USA). Blood samples were collected in EDTA vacutainers then centrifuged at 2,000 g for 20 min at 4°C. Plasma aliquots were stored until assay. On the day of assay, plasma samples were thawed and then filtered using a 10,000 MWCO Amicon Ultra filter (Millipore) and centrifuging samples at

14,000 g for 30 min at 4°C. Nitric oxide is converted to nitrate and nitrite in the cell. Nitrate in the sample is converted to nitrite in presence of nitrate reductase and cofactors. Following that reaction, the colorimetric detection of nitrite is made possible as a colored azo dye product of the Griess reaction absorbs visible light at 540 nm. The Griess reagents used were N-(1-naphthyl) ethylenediamine in 2 M hydrochloric acid and sulfanilamide in 2 M hydrochloric acid. Inter- and intra-assay coefficients of variation from preliminary trials were 7.6% and 10.6%, respectively [129].

#### *Superoxide Dismutase*

SOD activity was measured by commercially available assay kit (Cayman Chemical, Ann Arbor, MI). Blood samples were collected in sodium-heparin vacutainers then centrifuged at 2,000 g for 20 min at 4°C. Plasma aliquots were stored until assay. On the day of assay, plasma samples were diluted 1:5 in sample buffer (50mM Tris-HCl, pH 8.0). Tetrazolium salt radical detector solution was used to detect superoxide radicals generated by hypoxanthine and xanthine oxidase. One unit of SOD activity is defined as the amount of enzyme needed to exhibit a 50% dismutation of the superoxide radical. Cytosolic, extracellular and mitochondrial SOD activity (total SOD activity) were measured. Inter- and intra-assay coefficients were 5.9% and 12.4%, respectively [129].

#### *8-isoprostane PGF<sub>2α</sub>*

Lipid peroxidation was assessed by the measurement of free 8-isoPGF<sub>2α</sub> in plasma using a commercially available EIA kit (Cayman Chemicals, Ann Arbor, MI). Plasma samples for 8-isoPGF<sub>2α</sub> were collected in a .008% BHT solution prior to storage. Solid phase extraction using C-18 cartridges (Daigger) and a vacuum manifold were used to remove particulates. Precipitation of proteins using ethanol and acidifying to pH 4.0 was necessary prior to passage through C-18 cartridges. Cartridges were activated by passing 5 ml methanol followed by 5 ml UltraPure water. The plasma sample (1 ml) was then be passed through the cartridge followed by 5 ml UltraPure water and 5 ml HPLC grade hexane. Once the cartridges dry, the 8-isoprostane sample will be eluted with 2 ml ethyl acetate containing 1% methanol. Prior to assay,

samples stored in the 2 ml ethyl acetate solution were evaporated to dryness and reconstituted in .5 ml of EIA buffer (Cayman Chemicals, Ann Arbor, MI). Plasma 8-isoPGF<sub>2α</sub> levels were measured using a commercially available enzyme immunoassay kit according to the manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI). Baseline and final samples were assayed in duplicate. Inter- and intra-assay coefficients were 8.4 and 12.2, respectively.

#### *2,3-dinor-6-keto Prostaglandin F<sub>1α</sub>*

Measurement of 2,3-dinor-6-keto Prostaglandin F<sub>1α</sub> was measured in 24-hour urine samples. On the day of assay, a liquid-liquid extraction protocol using ethyl acetate was used to purify the samples. The urine samples were acidified using equal volumes of 1M citrate buffer (pH 4.0) prior to the addition of 4 volumes of ethyl acetate. The samples were then vortexed 2x10 seconds, remove the top fraction (approximately 1 ml), place into a clean test tube and repeat a total of 3 times. Purified samples were then evaporated to dryness under a gentle stream of nitrogen. Once dry, samples were re-suspended in .5 ml of assay buffer (Cayman Chemicals, Ann Arbor, MI) and urinary 2,3-dinor-6-keto Prostaglandin F<sub>1α</sub> will be measured using a commercially available enzyme immunoassay kit according to the manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI). Baseline and final samples were assayed in duplicate. Inter- and intra-assay coefficients were 7.7 and 9.4, respectively.

#### Statistical Analyses

Participant's data will be used if they reach at least 70% compliance with the exercise intervention. Prior to all analyses, the Kolmogorov-Smirnov test was performed to test the data for normality. To address Specific Aim 1, Pearson correlations were used to determine if there were any significant correlations between VO<sub>2max</sub> and the indices of blood vessel health and function. Partial Pearson correlations were used to control for the influence of obesity.

