Sensitivity of the Vasoactive Range in Determining Aerobic Fitness

Preston L. Bell

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Sensitivity of the Vasoactive Range in Determining Aerobic Fitness

by

Preston Bell

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The University of Southern Mississippi
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in the School of Kinesiology

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Approved by

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Department of Kinesiology

Ellen Weinauer, Ph.D., Dean
Honors College
Abstract

Previous work has demonstrated a direct relationship between aerobic fitness and indices of vasodilatory function (i.e., Flow-mediated Dilation; FMD). Importantly, recent evidence suggests that vasoconstrictor function to reductions in blood flow (i.e., Low-Flow Mediated Constriction; L-FMC), as well as during sympatho-excitation (i.e., Cold Pressor Test), may compliment the FMD measure, thus, providing an overall range of vascular responsiveness. The purpose of this thesis project was to test the hypothesis that vasoactive range indices (peak vasodilation + nadir vasoconstriction) are sensitive to aerobic fitness levels in healthy young men. Fourteen males (age: 22±4 yrs) were recruited, and divided evenly into a high (HF) vs. low (LF) aerobic fitness group, quantified via YMCA cycle ergometry (VO2 peak extrapolation), and a 3-min step test (1-min HR recovery). Duplex Doppler-ultrasound was used to assess brachial artery responses to the following physiological stimuli: FMD, L-FMC, CPT, and local heating. Vasodilatory responses were calculated from the peak change in artery diameter, and vasoconstrictor responses were determined from the nadir values in response to stimuli, respectively. VO2 peak (HF=55±10 vs. LF=38±6) and HR recovery (HF=38±12 vs. LF=24±9 beats) were greater in the HF group (P<0.05). All vasoactive range indices were similar between groups; however, L-FMC change tended to be greater in HF (HF=0.1±0.06 vs. LF=0.02±0.07mm, P=0.057). A correlational analysis revealed an inverse relationship between L-FMC and HR recovery (r=-0.653, P=0.02). Collectively, these findings suggest that vasoactive range indices are not sensitive to aerobic fitness in healthy young men; however, high fit individuals may exhibit greater vasoconstrictor function to reductions in blood flow.

Key Terms: Aerobic, Vasoactive, Cardiorespiratory, and Vascular
Dedication

To God:
For your Glory.

To my lovely fiancée, Nicolet:
Thank you for always encouraging me and helping me in stressful times.

To my supportive family:
Thank you for pushing me to be the best I can be.

And, to my Paw-Paw, Jimmy Rowell:
May this research help those at risk of developing cardiovascular disease.
Acknowledgements

What a privilege it has been to learn from Daniel Credeur, my thesis advisor. I could not have completed this thesis without his patient, and well-calculated guidance. Thank you for all that you have done. His work in this thesis is deserving of many thanks.

Also, thank you to my brothers in Pi Kappa Phi who were willing to participate in this research.
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<tr>
<td>AUC</td>
<td>Area-Under-the Curve</td>
</tr>
<tr>
<td>BIA</td>
<td>Bioelectrical Impedance Assessment</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>CPT</td>
<td>Cold Pressor Test</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>FMD</td>
<td>Flow Mediated Dilation</td>
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<td>HF</td>
<td>High Fit</td>
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<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>L-FMC</td>
<td>Low-Flow Mediated Constriction</td>
</tr>
<tr>
<td>LF</td>
<td>Low Fit</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximum Voluntary Contraction</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
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</table>
Chapter 1: Introduction

Cardiovascular disease (CVD) is considered the number one cause of morbidity and mortality in the United States (Prevention 2014). Disruption within the vascular endothelium is believed to be an early initiating event in CVD development, including atherosclerosis [1-3]. The technique known as flow-mediated dilation (FMD) is considered the non-invasive ‘gold standard’ for assessing vascular endothelial function in humans [3, 4]. This technique examines an artery’s vasodilatory responsiveness to an increase in blood flow-induced shear stress [4, 5], which is mediated through temporary circulatory arrest (5 mins) of a limb using a pneumatic cuff inflated to a supra-systolic pressure (e.g., 220 mmHg). Following cuff release, artery diameter and blood velocity changes are examined via Doppler-ultrasonography [4].

