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A PRE AND POST EXERCISE COMPARISON OF THREE ASSESSMENT TOOLS COMMONLY EMPLOYED TO ASSESS VASCULAR FUNCTION

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A thesis submitted in partial fulfillment of the requirements for the degree of Masters in Science at Virginia Commonwealth University

by

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List of Abbreviations

AI: augmentation index

AUC: area under the curve

BAD: brachial artery diameter

BL: baseline

BMI: body mass index

BP: blood pressure

FBF: forearm blood flow

FMD: flow mediated dilation

HR: heart rate

MAP: mean arterial pressure

PE: post-exercise

PO: post-occlusion

PR: pre-exercise

RHI: reactive hyperemic index

VL: blood velocity

VR: vascular reactivity

Abstract

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Clinical research efforts have recently focused on the detection of subclinical indicators and precursors of cardiovascular disease (CDV), including endothelial dysfunction (ED). Several vascular assessment tools have been developed as a means of detecting early alterations in vascular responses and subsequent characterization of ED. However, three of the most commonly employed techniques have several methodological differences, including the fact that each technique evaluates a different component of the vasculature. Flow mediated dilation (FMD) via ultrasound evaluates vascular function of the large conduit vessels such as the brachial artery. Strain gauge plethysmography (SGP) measures vasodilatory capacity of resistance vessels by assessing forearm blood flow. Lastly, Peripheral artery tonometry (PAT) evaluates the ratio of pulse wave amplitude (PWA) by assessing the microcirculation of the fingers. While these measures are typically performed during a resting condition, there has been recent interest in

evaluating vascular responses following an acute bout of exercise. The documents contained herein include a review of the relevant literature related to mechanisms of vessel function, assessment of vascular dysfunction using non-invasive tools, and vascular responses brought on by different types and intensities of exercise. Additionally, a manuscript is included which details a pilot research study designed to compare the pre- and post-exercise reactive hyperemia responses to 5 minutes of occlusion as evaluated by SGP, FMD and PAT in apparently healthy, untrained males. Relationships among the primary outcome variables obtained from the three techniques both before and after exercise are also evaluated. It is our hope that this initial pilot study in apparently healthy subjects will provide a foundation on which to develop a vascular function assessment protocol that will improve early detection of ED and therefore earlier intervention and ultimate reduction of CVD mortality.

I. INTRODUCTION

Cardiovascular Disease (CVD) continues to be the leading cause of death worldwide (He & MacGregor, 2009). Recently, researchers have shifted their attention to recognizing the preliminary signs of CVD, in hopes of preventing and reversing the early development of vascular abnormalities. Subclinical indications of CVD include vessel wall abnormalities and delayed vascular responses. Vascular reactivity refers to the morphological and physiological changes that blood vessels undergo in response to a stimulus. This self-regulation of tone and adjustment to changes in blood flow are endothelium-dependent, and imperative to normal vascular function. Impaired endothelium-dependent vasodilation is the main manifestation of poor vascular reactivity, and could predict the risk of cardiovascular incidents (Schächinger, Britten & Zeiher, 2000). In fact, endothelial dysfunction (ED) has been shown to be a stronger predictor of cardiovascular disease than classic risk factors for coronary artery disease such as hypertension, hypercholesterolemia, smoking, family history, and evidence of atherosclerosis incidents (Schächinger, Britten & Zeiher, 2000). ED is defined as the progressive development of irregular vascular function, such as abnormal vessel tone, loss of the endothelium's atheroprotective properties, and most notably, diminished nitric oxide (NO) availability (Vane, Anggård & Botting, 1990, Quyyumi, 1995). Fortunately, ED is considered to be a treatable stage of CVD development.

Cardiovascular disease treatments range from medicinal regimens to high risk invasive surgical procedures. Angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs) and beta-adrenergic antagonists are commonly

used drug therapies for hypertension, coronary artery disease and other vascular complications (Gustafsson, Segura & Ruilope, 2010). Although mostly effective at preventing cardiovascular events, some patients on such therapies will still develop heart failure regardless of the type of drug therapy (Verdecchia, et al., 2009). Invasive procedures also known as percutaneous coronary interventions (PCIs) include angioplasty and stenting (Hashemi, et al., 2010). PCIs are common and effective procedures in the treatment of various cardiovascular diseases, yet these invasive procedures are risky and recovery can be an arduous process (Hashemi, et al., 2010). The ineffectiveness and risk associated with cardiovascular disease treatment accentuates the importance of early detection of ED, thereby preventing the full development of cardiovascular disease.

Three commonly employed clinical and research tools to evaluate vascular function and detect ED include brachial artery flow-mediated dilation (FMD) via ultrasound, venous occlusion strain-gauge plethysmography (SGP), and peripheral artery tonometry (PAT). All three techniques utilize a pressure cuff that is inflated to suprasystolic pressure (240mm Hg) for five minutes, substantially minimizing blood flow to the limb. Following the rapid release of the cuff, oxygenated blood rushes into the hypoxic area inducing rapid vasodilation in a process known as reactive hyperemia. The extent of the vessel's reaction to this process, as well as the time frame in which it occurs, are markers of vasomotor function (Corretti, et al., 2002 & Dhindsa et al., 2008). While all three tools have shown a strong ability to evaluate specific parameters related to vascular health, they do so by evaluating different components of the cardiovascular system. By

combining their different diagnostic abilities, early CVD detection and prevention may be substantially enhanced.

Brachial artery flow-mediated dilation (FMD) evaluates vascular function of the large conduit vessels such as the brachial artery, which allow for increased blood flow to large areas (Segal & Durling, 1986). FMD utilizes high resolution ultrasound imaging to scan and measure the diameter of the brachial artery at rest and during hyperemic conditions. The magnitude and speed of the brachial artery diameter change is indicative of endothelial function, which is impaired in patients with coronary atherosclerotic lesions (Zeiher, Schächinger & Minners, 1995). Moreover, recent research has further pinpointed the location that the FMD technique is evaluating. It was previously believed that impaired FMD was reflective of abnormal microcirculation, but more recent studies have found that it directly indicates atherosclerotic processes occurring in the conduit brachial artery (Padilla et al., 2009). The CVD predictive ability of FMD has been extensively explored in many healthy and clinical populations, and although confounding results have been found, there is a general agreement that brachial artery FMD is a useful tool in the classification of subjects into low, intermediate, or high-risk CVD categories (Yeboah et al., 2009).

Venous occlusion strain gauge plethysmography (SGP) has been utilized to assess forearm blood flow as a marker for vascular function. A mercury-filled strain gauge is wrapped around the thickest portion of the forearm, and it senses the magnitude of forearm diameter change during a time when venous outflow is occluded, which allows calculation of arterial inflow at baseline and after an occlusive stress. The reactive hyperemia induced forearm blood flow (FBF) responses denote vasodilatory capacity of

resistance vessels, an indicator of vascular health (Patterson, 1955). SGP has proven effective at detecting ED. (Shechter, Matetzky, Arad, Feinberg & Freimark, 2009).

Peripheral artery tonometry (PAT) is an innovative technique recently developed to detect ED by determining pulsatile blood volume changes in the fingers. In contrast to the assessment tools mentioned above, PAT assesses pulsatile capillary blood flow, which is responsible for blood flow for localized areas such as the fingers and toes (Hamburg & Benjamin, 2009). Specifically, PAT evaluates the ratio of pulse wave amplitude (PWA) during reactive hyperemia compared to baseline values to determine the degree of ED (Heffernan, et al., 2010). The PWA ratio, also referred to as the PAT Reactive Hyperemic Index (RHI), is becoming an accepted measure of microvascular ED. Moreover, it has been found to be significantly related to the widely accepted criterion for ED evaluation, brachial artery FMD (Heffernan, et al., 2010).

The protective effects of exercise on the cardiovascular system in particular have been the focus of research in the last few decades. The importance of physical activity on general health has been well recognized and advocated by health organizations such as the American College of Sports Medicine and the American Heart Association. The Surgeon General recommends 30 minutes of moderate intensity physical activity on most days of the week to acquire the beneficial health effects of exercise (www.cdc.gov). Some of these beneficial effects include but are not limited to improvements in CVD factors such as high blood pressure, LDL cholesterol, triglycerides and body weight (Paffenbarger, Jung, Leung & Hyde, 1991). Exercise has also been shown to increase insulin sensitivity in healthy subjects and decrease insulin resistance in obese and type 2 diabetes patients (Scheen & Paquot, 2001).

The effects of an acute bout of exercise on vascular function in several different populations have been evaluated utilizing SGP, PAT and FMD. Using SGP to assess vasodilatory responses, it was found that FBF was significantly higher following an acute bout of exercise when compared to resting FBF in both endurance and resistance trained athletes (Baynard , Miller, & Fernhall, 2003). This same study determined that one bout of acute maximal exercise increased vasodilatory capacity in both groups. Significant increases in brachial artery diameter have been detected by FMD via ultrasound following an acute bout of low intensity exercise, and even greater increases after an acute bout of high intensity exercise in overweight active and inactive men. (Harris, Padilla, Hanlon, Rink & Wallace, 2008). Similar findings were observed in studies investigating the effects of exercise on vascular responses evaluated by PAT (Levin, et al., 2001). In subjects with coronary artery disease (CAD), altered peripheral vascular responses were detected during and three minutes following a routine exercise protocol (Levin, et al., 2001). Altered peripheral vascular responses included abnormal PAT indices, increased vasoconstriction during the acute exercise bout, as well as less vasodilation following the exercise.

Although all three techniques have shown to successfully detect exercise induced vascular changes in different vessel types, all three techniques have not been compared and contrasted pre and post-acute exercise. Furthermore, some studies have found diminished vascular function following an acute bout of exercise in diseased populations (Sylvestro et al.; Mcgowan, et al., 2006). Since the stress applied to the cardiovascular system by exercise may alter vascular responses to an occlusive stress, assessment of

vascular function after a quantitative exercise stress may provide additional predictive value related to the health of the vasculature.

This document has been organized to include a thorough review of the relevant literature related to vessel function and assessment of vascular dysfunction using non-invasive tools. Additionally, vascular responses brought on by different types and intensities of exercise will be presented. The review of literature is followed by a manuscript titled "A pre and post-exercise comparison of three assessment tools commonly employed to evaluate vascular function", which details a pilot research study designed to compare the pre- and post-exercise reactive hyperemia responses to 5 minutes of occlusion as evaluated by FMD, SGP, and PAT. For each assessment tool, pre- and post-exercise vascular function responses were evaluated in apparently healthy, untrained, non-obese males. Additionally, relationships among the primary outcome variables obtained from the three techniques both before and after exercise were evaluated.

II. REVIEW OF RELEVANT LITERATURE

The vascular system is composed of the vessels of the body, including the arteries and veins and its main purpose is to circulate blood to all body tissues. The task of providing tissues with sufficient oxygenated blood to meet metabolic demands is of primary importance, and it emphasizes the need for a comprehensive understanding of vascular function. Unfavorable changes in endothelial function and arterial stiffness, hence changes in vascular function, are thought to be predictors of the development of cardiovascular disease (Wilson, 2005). Detecting these changes may be beneficial for early detection and prevention of cardiovascular disease, as well as the determination of the prognosis for future cardiovascular events. Several non-invasive tools have been developed and are utilized to evaluate vascular function including brachial artery flow-mediated dilation (FMD) via ultrasound, venous occlusion strain-gauge plethysmography (SGP), and peripheral artery tonometry (PAT) (Alomari et al., 2004; Wilson, 2005)

The following is a review of the scientific literature related to vascular function, molecular mechanisms related to vascular tone, pathogenesis of atherosclerosis, and the clinical assessment of vascular function utilizing non-invasive tools. The review is structured to 1) provide background on the function of the endothelium, 2) provide a detailed description of commonly employed non-invasive tools for assessing vascular function and their utilization as prognostic indicators of cardiovascular disease, and 3) provide a review of alterations in vascular function

brought about by acute and chronic exercise training which may influence vascular function.

The Endothelium and Vascular Function

The interior surface of all blood vessels is lined by a single layer of endothelial cells that function as a metabolically active barrier between blood in the vessel lumen and the blood vessel wall (Terzuoli, Marinello, Frosi, Ciari, & Porcelli, 2008). Endothelial tissue can also be found in the interior surface of lymphatic vessels and the heart. An important feature of the endothelium is its ability to detect changes in blood flow hemodynamics as well as to react to these changes by making appropriate modifications to return to vascular homeostasis (Simionescu & Antohe, 2006). The endothelium-dependent response to these changes in hemodynamics includes the secretion of both vasodilating substances (e.g., nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF)) and vasoconstricting substances (e.g., Endothelin 1 and Angiotensin II), which directly affect vascular tone and vascular reactivity (Verma & Anderson, 2002).

In addition to being an endothelium-derived relaxing factor, NO also contributes to vessel homeostasis by regulating vascular function. Derived from L-arginine, NO possesses essential properties that maintain endothelial function and vascular reactivity, such as hindering platelet aggregation and adherence to blood vessel walls (Ignarro, 1989). In fact, reduced NO bioavailability is a primary contributor to endothelial dysfunction (Yao et al., 2010). Endothelial dysfunction is considered to be the earliest clinical indication of risk for cardiovascular diseases, and is closely related to a series of severe chronic and acute illnesses such as arthrosclerosis, multi-vessel coronary

artery disease, diabetes mellitus, hypertension, and stroke (Kitta et al., 2009). Opposing the vasorelaxing effects of NO is an active vasoconstricting peptide, Angiotensin II (ATII) (Johren, Dendorfer, & Dominiak, 2004). ATII is a potent vasoconstrictor which works directly on the vascular system through G-protein coupled receptors, or indirectly by facilitating aldosterone and norepinephrine release (Johren, Dendorfer, & Dominiak, 2004). An increase in expression of ATII Type 1 receptors (AT_1R) has been strongly linked to atherosclerosis (Libby, 2002). Moreover, it has been found that over-expression of the ATII Type 2 receptor (AT_2R), which opposes the effects of AT_1R, reduces atherosclerotic lesion formation, playing a protective role against cardiovascular disease (Hu et al., 2008). In addition, studies have found that ATII regulates the activity of endothelial nitric oxide synthase (eNOS), the enzyme that catalyzes the formation of NO from L-arginine (Kawashima & Yokoyama, 2004). A deficit of this important enzyme has been observed in patients with atherosclerosis, as it reduces NO bioavailability (Kawashima & Yokoyama, 2004). The vascular role of ATII has been thoroughly investigated, and its activity has been selectively opposed by AT_2R receptor activation, involving the endothelial dependent bradykinin pathway and NO production (Hannan, Davis, & Widdop, 2003).

