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THE EFFECT OF OXIDATIVE STRESS ON CUTANEOUS VASCULAR FUNCTION IN HEALTHY AFRICAN-AMERICAN AND CAUCASIAN ADULTS

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by

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ABSTRACT

THE EFFECT OF OXIDATIVE STRESS ON CUTANEOUS VASCULAR FUNCTION IN HEALTHY AFRICAN-AMERICAN AND CAUCASIAN ADULTS

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African-Americans have known impairments in vascular function as compared to their Caucasian counterparts. Dysfunction within the microvascular circulation, more specifically the cutaneous circulation, mirrors the systemic circulation and is indicative of risk for future cardiovascular disease. We aimed to test the hypothesis that acute pharmacological inhibition of superoxide can restore cutaneous microvascular responses to local heating. Healthy, college-aged males (African-Americans n=4, 23±2 years old; Caucasians n=4, 24±3 years old) were recruited from The University of Texas at Arlington. Prior to the infusion of drugs, each subject had baseline blood pressure and brachial artery flow mediated dilation (FMD) measurements. African-Americans had significantly higher baseline mean arterial pressure (P=0.038), but no significant difference in FMD (P=0.952).

Each subject was equipped with four microdialysis fibers with each site containing either Tempol (superoxide scavenger), Apocynin (blunts oxidative stress from NADPH oxidase), Allopurinol (blunts oxidative stress from xanthine oxidase), or lactated Ringer solution (control). Each site was also equipped with local heating elements and laser Doppler probes to measure red blood cell flux, expressed as percent cutaneous vascular conductance (CVC) max. A two-way ANOVA (SigmaStat 3.11, Chicago, IL) with $\alpha=0.05$ revealed significance in both CVC and nitric oxide contribution between the groups ($P=0.008$, $P=<0.001$ respectively). There was a significant difference between African-Americans and Caucasians at the site perfusing Ringers ($P=0.002$), but sites containing Tempol, Allopurinol, and Apocynin showed no significant difference. This suggests that reducing the circulating superoxide and superoxide production restores vascular responses in African-Americans, and supports the hypothesis that oxidative stress contributes to microvascular dysfunction seen in African-Americans.

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CHAPTER 1

INTRODUCTION

1.1 The Problem

African-American individuals possess a significantly increased risk for cardiovascular disease, obesity, and diabetes when compared to Caucasians. Another significant risk factor, which can be considered a precursor to other diseases, seen in African-Americans is hypertension. Compared to Caucasians, African-Americans have both a higher occurrence and earlier onset of hypertension.[1] A contributing mechanism to these risks is vascular endothelial dysfunction. African-Americans can elicit impaired vascular function as early as young adulthood, increasing their risk for adverse cardiovascular events in the future. Endothelial vascular dysfunction observed in the African-American population is not seen in their Caucasian counterparts. Impaired microvascular function also highly correlates with symptoms associated with metabolic syndrome and is a known step in the atherosclerotic process, allowing it to be a mirror in which cardiovascular health can be evaluated. Pathologically, this impairment can be detected as increased levels of endothelin 1, a vasoconstrictor, and a decreased flow mediated dilation response in the brachial artery.[2] In previous research, it is stated that a causal relationship exists between dysfunctional nitric oxide-dependent vasodilation responses and insulin resistance, and vice versa. In clinical studies, those who were given medication to improve insulin sensitivity and endothelium-dependent vasodilation elicited fewer cardiovascular events than are typically observed in insulin resistant individuals.[3]

Another mechanism by which these disparities are thought to occur is reduced nitric oxide bioavailability. Hypertension is a known factor that reduces the amount of nitric oxide, a potent vasodilator, within the vascular system. One method that is commonly used to measure microvascular function is induced reactive hyperemia. Hyperemia in response to rapid heat changes is highly dependent on nitric oxide and endothelial derived hyperpolarizing factors (EDHF). Nitric oxide is known to be the primary agent involved in vasodilation up to 40ºC; higher temperatures elicit a stronger dependence on EDHF.[1,5] The cutaneous circulation is an easily accessible vascular bed that mirrors the systemic circulation, and therefore provides an effective means to measure nitric oxide bioavailability and vascular function with minimal risk to the subjects. While the exact mechanisms for impaired cutaneous vascular function in African-Americans is relatively unknown, cutaneous microdialysis provides a means by which researchers can assess mechanisms commonly associated with systemic vascular disease.

1.1.1 Purpose

The purpose of the study is to evaluate racial differences in microvascular function as measured by nitric oxide-dependent vasodilation. Other studies have assessed the impairment of nitric oxide bioavailability via oxidative stress in diseased populations, as well as at-risk populations. Recent findings suggest an impairment in the effects of nitric oxide on endothelial smooth muscle in African-American adults.

1.1.1.1 Research Questions

Given the results seen in previous literature, it is hypothesized that:

1) African-Americans elicit impaired skin blood flow response to local heating as compared to Caucasian counterparts.

2) The inhibition of superoxide, a form of oxidative stress, will restore the local heating response in African-American individuals.
3) Inhibition of superoxide sources—such as NADPH-oxidase and xanthine oxidase—will restore the local heating response in African-Americans.

1.2 Significance

According to the American Heart Association, cardiovascular disease is the leading cause of death in the United States. African-Americans are known to have an increased risk for cardiovascular disease and stroke as compared to their Caucasian counterparts. The most common conditions noted to increase an individual's risk for cardiovascular disease are obesity, diabetes, and hypertension. African-Americans have both a higher prevalence and earlier onset of hypertension compared to Caucasians, increasing their risk for cardiovascular disease. This could be due to a variety of factors including chronic stress exposure, stress-induced blood pressure reactivity, vasoactive substance release, and impaired sodium handling.[4] In the United States, individuals are at high risk for developing cardiovascular disease, as well as other risk factors including diabetes, obesity, and hypertension. Higher prevalence of these diseases significantly increases the population's risk for all-cause mortality. Since this is such a prevalent issue in the United States, understanding the etiology—especially among races—could enhance the clinical treatment and prevention of this disease. Also, the use of the microvascular system to study systemic disease processes is a developing field. The information from this study would contribute to preexisting and ongoing research within this field.

1.3 Limitations

One of the limitations of the study would be subject participation. Most of the subjects recruited were students attending The University of Texas at Arlington; however, some outside subjects were recruited via organizations and word of mouth. Participation was limited by subject willingness to participate. Also, there are some limitations to controlled factors in the study. While subjects were matched for BMI and waist-to-hip ratio, they were not matched for socioeconomic status, diet, or fitness levels. Also, this study included a small sample size, offering opportunity for a large range of subject variability. That being said, the results of the study could be interpreted as a proof of concept and a framework for future studies. While it is generally accepted that the microvascular system mirrors the systemic vasculature in an individual, there is a possibility that any impairments in the microvascular system does not reflect the subject's systemic vasculature. Finally, there is also a possibility that the subject has an underlying issue in the systemic vascular system that is not reflected in the microvasculature.