To address Specific Aim 2, Pearson correlations were used to examine the strengths of relationships between VO<sub>2max</sub> and indices of blood vessel health and function after the exercise intervention. Paired T-tests were used to determine change, if any, in all calculated variables (clinical characteristics, biomarkers, VO<sub>2max</sub>). Pearson correlations were used to determine the strength of relationships between the percent change in all variables. Partial Pearson

correlations were used to control for the influence of obesity on the relationships between variables after the intervention. For the ratio and interval data following the normal distribution, the most common descriptive statistics is mean and standard deviation (SD). Descriptive values are presented as mean $\pm$ SD. A value of  $P<.05$  will be used to determine statistical significance. Statistical analysis was performed using SPSS 20.0.

## CHAPTER 4

### RESULTS

#### Pre-Intervention Analyses

##### *Participant Demographics and Clinical Characteristics*

The group of middle-aged participants was made up of 19 females and 3 males with ages ranging from 41 to 64 years (mean age  $52.3 \pm 6.3$  years). Nine of the 19 females were post-menopausal and were not on a hormone replacement therapy (HRT). Clinical characteristics and demographic information for the study group are shown in Table 2 below. Almost half of the participants were pre-hypertensive (N=10, 45.5%) (Table 3). Mean BMI for the group was  $29.9 \pm 4.8$  kg/m<sup>2</sup> at baseline which would classify the group as pre-obese according to the WHO guidelines [130]. Both the men and the women had  $VO_{2max}$  levels that fell below the normal range for their age and sex [124].

Table 2. Demographic and clinical characteristics

	Pre-Intervention	Post-Intervention
Age (years)	$52.3 \pm 6.3$	
TC (mg/dl)	$190 \pm 26.8$	$184.1 \pm 27.8$
Trig (mg/dl)	$85.8 \pm 40.8$	$67.9 \pm 22.4$
HDL (mg/dl)	$63.8 \pm 15.3$	$62.6 \pm 12.6$
LDL (mg/dl)	$108.9 \pm 24.1$	$108 \pm 25.1$
fGLU (mg/dl)	$92.5 \pm 10.1$	$88.8 \pm 10.8$
CRP (mg/L)	$3.6 \pm 3.2$	$3.5 \pm 3.3$
SBP (mmHg)	$122.2 \pm 10.3$	$121.3 \pm 12$
DBP (mmHg)	$77 \pm 5.9$	$78.2 \pm 7.9$
Height (cm)	$166.8 \pm 6.4$	$167.1 \pm 7$
Weight (kg)	$83.8 \pm 18$	$82.57 \pm 15.9$
BMI (kg/m <sup>2</sup> )	$29.9 \pm 4.8$	$29.5 \pm 4.3$
BF (%)	$39.9 \pm 9.4$	$39.4 \pm 7.5$

Data are presented as Mean  $\pm$  SD.

TC indicates total cholesterol; Trig, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; fGLU, fasting blood glucose; CRP, c-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; BF, body fat.

Table 3: HTN Status of Participating Subjects

		Baseline		Final	
		N	%	N	%
Normal	SBP <120 and DBP <80	10	45.5	11	50
Pre-HTN	SBP 120-139 or DBP 80-89	10	45.5	8	36.4
Stage-1 HTN	SBP 140-159 or DBP 90-99	2	9.1	3	13.6
Total		22	100	22	100

*Baseline Associations: Aerobic Capacity, Clinical Characteristics and Biomarkers*

There was no significant correlation between the plasma and urinary biomarkers and VO<sub>2max</sub> aside from CRP. There was a significant negative correlation between VO<sub>2max</sub> and CRP (r= -.531, p= .002). CRP was also found to have a significant positive association with the marker of lipid peroxidation, 8-isoPGF<sub>2α</sub> (r= .875, p= .002) and a significant negative association with the vasodilator NO (r= -.348, p= .038). There was also a significant positive association between the concentrations of cardioprotective NO and PGI<sub>2</sub> (r= .414, p= .025) as well as a significant positive relationship between SOD and BMI (r= .456, p= .011) (Table 4).