The concept of FMD implies that increases in arterial shear stress induce endothelial cells to produce and release vasodilatory compounds, most notably nitric oxide, which result in smooth muscle relaxation with subsequent vasodilation (i.e., increased vessel diameter) of an artery [6]. The response is typically presented as a maximal percentage increase [7] from resting diameter measurements, along with shear-rate, which is calculated using the blood velocity data [4]. Importantly, FMD has high prognostic value such that for every 1% change in the measurement translates to an approximate 13% change in CVD related events, such as myocardial ischemia and stroke [6]. However, novel assessment strategies beyond this traditional FMD approach have the potential to provide further mechanistic insight into CVD risk, as well as overall cardiovascular health and fitness.

Recent evidence suggests that assessing an artery’s vasoconstrictor responsiveness to various physiological stimuli may also be clinically relevant [5, 8-11].
Importantly, a diminished vasoconstrictor response of an artery may lead to alterations in blood flow distribution, as occurs during physical stress (e.g., exercise) [9]. For example, during strenuous exercise blood flow is redistributed to metabolically active skeletal muscle through dilation of these vascular beds, and constriction in non-contracting tissue (e.g., visceral organs, and non-active skeletal muscle). A phenomenon known as low-flow mediated constriction (L-FMC), has recently emerged as a measure of artery vasoconstrictor responsiveness during pneumatic cuff occlusion, as would be carried out during an FMD test [7]. The nadir diameter induced by the low-flow and shear is expressed as a percentage decrease in artery diameter from baseline to pre cuff-inflation [7], and is thought to represent an index of resting vascular tone [8]. While FMD provides information pertaining to shear stress-induced vasodilation, the L-FMC response provides supplementary information regarding the roles that shear stress and arterial tone have on resting artery diameter [7].

Pairing FMD with L-FMC yields a vasoactive range [8]. This concept was originally reported by Black et al., who determined maximal artery vasodilation following FMD and localized heating of the hand, and nadir artery diameters were obtained via L-FMC [8]. The authors suggested that use of a vasoactive response range allows for standardization of resting diameter by calculating arterial tone from the minimum and maximum diameter values obtained [8]. Arterial tone is thought to reflect the influence of sympathetic nerve activity [8, 10]. Artery vasoconstrictor responses can also be assessed non-invasively using sympatho-excitatory procedures. Tests which activate the sympathetic nervous system to induce a vasoconstrictor responses in an artery (e.g., cold pressor test; CPT), result in alpha-adrenergic mediated vasoconstriction.
(i.e., decrease in artery diameter) [10, 12]. For example, the CPT consists of submersion of a subject’s limb (e.g., hand or foot) in a cold water for 1-2 minutes while measuring diameter in the opposite limb using Doppler-ultrasound before, during and after limb submersion [12].

While the vasoconstrictor and vasodilator responses measured independently of each other can provide valuable information, the combination of the two responses may provide a more expansive ‘barometer’ of vascular health. The “vasoactive range” proposed by Black et al. [8] accomplishes this to a degree, whereas, other studies have assessed vasoactive responses using FMD and a sympatho-excitatory maneuver, such as the CPT. This latter model, termed, “vascular operating range”, represents an overall range by which a conduit artery operates in response to increased shear and sympathetic activation [9, 12]. Welsch et al. [12] indicate that the vascular operating range may lead to the development of a more representative depiction of vascular health and fitness. Nonetheless, the relationship between any proposed vasoactive range index (i.e., vasodilation + vasoconstriction) and cardiovascular health and fitness is currently unknown.

Therefore, the purpose of the present study is to determine the sensitivity of the vasoactive range in determining cardiovascular fitness and health. It is hypothesized there will be a direct relationship between the vasoactive range and aerobic fitness levels in healthy young individuals.
Chapter 2: Literature Review

A. Background

Cardiovascular disease (CVD) is a considerable threat to public health in the United States, given this disease takes the largest toll on human death each year (CDC 2014). Dysfunction within the vascular endothelium is considered a precursor to this threat, and can serve as an early indicator of CVD development, including atherosclerosis and coronary artery disease [1-3, 5]. Noninvasive assessments of vascular endothelial function can be of important clinical value, thus, research efforts have focused on developing noninvasive biomarkers of vascular health. The purpose of this literature review will be to focus a discussion on some current methodologies used to assess vascular endothelial function in humans. In this, the functional indices, validity/reliability, and limitations of each assessment strategy will be presented to delineate the most accurate and up-to-date practices for current researchers. Finally, the clinical utility of a vasoactive range will be questioned in the form of a proposal of study.