CHAPTER 2

LITERATURE REVIEW

2.1 The Microvascular System

Within the cardiovascular system, blood flow can be broken into multiple different classifications. A specific area of focus is the microvascular system, which consists mainly of arterioles, capillaries, and venules. Blood flow within vasculature is the result of perfusion pressure gradient, or the difference between arterial and venous pressures, and conductance in the vascular tree—determined by the size and number of vessels as well as the resistance. Regulation of blood flow in order to maintain homeostasis is controlled by constriction and dilation of the vascular smooth muscle; these changes in resistance are known as vasomotor or vascular tone.[5] One specific division of the microvascular system, the endothelium, consists of a network of flat, polygonal cells held together by a web of connective tissues. Capillaries along the endothelium contain junctional strands that allow certain molecules to permeate the barrier and diffuse into the endothelium. Calcium channels are abundant in the endothelium due to calcium's large role in regulatory functions within the endothelium. Unlike muscle or nerve cells, endothelial cells do not possess excitability, the ability to generate an action potential. Instead, endothelial cells possess receptor-operated channels that increase the intracellular calcium levels when stimulated by an agonist.[6-7]

Among many of its other functions, the endothelium acts as a modulator of vascular tone. Changes in this tone are essentially due to changes in either calcium concentration or

sensitivity brought about by vasoactive factors. Vasoactive factors can be divided into two main categories—those that increase depolarization of the vascular smooth muscle or chemical factors. Chemical factors typically act on receptors or use second messengers, but some of the endothelial chemicals, namely nitrates, act directly on enzyme activity.[5] One of the most common chemical factors within the endothelium is prostacyclin, a vasodilator. Another prominent substance is nitric oxide (NO), an endothelium-derived gas with strong vasodilator properties. Nitric oxide was originally identified as "endothelium-derived relaxing factor" or EDRF. NO can override the actions of several vasoconstrictors, including endothelin. Endothelium-derived Hyperpolarizing Factor (EDHF) is another vasodilator discovered within vessels in which the prostacyclin and NO production was blocked; it primarily acts on smooth muscle and is the primary vasodilator at increased temperatures.[6-7]

Vascular smooth muscle plays a vital role in the regulation of total peripheral resistance, arterial and venous tone, as well as distribution of blood throughout the body. The endothelium and smooth vascular muscle have many close interactions, as seen by the myoendothelial junctions in arterioles—parts of endothelial cells that project into the vasculature. Peripheral circulation is modulated by dual control, meaning both the central nervous system and local tissues can act as a regulator. Unlike vessels within the systemic circulation, vessels within the cutaneous circulation, namely arterioles, are not supplied by parasympathetic vasodilator nerves. These vessels exhibit some basal tone and are highly sensitive to vasodilating agents.[7]

Many of the vascular resistance changes that take place are due to a change in perfusion pressure, or arterial blood pressure. This occurs to maintain constant blood flow

and is referred to as autoregulation. The phenomenon of autoregulation can best be explained by the myogenic mechanism. According to this concept, vascular smooth muscle contracts in response to increased pressure—or increased stretch—and relaxes in response to a decreased pressure; the myogenic mechanism is a response independent of the endothelium. This mechanism allows "down-stream" vasculature to be protected from increases in arterial pressure.[8] Endothelium itself, however, is also a known regulator of blood flow. An increase in the longitudinal pressure of a vessel causes vasodilation; this is thought to occur because of an increase in shear stress, which, in turn, increases nitric oxide production.[7] Shear stress, however, can induce vasodilation without other mechanisms at hand. Flow-induced vasodilation occurs with change in intraluminal flow without respective changes in intraluminal pressure.[5] One of the main factors in regulation of local blood flow is metabolites produced by the tissues. Any change causing an inadequate oxygen supply will increase the production of vasodilator metabolites, which are released directly from the tissue. There are two mechanisms to illustrate this concept—active hyperemia, increased blood flow caused by increased tissue activity, and reactive hyperemia, increased blood flow immediately following a period of occlusion. Duration of reactive hyperemia is directly proportional to the duration of occlusion. Immediately after occlusion ceases, resistance vessels dilate and cause a cascade of vasodilation in the microcirculation, significantly increasing blood flow to the area.[5-7]

2.1.1 The Function Of Nitric Oxide In Cutaneous Circulation

Nitric oxide (NO), previously known as endothelium-derived relaxing factor (EDRF), is thought to be the most important endothelial-mediated vasodilator. The production and release of NO is often caused by other agents including acetylcholine,

adenosine triphosphate, bradykinin, serotonin, substance P, and histamine. Nitric oxide stimulates guanylyl cyclase to increase cyclic guanosine monophosphate (cGMP), which relaxes the smooth muscle by decreasing levels of free calcium.[7] Nitric oxide reduces mitochondrial activity, which reduces inflammation and the production of reactive oxygen species. In turn, this reduces cardiac factors that produce dilating substances, decreasing platelet activation. Such actions protect against thrombosis and cardiac or vascular inflammation. Other functions of NO include contributing to gap formation in venules during the inflammatory process, inhibiting myocyte proliferation, inhibiting platelet aggregation, and inhibiting transcription of leukocyte-binding adhesion molecules; all of these actions act to prevent atheroma.[8]

Nitric oxide is produced from L-arginine and oxygen by the enzyme endothelial nitric oxide synthase (eNOS). Other compounds that are analogous to arginine, such as nitroarginine methyle ester (NAME), compete with arginine to bind with eNOS and can, therefore, block NO production. eNOS stimulation can be enhanced by acetylcholine and inflammatory mediators.[6] Phosphorylation activates eNOS and can be stimulated by shear stress, chemical factors, or an increase in intracellular calcium. While nitric oxide is continuously produced, it is degraded within seconds by reacting with superoxide anions or diffusing into the blood and binding with hemoglobin.[5]