Table 4. Pearson Correlations

Variables		Pre-Intervention		Post-Intervention		Pre-Intervention		Post-Intervention	
		r	P value	R	P value	r	P value	r	P value
		No Covariate				Co-varied for Obesity			
CRP	VO <sub>2max</sub>	-0.531	0.002*	-0.491	0.005*	0.001	0.499	-0.474	0.051
CRP	NO	-0.348	0.038*	-0.048	0.413	0.005	0.496	-0.366	0.11
CRP	8-isoPGF <sub>2a</sub>	0.875	0.002*			0.816	0.024*		
NO	PGI <sub>2</sub>	0.414	0.025*	0.2	0.187	-0.17	0.374	-0.085	0.391
SOD	BMI	0.456	0.011*			0.608	0.003*		

VO<sub>2max</sub> indicates aerobic capacity; CRP, C-reactive protein; VCAM-1, vascular cell adhesion molecule-1; SOD, superoxide dismutase; NO, nitric oxide; PGI<sub>2</sub>, prostacyclin (measured by urinary metabolite 2,3-dinor-6-keto Prostaglandin F<sub>1α</sub>); BMI, body mass index; 8-isoPGF<sub>2a</sub>, 8-isoprostane PGF<sub>2a</sub>. \*p < .05

Post-Intervention Analysis

*Post-Intervention Associations: Aerobic Capacity, Clinical Characteristics and Biomarkers*

The data in Table 5 represent the observed changes in biomarker concentration or activity, change in VO<sub>2max</sub> and difference, if any, in clinical characteristics after the 6-month exercise intervention. Notable changes were observed with an increase in the concentration of cardioprotective NO (pre 24.1 ± 8.8 μmol/L, post 37.2 ± 15.6 μmol/L, p= .002) and a decrease in

pro-atherogenic VCAM-1 (pre  $632.6 \pm 179.1$ , post  $525.1 \pm 148.5$ ,  $p = .011$ ) (Table 5). Additionally, the association between  $VO_{2max}$  and CRP remained significant following the AEXT intervention ( $r = -.491$ ,  $p = .005$ ). After controlling for obesity, a negative association remained although not statistically significant ( $r = -.474$ ,  $p = .051$ ). Pearson correlations were used to observe the relationships between the percent of change in all variables. The analysis did not reveal any significant correlations between any of the primary variables listed below in Table 5.

Table 5. Changes in  $VO_{2max}$  and Biomarkers Pre- to Post-intervention

	Pre-Intervention	Post-Intervention	P value
$VO_{2max}$ (ml/kg/min)	$25.3 \pm 3.9$	$28.7 \pm 5.8$	0.001*
CRP (mg/L)	$3.6 \pm 3.2$	$3.5 \pm 3.3$	0.111
VCAM-1 (ng/ml)	$632.6 \pm 179.1$	$525.1 \pm 148.5$	0.011*
SOD (U/ml)	$4.0 \pm 2.7$	$5.2 \pm 2.7$	0.131
NO ( $\mu\text{mol/L}$ )	$24.1 \pm 8.8$	$37.2 \pm 15.6$	0.002*
$PGI_2$ (pg/ml)	$3134.1 \pm 2551.0$	$2351.9 \pm 1719.1$	0.167

All data are presented as Mean  $\pm$  SD.

$VO_{2max}$  indicates aerobic capacity; CRP, C-reactive protein; VCAM-1, vascular cell adhesion molecule-1; SOD, superoxide dismutase; NO, nitric oxide;  $PGI_2$ , prostacyclin (measured by urinary metabolite 2,3-dinor-6-keto Prostaglandin  $F_{1\alpha}$ ). All data are presented as Mean  $\pm$  SD. \* $p < .05$ .

## CHAPTER 5

### DISCUSSION

#### Introduction

Findings reported by Pahor et al. suggest that traditional risk factors do not adequately explain the racial disparity in CVD and goes further to suggest that new risk factors are needed to improve the risk factor stratification algorithms [131]. Elevated levels of VCAM-1, CRP and 8-iso PGF<sub>2α</sub> are evidence of endothelial activation. Once activated, the endothelium must compensate by increasing antioxidant concentrations to scavenge excess reactive oxygen species along with factors that promote vasodilation and inhibit thrombosis. Early in the development of CVD and in sedentary individuals, there is evidence of an imbalance in these vasoactive compounds. Increased laminar shear stress caused by aerobic exercise training can reverse endothelial activation and restore endothelial health in patients with CVD, diabetes and obesity. The degree of improvement is dependent on the initial degree of damage. For example, a pre-diabetic person may have a more optimal response to AEXT than an individual with advanced cardiovascular disease. As previously mentioned African Americans are at elevated risk for cardiovascular disease and have impaired endothelial function compared to Caucasians. Racial differences in endothelium-dependent vasodilation have been found in a variety of populations including young individuals and adults. This may be due to greater basal levels of oxidative stress, decreased nitric oxide bioavailability or greater risk factor burden in African Americans. Studies have shown that exposing the endothelium to shear stress, in vitro and in vivo, has resulted in cardioprotective adaptations to the vasculature [23-24,29-34,52].