B. Current Noninvasive Vascular Assessment Strategies

The following five assessment strategies will be discussed: 1) Flow Mediated Dilation, 2) Low-Flow Mediated Constriction (L-FMC), 3) the Cold Pressor Test (CPT), 4) the “vasoactive range”, and 5) the “vascular operating range”.

Flow Mediated Dilation

Flow mediated dilation (FMD) is considered the non-invasive ‘gold standard’ for assessing vascular endothelial function in humans [3, 4]. This technique examines an artery’s vasodilatory responsiveness to an increase in blood flow-induced shear stress [4].
In this technique, a pneumatic cuff (220 mmHg) is inflated to occlude an artery – the brachial artery in this study – for a duration of five minutes [4], while baseline (resting) diameter is measured for 1-2 minutes prior to occlusion cuff inflation [4]. While the artery is occluded, a high-resolution Doppler-ultrasound probe is positioned over the artery, proximal to the cuff. Following deflation of this cuff, a hyperemic response ensues, characterized by a large increase in blood velocity and shear stress on the artery wall.

Shear stress is the tangential force of blood flowing across the artery lumen, and is a potent stimulant for vasodilation [4]. This shear stress induces the endothelial cells to produce chemicals via signal transduction. These chemicals, including nitric oxide, result in the relaxation of the smooth muscle cells within the artery [10]. This process is monitored by the Doppler-ultrasound machine to obtain vessel diameters and blood velocity signals. These data are typically analyzed using specialized edge-detection software [4]. The FMD response is presented as a maximal percentage increase in diameter [7], along with shear stress, which is calculated using velocity and diameter data [4]. Shear stress is derived using the following formula: 

\[ \frac{4 \eta V_m}{D} \]

where \( \eta \) is blood viscosity, \( V_m \) is the average blood velocity, and \( D \) is the average artery diameter [13]. In humans, shear stress is estimated using the shear rate calculation: 

\[ 2(2 + n)V_m \cdot D^{-1} \]

where \( n \) represents the shape of a parabolic velocity profile (\( n=2 \)), a normal assumption when determining shear rate [14].

Previous work has shown that normalizing FMD to shear rate area-under-the-curve (AUC) may help to account for inter-subject variability [13]; however, the most accurate method of FMD normalization is still under investigation [4]. Importantly, the
FMD technique has high prognostic value, such that for every 1% increase in FMD translates to an approximate 13% decrease in CVD related events, such as myocardial ischemia and stroke [6].

Current research shows that the FMD technique is a valid and reliable index of vascular endothelial function in humans. For example, previous work has demonstrated that FMD in the brachial artery strongly correlates with endothelial function in coronary arteries [15, 16], as well as coronary artery stenosis [17]. The FMD technique has also been shown to be highly reliable when performed under controlled experimental conditions. For example, intra-class correlation coefficients of 0.92, 0.94, and 0.90 for days, testers, and readers, respectively, have been reported for brachial artery FMD [11]. Several studies have utilized the FMD technique [3, 8, 10, 12, 16, 18], and recently, an updated guidelines consisting of methodological considerations have been published for this technique [4, 19]. However, a primary limitation to the FMD technique is that it examines only one aspect of vascular function, vasodilator capacity.

**Low-Flow Mediated Constriction**

Low-flow mediated constriction (L-FMC) is unique way to examine vasoconstrictor responses of large conduit arteries. Similar to FMD, the L-FMC is assessed using Doppler-ultrasound; in fact, it can be performed during the FMD test [7]. In the final 30 seconds of cuff occlusion – when blood flow and shear stress are minimal – the artery diameter is measured to account for any change from baseline that may have occurred during cuff-occlusion [7]. The precise underlying mechanism of L-FMC is unclear; however, a low shear-mediated imbalance between vasodilatory (e.g., nitric oxide or prostaglandin formation) and vasoconstrictor (endothelin-1 and sympathetic
nervous activity) factors may be playing a role [7, 20]. This L-FMC is typically expressed as a percentage decrease in arterial diameter [7]. Thus, L-FMC provides supplementary information in regards to the role shear stress has on baseline artery diameter conditions, such as arterial tone [7].