2.1.2 Measures Of Cardiovascular Function

There are a multitude of tests available to measure cardiovascular function both systemically and in the specific circulations, such as cutaneous circulation. Primary measures of microvascular function include temperature-controlled vasodilation, or vasoconstriction, and induced reactive hyperemia. One of the main functions of human

skin is to regulate core temperature; because of this, cutaneous vascular tone has an inverse relationship to ambient temperature. Thermoregulation in the skin is controlled by the cutaneous sympathetic nervous system; this process combines both local and reflex factors to modulate heat transfer and sweating.[9] The initial increase in skin blood flow is due to withdrawal of sympathetic vasoconstrictive tone followed by reflex nerve activity. Initiation of the reflex activity releases several vasodilators including prostanoids, histamine receptors (H1), neurokinin-1 (NK-1), and transient receptor potential villanoid type 1 (TRPV-1). The release of these factors in turn increases nitric oxide activity, suggesting an interaction between sensory nerves and NO.[10] If the skin is locally heated, the arterioles, venules, and small veins dilate. Heat-induced vasodilation is partially due to increased eNOS activity. During heat stress, skin perfusion can increase to as much as 7-8 L/min, causing an increase in cardiac output as well as vasoconstriction in inactive areas to maintain arterial blood pressure.[6-7] Gradually increasing the temperature of the skin to 39ºC was found to be the most effective in isolating nitric oxide-dependent vasodilation. However, at temperatures greater than 40ºC, endothelial-derived hyperpolarizing factors have a greater role.[1]

Flow mediated dilation (FMD) is a vital measurement in microvascular function. This metabolically controlled vasodilation selectively dilates vessels considered to be "up-stream" in order to supply the areas in need. Most often, the dilating substance during this phenomenon is nitric oxide that is released by an increase in shear stress. The amount of FMD in an individual is inversely related to their risk for future cardiovascular disease. This test provides a mechanism by which the macrovascular and microvascular systems can be linked. Often applied as a subset of FMD, reactive hyperemia is another crucial test

to evaluate microvascular function. If blood flow is occluded, whether for a few seconds or a few minutes in duration, the flow immediately after occlusion is greater than that before the occlusion. However, the blood flow returns to baseline after a given time; this occurrence is reactive hyperemia. The longer the occlusion, the greater the peak flow once occlusion is released. When metabolites build up in the occluded area due to a lack of oxygen, resistance decreases in the arteries supplying the arterioles in that area. Vasodilation starts in the microvessels then propagates to the systemic vessels. This also increases the shear stress once occlusion is released and further promotes vasodilation.[7,9] Reactive hyperemia, when compared with the Framingham risk model and venous blood analysis of cholesterol, GFR, and fasted glucose, highly correlated with future cardiac events. The reactive hyperemia index (RHI) is highly predictive when evaluating for atherosclerotic processes.[11]

2.2 Microvascular Dysfunction

Microvascular disease and microvascular dysfunction are interchangeable terms with a broad spectrum of implications. Dysfunction can range from inadequate tissue perfusion to impaired endothelial-dependent vasodilation.8 Endothelial dysfunction has been highly associated with vascular inflammation, lipid deposits, thrombosis, and coronary dysfunction in previous studies.[12] Endothelial dysfunction is commonly attributed to reduced nitric oxide (NO) bioavailability. When the bioavailability of nitric oxide is reduced, whether it is from reduced production or impaired signaling, the risk for cardiovascular disease significantly increases. Several diseases linked with limited NO bioavailability include hypertension, atherosclerosis, stroke, and heart failure. The mechanism by which this occurs is related to an increase in reactive oxygen species (ROS)

produced primarily by NADPH oxidases. Reactive oxygen species can inhibit NO function by inactivating NO itself or by oxidizing the NO receptor, guanylyl cyclase.[13] Another mechanism by which reactive oxygen species can be produced is by endothelial microparticles. There is no current evidence that the microparticles produce ROS directly, but they are thought to induce ROS production within an endothelial cell via an NADPH oxidase-dependent pathway. Microparticles within the endothelium are anuclear vesicles and are highly indicative of vascular dysfunction and are often used to predict the risk of future cardiovascular events. These vesicles are part of the communication system between the endothelium, vascular smooth muscles, immune cells, and fibroblasts, and aid in the processes regulated by the endothelium such as blood flow regulation, coagulation, cellular trafficking, and inflammation responses.[14]

2.3 Racial Differences In Microvascular Function

In cases of cardiovascular disease, African-Americans possess a disproportionately higher morbidity than Caucasian-Americans. While some of the cases can be attributed to higher levels of risk factors in African-Americans—such as higher rates of obesity, diabetes, and smoking—there is reason to believe there is a pathological reason for this occurrence.[15] African-Americans are known to have both an earlier onset as well as a higher prevalence of hypertension as compared to their Caucasian counterparts. Some contributing factors to this pathology include chronic stress exposure, stress-induced blood pressure reactivity, vasoactive substance release, and sodium handling.[4,6] In addition to decreased NO bioavailability, another mechanism by which African-Americans can elicit an impaired response is decreased blood vessel elasticity. Elasticity and compliance allows vessels to compensate for pulsatile waves of blood and transform it into a smooth linear

flow. Causes of this decrease in compliance can include arterial stiffening and decreased microvascular reactivity.[16] African-Americans also elicit a higher rate of arterial stiffening; this can significantly increase cardiovascular disease risk. Arterial stiffening can worsen hypertension, which is highly prevalent in African-Americans, in a positive feedback mechanism; chronic elevation in arterial pressure leads to arterial wall thickening and remodeling.[15] In previous studies, treating these individual mechanisms pharmacologically elicit little improvement in the overall risk factors.

On average, African-Americans elicit impaired vascular function on both non-invasive and invasive tests. Compared to Caucasians, African-Americans have a lower reactive hyperemia index—as measured by flow mediated dilation—and greater arterial stiffness, as measured by pulse amplitude tonometry. In previous studies, these dysfunctions in both arterial tone and elasticity have been present in healthy subjects with no current cardiovascular disease risk factors, suggesting a mechanistic explanation to increased rates of cardiovascular disease (CVD) in African-Americans.[15-16] To further assess young and otherwise healthy African-Americans with no current CVD risks, carotid blood pressure can be measured. Carotid blood pressure is proven to be more indicative of future disease processes than peripheral (brachial) blood pressure. Compared to Caucasians, African-Americans have elevated central blood pressures, while the peripheral blood pressure is relatively the same. This effect is thought to be due to vascular stiffness and reduced pulse pressure amplitude.[16]

Compared to Caucasians, African-Americans tend to have lower concentrations of circulating antioxidant scavengers and increased levels of oxidative stress biomarkers. Oxidative stress biomarkers possess a direct relationship with elevated blood pressure in

otherwise normotensive males. African-Americans with blood pressure impairments also elicited abnormal lipid peroxidation; Caucasians, on the other hand, have normal lipid peroxidation and abnormal DNA repair.[4] Another method to monitor oxidative stress biomarkers is by measuring plasma levels of cystine and glutathione. Cysteine is a common extracellular aminothiol—responsible for oxidizing proteins—that readily reacts with oxidants and is converted to cystine. Glutathione, on the other hand, is an intracellular antioxidant that regulates the cell's oxidation-reduction (redox) reactions and is converted to glutathione disulfide. With increasing levels of oxidative stress, it is expected to observe higher levels of cystine and lower levels of glutathione; these levels also indicate increased risk for sudden cardiac death. These abnormal findings are more attenuated in African-Americans than in their Caucasian counterparts.[17]

CHAPTER 3

METHODOLOGY

3.1 Experimental Design

This study consisted of independent subject t-tests and a two-way ANOVA test between subject groups. The alpha level set for all the analyses was $p<0.05$. The skin blood flow, reactive hyperemia, and lab results were evaluated between African-American and Caucasian-American groups. Analysis of the variables was determined as the effect of the drug at each site as compared to the control site in both subject groups. The study consisted of a single laboratory visit lasting approximately six hours in duration.