Another potential mechanism contributing to the development of endothelial dysfunction involves the signaling G protein GTPase RhoA and its target Rho kinase (ROCK), which is directly involved in T-cell transendothelial migration, thrombus formation, and regulation of the endothelial barrier integrity (Yao et al., 2010). Detrimental effects on endothelial function are observed with the overactivation of RhoA/ROCK resulting in reduced eNOS expression, hence a decreased NO

bioavailability. Furthermore, ROCK inhibitors reverse this effect, upregulating eNOS expression and NO production (Yao et al., 2010)

Disruption of the activity of vasoactive substances such as NO and ATII can severely alter vascular balance and may lead to endothelial dysfunction. In addition to the endothelium, these vasoactive substances also target the smooth muscle cell layer of the vessel wall, causing vasoconstriction or vasodilation. The role of smooth muscle cells in vascular function is critical to maintaining vascular health, and their interaction with vasoactive substances will be further explored below.

Smooth Muscle Cells and their role in Vascular Function

Surrounding the endothelial layer is a thick layer of smooth muscle cells constituting the media layer of the vessel wall. Vasoconstriction and vasodilation are mediated by the tonic vascular smooth muscle cells, which behave as multiple, independent units resulting in graded changes in vascular tone (Fisher, 2010). Specifically, NO diffuses into the media, and binds to the guanylyl cyclase receptors starting a cascade of events resulting in an increase in the second messenger cGMP. Subsequently, this reaction arbitrates vascular processes such as vasorelaxation, inhibition of platelet activation and migration (Bassil & Ananda-Srivastava, 2007). The dual effects of vasoactive substances on both the metabolically active endothelium and on vascular smooth muscle cells compound the significance of their role on vascular homeostasis and prevention of vessel-related pathogenesis.

Blood vessel types and their mechanisms

There are three major types of blood vessels which differ greatly in size, function and blood flow control mechanisms. SGP evaluates vascular reactivity of

resistance vessels, FMD focuses on conduit vessels, and Endo-PAT assesses the capillaries. Resistance vessels are 50µm or smaller in diameter and are in command of regional blood flow, whereas conduit vessels such as the brachial artery are much larger with diameters of several millimeters, allowing for increased blood flow to large areas (Segal & Durling, 1986). During exercise, blood flow to the muscles is mainly dictated by resistance vessels, which occurs via a NO-mediated function, as well as cardiac pump capacity, muscular pump, and the sympathetic system (Saltin B., Radegram G., Koskolou M., Roach R., 1998). The significantly smaller capillaries are responsible for microcirculation, providing blood flow for localized areas such as the fingers and toes. Digital vascular function at rest and during hyperemic responses has been thoroughly investigated and directly correlates to vascular function in other regions (Hamburg & Benjamin, 2009). The differences and similarities in vascular reactivity for each vessel type following a stressor require further investigation.

Hormonal and Gender effects on Vascular function

Hormones closely linked to the menstrual cycle, such as estrogen and progesterone, have a direct effect on the endothelium (Lynn, McCord, & Halliwill, 2006). Estrogen receptors are found on the endothelium and estrogen binding elicits NO release, augmenting a common endothelium relaxing mechanism (Deroo & Korach, 2006). Elevated estrogen levels in females, and their enhancing effects on endothelium relaxing mechanisms further denote gender-related differences in vascular function. These hormonal differences may be advantageous for cardiovascular health in menstruating females when compared to males. Additional findings state that endothelial progenitor cells implicated in cardiovascular homeostasis were found to

oscillate in phase with the menstrual cycle in ovulating women (Fadini et al., 2008). It is hypothesized that endothelial progenitor cells migrate to the peripheral circulation during the different phases of the menstrual cycle, and higher levels are found in ovulating women than in post-menopausal women and males (Fadini et al., 2008). Future vascular function studies should include females during the various menstrual cycle phases in order to apply the findings to benefit a larger segment of the population with the incremental knowledge of endothelial dysfunction detection and treatment. Additionally, the vasorelaxing effects of estrogen should be further investigated as a potential hormonal therapy for CVD treatment.

In addition to gonadal steroids, other important hormones alter vascular mechanisms. Acute augmentations of growth hormone (GH) not only provoke endothelium dependent vasodilation marked by increased NO synthesis, but also increased sensitivity to vasodilating agents (Napoli et al., 2003). This study also suggests the potential therapeutic role of GH on vascular pathogenesis.

Assessment of Vascular function

Assessment of endothelial dysfunction has become increasingly important as the prevalence of CVD has dramatically risen in the last several decades. There are several non-invasive assessment tools available to evaluate vascular function. Three of the most commonly utilized assessment tools are flow mediated dilation (FMD), strain gauge plethysmography (SGP), and peripheral artery tonometry (PAT). All three measurement techniques attempt to occlude all blood flow to the limb with a pressure cuff inflated to supra systolic pressures, creating a hypoxic state. Reactive hyperemia, a compensatory vascular increase in blood flow to the occluded area to reestablish

oxygen blood to ischemic tissues, occurs as a result of the deflation of the pressure cuff (Pyke & Tschakovsky, 2007). How quickly the vessels react to these changes reveal the health of the vasculature. Although all three assessment tools evaluate vascular health, they do so by assessing the vascular reactivity of different types of vessels. SGP evaluates vascular reactivity of resistance vessels, FMD focuses on conduit vessels, and PAT assesses vascular function of the microcirculation. To date, there have been no studies in which vascular function has been evaluated across different vessel types at rest and following an acute exercise bout.

Abnormalities in the vasodilatory and vasoconstrictive abilities of vessels are a feature of endothelial dysfunction, a precursor of more serious vascular diseases such as atherosclerosis, heart disease, and diabetes (Vogel, 2001). Furthermore, it has been found that endothelial dysfunction is significantly more prevalent in diseased populations such as patients with Diabetes Mellitus and Coronary Artery Disease (CAD) (Tagawa et al., 2004), highlighting the need for reliable endothelial dysfunction evaluation tools for early detection and prevention of cardiovascular disease and other related illnesses in already vulnerable, unhealthy populations.

Vascular function and its homeostasis require intricate interactions and co-relationships amongst different cell types, hormones, vasoactive substances, and it is affected by diurnal variations. It is imperative to take these relationships into account, and to control for them during vascular function studies as they may represent confounding variables. The next few sections will explore in detail the techniques and measurement issues related to FMD, SGP, and PAT.

Flow mediated dilation. Brachial artery flow-mediated dilation (FMD) is a frequently used method which employs high resolution ultrasound to measure brachial artery diameter (Verma, Buchanan, & Anderson, 2003). FMD measures macrovascular reactivity and is used to identify subclinical CVD markers in at risk patients (Dhindsa et al., 2008). Although a trained ultrasonographer is necessary in order to effectively assess vascular function with FMD, a study found difficulties finding the brachial artery in only 3% of the population being studied, obtaining FMD values for 97% of the subjects (Lind, Fors, Hall, Marttala & Stenborg, 2005). Initial scanning of a segment of the brachial artery at rest provides a baseline vessel diameter which is subsequently compared to a post-occlusion reactive hyperemic diameter. In addition to producing reactive hyperemic vasodilation following the pressure cuff release, several studies have utilized artificial means to induce endothelium-independent vasodilation, such as the administration of sublingual nitroglycerin as a means of determining maximal vasodilatory capacity (Lierberman et al., 1996; Lierberman et al., 1994). Although an efficient method to produce vasodilation, nitroglycerin-induced vasoactivity does not reflect an individual's vascular health.

FMD has been found to be a valuable tool in the early detection of cardiovascular disease risks, and it is considered the gold standard for diagnosing endothelial dysfunction (Celermajer et al., 1992; Harris, Padilla, Rink, & Wallace, 2006; Peretz et al., 2007). This technique has been successfully used in a vast range of populations including children and adults in attempts to detect these preclinical cardiovascular disease precursors (Al-Qaisi, Kharbanda, Mittal & Donald, 2008). Additionally, FMD has been found to be a promising CVD detection tool in

asymptomatic patients when reactive hyperemia values stray from expected values for healthy individuals (Celermajer et al., 1992). In a 2005 study, Lindt et al. compared FMD to other invasive and non-invasive vascular function assessment tools and reported average values measured by these techniques in healthy young and healthy elderly populations. The healthy young group had lower brachial artery diameter and maximal diameter, and higher diameter change and % FMD when compared to the elderly healthy group (Lind, Fors, Hall, Marttala & Stenborg, 2005). Noteworthy values reported in this study for the healthy young group include baseline brachial artery diameter (3.1 ± 0.5mm), maximal diameter (3.4 ± 0.4mm) and a 9.4% FMD change representing the percent change in artery diameter at baseline and following the occlusion. The same study also demonstrated that FMD values decreased with age. Other important relationships found when investigating the association of FMD in healthy men were that FMD is directly related to resting wall shear stress, and that a negative and direct correlation exists between shear stress determinants such as arterial diameter and flow velocity. (Gnasso, 2001). In epidemiology studies, brachial artery FMD has consistently demonstrated debilitated vascular functions in subjects exhibiting cardiovascular disease risk factors. Significantly lower brachial artery FMD was found in moderately obese pre-pubertal boys without insulin resistance, both at rest and during exercise. (Karpoff et al., 2009). A subsequent study reiterated the ability of FMD via ultrasound to detect endothelial dysfunction, as it was found that this method successfully identified the functional state of the endothelium in patients with cardiovascular disease risk factors (Atkov, Balahonova & Pogorelova, 1998).

The addition of exercise as a stressor to the cardiovascular system was assessed with FMD in several studies. The results indicated that highly endurance-trained athletes have larger arterial diameter but similar endothelial-dependent vasodilation when compared to untrained controlled subjects (Rognmo et al., 2008). Additionally, it has also been reported that NO-dependent vascular mechanisms occur in 45 to 60 seconds in healthy individuals following FMD caused ischemia (Celermajer et al., 1992), and the response to acute endurance exercise measured by FMD has shown to be highly dependent on the time following exercise. Obtaining vascular function assessment in a timely manner is therefore a necessary factor for valid post-exercise vascular function evaluation.

Although the evidence presented above has shown FMD to efficiently assess vascular function, studies comparing FMD to other vascular function assessment tools have found contradictory results. When FMD was compared to increases in forearm blood flow obtained by SGP, both assessing peripheral vascular endothelial vasodilatory capacity, it was found that there was no significant correlation between the two (Eskurza, Seals, DeSouza, Christopher & Tanaka, 2001; Gori at al. 2006). The reasoning behind this discrepancy was attributed to the different macro and microvascular properties influencing each technique (Dhindsa et al., 2008). The same theory was applied to the lack of association between FMD and reactive hyperemic flow, and FMD and sheer stress, although both factors are known stimuli for FMD (Dhindsa et al., 2008). On the contrary, a 2007 study found that reactive hyperemia and FMD were significantly reduced in patients with cardiovascular disease risk factors (Huang et al., 2007). This method's reproducibility and repeatability have been

previously established, and it was found that FMD possesses a very low coefficient of variation for in vivo measurements for flow mediated dilation (Sorensen et al., 1995) and a coefficient of repeatability of 4% for baseline brachial diameter measurements (Rognmo et al., 2008).

While this method is non-invasive and clinically useful, it has several limitations for clinical use including the significant cost of the equipment and the need for a trained ultrasonographer. Additionally, the lack of a consistent protocol for the utilization and interpretation of FMD has also contributed to variations in clinical findings. For example, a recent study found an 8.9% difference in magnitude of brachial artery flow mediated dilation following proximal occlusion of the upper arm versus distal occlusion of the forearm (Peretz et al., 2007). It is therefore warranted to develop a standard pneumatic cuff occlusion location for the clinical utilization of FMD. Furthermore, differences in day to day variability in vessel diameter due to biological circadian rhythms have also been observed (Verma et al., 2003) and a diurnal effect due to increased sympathetic nervous system activation has been proven to influence FDM, providing erroneous, decreased readings (Hijmering et al., 2002). It is important to note that no effects have been found on FMD readings due to repetitive bouts of reactive hyperemia up to a two hour period; providing flexibility for different research designs (Harris et al., 2006). Implementation of a new FMD vascular function assessment protocol should take the previous potential measurement issues into consideration in order to minimize measurement error.