In this study, we examined how vasoactive biomarkers responded to AEXT in a sedentary, pre-hypertensive African American population sample. To address Specific Aim 1, at baseline, we found that higher VO<sub>2max</sub> was strongly associated with lower CRP concentration, an indicator of CV event risk and systemic inflammation. Hypothesis 1 was accurate with respect to the correlation of CRP and VO<sub>2max</sub> but did not hold true for associations with the other biomarkers of endothelial activation. Specific Aim 2 was to determine if 6 months of AEXT would modify the concentrations of endothelial biomarkers of activation in association with an increase in VO<sub>2max</sub>.

Following AEXT, there was increased NO concentration and decreased VCAM-1 concentration, although these biomarkers were not directly related to the improvement of  $VO_{2max}$ . To address hypothesis 2, the association of CRP and  $VO_{2max}$  remained significant following 6 months of AEXT with and without the influence of obesity. The lack of significant change in other biomarkers could be due to an inappropriate intervention (exercise mode, intensity, duration) or the healthiness of the study sample population. Aside from blood pressures classifications in the pre- and Stage-1 hypertensive ranges, the study sample was free of overt disease.

#### Pre-Intervention Findings

Apparently healthy, middle-aged African Americans participated in a 6-month AEXT intervention. Traditional screening tests prior to beginning AEXT identified participants that were at risk for developing diabetes and HTN. Wei et al. report that men with low  $VO_{2max}$  similar to the current group of participants may have up to a 1.9-fold increased risk of developing impaired fasting glucose compared to men with high  $VO_{2max}$  [132]. In addition to screening tests, Framingham risk score was calculated for all participants prior to and following the intervention. The mean Framingham risk score for the group was  $\leq 1\%$  chance of a CV event in the next 10 years.

The current group of participants were borderline obese according to standards set forth by the World Health Organization [130]. The prevalence of overweight and obese individuals is higher in ethnic minorities [13,130]. Obesity has been characterized as a state of low-grade inflammation. An abundance of white adipose tissue is related to an elevation in adipokines which could have an inhibitory effect of insulin's anti-inflammatory function. Increased adipokine release will also elicit a release of inflammatory oxidative stress markers [133-134]. Based on information obtained by BMI and BIA, our participants had substantial fat mass. This may, in part, explain the levels of inflammatory and oxidative stress markers observed in this sample.

Although normal clinical ranges for most plasma and urinary biomarkers that were investigated in this study have not been established, a review of available literature made a relative comparison possible. The plasma levels of biomarkers 8-isoPGF<sub>2 $\alpha$</sub>  and VCAM-1 were higher than the normal ranges reported by Wang et al. [135] and Deneva-Koycheva et al. [136]

both before and after the intervention. SOD and PGI<sub>2</sub> fell below ranges determined to be normal by Winnefeld et al. [137] and Kakutani et al. [138] both before and after AEXT. NO levels remained within normal ranges set forth by Ghasemi et al. [139] in pre-intervention and post-intervention analyses. Comparisons were made with studies that used similar methods to those used in the current study. The study populations differed demographically. Additional research is needed in order to accurately determine clinically relevant concentrations for each biomarker. To date, CRP is the only biomarker in clinical use.

#### *Relationship Between VO<sub>2max</sub> and CRP*

Data reveals that African American men (2.1 mg/dl) and women (3.5 mg/dl) have higher CRP levels than Caucasian men (1.7 mg/dl) and women (3.2 mg/dl) [148]. Khera et al. and others show supporting evidence that women have higher CRP levels than their male counterparts and further states that CRP is highly correlated with obesity [149-150]. There is a strong relationship between disruptions in endothelial health and pro-inflammatory conditions. Chronic pro-inflammatory conditions (including obesity) can have significant implications in the development and progression of CVD. There was a significant negative correlation found between VO<sub>2max</sub> and CRP ( $r = -.498$ ,  $p = .009$ ) which suggests those participants who were less aerobically fit had higher circulating inflammatory markers prior to the intervention (Specific Aim 1), consistent with findings by Fantuzzi [134]. This was also substantiated by studies by Geffken et al. and Taaffe et al. [140-141].

The negative association between CRP and PGI<sub>2</sub> corresponds with findings from Venogupal et al. that demonstrate CRP inhibits PGI<sub>2</sub> release by nitration of the prostacyclin synthase [73]. Several studies have determined that CRP is implicated in increasing concentrations of O<sub>2</sub><sup>-</sup>, the inducible form of NO synthase, leading to NO release. The high affinity for O<sub>2</sub><sup>-</sup> to bind with NO increases the ONOO<sup>-</sup> concentration. The abundance of ONOO<sup>-</sup> allows nitration of prostacyclin synthase, inhibiting its activity [73-75].