3.2 Subjects

Eight healthy, college-aged males were recruited from The University of Texas at Arlington for the study (Table 3.1). Four African-American males (age 23.25±1.64 years old) volunteered to participate in the study. Four Caucasian males (age 23.75±3.03 years old) were then similarly matched based on age, body mass index (BMI), and waist-to-hip ratio (WHR).

Table 3.1: Subject Demographics

	African-American (n=4)	Caucasian (n=4)	P Value
Age (years)	23.25±1.64	23.75±3.03	0.810
BMI	26.21±1.86	24.28±2.97	0.378
WHR	0.774±0.039	0.786±0.035	0.726

3.3 Instrumentation

3.3.1 Microdialysis

Four semipermeable microdialysis fibers—approximately 1/100th an inch in diameter—with microscopic holes were placed in the dermal layer of skin on the ventral surface of one forearm. These were placed using aseptic technique with 25-G needles. Once the needles were placed in the dermal layer of skin, the microdialysis fibers were threaded through the lumen of the needle, after which the needle was removed while leaving the fibers in place. The fibers were secured with tape and perfused with Lactated Ringer's solution until the beginning of drug perfusion. This was continued for approximately 60 minutes to allow hyperemia associated with the needle stick to subside.

Local skin heaters covering an area of approximately 0.78cm^2 were placed directly over each membrane site and secured with tape. The red blood cell flux at each site was measured in real time via a laser Doppler flow probe; these were seated in the center of each local heater.

3.3.2 Cardiac Rhythm/Heart Rate

An electrocardiogram was placed on a monitor for each subject, and both heart rate and cardiac rhythm were continuously obtained throughout the study (CardioCard, Nasiff Associates, Central Square, New York).

3.3.3 Arterial Blood Pressure

Blood pressure was intermittently obtained by auscultation of the brachial artery of the nonexperimental arm via electrosphygmomanometry (Tango+, SunTech, Raleigh, NC). Mean arterial pressure (MAP) was calculated as the diastolic blood pressure plus one-third

of the pulse pressure. The blood pressure cuff was inflated approximately 20mmHg over the subject's systolic blood pressure during the intermittent measurements.

3.3.4 Cutaneous Blood Flow

Cutaneous blood flow was obtained from the forearm equipped with microdialysis fibers via laser Doppler flowmetry using an integrating probe (MoorLab Laser Doppler Perfusion Monitor, Moor Instruments). The laser emitted penetrates the skin approximately 1mm and reflects off red blood cells at or near the surface of the skin. The signal returns to the probe with an index of the speed and number of red blood cells in the path of the laser, which is used to calculate red blood cell flux. The cutaneous vascular conductance was calculated from the ratio of laser Doppler flux to MAP.

3.3.5 Local Heating Elements

The local heating elements—also containing the laser Doppler—were attached directly over each membrane site. These local heating elements (3cm diameter, Periflux 5020, Probe 450, Perimed) were heated to 39ºC for at least 30 minutes to obtain maximal skin blood flow.

3.3.6 Blood Specimen

A blood specimen was obtained via venipuncture in the antecubital vein under sterile technique by personnel trained in phlebotomy. Blood was collected for analysis of glucose, blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR), and lipids. The blood drawn did not exceed 100mL and was stored using a coding system in a -80ºC freezer until analysis.

3.3.7 Medical History Questionnaire

Prior to analyzing data, subjects completed a medical history questionnaire. This was used to further match African-American subjects with Caucasian counterparts of similar height, weight, age, and sex.

3.3.8 Drug Perfusion

A formal Investigative New Drug (IND) application was obtained prior to the study. Tempol, L-NAME, and Apocynin do not currently have FDA approval; SNP is currently approved for use in acute hypertension, congestive heart failure, and hypotension during surgery. Allopurinol is currently approved for use in gout, congestive heart failure, hyperuricemia, and Lieshmaniasis. All drugs were purchased from Sigma-Aldrich and have been injected via cutaneous microdialysis in previous studies. The drugs are stored in their solid form according to the manufacturer recommendations in cabinets or the freezer in ERB 187A. On the day of the study, the drugs were weighed to the nearest 0.01mg using an analytical balance (Mettler-Toledo XS-64). Once placed in a tube, the appropriate amount of Lactated Ringer's solution was added to the tube and vortexed for approximately 30 seconds. Once mixed, the drug solutions were filtered through a syringe filter and placed in a 1mL syringe; the syringe was protected from light until use.

The dosages of the drugs were determined according to prior studies. The following doses were used: 10μM Tempol, 100μM Apocynin, 10μM Allopurinol, 28mM SNP, and 10mM L-NAME. The drugs were perfused through the microdialysis fibers at the rate of 2μL/min via an infusion pump (Harvard Apparatus, Pump 11). According to the concentration, time of infusion, and infusion rate, the amount of each drug given

throughout the study is as follows: 0.21µg Tempol, 1.99µg Apocynin, 0.17µg Allopurinol, 0.16µg L-NAME (at each site), and 0.5µg SNP (at each site).

3.3.9 Flow Mediated Dilation

Flow mediated dilation was measured non-invasively in the brachial artery. A Doppler ultrasound machine (Logiq P5, General Health Care) equipped with a high-definition linear array transducer was used to obtain brachial artery diameter and blood flow while the subject was in a supine position. A longitudinal image of the brachial artery was obtained 5-10cm proximal to the antecubital fossa, and a blood pressure cuff was placed on the subject's forearm 3-5cm distal to the antecubital fossa. Once baseline recordings were obtained, the cuff was inflated to 220mmHg for 5 minutes. After cuff deflation, ultrasound-derived measurements of the brachial artery diameter and blood flow was obtained for 3 minutes.

3.3.10 Statistical Analysis

Statistical Analysis was performed by a statistical software package (SigmaStat 3.11, Chicago, IL). Descriptive statistics were used for the analysis of the mean, standard deviation, and coefficient of variation for bodyweight (kg) and age (year). Analysis of the effect of the drug solution in each microdialysis site with respect to the control site (Ringer's) in each of the subject groups (Caucasian and African-American) was performed utilizing independent student t-tests and two-way analysis of variance tests (ANOVA). Following the two-way ANOVA, an appropriate multiple comparison post-hoc analysis was performed. The alpha level for all analyses was set at $P<0.05$.