Strain Gauge Plethysmography. Another common technique to assess vascular function is strain gauge plethysmography (SGP), which is utilized to evaluate both

baseline and post-occlusion forearm blood flow. In this method, a pressure cuff is placed on the upper arm and the wrist. Inflating the proximal cuff creates an upper arm occlusion (~40 mmHg) and produces sufficient pressure to occlude venous blood outflow from the limb, yet arterial inflow is unaffected. As arterial blood continues to enter the limb and venous return is impeded, the volume of the limb increases. The wrist cuff is inflated to a suprasystolic pressure to prevent blood flow into the hand. The change in limb circumference is assessed by a mercury-filled strain gauge placed around the forearm. Following a determination of baseline forearm blood flow, blood flow is occluded by inflating the upper arm cuff to a suprasystolic values (>200 mmHg). After five minutes of occlusion, the cuffs can be quickly deflated and forearm blood flow can be assessed during the period of reactive hyperemia. The reactive hyperemic blood flow response is often quantified as an area under the curve during the three minute period immediately following release of the upper arm cuff (Bousquet-Santos, Soares, & Nóbrega, 2005). SGP's ability to measure vascular function, reproducibility, and relationships between different physiological findings were assessed. Mercury-filled SGP was found to be a reproducible vascular assessment tool, as established by the evaluation of forearm arterial and venous indexes both at rest and post a 5 minute brachial artery occlusion at 240mm Hg on thee different occasions (Alomari et al., 2004). Some notable findings regarding physiological relationships found in the previously mentioned study include the association between handgrip strength, venous capacitance and outflow; as well as the suggestion of a link between venous function and exercise performance (Alomari et al., 2004). In agreement to the general concept that exercise enhances a variety of cardiovascular parameters, baseline

FBF and vascular reactivity were appreciably improved following an acute maximal aerobic exercise bout.

Due to the proven reliability of this assessment tool, SGP has been used to test the assumed physiological effects of various substances on the vasculature. The nonessential amino acid L-arginine, a precursor to NO, has been found to increase blood flow in healthy individuals (Campbell, La Bounty & Roberts., 2004) and is being marketed as a supplement that increases nutrient and oxygen delivery to muscle, increasing muscle size during and following resistance exercise. Using SGP, it was found that FBF was unchanged following the ingestion of L-arginine prior to resistance exercise; contradicting the marketing claims of the vascular enhancement faculties of L-arginine supplements (Fahs, Heffernan & Fernhall, 2009).

The combination of SGP with other vascular assessment tools has been used in some clinical studies to assess CVD risk factors. Merging the SGP and FMD techniques, researchers used brachial artery blood velocity values measured by FMD and forearm blood flow values determined by SGP to calculate brachial artery diameter in a young healthy population. A strong correlation was found between the two methods at rest. Moreover, forearm heating-induced increases in forearm blood flow and blood flow velocity were accompanied by large increases in brachial artery diameter (Sinoway, Hendrickson, Davidson, Prophet & Zelis, 1989). Yet when increases in forearm blood flow were compared to changes in FMD, both assessing peripheral vascular endothelial vasodilatory capacity, it was found that there was no significant correlation between the two. (Eskurza, Seals, DeSouza, Christopher & Tanaka, 2001; Gori et al. 2006). The combination of more than one vascular function

assessment tool should be further investigated, as it may strengthen CVD diagnostic and preventive abilities.

Peripheral artery tonometry. More recently, a clinical assessment tool utilizing peripheral artery tonometry (PAT) technology and marketed under the brand name Endo-PAT® has been developed to assess microcirculation in the fingers as a means of evaluating vascular function. This technique utilizes a PAT signal obtained via a modified plethysmography finger probe that measures blood volume changes before and after 5 minutes of upper arm occlusion. While there is a significant expense for the Endo-PAT® unit and the single use finger probes, the use of the unit does not require extensive expertise or training to operate (Selamet Tierney, 2009). Latex finger probes are placed on index fingers of both hands and following an initial baseline assessment, blood flow is assessed for five minutes following a five minute upper arm occlusion. Since this technique uses a latex finger cuff and assesses blood flow at the nail bed, PAT cannot be utilized on patients who are either allergic to latex or have long or acrylic finger nails. According to the manufacturer's website, a PAT score of 1.67 or lower indicates a need for medical assessment of the cardiovascular system, a score between 1.68 and 2.0 reflects a need for a reduction of lifestyle CVD risk factors such as smoking and being overweight, whereas a score of 2.1 or higher signifies a healthy endothelium (http://www.itamar-medical.com/). A recent study observed acceptable reproducibility and reliability of the PAT technique with insignificant within-subject variability in healthy adolescents (Selamet Tierney, 2009).

Endo-PAT has been used to measure endothelial function differences in several chronic disease populations, including those with high global cardiovascular risk

(HCVR) and human immunodeficiency virus (HIV). The author's primary observation was that patients with HCVR and HIV have diminished endothelial function when compared to healthy subjects (Ferre, Plana, & Coll, 2007). Several epidemiologic studies have found that people who work for long shifts are at a heightened risk for cardiovascular disease due to arterial hypertension, hyperlipedimia and metabolic syndrome (Knutsson, Jonsson, Akerstedt & Orth-Gomer, 1986; Sookoian et al., 2007; Suwazono, et al., 2008; Dochi, et al., 2009). More recently, these findings were confirmed by lower PAT scores in shift workers when compared to non-shift workers, indicating endothelial dysfunction (Suessenbacher, et al., 2011).

When comparing this technique to the gold standard vascular function assessment tool, FMD, conflicting results have been found. In a vascular function assessment tool comparison study, it found that PAT does not have a high correlation with FMD (Bianchini et al, 2006). In contrast, reduced PAT hyperemia ratios were consistently observed in endothelial dysfunction patients with reduced brachial artery FMD (Kuvin et el., 2003) and in healthy patients, higher FMD values were accompanied by higher PAT hyperemia ratios (Kuvin et el., 2003). As noted in this study, FMD and PAT have contrasting macro and micro vascular properties, yet these techniques were found to be strongly associated with each other, challenging the author's hypothesis that no correlations were expected between the two tools (Dhindsa et al., 2008). The evidence presented above suggests that PAT could be a complementary approach to aid in the detection of patients at risk for CVD, and may even be used as an alternative method, as PAT is more user friendly than FMD.

The Effects of Exercise on Vascular Function

A sedentary lifestyle is closely linked to an increased risk for heart disease, whereas exercise, both aerobic and anaerobic, has been found to produce favorable outcomes on cardiovascular health. In addition to being a successful cardiovascular disease preventative measure, exercise can also be a crucial component of cardiac rehabilitation following myocardial infarction. (Chandrashekhar & Anand, 1991). In fact, exercise significantly reduces systolic and diastolic blood pressure, which is turn decreases cardiac mortality rate by 5-9% (Ohkubo et al., 2001).

During exercise, vascular adaptations occur to meet the working muscle's significantly higher metabolic needs. These adaptations will differ accordingly with various exercise types and intensities. Some of these adaptations include increased blood pressure, blood flow and shear stress (Tanaka, H., 2006). Shear stress is the parallel frictional force that blood applies to the vessel wall as it flows, and it is closely linked to endothelium dependent vasodilation due to increased NO production (Woodman, Price, & Laughlin, 2005). Along with an increase in shear stress, dynamic low-intensity handgrip exercises have been shown to intensify flow mediated dilation of the brachial artery in healthy subjects further supporting the link between shear stress and increased vascular function (Mcgowan, C. et al., 2006; Tanaka, H., 2006). In fact, a linear relationship exists between FMD and shear stress, demonstrating a dose response depending on the amount of stimulus inflicted by shear stress (Rognmo et al., 2008). Additionally, Rognmo et al reported a negative correlation between FMD resting artery diameter; therefore smaller vessels will experience greater shear stress and greater FMD responses. Furthermore, at the onset of exercise, there is a rapid increase in blood velocity and therefore blood flow as observed via ultrasound Doppler

(Shoemaker, Hodge, & Hughson, 1994). Some of these responses are observed only at specific exercise intensities , as acute moderate-intensity exercise was shown to increase blood pressure and FBF, while acute mild-intensity exercise did not cause these changes in young healthy males (Goto et al., 2007). Although this study was unable to test this relationship during high-intensity exercise due to large body movements, it was determined that the vasodilation observed during moderate-intensity exercise occurred due to an increase in NO bioavailability. Exercise induced vascular improvements have been found to occur throughout the entire vasculature, and are not limb specific. Enhanced FMD, vascular remodeling and vasodilatory capacity of conduit and resistance vessels of the upper limbs were reported following predominantly lower limb exercises (Walther et al., 2008).

Chronic exercise training. The beneficial effects of chronic exercise on vascular function have been thoroughly studied and are well understood. The advantageous effects of aerobic exercise include but are not limited to improved oxygen uptake from working muscle and cardiac output while resistance exercise increases bone density and muscular strength (Rubattu, 2007). Several studies have observed significant improvements in vascular health, specifically changes in vessel structure following endurance and resistance exercise training. Following a 2 hour per day, 6 day endurance exercise training program, a significant decrease was observed in central and peripheral arterial stiffness evaluated via peripheral pulse wave velocity (PPWV) in healthy young males (Currie, Thomas, & Goodman, 2009). Arteries, composed of elastin and collagen, are prone to lose their elasticity, which negatively affects vascular function. The significant decreases in arterial stiffness observed in the previously

mentioned study were due to adaptive responses of the body to a short term exercise program. Vascular adaptations to long term exercise training have also been evaluated. Although a 2-year long endurance and strength training program resulted in an improved cardiovascular risk profile in patients with Diabetes Mellitus (DM), there were no indications of significant improvement in arterial stiffness (Loimaala et al., 2009). The observed discrepancy between short-term and long-term exercise training effects on arterial stiffness may not be due to the length of the exercise program, but rather the decreased baseline levels of elastin in the vessels of patients with DM (Loimaala et al., 2009). In a recent study that employed FMD via ultrasound following an acute bout of resistance exercise on 10 healthy, moderately active men, an increase in blood flow, therefore in vasodilatory capacity, was detected (Collier et al., 2010). As shown through these studies, resistance exercise indeed enhances several aspects of vascular function.

Acute exercise training. More recently, the greater number of studies have evaluated the effects of acute bouts of exercise, and the results have been inconsistent. Several studies have found significant improvements in cardiovascular function following a bout of acute exercise in different diseased and healthy populations. Immediately following an acute bout of aerobic exercise, it was found that flow mediated dilation increased in obese patients with impaired vascular function (Zhu et al., 2010) and impaired microvascular endothelial function was restored by an acute bout of lower limb exercises in post-surgical varicose vein patients (Klonizakis, Tew, Michaels, & Saxton, 2009). Acute exercise resulted in a nearly two-fold increase in brachial artery flow mediated dilation, as well as significant increases in total peripheral conductance

in healthy sedentary normotensive postmenopausal women, yet these changes were not observed in premenopausal women (Harvey et al., 2005). In a more recent study, a two-fold increase in FBF was observed following a single bout of aerobic exercise, and a similar increase was seen after a single bout of resistance exercise in a group of healthy, moderately active young men (Collier et al., 2010). Another recent study utilized FMD to compare the effects of different intensities of acute exercise bouts on post-prandial decreases of endothelial function. The study found that moderate intensity exercise preceding a high-fat meal diminished endothelial dysfunction in comparison to control subjects who did not endure the single bout of moderate intensity exercise. In addition, this study found that an acute bout of high intensity interval exercise exceeded the protective effects of the acute bout of moderate intensity, significantly increasing flow mediated dilation regardless of the post-prandial lipedemia (Tyldum, 2009).

As exercise intensity increases, the working muscles, including the heart, require increased oxygen use and delivery to meet increasing demands. Forearm blood flow and vascular reactivity have been assessed by SGP in healthy, sedentary subjects following an acute maximal exercise test, with both FBF and vascular reactivity being significantly enhanced (Bousquet-Santos, Soares, & Nóbrega, 2005). Moreover, vascular reactivity was still elevated 60 minutes post-exercise, indicating subacute effects of a bout of maximal aerobic exercise on vascular function (Bousquet-Santos, Soares, & Nóbrega, 2005).

Despite the robust evidence of the beneficial effects of acute bouts of exercise on cardiovascular health, several studies have found opposing results. Acute exercise

was found to aggravate endothelial dysfunction, marked by a decreased in flow mediated dilation in patients with intermittent claudication (Sylvestro et al., 2002).

Similar findings marked by a decrease in brachial artery FMD were seen following an acute bout of isometric handgrip exercises in patients medicated for hypertension (Mcgowan, C. et al., 2006). It is a strong possibility that the contradicting findings from the previously mentioned studies may be population and disease specific. Further investigation of the effects of acute bouts of different intensities on endothelial dysfunction in healthy, unmedicated populations is clearly warranted.

Acute exercise, although beneficial to cardiovascular health in specific populations, seems to also be a stressor to the cardiovascular system, aggravating already existing risk factors and diseases in others (Sylvestro et al., 2002). Application of this concept in CVD detection and prevention efforts has been successful, and is now a pivotal part of diagnostic testing. CVD diagnostic testing consisted of testing patients with an electrocardiogram (EKG) test at rest. EKGs have been the gold standard diagnostic test for CVD detection as they provide relevant prognostic information such as cardiac abnormalities including left ventricular hypertrophy, Q waves, ST segment changes, left bundle branch block, atrial fibrillation, and other factors associated with CVD (Möhlenkamp, Wieneke, Sack, Erbel, 2007). In fact, performing myocardial perfusion imaging at rest within 2 to 6 hours of pain chest symptoms detects patient's risk level for acute myocardial infarction (Tatum et al., 1997).

As demonstrated above, exercise-induced vascular function alterations vary in type and magnitude. This is particularly important in diseased populations, which have shown diminished vascular function following an acute bout of exercise (Sylvestro et al.;

Mcgowan, et al., 2006). The ability of exercise to stress the cardiovascular system results in altered vascular responses, which may provide additional prognostic data related to the health of the vasculature. Moreover, challenging the cardiovascular system to a safe point may highlight otherwise unseen cardiovascular abnormalities aiding in early detection of CVD precursors, and even preventing the development of CVD altogether. Our study attempts to evaluate three commonly used tools that normally evaluate vascular function at rest, following a moderate intensity acute bout of aerobic exercise. It is our hope that the additional exercise-induced stress on the cardiovascular system may further reveal signs of patients with an elevated risk for CVD to decrease their propensity to development, rather than detect the existence of the disease considered the world's largest killers.