CRP was also found to have a significant positive correlation with the marker of lipid peroxidation, 8-isoPGF<sub>2α</sub> ( $r = .875$ ,  $p = .002$ ). This relationship has been documented previously by Cottone et al. in both hypertensive and normotensive populations [74-75]. Urinary excretion of

8-isoPGF<sub>2α</sub> is elevated in patients at risk for future CV events. CRP predicts CV outcome.

Oxidative stress is considered to be a vital step in endothelial activation. A change in endothelial phenotype characteristic of endothelial activation involves increased permeability of endothelial cells and adhesion cell-leukocyte interactions.

#### Post-Intervention Findings

There were no significant changes in clinical characteristics following the exercise intervention although two of the four participants were re-classified from pre-diabetic to normal because their fasting blood glucose fell below 100 mg/dl. Mild to moderate physical activity levels have been associated with a lower risk of developing diabetes or pre-diabetes [132]. Both SBP and DBP remained consistent from pre- to post-intervention. There is ample evidence of AEXT causing a reduction in BP [18-23,30,35]. The largest changes in BP were seen in study populations that had baseline BP in the Stage-1 or Stage-2 hypertensive ranges. Individuals with BP in those ranges may expect a drop of up to 8 to 10 mmHg and 6 to 10 mmHg in SBP and DBP, respectively, due to the independent effect of AEXT [142]. The exact mechanism of how AEXT helps to lower high blood pressure is unclear, however it is hypothesized that biochemical, neural and hormonal changes in the blood vessel walls induce both acute and long-term vasodilation. The lack of significant change in BP over the course of the study could be attributed to the majority of the study population (20 of 22 participants) being classified as normotensive and pre-hypertensive prior to the intervention.

The study group had no change in BMI or percent body fat, indicators for obesity. The beneficial effects of exercise have been well documented. Some beneficial effects including alterations in BP, diabetic status and a decreased resting heart rate, are commonly associated with a decrease in adiposity [15,20-21,24,47-49]. The lack of change in overall adiposity may in part explain the lack of significant alterations in some of the inflammatory biomarkers. The impact of obesity on CRP levels is discussed in greater detail below.

We were unable to calculate whether the exercise intervention had an influence on 8-isoPGF<sub>2α</sub> due to insufficient data. Fearheller et al. examined effects of moderate intensity AEXT in an African American population and reported increased urinary 8-isoPGF<sub>2α</sub> levels in a group

following the 6 month intervention [85] which is in contradiction to other reports [90-91]. Kelly et al. and Luk et al. both report no change in 8-isoPGF<sub>2α</sub> following a high intensity exercise intervention, despite both groups improving exercise capacity [143-144]. There is limited data concerning exercise-mediated changes in an African American population.

#### *Post-Intervention CRP Concentration*

The study was designed to keep body mass consistent throughout the duration of the study so as to minimize independent effects of weight loss. This may, in part, explain the lack of significant change in CRP considering the evidence that CRP is induced by adipokines released by white adipose tissue. Evidence from diet and exercise weight loss studies reveal weight loss-induced reductions in CRP [82,140-141,145]. Both pre-intervention and post-intervention analysis revealed a significant negative correlation between CRP and VO<sub>2max</sub>.

#### *VO<sub>2max</sub> Response to AEXT and Relationship to CRP*

Statistical increases in VO<sub>2max</sub> were observed in the group of participants following the AEXT. Change in VO<sub>2max</sub> ranged from -15% to 44% (mean change 9.2±14.5%), following a Gaussian distribution. Bouchard et al. [146-147] reported similar responses to exercise training in various study populations that had mean values ranging from 8.7±10.5 to 18.9±10.3%. Bouchard et al. data suggest there may be genetic variants responsible for a person's response to exercise. This data should be interpreted with the consideration that the populations differed in the HERITAGE family study and the current population sample [146]. An increase in VO<sub>2max</sub> signifies that the intensity and duration of the exercise intervention was appropriate to improve CV health. Bouchard et al.[147] later investigated adverse responses to exercise in varied study populations in which subjects experienced changes in an opposite, unfavorable direction compared to the expected beneficial effects [147]. In the current study, this trend has been observed for several of the biomarkers of endothelial activation, including CRP. It was beyond the scope of the current study to ascertain whether this was due to genetic variants.

The significant negative relationship between VO<sub>2max</sub> and CRP remained significant following the AEXT despite any difference in CRP concentration. Interestingly, this correlation

was the only association that remained significant when we controlled for obesity. This observation leads to the assumption that the significant associations that were observed between variables (other than  $VO_{2max}$  and CRP) would not exist without the influence of adiposity.