3.4 Procedures

The subjects presented to the laboratory (ERB 186) in the morning status post an overnight fast. Each subject was then given informed consent documents and presented with a health history questionnaire. Basic measurements, such as height and weight, were then taken, and the subject began resting in a semi-recumbent position. Flow mediated dilation testing was then conducted on the left brachial artery of the subject. Once an image was obtained on the Doppler ultrasound, 2 minutes of baseline data was collected. The cuff was then inflated to suprasystolic pressures for 5 minutes; 3 minutes of data was collected post cuff release (see 3.3.9). The subject's non-dominant forearm was then placed at the level of their heart and instrumented with four microdialysis fibers (see 3.3.1). Each site was then taped in place and equipped with a thermal heater and laser-Doppler probes directly above the perfusion site. The sites were flushed with Lactated Ringer solution, and the subject was then given 60-90 minutes for the trauma associated with the needle puncture to subside. Each microdialysis site was randomly assigned either Lactated Ringer solution, Allopurinol, Apocynin, or Tempol. Baseline data was collected with local heaters set to 33°C. After 10 minutes of baseline data collection, the local heating units were increased to 39° C for a period of 60 minutes. After a 5-minute plateau in skin blood flow, all microdialysis sites were perfused with 20 mM of the NOS inhibitor NG-nitro-L-arginine methylester (L-NAME), allowing the calculation of percent nitric oxide contribution. Once blood flow stabilized following L-NAME infusion (about 30 mins.), local temperature was increased to 43 ºC and 28 mM of sodium nitroprusside (SNP) was perfused through each site to induce maximal vasodilation.

CHAPTER 4

RESULTS

4.1 Subject Characteristics

A total of four African-American subjects (n=4) and four Caucasian subjects (n=4) were matched identically for age, waist-to-hip ratio (WHR), and BMI. Demographics and characteristics can be seen in Table 4.1. The mean age for blacks was 23±2, with Caucasians having a mean of 24±3. The African-American group was slightly younger and had a significantly higher mean arterial pressure (MAP). However, the blood values obtained for both groups were not significantly different. Lab results can be seen in Table 4.2.

Table 4.1: Subject Demographics And Characteristics

	African-American (n=4)	Caucasian (n=4)	P Value
Age	23±2	24±3	0.810
Height (cm)	176.1±6.9	180.7±2.7	0.330
Weight (kg)	81.7±10.7	79.4±10.7	0.803
BMI	26.2±1.9	24.3±3	0.377
WHR	0.77±0.04	0.79±0.03	0.726
Systolic Blood Pressure (mmHg)	123±7	121±3	0.663
Diastolic Blood Pressure (mmHg)	68±4	58±6	0.055
Mean Arterial Pressure (mmHg)	86.3±1.8	78.7±4.7	0.038

Table 4.2: Subject Blood Analysis Results

	African-American (n=4)	Caucasian (n=4)	P Value
Glucose (mg/dl)	92.7±3.4	84.5±6.1	0.136
eGFR (ml/min/1.73)	105.7±18.5	105.3±8.6	0.975
Cholesterol, total (mg/dl)	169.7±57.0	169.8±41.0	0.999
Triglycerides (mg/dl)	74.7±24.3	113±55	0.385
HDL (mg/dl)	62.3±1.7	52.0±18.3	0.449
VLDL (mg/dl)	15.0±5.0	22.8±11.1	0.384
LDL (mg/dl)	92.3±51.3	95.0±24.2	0.941

4.2 Flow Mediated Dilation Results

Flow mediated dilation (FMD) Doppler videos were analyzed on Cardiovascular Suite. From the data analyzed combining pre-occlusion and post-occlusion, measurements for mean diameter, anterior shear, retrograde shear, mean shear, oscillation factor, time to peak, shear area under the curve (velocity response post-occlusion), shear max, %FMD, and FMD/area under the curve were calculated. After conducting a two-tail, paired equal variance t-test, none of the measurements showed significant differences between African-Americans and Caucasians (Table 4.3).

Table 4.3: Subject Flow Mediated Dilation Results

	African-American (n=4)	Caucasian (n=4)	P Value
Mean Diameter (mm)	3.87±0.71	4.18±0.81	0.636
Anterior Shear ($sec.^{-1}$)	364.6±317.3	305.5±102.0	0.769
Retrograde Shear ($sec.^{-1}$)	-5.89±10.0	-8.27±9.35	0.774
Mean Shear ($sec.^{-1}$)	358.7±320.8	297.2±107.3	0.764
Oscillatory Index	2.9±4.8	3.2±3.9	0.928
Time to Peak (sec.)	68.9±29.6	43.0±21.7	0.268
Shear Max ($sec.^{-1}$)	723.2±110.8	1192.2±327.6	0.057
FMD%	6.21±1.64	6.39±4.46	0.952
FMD/AUC	0.23±0.08	0.16±0.06	0.291

From this data, post-occlusion data was separately analyzed in order to measure the reactive hyperemia response between subjects. Using the velocity and diameter values obtained during FMD analysis, flow was calculated. With this subset of data, the baseline flow, area under the curve (AUC) for flow, peak flow, baseline velocity, AUC for velocity, and peak velocity were calculated. The African-American subjects (n=4) showed significant differences in measurements for AUC flow, AUC for velocity, and peak velocity. There were no significant differences in baseline data (Table 4.4).

Table 4.4: Subject Reactive Hyperemia Results

	African-American (n=4)	Caucasian (n=4)	P Value
Baseline Flow (mL/min)	297.0±343.1	233.7±76.8	0.766
AUC (mL)	331.7±104.6	1115.7±442.3	0.024
Peak Flow (mL/min)	541.6±285.7	991.2±451.2	0.195
Baseline Velocity (cm/min)	36.0±36.3	28.3±4.9	0.731
AUC (cm)	2669.8±192.0	7432.7±1306.1	0.00078
Peak Velocity (cm/min)	71.7±17.6	117.0±18.8	0.023

4.3 Microdialysis Results

Red blood cell flux collected continuously from the laser Doppler probes during each study was used to calculate the percent cutaneous vascular conductance (CVC) max, with the maximal value being obtained with heat at 43ºC and infusion of SNP. The percent CVC max values for each site were compared between subject groups, as well as between sites, using Sigmastat. In the African-American subject group, the control site (Ringers) obtained the lowest percent CVC max (41.3±6.5), while the site perfusing Apocynin obtained the highest percent CVC max (79.0±6.2). In the Caucasian group, however, the Ringers (control) site obtained the highest percent CVC max (73.1±4.4). The lowest percent CVC max observed in the Caucasian group occurred at the site perfusing Apocynin (61.2±6.9), as seen in Figure 4.1 (see Appendix A Figure 1 and Figure 2). Data for each site between groups passed a normality test (Shapiro-Wilk, P=0.289) and an equal variance test (P=0.362). The groups were compared in a two-way ANOVA, with a significant difference between the African-American and Caucasian groups (P=0.008). Each site was

compared pairwise in the post-hoc analysis using the Tukey Test, with a significant difference between groups at the control site (Ringers, P=0.002). There was no significant difference between African-Americans and Caucasians at any of the sites perfusing drugs (Table 4.5).