III. ABSTRACT

Background: Endothelial dysfunction (ED) is one of the earliest subclinical indicators of impaired cardiovascular health and several non-invasive tools have been developed to evaluate vascular function, including strain gauge plethysmography (SGP), brachial artery flow-mediated dilation (FMD) via ultrasound, and peripheral artery tonometry (PAT). While these tools have extensively been studied during a resting condition, the responses following acute exercise are not as well characterized. **Purpose**: The purpose of this study was to compare the pre- and post-exercise vascular function values obtained with SGP, FMD, and PAT. Relationships among the primary outcome variables obtained with each assessment tool were also evaluated. **Methods**: Vascular function was assessed in 17 sedentary, apparently healthy male subjects (24±4 yrs; 24.5±3.2 kg/m^2) at rest and following an acute submaximal exercise bout with SGP, FMD, and PAT. **Results**: During rest, post-occlusion reactive hyperemia resulted in significant ($p<0.05$) increases in forearm blood flow (FBF; 2.13±1.03 vs 6.35 ± 2.90 mL/min/100 mL tissue) and area under the curve (AUC; 226.77 ± 111.20 vs 588.22 ±283.33 mL/min/100 mL) as determined by SGP. Brachial artery diameter (BAD) as assessed with FMD was increased by 5.3% ($p<0.05$). Resting reactive hyperemia index (RHI) as assessed by PAT was observed to be 1.73±0.34. Significant exercise-induced increases ($p<0.05$) were observed in baseline and post-occlusion FBF and baseline AUC values utilizing SGP. Additionally, FMD baseline blood velocity was significantly increased (91.8±11.1 vs 108.0±17.1 cm/sec, $p<0.05$) and the PAT augmentation index (AI) was significantly more negative (-8.8 ±9.4 vs -18.9±8.4%, $p<0.05$) after exercise. There were no significant correlations observed among the primary outcome measures obtained from each

assessment technique. There was, however, a moderate correlation between pre-exercise vascular reactivity as assessed by SGP and change in blood velocity as assessed by FMD ($r= 0.566$, $p= 0.035$). **Conclusions**: The addition of an exercise stress to vascular function assessment may offer greater insight into the health of the vasculature. This initial study was undertaken to further evaluate the pre- to post-exercise responses obtained using three commonly employed vascular function assessment techniques in healthy individuals. Additional research as to the value of the addition of an exercise stress to vascular function assessment in individuals with traditional cardiovascular disease risk factors or known cardiovascular disease is warranted.

VI. INTRODUCTION

The development of cardiovascular disease (CVD) is a gradual process consisting of morphological and physiological vessel changes that slowly debilitate the vasculature (Vane, Anggård & Botting, 1990, Quyyumi, 1995). CVD treatment has been thoroughly investigated, and research efforts have recently shifted to further understanding prevention as well as early detection of subclinical indicators of CVD, including endothelial dysfunction (ED). ED is defined as the progressive development of irregular vascular function, such as abnormal vessel tone, loss of the endothelium's atheroprotective properties, and most notably, diminished nitric oxide (NO) availability (Vane, Anggård & Botting, 1990). NO contributes to vessel homeostasis by regulating vascular function and mediating endothelium-dependent vasodilation (Verma & Anderson, 2002). As a precursor of CVD, ED has proven to be treatable and even reversible by the implementation of drastic lifestyle changes such as smoking cessation, healthy eating habits and the adoption of an exercise regimen (Chandrashekhar & Anand, 1991).

Exercise has been found to produce favorable outcomes on cardiovascular health. In fact, exercise significantly reduces systolic and diastolic blood pressure, which in turn decreases cardiac mortality rate by 5-9% (Ohkubo et al., 2001). Chronic exercise is linked to an extensive range of health benefits but the effects of acute exercise are less clear. Supplementary exercise-induced effects include changes in heart rate and cardiovascular responses such as blood pressure, blood flow, and shear stress (Tanaka, 2006), which are known to improve following exercise training. The effects of an acute bout of exercise on these cardiovascular responses have been found

to vary, and seem to be population and exercise intensity specific (Zhu et al., 2010 & Sylvestro et al., 2002).

As the prevalence of CVD has dramatically risen in the last several decades (He & MacGregor, 2009), clinicians have become increasingly interested in obtaining valid and reliable measures of vascular function as a means of quantifying ED. Several non-invasive assessment techniques have been developed to evaluate vascular function including flow mediated dilation (FMD), strain gauge plethysmography (SGP), and peripheral artery tonometry (PAT). These techniques assess vascular function by evaluating reactive hyperemic responses to a given occlusive stress; however, each technique evaluates post-occlusion changes in different areas of the vasculature. Additionally, studies employing these techniques have primarily provided data on vascular function at rest; however, recent research has also evaluated vascular function following an acute exercise bout. Some of these findings observed during an acute bout of exercise include significant increases in brachial artery flow mediated dilation, as well as significant increases in total peripheral conductance (Harvey et al., 2005) and a two-fold increase in FBF following a single bout of aerobic exercise (Collier et al., 2010).

Acute effects of exercise were recently investigated in post-surgical varicose vein patients with ED. The study found that impaired microvascular endothelial function was restored by an acute bout of lower limb exercises (Klonizakis, Tew, Michaels, & Saxton, 2009). Despite the robust evidence of the beneficial effects of acute bouts of exercise on cardiovascular health, several studies have found conflicting results. Acute exercise was found to aggravate endothelial dysfunction, marked by a

decreased in flow mediated dilation in patients with intermittent claudication (Sylvestro et al., 2002). Similarly, findings marked by a decrease in brachial artery FMD were observed following an acute bout of isometric handgrip exercises in patients medicated for hypertension (Mcgowan, C. et al., 2006). The discrepancies observed may be population specific, as diminished vascular function following exercise seems to be more predominant in diseased populations.

The augmented stress induced on the cardiovascular system by exercise is the basis for including graded exercise testing as a means of detecting CVD (Lear, Brozic, Myers & Ignaszewski, 1999). As with traditional cardiovascular exercise testing, the inclusion of exercise as a stressor during assessment of vascular function with tools such as SGP, FMD and PAT may aid in the recognition of early signs of CVD development and other cardiovascular abnormalities otherwise undetected by assessment at rest. However, there is currently no consensus on which assessment tool is most appropriate for a pre- and post-exercise evaluation of vascular function. Therefore, the purpose of this pilot study was to evaluate pre- and post-exercise reactive hyperemic responses utilizing three common vascular function assessment tools: SGP, FMD, and PAT. Additionally, this study sought to evaluate the relationships among the primary outcome variables associated with each technique both before and after an acute bout of moderate intensity aerobic exercise.

VII. METHODS

Subjects. Apparently healthy sedentary subjects aged 19-35 years with body mass index (BMI) values <30 kg/m^2 were recruited to participate in the study. Subjects were asked to attend three research trials (SGP, FMD and PAT); randomly assigned with at least 48 hours in between trials, to assess vascular function both at rest and following 20 minutes of moderate intensity aerobic exercise. All subjects voluntarily participated in the study and gave written informed consent. Each subject completed a brief medical history questionnaire and the short form of the International Physical Activity Questionnaire (IPAQ-short) prior to beginning the study. Upon arriving for each trial, each subject's weight was obtained to the nearest 0.25 kg and height was obtained to the nearest 0.25 cm. Subjects were subsequently placed in a supine position for a minimum of 30 minutes prior to any assessments. Subjects were instructed not to consume food after midnight the night before participating in each trial, although moderate water consumption was allowed until the time of the trials. Additionally, subjects were asked to refrain from alcohol and tobacco consumption for a minimum of 24 hours, and to refrain from exercise for a minimum of 48 hours prior to each trial. All procedures were approved by the VCU Institutional Review Board

*Exclusion Criteria***.** Exclusion criteria for this study included any history of cardiovascular or metabolic disease, or if the subject was taking any medication that might affect cardiovascular hemodynamics, vascular function, or vascular responses to exercise. Females were excluded from this pilot study as varying hormone concentrations during the phases of the menstrual cycle have been shown to alter vascular

function (Deroo & Korach, 2006). Subjects were also excluded if they had a latex allergy since the assessment of vascular function with the PAT unit requires contact with an inflatable finger cuff made from latex. Lastly, since exercise training is thought to alter vessel structure and possibly vascular function, the current study sought to recruit subjects that did not meet the Surgeon General's recommendation of 30 minutes of moderate intensity physical activity 5 days per week as determined by the IPAQ-short.

Assessment of Vascular Function

All vascular function measurements were performed in the morning (~7:00 AM), in a quiet environment and at controlled ambient temperatures both at rest and following a 20 minute bout of submaximal exercise.

Strain-gauge plethysmography. Forearm blood flow was assessed using mercury-in-rubber strain-gauge plethysmography (SGP; Model AI6, D.E. Hokanson, Inc., Bellevue, WA). Methods utilized for forearm blood flow measures were similar to those previously reported by Bousquet-Santos et al. (2005). A pressure cuff was placed around the upper right arm. A smaller wrist pressure cuff was placed around the right wrist to occlude hand circulation. A mercury-filled strain gauge was placed around the widest part of the forearm, 10 cm distal to the olecranon process. The arm was supported by foam supports at a level approximately 10° above the heart. Electrodes were placed on the subject's chest for electrocardiogram (ECG) acquisition. Baseline forearm blood flow (FBF) measurements were taken by inflating the wrist cuff to 250 mmHg and then rapidly inflating the upper arm cuff to 40 mmHg for 4-s to occlude venous outflow, followed by an 8-s deflation, during each 12-s cycle. Nine cycles were recorded to determine the average rate of volume change during venous occlusion (ml/100 ml of forearm tissue

volume/min). Following baseline measures, the upper cuff was inflated to 240 mmHg for 5 minutes to occlude blood flow and subsequently induce reactive hyperemia. Upon cuff deflation, FBF was measured for another nine cycles. FBF trials were analyzed using the Hokanson AI6 Software version 2.2.4, and subsequently exported to a spreadsheet for further analysis. The values obtained at rest and following the acute submaximal exercise bout included baseline and hyperemic FBF (ml/100 ml of forearm tissue volume/minute), area under the curve (AUC), and vascular reactivity. AUC was calculated by the addition of the averages of every two consecutive FBF values over the 12 second cycle time frame. Vascular reactivity was calculated as the difference between AUC at baseline and during reactive hyperemia.

Flow mediated Dilation. Brachial artery diameter was assessed with high resolution ultrasound (HDI 5000, Phillips ATL; Oceanside CA) utilizing methods similar to those previously reported by Peretz et al. (2007). Approximately 15 minutes prior to the trial, a resting blood pressure measurement was obtained from the left arm. For the FMD trial, the right arm was comfortably placed in an extended position to allow for ultrasound scanning of the brachial artery at a location above the antecubital fossa. A pressure cuff was placed around the subject's forearm and ECG electrodes were placed on the subject's chest to obtain a corresponding ECG signal for synchronization of diameter measurements with the R-wave of the cardiac cycle. All baseline and post-occlusion trials were recorded to Super VHS videotape for later analysis. A longitudinal section of the brachial artery was located by a trained ultrasonographer and baseline brachial artery diameter and baseline Doppler blood velocity were recorded. Following the baseline measures, the forearm pressure cuff was inflated to 240 mmHg for five minutes to

occlude blood flow. At the end of the five minute occlusion period, the cuff pressure was quickly released and peak reactive hyperemic blood velocity was assessed and recorded for approximately 30 seconds. After 45 seconds, the assessment mode was quickly changed to record images of the vessel diameter for the next 2 minutes to obtain peak diameter during reactive hyperemia.

WinDVR software (InterVideo, Inc.) was utilized to capture baseline and post-occlusion images for analysis. Images for analysis of brachial artery diameter and blood flow velocity were captured at the end of diastole at the peak of the ECG R wave. Vascular regions of interest were delineated at the intima-lumen interface for brachial artery diameter measurements employing software developed by the Clinical Research Center (CRC) at Virginia Commonwealth University. Values obtained from each trial included baseline brachial artery diameter, 1 minute post-occlusion brachial artery diameter, average baseline blood velocity, and peak reactive hyperemic velocity. FMD was calculated by dividing the post-occlusion diameter change by the baseline diameter and expressed as the brachial artery diameter percent change [((maximal reactive hyperemic diameter – baseline diameter)/baseline diameter)*100]. Lastly, reactive hyperemic blood velocity change was calculated by dividing the post-occlusion blood velocity change by the baseline blood velocity [((maximal reactive hyperemic blood velocity – baseline velocity)/baseline velocity)*100]. Each assessed value was obtained by averaging 5 baseline and 5 post-occlusion images.

Peripheral Artery Tonometry. Pulsatile blood volume changes in the fingers were determined with peripheral artery tonometry (Endo-PAT2000, Itamar Medical). Methods utilized for determination of vascular function with PAT were similar to those previously

reported by Selamet and Tierney (2009). A pressure cuff was positioned around each subject's upper right arm and non-invasive pneumatic latex finger cuffs were placed on the right and left index fingers. Pulse wave amplitude was continuously recorded from both fingers. The finger cuffs were inflated and one minute of measurement of pulsatile changes were evaluated to ensure the absence of leaks in the cuffs. Subsequently, baseline measurements were obtained for 5 minutes in both the right and left index fingers. The pressure cuff was subsequently inflated to 240 mmHg to occlude blood flow for 5 minutes. Pulsatile flow was continuously assessed during the 5 minute occlusion period to ensure adequate occlusion of blood flow. Following the 5 minute occlusion period, the cuff was rapidly deflated and the pulsatile blood flow response to reactive hyperemia was assessed for an additional 5 minutes. The EndoPAT™ automatic analysis software was utilized to obtain the reactive hyperemic index (RHI) and the augmentation index (AI) for each trial. The RHI is the ratio of the average pulse wave amplitude measured over 60 seconds, starting one minute after cuff deflation, to the average pulse wave amplitude measured at the baseline (Syvänen, Korhonen, Partanen, & Aarnio, 2010). According to the Itamar Medical Manager of Cardiovascular Development, AI indicates the elasticity of the tunica media, an independent risk factor for CVD (Tirosh, R, personal communication, July 11, 2011). In addition, each trial was analyzed by manually selecting baseline, occlusion, and test areas as detailed by Selamet and Tierney (2009). Any extraneous artifact was also marked, if present. Test over baseline ratios (T/B) for both test and control arms were calculated by the EndoPAT2000™ software. The test arm ratio was subsequently normalized to accommodate any changes in the control arm during the trial.