Much debate has been had over the appropriate exercise intensity and modality to elicit changes in endothelial activation and health. Geilen et al. concluded that there is a frequency-dependent dose of moderate intensity exercise that will elicit improvements in endothelial health and function [47,50,145,152]. Several studies indicate regular exercise can lower CRP levels, and that in fact there is an inverse correlation between  $VO_{2max}$  and CRP levels in both men and women [80-81, 140, 142-145,148-156]. Some studies show a strong correlation between BMI and CRP which suggests interventions that promote weight loss would also elicit a decrease in CRP concentration [157-160]. This warrants further investigation in human populations, with emphasis on those at risk for CVD.

There is evidence that exercise can both cause and attenuate inflammation. There have been reports of exercise-induced reductions in CRP [82,145]. Similar to VCAM-1, CRP is induced by cytokines. Acute bouts of exercise increase plasma levels of cytokines. Acute bouts of exercise can cause muscle and connective tissue damage, and in some cases, plasma cytokines may increase in those unaccustomed to exercise. The damaging effects of exercise can be attenuated if exercise bouts are consistently repeated to allow for adaptation to the increased oxidative load that accompanies exercise [145]. Studies have demonstrated that CV exercise training may reduce markers of systemic inflammation. Vassilakopoulos et al. concluded that in untrained humans, oxidative stress is a major contributor for exercise-induced cytokine production [148]. Data showed that in elderly and young populations, a greater level of physical activity is associated with lower levels of plasma cytokines and CRP and that aerobic exercise training is effective in reducing CRP in middle to older aged populations [82].

A reduction in plasma cytokines is commonly associated with AEXT and in some instances it is accompanied by a decrease in CRP levels. Niklas et al. reported African Americans had significantly decreased cytokine concentration compared to Caucasians [82]. Exercise mode and intensity in the current study was drastically different in comparison to some

studies that report significant decreases in CRP. Niklas et al. incorporated aerobic training, strength training, stretching and balance and counseling sessions while Mattusch et al. had participants run 31 +/- 9 km at the beginning of the 9 month program to 53 +/- 15 km at the end [81-82]. Further investigation is needed to determine an optimal workload to elicit change in this population.

#### *Post-Intervention VCAM-1 Concentration*

VCAM-1 concentration decreased significantly ( $p=.011$ ) following the AEXT intervention. Oxidative stress and expression of VCAM-1 on vascular endothelial cells are early features in the pathogenesis of atherosclerosis and other inflammatory diseases. VCAM-1 is a direct associate of endothelial activation. Models using human umbilical vein endothelial cells were able to determine VCAM-1 gene expression is sensitive to oxidation-mediated signals [148-150]. Oxidative stress manifests when ROS overwhelm the antioxidant capacity of the cell. ROS produced by endothelial cells and neighboring smooth muscle cells as a result of paracrine and autocrine mechanisms by which cytokines produced by immune and/or endothelial cells induce production of ROS. Following a bout of exercise, cytokine-induced expression of adhesion molecules facilitates leukocyte infiltration, further enhancing ROS-induced oxidative damage. In the present study, VCAM-1 did not correlate with any other variables at baseline or at final despite significant changes in NO and  $VO_{2max}$  which indicate deactivation of the endothelium. It is likely that there is another mechanism responsible for the change in VCAM-1 expression in this sample of sedentary African Americans.

Moderate levels of laminar shear stress, which mimic moderate intensity exercise in vitro, have been reported to reduce VCAM-1 expression in endothelial cells. On the contrary, low and high levels of laminar shear stress resulted in increased expression of VCAM-1 [148-152]. The findings in this current study parallel those from Reza et al. [149] in which a decrease in VCAM-1 concentration was observed following a 12-week interval training program (VCAM-1 levels BL;  $501.10 \pm 26.53$ , F;  $393.30 \pm 24.45$  ng/ml). Reza et al. [149] also reported a significant increase in  $VO_{2max}$  along with a decrease in obesity (body fat percentage and BMI). The moderate exercise intensity in this study was appropriate to elicit an anti-atherogenic alteration in the endothelium.

### *Post-Intervention SOD Activity*

SOD activity was slightly improved but not statistically significant after AEXT. There may have been compensation by other  $O_2^-$  scavengers that reduced the amount of  $O_2^-$  available to bind to NO, forming ONOO<sup>-</sup> but that is beyond the scope of this study. Conflicting evidence is reported about AEXT mediated effects on SOD activity. In several animal and cell models, both acute and chronic exercise along with elevated levels of shear stress provoked an increase in SOD activity [34,152-153]. Some studies report SOD activity was unaffected by neither chronic nor acute exercise, but there were significant increases in other antioxidants (glutathione peroxidase, catalase) [153]. The lack of change in adiposity can provide a probable explanation for the lack of significant change in SOD activity. As previously stated, adipose tissue releases adipokines. They, in turn, increase the release of CRP and ultimately an increase in oxidative stress [82,140-141,145]. Without a validated normal range of values it is difficult to make an inference. Compensation by alternative endogenous antioxidants is a plausible assumption but confirming that is beyond the scope of this study.