Figure 4.1: Comparison of CVC Max Between Subject Groups at 39ºC Heating

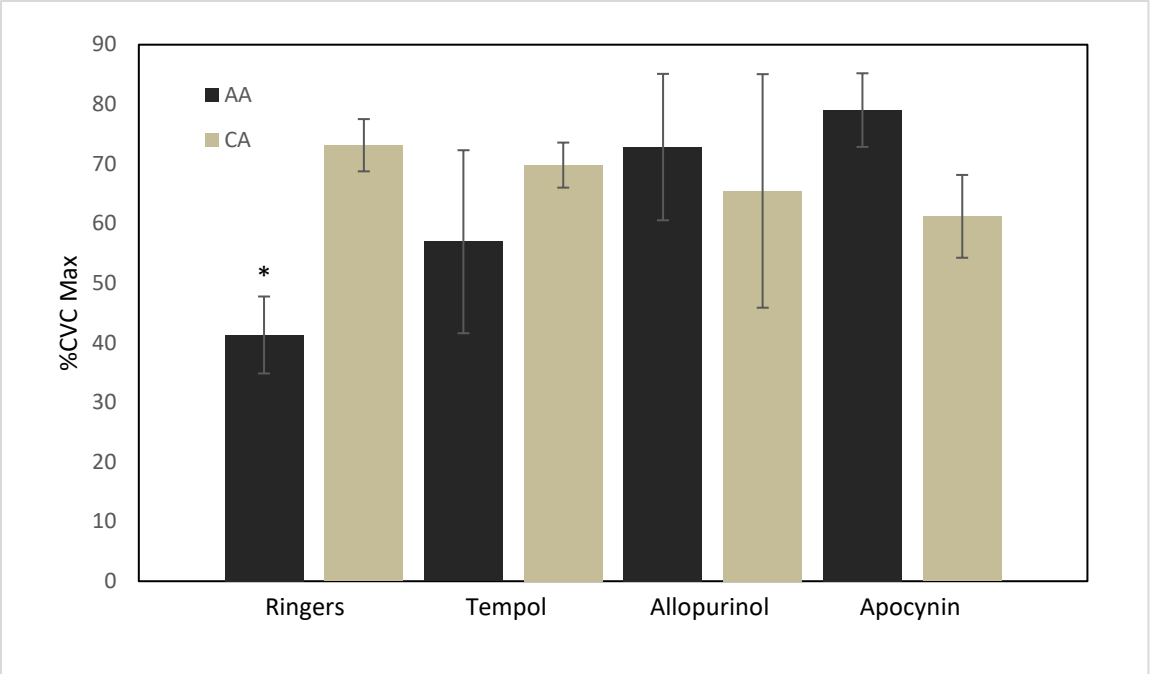


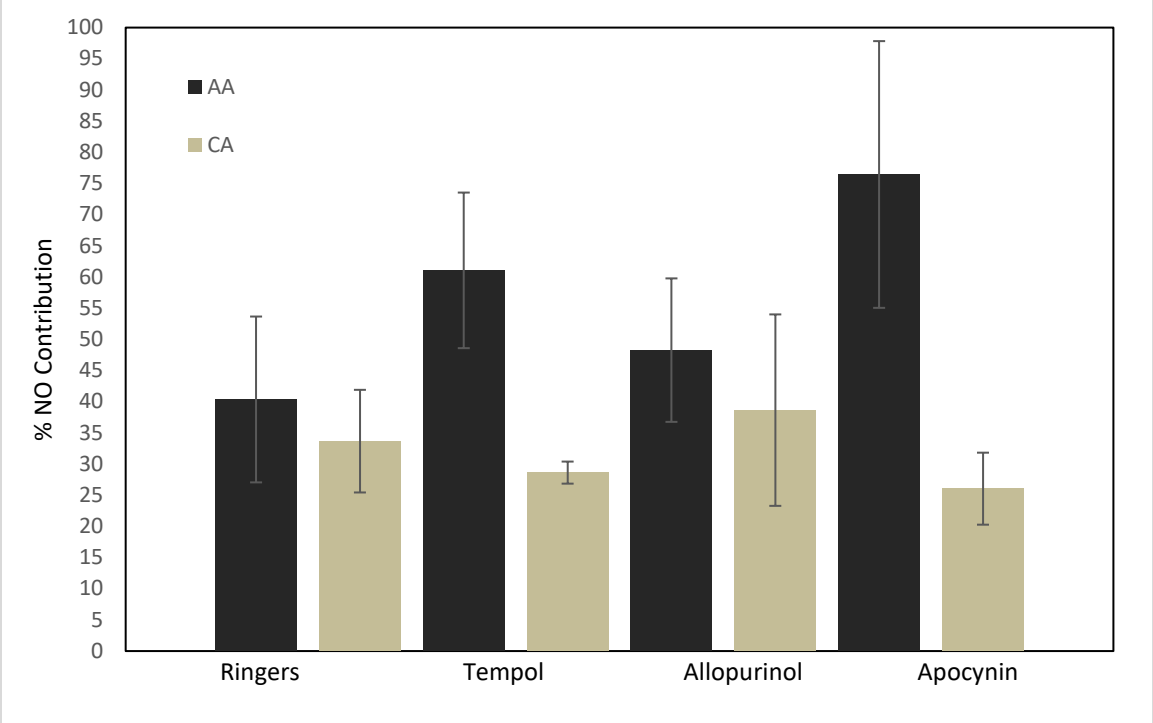
Table 4.5: Tukey Test Results—Comparison of CVC Max Within Perfusion Sites

Comparison	Site	q	P
AA vs. CA	Tempol	2.063	0.159
AA vs. CA	Ringers	5.108	0.002
AA vs. CA	Allopurinol	1.181	0.413
AA vs. CA	Apocynin	2.860	0.055

From the percent CVC max calculations, nitric oxide contribution for each site was calculated. This was done by dividing the control site (Ringers) value during 39ºC heating

by the L-NAME value for each drug. These values were compared between groups, as well as between sites using a two-way ANOVA and post-hoc Tukey Test. The data passed a normality test (Shapiro-Wilk, P=0.182) and equal variance test (P=0.622). Comparison of African-Americans and Caucasians yielded significant difference (P<0.001). Results can be seen in Figure 4.2.