Submaximal Exercise Bout. Following the pre-exercise assessment of vascular function, each subject participated in a 20 minute submaximal bout of treadmill exercise. The exercise began with a three minute warm up at 3.0 MPH and 0% grade. Following the brief warm up, the treadmill speed and grade was set to a workload that would elicit a heart rate equal to 75% of the subject's age-predicted maximum heart rate for the 20 minute exercise bout. Heart rate was continuously monitored throughout the exercise bout and minor adjustments in speed and/or grade were made and noted during the first trial. Subsequent trials utilized the same speed and grade to equate the workloads between the three vascular function trials. Upon completion of the 20 minute exercise bout, subjects were instructed to cool down for three minutes at 3.0 MPH, and 0% grade. Participants were immediately taken to an examination room for post-exercise vascular function measures. All post-exercise vascular function measures were commenced within 10 minutes of completion of the exercise bout.

Statistical Analysis. All data are represented as mean ± standard deviation. Paired samples t-tests were employed to compare baseline and post-occlusion means before and after exercise for each measure and pre- and post-exercise means for each vascular function measure. Additionally, Pearson Product Moment Correlations were employed to determine relationships among pre- and post-exercise values obtained from each assessment tool. The α-level was set at $p < 0.05$ for all statistical analyses.

VIII. RESULTS

Subjects

Seventeen apparently healthy sedentary male subjects (24 ± 4.14 years; 24.5 ± 3.23 kg/m^2) voluntarily participated in the study and underwent testing with SGP, FMD and PAT prior to exercise and following a moderate-intensity acute bout of aerobic exercise. Subject population characteristics are presented in Table 1.

Effects of exercise on hemodynamic measures

Average heart rate during the 20 minute exercise bout was 148 ± 2 beats/min. There were significant increases in mean arterial pressure (86.8 ± 9.98 mmHg vs 91.8 ± 2.34 mmHg; $p < 0.003$), systolic blood pressure (117.47 ± 7.4 mmHg vs 124.35 ± 8.61mmHg; $p < 0.006$) and diastolic blood pressure (71.71 ± 12.72 mmHg vs 75.76 ± 11.87 mmHg; $p < 0.014$) from pre- to post-exercise. Additional hemodynamic measures are found in Table 1.

Strain Gauge Plethysmography

Pre-exercise. SGP data were obtained on 15 subjects. Data from 2 subjects were excluded from the analysis due to equipment failure during one or more of the reactive hyperemia trials. Significant differences were observed between baseline FBF and post-occlusion FBF (2.13 ± 1.03 mL/min/100 mL tissue vs 6.35 ± 2.90 mL/min/100 mL; $p= 0.005$) representing reactive hyperemia-induced changes (Figure 1). Additionally, the calculated post-occlusion AUC values were significantly greater compared to baseline AUC values (226.77 ± 111.20 mL/min/100 mL vs 588.22 ± 283.99 mL/min/100 mL ; $p< 0.0001$). Pre-exercise vascular reactivity (VR) was determined to be 355.26 ± 203.39 mL/min/100 mL.

Post-exercise. During the post-exercise SGP trial, post-occlusion FBF values were significantly greater than baseline post-exercise values (3.0 ± 1.03 mL/min/100 mL tissue vs 7.61 ± 2.77 mL/min/100 mL; $p< 0.0001$) representing reactive hyperemia (Figure 1). Post-occlusion AUC values were also significantly greater than baseline values (375.34 ± 107.08mL/min/100 mL vs 703.71 ± 283.16 mL/min/100 mL ; $p< 0.0001$). Lastly, post-exercise VR was observed to be 366.68 ± 210.79 mL/min/100 mL.

Pre- to post-exercise changes in SGP. Pre- and post-exercise baseline and post-occlusion SGP values are provided in Figure 1. After exercise, baseline FBF was significantly greater than the pre-exercise baseline FBF (3.00 ± 1.03 mL/min/100 mL tissue vs 2.13 ± 1.03 mL/min/100 mL tissue; $p= 0.012$), and post-exercise post-occlusion FBF was also significantly greater than its pre-exercise counterpart (7.61 ± 2.77 mL/min/100 mL tissue vs 6.35 ± 2.90 mL/min/100 mL tissue; $p= 0.002$). Additionally, post-exercise baseline AUC was significantly greater than pre-exercise baseline AUC ($p= 0.008$) however, there was no significant difference observed between pre- and post-exercise post-occlusion AUC values and the calculated pre- and post-exercise VR were not significantly different. Pre-exercise post-occlusion FBF was significantly correlated to post-exercise post-occlusion FBF ($r= 0.763$, $p=0.002$) and post-exercise post-occlusion maximum FBF ($r= 0.753$, $p=0.003$). A significant correlation between pre-exercise and post-exercise post-occlusion maximum FBF was also observed ($r= 0.826$ $p= 0.001$). Additionally, pre-exercise VR correlated with post-exercise VR ($r= 0.742$, $p= 0.004$), as well as pre and post-exercise post-occlusion AUCs ($r= 0.745$, $p= 0.002$).

Flow Mediated Dilation

Pre-exercise. Pre-exercise FMD data were obtained on 16 subjects. One subject was excluded due to equipment malfunction during the pre-exercise FMD trial. Baseline brachial artery diameter and average blood velocity was determined to be 4.178 ± 0.37 mm and 91.839 ± 11.126 cm/sec, respectively. As expected during post-occlusion reactive hyperemia, there was a 5.3% increase in brachial artery diameter ($p < 0.0001$) (Figure 2) and a 71.9% increase in blood velocity ($p < 0.0001$). Pre-exercise FMD measure correlations are found in Table 3.

Post-exercise. Similar to the pre-exercise trial, there was a significant ($p < 0.0001$) increase of 4.9% in brachial artery diameter after the 5 minute occlusive stress (Figure 2). Post-exercise vascular measure correlations obtained with FMD can be found in Table 3.

Pre- to post-exercise changes in FMD. There were no significant differences observed between values obtained for baseline brachial artery diameter, post-occlusion brachial artery diameter, FMD percentage, or post-occlusion reactive hyperemic blood velocity during the pre- and post-exercise trials. Post-exercise baseline blood velocity was, however, significantly ($p < 0.0001$) greater than pre-exercise values. Pre- and post-exercise vascular function measures obtained by FMD are found in Figure 2. A strong positive correlation was found between baseline BAD values pre- and post exercise ($r = 0.976$; $p < 0.0001$) as well as between pre- and post-exercise post-occlusion BAD values ($r = 0.956$, $p < 0.0001$). The correlation coefficient between pre-exercise and post exercise FMD was 0.753 ($p = 0.001$). Correlation data for FMD measures are included in Table 3.

Peripheral Artery Tonometry

Pre-exercise. PAT data were obtained on 16 subjects. The data for one subject was excluded due to an equipment malfunction. Pre-exercise RHI values were determined to be 1.73 ± .34. Manual calculation of the RHI value resulted in a slightly lower mean value of 1.32 ± 0.335. Mean AI value at rest was found to be -8.77 ± 9.35%.

Post-exercise. Post-exercise RHI values were determined to be 1.55 ± 0.191. Manual calculation of the post-exercise RHI value resulted in a mean value of 1.10 ± 0.29. Mean AI value following exercise was determined to be -18.92 ± 8.41%.

Pre- to post-exercise changes in PAT. There was no significant difference observed between the computer-determined RHI values obtained pre- and post-exercise. There was, however, a pre- to post-exercise significant difference ($p= 0.022$) in the manually-calculated RHI values. Additionally, there was a significant difference observed in the AI values ($p<0.001$). Significant correlations were observed for AI values obtained at rest and AI values after exercise ($r=0.627$, $p= 0.022$).

Relationships among Vascular Assessment Tools

There were no significant correlations observed among the primary outcome measures obtained from each assessment technique. There was, however, a moderate correlation between pre-exercise VR as assessed by SGP and pre-exercise change in blood velocity as assessed by FMD ($r= 0.566$, $p= 0.035$). Correlations between measures obtained by SGP, PAT and FMD are included in Table 4.

IX. DISCUSSION

The endothelium plays a central role in the preservation of vascular homeostasis. In fact, an impaired endothelium is the initial manifestation of CVD development, occurring long before any of the well-recognized epidemiologic characteristics of the onset of the disease (Wilson, 2005). Although several endothelial function assessment tools have been developed, they are yet to be fully evaluated as valid tools for the early detection of CVD (Peretz et al., 2007; Gori et al., 2006 & Selamet Tierney, 2009). In this pilot study, inter-relationships among different vascular function techniques were evaluated on the same subjects prior to exercise and following an acute bout of moderate intensity exercise. While all three techniques (FMD, SGP and PAT) in this study have been developed to assess vascular function following blood flow occlusion and subsequent post-ischemic reactive hyperemia, each technique functionally evaluates a different component of the vasculature to provide an indication of vascular health.

In contrast to the thoroughly investigated effects of chronic exercise on vascular function, research on the effects of an acute bout of exercise on vascular hemodynamics is sparse. In the current study, we have observed increases in forearm blood flow, forearm blood flow area under the curve, brachial artery diameter, and blood velocity both before and after an acute bout of moderate intensity aerobic exercise in response to a 5 minute occlusive stress. Additionally, we found significant differences between pre-exercise and post-exercise values for baseline forearm blood flow, baseline forearm blood flow area under the curve, and post-occlusion forearm blood flow values obtained from

SGP. Moreover, we observed an increase in baseline blood velocity as assessed by FMD, as well as a decrease in AI% as assessed by PAT. Lastly, we found no significant correlations among the primary outcome measures obtained from SGP, FMD, and PAT either before or after exercise.

Pre-exercise vascular function. Pre-exercise vascular function values obtained from each method are similar to those previously reported (Alomari et al., 2005; Peretz et al., 2007 & Bonetti et al., 2004). Pre-exercise SGP resulted in increased FBF and AUC values following a 5 minute occlusion when compared to their baseline counterparts. AUCs were calculated as a flow-time index. These findings are comparable to those of Alomari et al., who found similar increases in post-occlusion FBF in repeated trials, as well as those of Bousquet-Santos et al, which measured FBF pre- and post-exercise (Alomari et al., 2005; Bousquet-Santos & Soares, Nobrega, 2005).

Pre-exercise flow mediated dilation via ultrasound revealed significant increases in blood velocity and BAD values following the 5 minute occlusive stress. These results are in agreement with those of Peretz et al., who found significant increases from baseline BAD to post-occlusion BAD during a resting condition using both upper-arm and forearm occlusion (Peretz et al., 2007). Comparing our results to those reported by other similar studies, we found that BAD values for our study seem to be slightly higher, yet our FMD values are lower than FMD reported by others (Harvey et al., 2005; Aizer et al., 2009, Okamoto, Masuhara & Ikuta, 2007; Rogmo et al., 2008). Larger brachial artery diameters were obtained with forearm occlusion than upper arm occlusion. Distal occlusion has been recommended over proximal occlusion, as it produces a dilatory response mainly dependent on NO.

Vascular function assessment by PAT does not produce separate baseline and post-occlusion values. Rather, the system software automatically calculates a reactive hyperemia index (RHI) as well as an augmentation index (AI). Additionally, test/baseline (T/B) ratios can be calculated by the system software following the manual demarcation of baseline, occlusion and test areas for both the control and the test (occlusion) arms. Significant differences were found between T/B1 (test arm) and T/B2 (control arm) reflecting reactive hyperemia-induced vascular changes. The mean resting RHI value in the current study was found to be 1.73 ± 0.34, values which reflect a healthy endothelium as expected from an apparently healthy population. These values are similar to those found by Bonetti et al., who reported a RHI value of 1.78 ± 0.08 in subjects with normal coronary endothelial function (Bonetti et al., 2004). The average pre-exercise AI value, which represents vessel elasticity, was -8.769 ± 9.347. While very few studies have measured AI, this measure may become a variable of greater interest for future studies, as AI is considered an independent CVD risk factor (Tirosh, R, personal communication, July 11, 2001).

Post-exercise vascular function. Post-exercise vascular function responses as detected by the SGP, FMD and PAT were variable in comparison to pre-exercise values. As observed during pre-exercise measurements, significant increases were also observed in FBF and FBF AUC values as assessed by SGP from baseline to post-occlusion following the acute exercise bout. These findings are similar to those of Bousquet-Santos et al., who also observed significant increases in baseline and post-occlusion blood flow following exercise. However, in contrast to their findings that reported an augmentation in VR shortly after, and 60 minutes after exercise, we did not observe a significant

increase in this value. As previously mentioned, AUC represents a flow-time index calculated from baseline and post-occlusion FBF measures respectively. Specifically, AUC is calculated by adding the means of each pair of two consecutive FBF measures, each pair which has been divided by the length of each cycle (12 seconds). Elevations in FBF due to exercise will in turn result in elevated AUC values. Due to this elevation following exercise, non-significant differences in VR are not entirely surprising since augmentations in AUC will in turn decrease post-exercise VR values. Although the SGP methodology of the current study was modeled after the technique of Bousquet-Santos et al., their exercise intervention consisted of an acute bout of maximal rather than moderate intensity exercise. The difference in exercise intensities between the two studies may be the cause of the discrepancy in VR findings, as higher exercise intensities are known to cause greater acute vascular responses (Harris, Padilla, Hanlon, Rink & Wallace, 2008).