In terms of reliability, L-FMC is a highly reproducible measure. In fact, previous work demonstrates that the intra-class correlation and range of variation for this measurement are 0.8 and 1.1%, respectively [7]. While L-FMC can serve as an index of resting vascular tone, it does not take into account vasoconstrictor responses elicited directly from sympathetic nervous system activation, as would occur during physical stress.

**Cold Pressor Test**

An artery’s vasoconstrictive response may also be elicited from sympathetic nervous system activation. For example, the cold pressor test (CPT) is a non-invasive test that activates the sympathetic nervous system to induce vasoconstriction via stimulation of alpha-adrenoreceptors in an artery [10, 12, 21]. The CPT consists of submersion of a subject’s limb (e.g., hand or foot) in cold water for a period of time (1-2 minutes) and measuring the diameter of the opposite limb during and after submersion with Doppler-ultrasound [12]. Previous research has shown that CPT induces a large increase in sympathetic nerve activity, even more so than other symptho-excitatory maneuvers (e.g., mental stress tasks or lower-body negative pressure) [10]. Importantly, cardiovascular responses to the CPT, such as heart rate and blood pressure, are sensitive in detecting coronary artery disease risk [5].
Current literature indicates that the CPT test can be a valid and reliable maneuver to increase sympathetic nervous system activity. For example, Credeur (2012) performed a test-retest protocol for heart rate and artery diameter responses to the CPT and obtained an intra-class correlation coefficient of 0.90 [9]. Furthermore, the blood pressure reaction to the CPT was reported to be a reproducible quantification of cardiovascular responses to stress over a standardized time interval [22]. Other studies have demonstrated that the CPT test can maintain high long-term stability as a cardiovascular stressor [21, 23]. One limitation to the CPT is that the cold nature of the water may be uncomfortable for some subjects, but manageable for others. Nevertheless, Dyson (2006) found that the pain seemed to subside to numbness in subjects after two minutes of limb submersion [10]. Thus, 2-minutes of CPT is a sufficient duration to elicit reflex-sympathetic activation. 

**Vasoactive Range**

The combined result of FMD plus L-FMC yields a vasoactive range, which was originally reported by Black et al. [8]. In this work, posterior-tibial artery dimensions were examined during cuff-occlusion, during reactive hyperemia, and also, following local heating of the leg (44°C water for 10 minutes). Local heating induces increases in skin blood flow, with subsequent vasodilation of larger upstream conduit arteries, in a biphasic manner; first, a rapid increase in vessel diameter within the first 3-4 minutes, followed by a further incremental increase up to a maximal response (≥10 minutes of local heating) [24]. Importantly, this maximal diameter response may reflect the physiological ceiling of the vasoactive range, similar to a diameter response achieved during pharmacologically-induced vasodilation (e.g., nitroglycerine) [8, 19]. Utilizing
this vasoactive range allows for standardization of resting diameter by calculating arterial tone from the minimum, baseline and maximum diameter values [8].

Arterial tone is, in essence, a reflection of resting artery diameter position within its vasoactive range, which is mediated through a balance between vasodilatory (e.g., shear stress) and vasoconstrictor (e.g., sympathetic nervous activity) influences [8]. Importantly, from a clinical stand-point, the combined vasoactive response (FMD + L-FMC) may be related to cardiovascular risk, such that individuals with multiple risk factors (e.g., hypertension and obesity), experience a smaller response range [25].

While fewer studies have reported on the vasoactive range [8, 14, 26, 27], the combined FMD + L-FMC appears to be a valid index of cardiovascular health. For example, it has been demonstrated that overall vasoactive reactivity is more sensitive in detecting cardiovascular disease risk, as compared to FMD or L-FMC alone [25, 27]. The vasoactive range has also been shown to be sensitive to training status. For example, healthy young individuals demonstrated a greater L-FMC and vasoactive range, but not FMD, post interval training [28].