Figure 4.2: Comparison of NO Contribution Between Groups at 39ºC Heating



CHAPTER 5

CONCLUSION

African-Americans were found to have significant impairments in their skin blood flow response to local heating that were attenuated by reducing superoxide and superoxide production via antioxidants. African-American subjects also elicited impaired reactive hyperemia responses, suggesting a systemic dysfunction in their vasculature. Compared to Caucasian individuals who were similarly matched for age, BMI, and fitness levels, African-American subjects had a greater mean arterial pressure, but similar glucose levels and lipid profiles. Since the mean arterial pressure is more of an indicator of overall peripheral resistance, it is more indicative of risk for future cardiovascular events. Another study noted greater central blood pressure in African-American subjects, but comparable blood pressure measurements taken from the brachial artery. In the study, the greater central blood pressure correlated with increased central arterial stiffness, as well as microvascular dysfunction.[16] Within the subject population, African-Americans were found to have microvascular dysfunction in the drug perfusion protocol while the Caucasians displayed a response within the normal limits. Between the subject groups, there was no significant difference in the FMD results and baseline data. However, the African-American group had a significantly lower flow and velocity AUC post-occlusion. This suggests that this subject population had less tissue perfusion following occlusion. Also, the African-American subjects elicited a significantly lower peak velocity post-occlusion, suggesting a higher amount of resistance within their vasculature. These blunted

responses seen in African-Americans post-occlusion are consistent with previous research. Another study evaluated blood flow before and after occlusion in the forearm of young, healthy African-Americans and Caucasians. Between groups, there was no difference in baseline forearm blood flow, but the African-American population had reduced blood flow responses post-occlusion. This is consistent with findings from previous studies and indicates impaired endothelial vascular dysfunction.[2] However, some previous studies revealed a correlation between impaired microvascular dysfunction and elevated total cholesterol and triglycerides. Within our subject population, there were no significant differences within the lipid panel. This could also be an indication that the dysfunction in our subjects was limited to the microcirculation and had minimal systemic effects.

Using the value for maximal vasodilation, each phase of the study could be expressed as a percent of the cutaneous vascular conductance at a maximal state. Values obtained during heat at 43ºC and SNP infusion were considered maximal vasodilation. Data collected from each site when heated to 39ºC was normalized to the heat and SNP phase in order to calculate the percent maximal CVC. Previous studies analyzed the heating protocols to assess which temperature was adequate to elicit a nitric oxide-dependent vasodilation response in the cutaneous vasculature. Comparing local heating at 36ºC, 39ºC, and 42ºC, it was concluded that gradual heating to 39ºC elicited the strongest vasodilation response attributable to NO. Temperatures below required L-NAME to attenuate the response, and temperatures above are thought to have a greater contribution from EDHF's.[1,20] In the Caucasian subject population, the control site endorsed the highest percentages of CVC max throughout the study. The other three sites, perfused with superoxide inhibiting drugs, elicited much lower vasodilation as compared to the control.

This was especially true of the site perfusing Apocynin, a drug that blocks superoxide production via the enzyme NADPH oxidase. In the African-American subject population, however, the control site endorsed the lowest percentage of CVC max throughout the study. The sites perfusing drugs inhibiting superoxide had an attenuated response to local heating, with the site perfusing Apocynin possessing the highest percent CVC max throughout the study. The control site in Caucasian subjects and the Apocynin site in the African-American subjects were comparable, suggesting a role of oxidative stress—in the form of superoxide—in the blunted cutaneous vascular response seen in African-Americans. Another study evaluated reactive oxygen species (ROS) produced by Angiotensin II. This form of oxidative stress can take two different pathways, NADPH or xanthine oxidase. In the study, Apocynin, Allopurinol (blocks xanthine oxidase), and Tempol (inhibits superoxide) were perfused during local heating protocol. It was found that Allopurinol and Tempol both attenuated the local heating response, suggesting a role of both the NADPH and xanthine oxidase pathways in cutaneous vascular control.[18] Other previous studies have assessed the effect of Tempol on nitric oxide bioavailability. In these studies, it was found that Tempol causes vascular changes during local heating protocol that are NO-dependent, suggesting that Tempol increases nitric oxide bioavailability.[19] Theoretically, reduction of oxidative stress would promote an increase in nitric oxide bioavailability. There was a significant difference in NO contribution at each site within our subject population, implying that the drugs reducing superoxide play a role in increasing NO bioavailability.

Some limitations of the study could include a smaller sample size with a large amount of variance. Future studies could include larger subject samples matched for body

fat percentage and general eating habits in order to decrease a portion of the variance that can occur.

With a limited sample size and a large range of variability in the sample size, the subjects still elicited significant differences in cutaneous vascular function. African-American subjects were found to have impaired responses to local heating and blunted reactive hyperemia responses while the Caucasian subjects had responses within the normal limits. However, with the perfusion of superoxide scavenging or inhibiting drugs, African-Americans had an attenuated response to local heating that was comparable to the Caucasian subjects. Reactive hyperemia findings in the African-American subject group suggest that the findings are not limited to the cutaneous circulation, but can be seen systemically as well.