Significant increases in post-occlusion BAD and velocity from their respective baseline values were detected by FMD following exercise. These findings are in agreement to those of Harvey et al., in which vascular function was assessed in post-menopausal women (Harvey et al., 2005). Unlike Harvey et al., we found no statistical significance in the difference between resting and post-exercise values for BAD, FMD and also post-occlusion blood velocities. In addition to the previously mentioned study, other studies that reported significant increases in BAD and improvements in FMD following similar exercise protocols in diseased and healthy populations (Padilla et al., 2009, & Zhu et al., 2007). In contrast, other studies revealed that acute exercise was found to aggravate endothelial dysfunction, marked by a decreased in flow mediated dilation in patients with intermittent claudication (Sylvestro et al., 2002). Similar

decreases in brachial artery FMD were seen following an acute bout of isometric handgrip exercises in patients medicated for hypertension (Mcgowan, C. et al., 2006). Although these discrepancies may be population or disease specific, further investigation of NO-dependent vasodilation responses after exercise is certainly warranted. Additionally, standardization of FMD methodology is of paramount importance in order to allow comparisons among published studies.

Analysis of the post-exercise data obtained via PAT revealed significantly different values in AI but not in RHI. The post-exercise mean AI% value was found to be -18.923 ± 8.411%, a value indicative of healthy, elastic vessels. T/B1 representing the occluded arm was slightly higher than the T/B2 ratio for the control arm, demonstrating the effects of blood flow occlusion. The T/B ratios have no clinical value but are have been utilized for research purposes to look at changes in blood flow in comparison to the control arm. Comparing pre-exercise to post-exercise values obtained by PAT revealed statistically significant differences between resting and post exercise T/B1 ratios of the test arms, reflecting altered reactive hyperemic responses to exercise. This significance was not observed between pre-exercise and post-exercise T/B2 ratios for the control arm. Interestingly, RHI values were lower following exercise, denoting endothelial dysfunction. There have been no studies assessing vascular function with PAT at rest and following exercise.

To our knowledge, there have been no studies comparing all three vascular function assessment tools pre and post-exercise. However, there have been several studies comparing different combinations of two of these tools both during a resting condition (pre-exercise) and following different exercise protocols. Unlike a limited

number of previous studies, we found no significant correlations among the primary outcome measures obtained from each assessment technique. Strong correlations were found between SGP and FMD in a study that utilized FBF and forearm volume to calculate diameter changes in the brachial artery (Sinoway, Hendrickson, Davidson WR Prophet & Zelis, 1989). These calculations were made under the assumption that blood that reaches the forearm travels mostly through the brachial artery. No such correlations were observed in our study prior to or after exercise. We did, however, find a moderate correlation between pre-exercise VR and pre-exercise changes in blood velocity as assessed by FMD. Our lack of correlation in vascular function measures by SGP and FMD has been supported by previous research. For example, when FMD was compared against SGP during a resting condition, it was found that there was no significant correlation between the two (Eskurza, I., Seals, D., DeSouza., Christopher A &Tanaka, H.,2001; Gori at al. 2006). A major difference between this study and the current study is the use of intrabrachial artery infusion of acetylcholine, commonly known for its vasodilatory properties as a stimulus for FBF. Although both methods assess peripheral vascular endothelial vasodilatory capacity, each method gauges vascular function of distinct vessel types, dictated by unique molecular mechanisms. NO controls vasoactivity of conduit vessels (FMD) via endothelial cells, yet FBF of the resistance vessels is partially (≈ 50%) controlled by smooth cells (Ferrara, et al., 2006).

Conversely, PAT evaluates the microcirculation of capillary networks. The differences in molecular mechanisms dictating vascular reactivity of different vessel types may explain the lack of significance in correlation analysis found in our study. In a comparison study, Bianchini et al. reported no correlation between vascular function

values obtained via PAT and FMD (Bianchini et al, 2006). However, Dihndsa et al, whose population was very similar to ours, found PAT and FMD to be strongly associated with each other (Dhindsa et al., 2008).

Several factors have been shown to influence the vascular function values obtained from the assessment tools employed in this study. These include the state of the vascular health of the population evaluated, methodology differences, and whether these assessments are performed at rest or following exercise (Harris, Padilla, Hanlon, Rink & Wallace, 2008; Gori at al. 2006; & Sylvestro et al., 2002). If post-exercise assessment is being performed, exercise intensity and the length of the exercise bout may be features worth further investigation.

Our results must be interpreted within the context of several study limitations. First, our population size was small, thereby limiting statistical power. Additionally, our population consisted of young, sedentary and apparently healthy individuals. In such populations, one can expect cardiovascular systems free of exercise-induced training effects, age-related deterioration, and disease. These characteristics are not representative of the general public, and thus may limit our ability to apply these findings. The use of an apparently healthy population in this pilot study limits the variability of vascular measures, reducing the likelihood of obtaining statistically significant differences. As SGP, FMD and PAT are manufactured to detect subclinical vascular abnormalities such as ED, future studies will benefit from the inclusion of a population possessing CVD risk factors to compare against a control population such as ours. Another noteworthy factor to consider is the exercise intensity required for significant vascular responses to occur, particularly in an apparently healthy population. Acute moderate-intensity exercise was

shown to increase blood pressure and FBF, while acute mild-intensity exercise did not cause these changes in young, healthy individuals (Harris, Padilla, Hanlon, Rink & Wallace, 2008). Incorporating an exercise bout of higher intensity than the submaximal exercise bout used in our study may intensify exercise-induced vascular responses. The additional stress that higher intensity exercise applies to the cardiovascular system may improve the effectiveness of endothelial dysfunction assessment with SGP, FMD and PAT in apparently healthy individuals. Alternatively, exercising the limb being assessed may yield more relevant vascular function findings. Limb specific exercises such as handgrip exercises have previously been successfully employed in the assessment of vascular function, and may be a worthy addition to the current study's methodology (McGowan et al., 2006). Although exercise is an effective addition to CVD diagnostic testing, as employed during stress tests, more specific exercise intensity parameters may be needed when attempting to detect pre-cursors in individuals without overt disease.

In this pilot study, reactive hyperemic vascular responses were investigated both before and after a submaximal aerobic exercise bout with SGP, FMD and PAT in a sedentary, apparently healthy population. Pre-exercise vascular responses were mostly similar to those found by other studies with similar populations; however, we observed variable responses in post-exercise measures. Exploring relationships among the vascular measures assessed by SGP, FMD and PAT before and after exercise may provide a more comprehensive insight into exercise-induced effects on the vasculature. Gaining an understanding of expected vascular responses for healthy individuals will aid in providing a foundation for vascular response evaluation of individuals with CVD risk factors and diseased populations. In our study, no correlations between the three tools

were found at rest and following exercise. Future research should consider the addition of populations at different CVD risk levels, as well as populations formally diagnosed with CVD to further investigate the diagnostic power and clinical use of an exercise stressor in conjunction with SGP, FMD and PAT to improve ED detection and CVD prevention.

X. TABLES

Table 1. Subject Demographics

Variable	Mean (±sd)
N	17
Age (years)	24.1 ± 4.14
Height (cm)	182.5 ± 6.93
Weight (kg)	81.5 ± 10.37
BMI (kg/m^2)	24.5 ± 3.23
Resting Systolic BP (mm Hg)	117.5 ± 7.40
Resting Diastolic BP (mm Hg)	71.7 ± 12.72
Resting MAP	86.81 ± 9.98
Post-exercise MAP	91.80 ± 9.58
Post-exercise Systolic BP (mm Hg)	124.4 ± 8.61
Post-exercise Diastolic BP (mm Hg)	75.8 ± 11.87
Exercise HR (bpm)	148 ± 2

Abbreviations: BMI, body mass index; BP, blood pressure; HR, heart rate; MAP, mean arterial pressure

Table 2. Pre and Post-exercise vascular function measures obtained from each of the three assessment tools

Measure	**Pre-Exercise**	**Post-Exercise**
SGP **(N= 15)**		
Baseline FBF (mL/min/100 mL tissue)	2.13 ± 1.03	3.00 ± 1.03*
Post-Occlusion FBF (mL/min/100 mL tissue)	6.35 ± 2.90	7.61 ± 2.77 *
Baseline AUC	226.77 ± 111.20	375.34 ± 107.08*
Post-Occlusion AUC	588.22 ± 283.33	703.71 ± 283.16 *
VR	355.26 ± 203.39	366.67 ± 210.79 *
FMD **(N= 16)**		
Baseline BAD (mm)	4 .178 ± 0.376	4.188 ± 0.399
Post-Occlusion BAD (mm)	4.397 ± 0.376	4.387 ± 0.374
FMD (%)	5.33 ± 3.02	4.88 ± 2.99
Baseline Blood Velocity (cm/sec)	91.839 ± 11.126	108.00 ± 17.09 *
Post-Occlusion Blood Velocity (cm/sec)	160.067 ± 21.39	172.73 ± 27.37
PAT **(N= 16)**		
RHI	1.73 ± 0.34	1.55 ± 0.19
AI (%)	-8.77 ± 9.35	-18.92 ± 8.41*

Abbreviations: BAD, brachial artery diameter; FMD, flow mediated dilation; RHI, reactive hyperemic index; AI, augmentation index; FBF, forearm blood flow; AUC, area under the curve; VR, vascular reactivity. *$p < 0.05$

Table 3. Correlation between vascular measures as assessed by FMD

	PE FMD	PE BL BAD	PE PO BAD	PR PO BAD
PR FMD	0.753** (P< 0.001)	-	-	-
PR BL BAD	-	0.976** (P< 0.000)	0.917** (P< 0.000)	0.944** (P< 0.000)
PR PO BAD	-	0.943** (P< 0.000)	0.956** (P< 0.000)	1

Abbreviations: PR, pre-exercise; PE, post-exercise; BL, baseline; PO, post-occlusion; post-exercise; BV, blood velocity ; FMD, flow-mediated dilation ; BAD, brachial artery diameter.
*P< 0.05; ** P< 0.01

Table 4. Correlation between vascular measures as assessed by SGP, FMD and PAT

	PR VL	PE FMD
PR VR	0.566* (P< 0.035)	0.564* (P< 0.045)
PE AI	-	-
PE RHI	- 0.272 (P< 0.346)	0.000 (P< 0.999)
PE VR	0.511 (P< 0.074)	0.087 (P< 0.788)

Abbreviations: PR, pre-exercise; PE, post-exercise; BL, baseline; PO, post-occlusion; post-exercise; VR, vascular reactivity ; VL, blood velocity ; FMD, flow-mediated dilation AUC, area under the curve.

XI.FIGURES

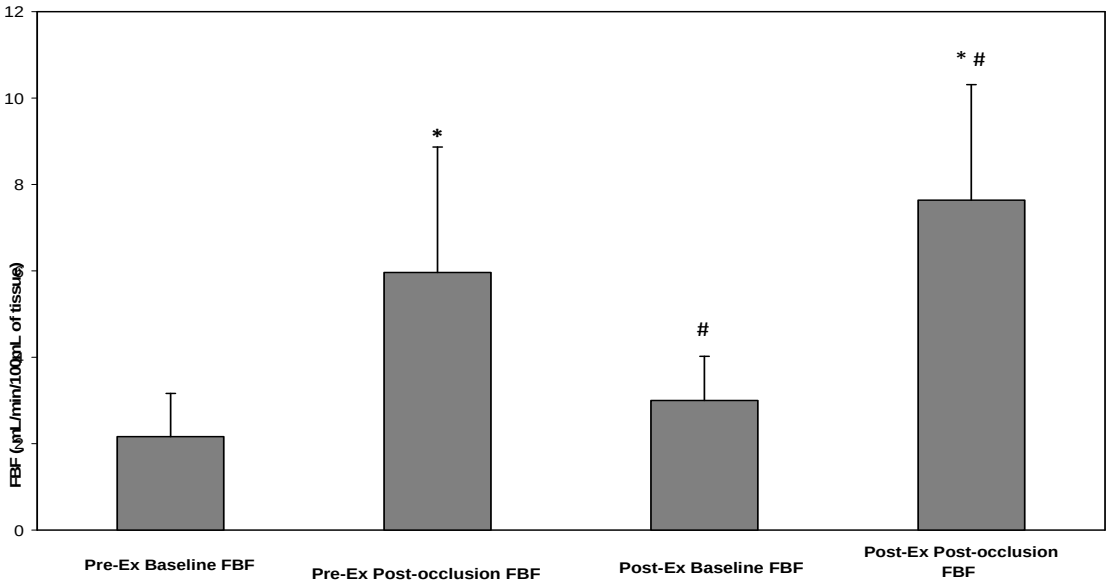

Figure 1. Changes between baseline and post occlusion FBF pre and post-exercise

* = post-occlussion significance, p< 0.05
# = post-exercise significance, p< 0.05