### *Post-Intervention PGI<sub>2</sub> (2,3 dinor 6-keto PGF<sub>1α</sub>) Concentration*

Urinary metabolites of PGI<sub>2</sub> were not significantly altered following AEXT. There was no significant relationship observed between PGI<sub>2</sub> and  $VO_{2max}$  prior to or following the AEXT. PGI<sub>2</sub> production and the signaling of its regulatory enzyme, COX reportedly declines with age [110]. However, AEXT enhances NO-mediated signaling of COX and improves the age-related decline [49]. The age related increase in ROS and decreased SOD expression could contribute to increased ONOO<sup>-</sup> and lipid peroxidation [140,145,153]. Quindry et al. revealed that AEXT does not elevate cardiac COX-2 activity which suggests there may be different mechanisms responsible for cardioprotection as a result of AEXT [154]. Assuming oxidative and inflammatory properties of CRP were unchanged following the AEXT, the lack of overall change in CRP concentration and the increase in available NO in the study sample suggests there would be more NO available to bind with  $O_2^-$  leading to inhibition of prostacyclin synthase. There was a slight decrease in the urinary metabolite (Table 5) of prostacyclin but it lacked statistical significance.

However, in our preliminary study findings, there was significance found between aerobic exercise and PGI<sub>2</sub> in a similar population. When probing for an explanation as to why this may have occurred, the primary difference was that one was an acute exercise stress while the other was an adaptive response to chronic exercise stress. The secondary difference was in the collection time of urine samples. In the preliminary study, urine was collected for 24-hours immediately following the acute bout of aerobic exercise. In an effort to reduce the rate of attrition, some tests were conducted simultaneously. In the present study, 24-hour urine collection was coupled with 24-hour BP monitoring which requires participants refraining from exercise the day prior to BP monitoring. Mitchell et al. observed in cultured endothelial cells that NO and PGI<sub>2</sub> do not necessarily follow the same time course [104]. This may, in part, explain the findings. Upregulation of prostacyclin synthase may only occur as an acute response to exercise.

#### *Post-Intervention NO Concentration*

In the current study, there was a significant increase in NO concentration following AEXT. NO release is mediated by flow-dependent mechanisms as well as receptor-mediated. Endothelial cells release NO in response to stimuli such as acetylcholine, Angiotensin II, DHEA and changes in arterial pressure. In endothelial cell models, NO responded to shear stress in a graded fashion. The increase could be attributed to the NOS substrate L-arginine [67,155]. Studies using a cultured endothelial cell model have provided evidence that in response to shear stress, L-arginine uptake by amino acid transporters is selectively increased in endothelial cells. The shear stress mediated NO formation depends heavily on extracellular L-arginine [155].

Once released, NO elicits vasodilation in the neighboring smooth muscle cells via increased intracellular cGMP (Figure 1). This endothelial biomarker inhibits platelet aggregation. African Americans reportedly have impaired nitric oxide production [43,44]. AEXT is a proven method for eliciting enhanced NO production in healthy populations as well as those with CVD. Several studies show a relationship between aerobic fitness and endothelium-dependent vasodilation suggesting changes in physical activity level will affect endothelial function. In the current study sample, the increase in NO could have, in part, been a compensatory mechanism

to account for the slight decrease in PGI<sub>2</sub> concentration following the AEXT. Together, NO and PGI<sub>2</sub> are vital vasoprotective substances.

There is evidence that aerobic exercise may attenuate the age related decline in endothelial function. Wray et al. [156] found impaired endothelial function in older and middle aged sedentary men; however in endurance trained individuals normal endothelial function was preserved. Furthermore, when the sedentary group was trained, there was a 30% increase in endothelium-dependent vasodilatation. Moderate intensity AEXT for at least 12 weeks was sufficient enough to cause significant increases in plasma nitrate and nitrite, indicating increased NO release [48-50].

#### Limitations

The lack of a control group limited any comparisons made to data reported from other studies, therefore limiting causal inference. It is impossible to determine whether changes in biomarkers were caused by AEXT or other systemic (non-random) factors. The participants in the current study were a subset of a larger study population. The target population of the larger study was middle-aged African Americans who were pre- and Stage-1 hypertensive but otherwise healthy. This sub-population was homogenous, lacking variation in co-morbidities that would be found in a larger group of middle-aged African Americans. Results may have greatly differed if the population was more diverse.