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APPENDIX A

ADDITIONAL GRAPHS

Figure A.1: Comparison of Percent CVC Max in African-American Subjects

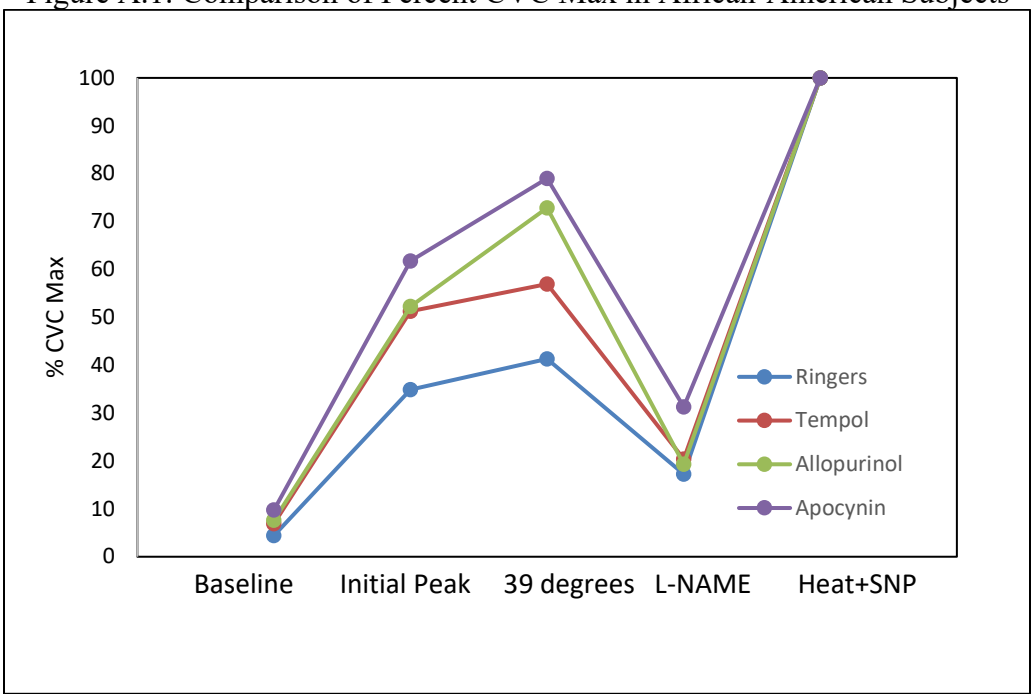
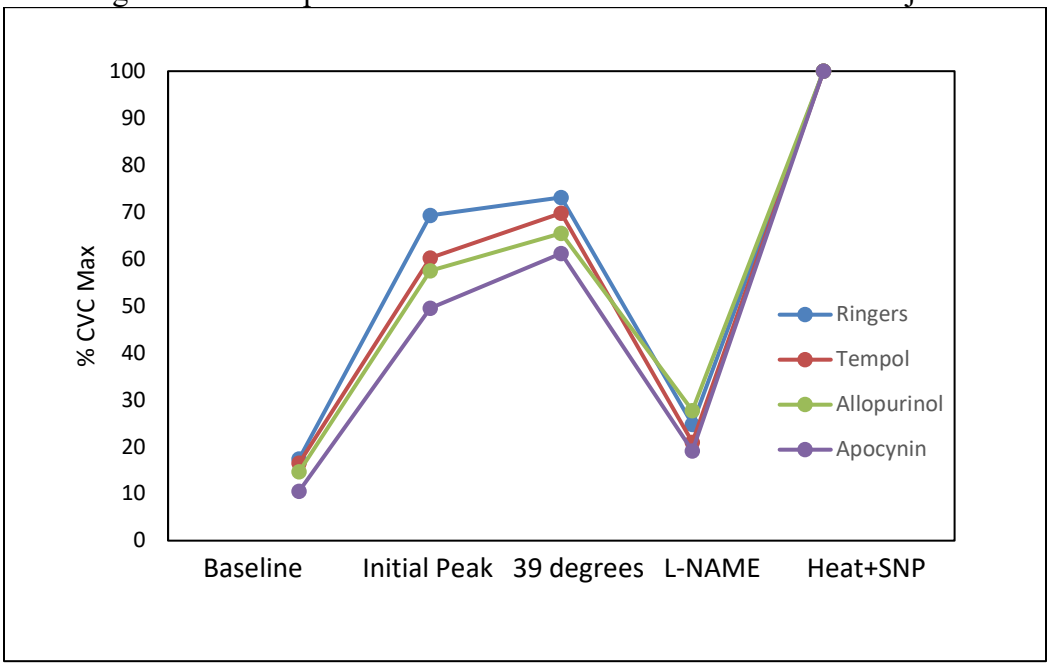


Figure A.2: Comparison of Percent CVC Max in Caucasian Subjects



APPENDIX B

HEALTH QUESTIONNAIRE

Health History Form

Date:_____
Subject ID: _____

Ethnicity (select one of the following):
- □ Hispanic or Latino
- □ Non-Hispanic or Latino

Race (select one of the following):
- □ American Indian or Alaska Native
- □ Asian
- □ Black or African American
- □ Native Hawaiian or Pacific Islander
- □ White
- □ I do not wish to disclose this information

Social History

My current exercise/activity level is: □Satisfactory □Unsatisfactory □I don't know
Type of exercise/activity:_____ Frequency:_____

My current weight is: □Satisfactory □Unsatisfactory □I don't know
I □currently □previously □ never use diet and/or exercise to lose/gain weight
I □currently □previously □ never use medication/supplements to lose/gain weight

Is caffeine part of your diet? □currently □previously; date stopped_____ □ never
Source of caffeine:_____ Frequency:_____

Tobacco use: □currently □ never □previously; year started_____ year quit_____
Type:_____ Amount:_____ Number of years:_____

Alcohol use: □never □occasionally/rarely □weekly □daily
Type:_____ Amount:_____

Illicit drug use: I □currently □previously □ never use elicit drugs (such as marijuana, PCP, crack, methamphetamines)
Type:_____ Last used:_____

Contraception/pregnancy risk (FEMALES ONLY):
I am currently using a reliable form of contraception:
□ YES □ NO □ I am not in a sexually active relationship

It is possible that I am pregnant □ YES □ NO
First day of your last menstrual period_____

Medical History

Allergies: (this includes food, medication, and/or latex) □ None Known

Allergy/Intolerance	Describe Reaction

Please mark the box if you have or have had any of the following conditions:

□ Asthma
□ chronic bronchitis/emphysema
□ other chronic lung disease
□ tuberculosis
□ high blood pressure
□ high cholesterol
□ diabetes
□ heart disease/chest pain
□ heart attack
□ racing heart/palpitations
□ abnormal electrocardiogram (ECG)
□ other heart trouble
□ bleeding/clotting disorder
□ headaches
□ seizures/epilepsy
□ stroke
□ thyroid disorder
□ ulcers
□ diverticulosis/diverticulitis
□ inflammatory bowel disease
□ bowel obstruction/ileus
□ gallbladder disease
□ liver disease
□ kidney disease
□ urinary problems
□ arthritis/joint problems
□ chronic infection
□ fainting spells
□ recurrent fatigue
□ cancer
□ anxiety/depression
□ alcohol/substance abuse
□ mental illness

Please list all surgeries and explain any "YES" answers below:

_____
_____
_____
_____

Medications: (current medications including over-the-counter, vitamins, and herbal supplements)

Medication	Dose/Amt	Frequency	Purpose

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BIOGRAPHICAL INFORMATION

Alexis “Lexi” McMillen is a senior Exercise Science major with minors in both Biology and Chemistry. She will graduate May 2017 with an Honors degree. She has been involved in several organizations on campus including The National Society of Collegiate Scholars, National Society of Leadership and Success, Medical and Dental Preparatory Association, and Operation Smile. She has also been active in the Honors College and served as an Honors College Advocate. Lexi has been involved in the research laboratories of Dr. Mark Ricard and Dr. Matthew Brothers during her undergraduate studies. Outside of school, Lexi has worked as an Emergency Department scribe and an inpatient phlebotomist at Methodist Mansfield Medical Center. She has also spent time volunteering as a cross country assistant coach and as a hospice volunteer for Brookdale Hospice Care. After graduation, Lexi will attend the Texas College of Osteopathic Medicine in Fort Worth to pursue a D.O/M.S. degree. In her free time, she enjoys endurance running and reading classic literature.