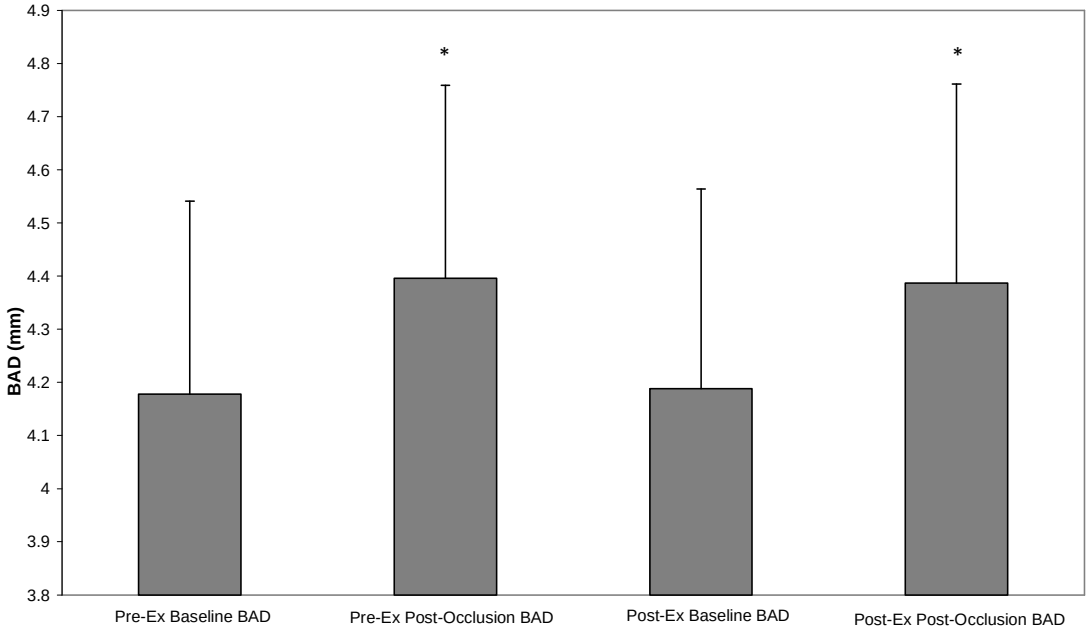

Figure 2. Pre and Post-Exercise Changes in Baseline and Post-Occlusion BAD

* = post-occlusion significance, p< 0.05

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APPENDIX A
IRB APPROVAL LETTER

VCU

Virginia Commonwealth University

MCV CAMPUS

Office of Research
Office of Research Subjects' Protection

Biotech Research Park, Bldg. 1
800 East Leigh Street, Ste. 114
P.O. Box 980568
Richmond, VA 23298-0568

(804) 828-0868 - Phone
(804) 828-1448 - Fax
(804) 828-1120 - TDD

DATE: March 9, 2010

TO: Ronald K. Evans, PhD
Department of Health & Human Performance
Box 842020

FROM: William E. Smith, PharmD, MPH, PhD
Chairperson, VCU IRB Panel A
Box 980568

RE: **VCU IRB #: HM12767**
Title: A Pre and Post Exercise Comparison of Three Assessment Tools Commonly Employed to Evaluate Vascular Function

On March 1, 2010, the following research study was ***approved*** by expedited review according to 45 CFR 46.110 Category 4. This approval includes the following items reviewed by this Panel.

RESEARCH APPLICATION/PROPOSAL: None

PROTOCOL: A Pre and Post Exercise Comparison of Three Assessment Tools Commonly Employed to Evaluate Vascular Function (VCU Research Plan; dated 01/27/10)

CONSENT/ASSENT:
- Research Subject Information and Consent Form (Version dated 01/27/10; 5 pages)

ADDITIONAL DOCUMENTS: None

This approval expires on February 28, 2011. Federal Regulations/VCU Policy and Procedures require continuing review prior to continuation of approval past that date. Continuing Review report forms will be mailed to you prior to the scheduled review.

The Primary Reviewer assigned to your research study is Varatharaj Mounasamy, MD. If you have any questions, please contact Dr. Mounasamy at vmounasamy@vcu.edu or 827-1332; or you may contact Stephan Hicks, IRB Coordinator, VCU Office of Research Subjects Protection, at hicksse2@vcu.edu or 828-9876.

Attachment – Conditions of Approval

Page 1 of 2

APPENDIX B

CONSENT FORM

RESEARCH SUBJECT INFORMATION AND CONSENT FORM

TITLE: A PRE AND POST EXERCISE COMPARISON OF THREE ASSESSMENT TOOLS COMMONLY EMPLOYED TO EVALUATE VASCULAR FUNCTION

VCU IRB PROTOCOL NUMBER:

INVESTIGATORS: Ronald K. Evans, Ph.D., Lorena P. Salom, B.S.

SPONSOR:

This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision. In this consent form, "you" always refers to the subject.

PURPOSE OF THE STUDY

The purpose of this study is to compare three different types of equipment that are commonly used to measure how blood vessels function by either measuring blood flow or changes in vessel size. We will be taking these measures while you are at rest and after 20 minutes of moderate intensity exercise on a treadmill.

DESCRIPTION OF THE STUDY

Blood vessels are lined with a single layer of cells called the endothelium that influences blood vessel diameter. Endothelial dysfunction is considered to be the earliest clinical sign of increased cardiovascular disease risk and early detection of changes in this cell layer may be beneficial for identifying individuals at higher risk for future cardiovascular events. Brachial artery flow-mediated dilation (FMD) via ultrasound, venous occlusion strain-gauge plethysmography (SGP), and peripheral artery tonometry (PAT) are commonly employed non-invasive tools for the evaluation of vascular function. Your participation in this study will last up to three weeks. Approximately 20 subjects will participate in this study.

PROCEDURES

If you decide to be in this research study, you will be asked to sign this informed consent form after you have had all of your questions answered. Whether or not you qualify for this study will be based upon your physical characteristics and medical history. The criteria for inclusion in this study are as follows: Sedentary males between 25-35 years of age with body mass index (BMI) values <30 kg/m^2. You will complete a medical history form prior to the beginning of the study. You may not have any history of cardiovascular or metabolic disease, or be taking any medication that might affect blood flow, blood vessel function, or the blood vessel responses to exercise. You will also be excluded if you have a latex allergy since the assessment of vascular

function with one of the techniques (PAT) requires contact with an inflatable finger cuff made from latex. Testing sites include the Health and Human Performance Lab at 3600 W. Broad St. and the General Clinic Research Center (GCRC) on the VCU Medical Center campus. Participation in this study will require that you visit the testing sites on three occasions approximately 1 week apart. During each visit, you will undergo baseline vascular function assessment with one of the techniques, followed by a submaximal exercise bout, and concluding with post-exercise vascular function assessment. Each visit will last approximately 2 hours.

The following measurements will be made during the study:

Anthropometric Measures. Your weight and height will be assessed with a scale and stadiometer. These measurements will be utilized to calculate your BMI. These measurements will be performed at 3600 W. Broad Street in the Health and Human Performance Laboratory on your first visit. These measures will take approximately 10 minutes to complete.

Blood vessel. Blood flow through your vessels will be assessed using three different techniques: Strain Gauge Plethysmography, Flow Mediated Dilation and Peripheral Artery Tonometry, each which will last approximately 35 minutes.

- Strain Gauge Plethysmography: Blood pressure cuffs will be positioned around your upper right arm and right wrist, and a mercury-in-rubber strain gauge will be placed around your forearm. During each trial, the wrist cuff will be inflated to a pressure of 240 mmHg prior to each measurement to occlude hand circulation. Baseline FBF will be determined by rapidly inflating the upper cuff to 40 mmHg for 4 seconds. Then, the upper arm cuff will be inflated to 240 mmHg to induce forearm ischemia for a period of 5 minutes. After 5 minutes of occlusion, the cuff will be released and FBF, as described above, will be determined during a 9 consecutive 12-second cycles.
- Flow Mediated Dilation: Brachial artery diameter at rest will be assessed with high resolution ultrasound. Following the resting measures, a blood pressure cuff will be positioned around your upper left arm and inflated to suprasystolic pressure to produce ischemia for a period of 5 minutes. Following cuff release, your vessel diameter will be reassessed
- Peripheral Artery Tonometry: A blood pressure cuff will be positioned around your upper right arm and finger cuffs will be placed on your right and left index fingers. Baseline blood flow changes will be measured for 5 minutes in both your right and left index fingers prior to inflating the blood pressure cuff on your right upper arm to approximately 200 mmHg to induce a 5 minute period of forearm ischemia. Following cuff release, your pulsatile blood flow changes will be reassessed.

Physical Activity Participation. Physical activity participation will be assessed at your first visit using a physical activity recall questionnaire. This questionnaire will ask you questions about the quantity and intensity of your physical activity over the previous 7 days. The questionnaire will take approximately 5 minutes to complete.

Submaximal Exercise Bout. The submaximal exercise bout will begin with a three minute warm up period on the treadmill at 3.0MPH, and 0% grade. You will then

exercise on the treadmill at 75% of your age-predicted maximum heart rate for twenty minutes. A brief cool down period of three minutes in length at 3.0MPH will follow the exercise bout. You will then be taken to an examination room for post-exercise vascular function measures.

RISKS AND DISCOMFORTS
Inflation of the cuff may cause discomfort, pain and tingling in your hands and fingers. As with any exercise participation, there is also the slight chance of an abnormal blood pressure response, fainting, and disorders of heart beat. In the event of a medical problem during testing, the technicians will administer emergency first aid and then activate the appropriate emergency medical system.

BENEFITS TO YOU AND OTHERS
You are not expected to receive any direct medical benefits from your participation in the study. You will not receive any monetary compensation for your participation. However, information from this study will help us better understand the similarities and differences between techniques that assess vascular function.

COSTS
There are no costs for participating in this study other than the time commitment for the three testing sessions.

ALTERNATIVE
This is not a treatment study. Your alternative is not to participate in this study.

CONFIDENTIALITY
Potentially identifiable information about you will consist of medical history forms, measures of blood vessel function, body mass index, and physical activity participation. Data are being collected for research purposes only. Your data will be identified by a unique identifier, your name, and stored in a locked research area in the Health and Human Performance Laboratory at 3600 W. Broad Street. All personal identifying information will be kept in password protected files and these files will be deleted within two years following the completion of the study. Access to all data will be limited to study personnel.

You should know that research data about you may be reviewed or copied by Virginia Commonwealth University. Personal information about you might be shared with or copied by authorized officials of the Federal Food and Drug Administration, or the Department of Health and Human Services (if applicable). Although results of this research may be presented at meetings or in publications, identifiable personal information pertaining to participants will not be disclosed.

COMPENSATION FOR INJURY

Virginia Commonwealth University and the VCU Health System have no plan for providing long-term care or compensation in the event that you suffer injury as a result of your participation in this research study.

If you are injured or if you become ill as a result of your participation in this study, contact a member of the research team immediately. A member of the study staff will arrange for short-term emergency care if it is needed.

Fees for such treatment may be billed to you or to appropriate third party insurance. Your health insurance company may or may not pay for treatment of injuries as a result of your participation in this study.

VOLUNTARY PARTICIPATION AND WITHDRAWAL

Your participation in this study is voluntary. You may decide to not participate in this study. Your decision not to take part will involve no penalty or loss of benefits to which you are otherwise entitled. If you do participate, you may freely withdraw from the study at any time. Your decision to withdraw will involve no penalty or loss of benefits to which you are otherwise entitled.

Your participation in this study may be stopped at any time by the study doctor or the sponsor without your consent. The reasons might include:

- the study staff thinks it necessary for your health or safety;
- you have not followed study instructions; or
- administrative reasons require your withdrawal.

QUESTIONS

In the future, you may have questions about your study participation. You may also have questions about a possible side effect, reaction to study medication, or a possible research-related injury. If you have any questions, complaints, or concerns about the research, contact:

Ronald K. Evans, PhD
Department of Health and Human Performance
804-828-7798
rkevans@vcu.edu

If you have questions about your rights as a research subject, you may contact:

Office of Research
Virginia Commonwealth University
800 East Leigh Street, Suite 113
PO Box 980568

Richmond, VA 23298
(804) 827-2157

You may also contact this number for general questions, concerns or complaints about the research. Please call this number if you cannot reach the research team or wish to talk to someone else.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions. Additional information about participation in research studies can be found at http://www.research.vcu.edu/irb/volunteers.htm.

CONSENT

I have been provided with an opportunity to read this consent form carefully. All of the questions that I wish to raise concerning this study have been answered.

By signing this consent form, I have not waived any of the legal rights or benefits, to which I otherwise would be entitled. My signature indicates that I freely consent to participate in this research study. I will receive a copy of the consent form once I have agreed to participate.

_____
Subject Name, printed

_____ _____
Subject Signature Date

_____
Name of Person Conducting Informed Consent Discussion / Witness (Printed)

_____ _____
Signature of Person Conducting Informed Consent Discussion / Witness Date

_____ _____
Investigator Signature (if different from above) Date

APPENDIX C

SUBJECT INSTRUCTIONS

Department of Health and Human Performance

A Pre and Post Exercise Comparison of Three Assessment Tools Commonly Employed to Evaluate Vascular Function

- Please no do consume food or drinks after midnight the night before participating in the study. Moderate water consumption is allowed.
- No alcohol or tobacco for a minimum of 24 hours prior to your appointment.
- Please refrain from exercise for a minimum of 48 hours prior to your appointment
- Please wear or bring comfortable clothes and shoes for your treadmill test.
- Please remove all fingernail polish on your index fingers prior to your visit.
- When you arrive at the GCRC, you will be placed in a room and allowed to rest for a minimum of 30 minutes prior to your testing session.

I. Directions to VCU Medical Center:

Arriving by I-64 West - coming from Virginia Beach, Williamsburg, etc.

1. Take Exit #190 for 5th Street and Downtown/Coliseum
2. Turn left at the 4th traffic light onto Marshall Street
3. Drive six blocks and turn left onto 11th Street
4. Drive one block and turn right onto Clay Street
5. Go one and one-half blocks to the Patient and Visitor Parking Decks E & S.

Arriving by I-64 East - coming from Charlottesville, I-81, etc.

1. Follow I-64 East which merges with I-95 South
2. Remain on I-95 south to Exit #74C for West Broad Street
3. Proceed West on Broad Street and follow directions given under "arrive by I-95 North"

Arriving by I-95 North - coming from Petersburg, North Carolina, etc.

1. Take Exit #74C for West Broad Street
2. Proceed West on Broad Street for three blocks to 11th Street, take right
3. Drive two blocks to Clay Street and turn right
4. Proceed one and one-half blocks to the Patient and Visitor Parking Decks E & S.

Arriving by I-95 South - coming from Washington, Fredericksburg, etc.