To date, clinically relevant reference values are lacking for the biomarkers (aside from CRP) that were studied. This limited the analyses that could be performed. Comparison of biomarker values was limited to those reported in similar population samples under similar conditions. Race/ethnic information was not always obtainable from research articles and may not have been representative of our study population.

The marker of lipid peroxidation, 8-isoPGF<sub>2α</sub> was omitted from final analysis. Of the participants that had 8-isoPGF<sub>2α</sub> measured at baseline, half (50%) did not complete the study. Despite the high correlation with CRP at baseline, data would not be valid to use in post-intervention analysis. Finally, there was insufficient data to conduct a risk or survival assessment.

Investigating multiple biomarkers simultaneously limited the number of participants that could be included in analysis.

### Concluding Remarks

Findings from pre-intervention data provided limited information in regards to discerning the best set of biomarkers associated with endothelial activation that are related to  $VO_{2max}$ . What was revealed was having a higher  $VO_{2max}$  was strongly associated with decreased concentrations of CRP; a marker of systemic inflammation that is highly associated with risk for CVD. This has implications for future studies investigating the prognostic value of biomarkers associated with endothelial health. Both  $VO_{2max}$  and CRP are used clinically to determine the progression and diagnosis of CVD.

It seems likely that other unknown cardioprotective mediators exist and may contribute to exercise-mediated alterations to the endothelium. Identifying biomarkers that can be modified by exercise is a focal point that should be examined. The use of biomarkers to augment traditional CV risk prediction is controversial. Measurement of biomarkers can provide additive information to traditional screening mechanisms. Of the endothelial biomarkers examined in this study, CRP is the only biomarker currently considered clinically relevant. The results of this study warrant further investigation in order to definitively discern which biomarker(s) would best supplement current clinical CVD screening methods for African Americans.

Post-intervention analysis suggests 6-months of moderate intensity AEXT is an appropriate intervention for elevating NO and decreasing VCAM-1 concentrations. This suggests there were cardioprotective modifications in the endothelial phenotype. The lack of significant change in SOD activity,  $PGI_2$  and CRP concentrations suggests moderate intensity AEXT is not a suitable mechanism to elicit improvements in all metabolic pathways that impact the state of the endothelium in previously sedentary African Americans which is contradictory to the responses observed in Native American and Caucasian groups [80].

Low  $VO_{2max}$  is linked to elevated risk for all-cause mortality and CV events [1,4,18-22]. Improvement in fitness levels is an important means to reducing mortality and morbidity.

Although CV risk involves non-modifiable risk factors (age, gender, family history, and race) clinicians should not only continue to encourage patients to engage in regular physical activity but in addition to prescribing exercise as a rehabilitative mechanism, they should begin prescribing exercise as a preventative “medicine”. Special consideration must be taken to tailor the prescription to the patient and specific to the ailment. This will require a better understanding of the impact of different exercise protocols (type, duration, and intensity) on endothelial biomarkers. Recommendations have been made to tailor exercise programs specifically for African Americans [161].

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**APPENDIX**  
INFORMED CONSENT

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

IRB Protocol #: 10831

Participant's Name  
and ID#:

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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 your 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<sub>2</sub> test:** VO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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 speed and the incline 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 you can 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 tablespoons) 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 feel 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 the 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 affect on your willingness to participate, continue your participation, or after your participation that may have an affect 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's signature

Date

Timeline	Visit	Procedure	Required
Month 1	<i>Orientation Visit</i>	1. Review medical history questionnaire, informed consent 2. Blood pressure taken.	1 hour time
Month 1	<i>Before Screening Visit 1</i>	12 hour overnight fast evening before screening visit 1	12 hours intake
Month 1	<i>Screening Visit 1</i>	1. Blood and urine sample drawn 2. Blood pressure taken.	1 hour time
Month 1	<i>Screening Visit 2</i>	Physical exam and exercise stress echo test	1 ½ hours time
Month 2	<i>Dietary Stabilization Period</i>	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

Month 2	<i>Before Baseline Testing</i>	12 hour overnight fast evening before	12 hours intake
		first visit. 1. Blood samples	monitoring <i>Several visits:</i>
Month 2	<i>Baseline Testing</i>	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	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 3 sessions a week
Month 9	<i>Before Final Testing</i>	12 hour overnight fast evening before	for 6 months 12 hours intake
Month 9	<i>Final Testing</i>	first visit of final testing. Repeat Baseline Testing	monitoring Same as baseline testing
			testing