1. Take Exit #74C to West Broad Street

2. Follow directions given under "arriving by I-95 North"

Arriving by U.S. Route 60 - coming from Lexington, etc.

1. Follow U.S. Route 60 which merges with 9th Street
2. Remain on 9th Street and go one block past Broad Street to Marshall Street and turn right
3. Drive two blocks and turn left onto 11th Street
4. Drive one block and turn right onto Clay Street
5. Go one and one-half blocks to the Patient and Visitor Parking Decks E & S.

- Valet parking is available for $5.00 at the entrances of the Gateway Building, ACC and North Hospital. Parking for the Children's Pavilion is available in the Pavilion's lower garage on 11th St. for $5.00.
- The VCU Medical Center has a Patient and Visitor Parking Deck located at 12th and Clay Streets. The Deck is open 24 hours daily. There is a charge for using this facility during the week.
- Reduced parking rates at the Visitor Parking Deck ($2.00 per visit) are available for patients and visitors that obtain parking validation at the Information Desks located on the first floor of ACC and Children's' Pavilion; ground floor of Gateway and Critical Care Hospital.
- Lost parking tickets for the Patient and Visitor Parking Deck will be charged $10.00 upon exit unless a $2.00 validation is presented.

vp Valet Parking **P** General Parking

- Arrive at the General Clinical Research Center (8th floor North Hospital) at least 15 minutes prior to your appointment time. North Hospital is accessible from the Gateway building (Main entrance). Go up the escalators; walk straight, all the way down the hallway (past the cafeteria), transition into North Hospital. Around the corner you will see a set of elevators. Go to 8th floor (GCRC).

II. Directions to 3600 West Broad Street (Health and Human Performance Laboratory):

Arriving by I-64 West - coming from Virginia Beach, Williamsburg, etc.

1. Take exit 70 towards Boulevard
2. Turn slight left to take the ramp toward VA-161
3. Turn left onto Hermitage Rd
4. Turn right onto Robin Hood Rd
5. Turn left onto Boulevard/VA-161 for 0.9 miles
6. Turn right onto W. Broad St/ US-250
7. 3600 W. Broad St is on the right after 0.6 miles

Arriving by I-64 East - coming from Charlottesville, I-81, etc.

1. Merge onto I-195 S via exit 79 toward US-60 W/Powhite Pkwy/ US 360W
2. Take the Hamilton St. exit toward US-33/US-250/BROAD ST
3. Take the ramp toward US-33/US-250/BROAD ST.
4. Turn left onto W. Broad St/US- 250
5. Arrive at 3600 W. Broad St on your left.

Arriving by I-95 North - coming from Petersburg, North Carolina, etc.

1. Merge onto I-195 N via exit 74 A toward VA-195-Toll/Downtown Expressway
2. Take exit toward US-33/US-250/Broad St
3. Turn slight right onto W. Clay St.
4. Turn right into Roseneath Rd.
5. Turn right onto W. Broad St/US-250/US-33
6. 3600 W. Broad St/ US-250/US-33

Arriving by I-95 South - coming from Washington, Fredericksburg, etc

6. Merge onto I-195 S via exit 79 toward US-60 W/Powhite Pkwy/ US 360W
7. Take the Hamilton St. exit toward US-33/US-250/BROAD ST
8. Take the ramp toward US-33/US-250/BROAD ST.
9. Turn left onto W. Broad St/US- 250
10. Arrive at 3600 W. Broad St on your left.

Arriving by U.S. Route 60 - coming from Lexington, etc

1. Merge onto I-64 E towards I-81/Staunton via exit 221 for 31 miles
2. Marge into I-195S via exit 186 towards Powhite Parkway
3. Take the Hamilton St. exit towards US-250/Broad St
4. Turn slight right onto N. Hamilton St.
5. Turn left onto W. Broad St.
6. Arrive at 3600 W. Broad St. on the left

- There is a parking lot in the back of the building. Please park in the last row, facing the highway.
- If you have any questions regarding your appointment, please do not hesitate to contact Lorena Salom at 240-476-7640. The General Clinical Research Center can be contacted at 828-9101.

APPENDIX D

RANDOMIZED SELECTION

1. SGP
2. Endo-PAT
3. Ultrasound
4. SGP
5. Endo-PAT
6. Ultrasound
7. SGP
8. Endo-PAT
9. Ultrasound
10. SGP
11. Endo-PAT
12. Ultrasound
13. SGP
14. Endo-PAT
15. Ultrasound
16. SGP
17. Endo-PAT
18. Ultrasound
19. SGP
20. Endo-PAT
21. Ultrasound
22. SGP
23. Endo-PAT
24. Ultrasound
25. SGP

APPENDIX E

STRAIN GAUGE PLETHYSMOGRAPHY DETAILED METHODS

STRAIN GAUGE PLETHYSMOGRAPHY DETAILED METHODS

Subject should rest in the supine position on stretcher for about 30 minutes prior to testing. Turn on arterial inflow system by switching power button to the ON position. The switch is located on the top right corner of the cart, behind the computer screen. Then, press the power button on the central processing unit to turn on computer. Using the mouse, double click on the green AI6 icon on desktop to open AI6 program.

Ask subject to raise their dominant arm, and to bend their elbow. Find olecranon process on the subject's dominant arm and measure approximately 10cm distal to the olecranon process to find widest part of the forearm and mark arm with a pen. Measure the circumference around the forearm of the dominant arm, and record. Select a strain gauge that is 2 to 3cms smaller than forearm circumference. Wrap large blood pressure cuff around the upper dominant arm tightly. A small blood pressure cuff should be tightly wrapped around dominant arm's wrist. This cuff will be inflated to suprasystolic pressures to completely occlude hand circulation during test; therefore isolating forearm circulation. Make sure both blood pressure cuffs are connected to the inflators on the cart. Carefully loop mercury-filled strain gauge around the widest part of the forearm, previously marked. The strain gauge strands should be even around the forearm, with both bands parallel to one another. Secure strain gauge in place by hooking the end of the mercury-filled band over the plastic head of the strain gauge. Place a small piece of adhesive tape over the plastic head to secure strain gauge to arm.

Plug in strain gauge to jack labeled “Strain Gauge 1” on the AI6 cart. The dominant arm should be raised approximately 10° above heart level by supporting the upper arm and hand with foam blocks. Avoid placing foam blocks on the forearm, as the strain gauge should not be touched. Ask the subject to be still and refrain from speaking, as this instrument is very sensitive to motion. Apply three electrodes to subject’s chest; two just below the clavicle on both sides. The third electrode should be placed on the left side, on the anterior axillary line, slightly above the navel. The skin should be prepped by briskly rubbing it with an alcohol pad. Shave off excess hair if necessary. Once all electrodes are applied, connect white Electrocardiogram (ECG) lead to the right upper electrode, the black lead to the upper left electrode, and the red lead to the lower left electrode.

Running the test:

Phase 1

Click on “Patient” on the top right corner of the screen, then on “Enter Demographics”. Fill out the required fields, Patient ID and Last Name. Click “Close”. This will store information on database. Check Pressure Settings box on the bottom right corner of the screen. Desired Wrist Pressure should be set at 250 mm Hg for total occlusion. Desired Venous Pressure should be set at 40mmHg. Click “SGP Balance” to manually calibrate and zero the plethysmographs. The AI6 will recalibrate the plethysmograph prior to each reading during arterial flow measurements. Press the “Start” button to begin test. Nine measurements will be recorded to determine the average rate of volume change during venous occlusion

(ml/100 ml of forearm tissue volume/min). Pause test following the end of the ninth measurement. This demarcates the end of Phase 1.

Phase 2:

1. Check “Ischemic Stress” box, located in the Pressure Settings box on the bottom right corner of the screen. Stress Pressure should be set to 240mm Hg, and the stress period should be set at 300 seconds. Click on the “Resume” button at the bottom right corner of the screen to start the ischemic stress period. The upper arm cuff will inflate to 240 mm Hg to induce forearm ischemia for a period of five minutes (300s). Following the five minute long Ischemic stress period, the cuff will be quickly deflated and forearm blood flow will be determined in the same manner as Phase 1. Allow for 9 post-ischemia test stages then click to End Test.

Repeat procedure following acute exercise bout.

APPENDIX F

FLOW MEDIATED DILATION VIA ULTRASOUND DETAILED METHODS

FLOW MEDIATED DILATION VIA ULTRASOUND DETAILED METHODS

The subject should be in the supine position, resting for thirty minutes prior to the start of the research trial. Position the subject so that the arm is in a comfortable position for imaging the brachial artery. Place large blood pressure around the forearm of test arm, and acquire a resting blood pressure measurement in the control arm. Apply three electrodes to subject's chest; two just below the clavicle on both sides. The third electrode should be placed on the left side, on the anterior axillary line, slightly above the navel. The skin should be prepped by briskly rubbing it with an alcohol pad. Shave off excess hair if necessary. Once all electrodes are applied, connect white Electrocardiogram (ECG) lead to the right upper electrode, the black lead to the upper left electrode, and the red lead to the lower left electrode.

The brachial artery should be located on the test arm by a trained ultrasonographer. Once the vessel has been located, data collection can begin. Press the record button for 10 to 12 heart beats, then press the "Pulse" button and correct the angle so that it is parallel to the vessel. Adjust the baseline to the appropriate values, then press HighQ. Continue to record for 10 to 12 beats for baseline flow. Press record again to pause recording. Inflate blood pressure cuff to 240mm Hg and start stop watch. At 4 minutes 30 seconds, press the record button. At 4 minutes 45 seconds, press pulse, correct the angle and press update. When the stopwatch marks 5 minutes, release the pressure from the blood pressure cuff, and press reset on the VCR timer. Continue to capture flow measurements until

flow start to noticeably drop, which will occurs in 20 to 25 seconds. Press the update button and pulse to get back to the vessel diameter screen. Continue to record the best image possible for 2 minutes post occlusion. Repeat procedure following the acute exercise bout.

APPENDIX G

ENDO PAT DETAILED METHODS

ENDO PAT DETAILED METHODS

Turn on Endo-PAT unit at least twenty minutes prior to test to allow machine to warm up. Turn on laptop and connect it to the Endo-PAT unit. Attach the fingertip like bio-sensors to apparatus. The bio-sensor probes are lined with latex; therefore subjects with latex allergies should be excluded from participating in the research study.

Click on Endo-PAT icon on the desk top. Once program has opened, a green EndoPAT™ icon should light up at the right bottom corner of the screen, indicating communication between the computer and the Endo-PAT unit. Click on "Patient ID" and fill out patient information.

Prior to the start of each research trial, subjects should rest in the supine position for thirty minutes. Resting blood pressure should be taken on the control arm approximately half way through the resting period. Check the subject's finger nails, they should be short and clean. Nails that protrude more than five mm from the fingertip should be trimmed. If there is nail polish on the nails, remove it with acetone. Remove all jewelry from the subject. Place finger probes on the subject's index fingers, and blue foam anchor around the middle finger. The foam anchor should be pushed down towards the root of the finger. Place blood cuff around the upper arm of the control arm. Both arms should be placed at the sides of the subject's body and elevated to heart level by resting the arms on the specialized blue cushions. The index fingers should be placed inside the finger holes at the top of the specialized cushions to avoid the finger probe to come in contact and provide support. Inflate finger cuff by pressing the inflate button on the Endo-PAT unit.

Press the yellow Standby button for one minute. Then, press the green “Go” icon to start recording data. Collect baseline data for a minimum of three minutes (says that should be for at least 5 to10 minutes?). Quickly inflate blood pressure cuff to 240 mmHg to occlude blood floor to the arm for a total of five minutes. Rapidly deflate blood pressure cuff and continue to collect data for another three minutes. Click the “Stop” button to end test.

Patient set up:

Prior to the start of each research trial, subjects should rest in the supine position for thirty minutes. Resting blood pressure should be taken on the control arm approximately half way through the resting period. Check the subject’s finger nails, they should be short and clean. Nails that protrude more than five mm from the fingertip should be trimmed. If there is nail polish on the nails, remove it with acetone. Remove all jewelry from the subject. Place finger probes on the subject’s index fingers, and blue foam anchor around the middle finger. The foam anchor should be pushed down towards the root of the finger. Place blood cuff around the upper arm of the control arm. Both arms should be placed at the sides of the subject’s body and elevated to heart level by resting the arms on the specialized blue cushions. The index fingers should be placed inside the finger holes at the top of the specialized cushions to avoid the finger probe to come in contact and provide support. Inflate finger cuff by pressing the inflate button on the Endo-PAT unit.

APPENDIX H

TREADMILL OPERATION

TREADMILL OPERATION

1) Turn on system. Button is on the right hand side of the system
2) Press Ctrl+ Alt+ Del
3) Password is “User”
4) Once the program has loaded, click on “next page” until reaching “TEST-DEMO” in the Select patient (local database) window, then press “Select”.
5) A window will come up that states “No Connection to BP”, Click OK
6) Press the blue Exercise button on the left side of the panel right above the keyboard. Then, click blue “Start Treadmill” button on the right side of the panel.
    a. Increase speed for warm up to 3.0 MPH for 3 minutes.
    b. Increase speed to reach 75% of the subject’s age predicted max HR, exercise bout lasts for 20 minutes.
    c. Decrease speed for cool down to 3.0 MPH for 3 minutes.
7) Press the red “Stop treadmill” button on the right hand side.
8) Press the blue “Test end” button on the left hand side of the panel.
9) Click Yes on the “Test end” window.
10) Click OK on the next window that appears.
11) Click OK on the BP window if it appears.
12) Click on the “Initial Screen” button on the right upper corner of the screen.
13) Click OK on the window that says “The patient ECG waveforms will no longer to displayed”
14) Press “Turn off Program”. Then click Yes on the “Do you want to terminate the program and shut down the device” window. This turns off the system.