Vascular Operating Range

Similar to the vasoactive range, the “vascular operating range” is a representation of overall artery function, but differs from the vasoactive range in that a sympatho-excitatory maneuver, the CPT, is used for the vasoconstrictor response instead of L-FMC. The vascular operating range represents a physiological limit by which a single conduit artery responds to vasodilatory and vasoconstrictor stimuli [9, 12]. Interestingly, this measure appears to be sensitive to exercise training status. For example, Welsch et al. found that the vascular operating range was larger in power trained athletes than in age-
matched controls [12]. The authors indicate that both vasodilator and vasoconstrictor responses are central to effective blood distribution during an exercise. Thus, vasoactive range indices may be sensitive discriminators of aerobic fitness. Importantly, the vascular operating range has been reported to be diminished in patients with cardiovascular disease, but localized exercise training can modulate this impairment [9].

D. Purpose and Hypothesis

While FMD has been reported to be a valid, reliable and sensitive marker of cardiovascular health, it is currently unknown whether examining an overall range of vascular responses (i.e., peak to nadir) to various physiological stimuli can provide a more comprehensive assessment of cardiovascular fitness and health. Thus, the purpose of the present study was to determine the sensitivity of vasoactive range indices in detecting aerobic fitness. It is hypothesized there will be a direct relationship between the vasoactive range and aerobic fitness levels in healthy young men.
Chapter 3: Methods

A. Study Design and Subjects

This study utilized a cross-sectional design. A total of 14 healthy male subjects between the ages of 18 and 35 years of age were recruited from the University of Southern Mississippi (USM), and the surrounding Hattiesburg, MS area. Participants were equally divided into a High Fit group (HF), and a Low Fit group (LF) based on aerobic exercise tests results performed during the pre-test screening.

B. Experimental Protocol and Procedures

All subjects signed an informed consent approved by the Institutional Review Board of the University of Southern Mississippi prior to completing any facet of the study. Participants also completed a medical history and physical activity level questionnaire. All screening and testing were carried out in the School of Kinesiology at USM. All testing was conducted in a dimly-lit, climate-controlled laboratory (temperature=21-23°C, humidity=50%).

Pre-Test Screening

Prior to experimental visits, a pre-test screening was performed to characterize fitness levels and to familiarize subjects with the laboratory and experimental procedures (Figure 1A). In addition, vascular scanning was performed with the ultrasound machine to ensure the attainment of quality images for ultrasound recordings. Height, weight, estimated percent body fat (determined by Bioelectrical Impedance- BIA), and resting blood pressure were obtained from all participants. Each participant underwent a series of
tests to determine physical fitness, including a handgrip strength test (dynamometry), a 3-min step test (heart rate recovery), and a YMCA cycle test (extrapolated VO₂ peak).

Figure 1. Schematic showing timeline for screening and experimental protocols: (A) Following subject arrival the informed consent, followed by anthropometrics and resting vitals were administered, then finally fitness testing. (B) Following initial set-up, vascular testing was performed including the FMD, CPT and Warm Bath. BIA, Bioelectrical Impedance Analysis; MVC, Max Voluntary Contraction; FMD, Flow-mediated Dilation; CPT, Cold Pressor Test.

Handgrip strength was assessed using a hand dynamometer (Jamar, Patterson Medical). The subject was instructed to perform a max voluntary contraction (MVC), standing with the dynamometer at their side and squeezing as hard as they could for approximately 3 seconds. This procedure was repeated for 3-5 trials on the dominant hand only. The average of the highest 3 values obtained was considered maximum handgrip strength.

Heart rate recovery from the YMCA 3-min step test (HR peak – 60 sec post-stepping) was carried out as outlined in the ACSM’s Guidelines for Exercise Testing and
Prescription [12]. For this test, participants were instructed to step on and off of a 12-inch box, altering legs, at a pace of 96 beats per minute set using a metronome, which translates to ~24 steps per minute [29]. Heart rate was read within 5 seconds of completion of the test using a heart rate monitor (Polar FS1), as well 1-minute after completion of stepping [29].

Following the step-test, a YMCA cycle test, which is a 12-min submaximal test, was performed using a leg ergometry unit (Monark, 828-E). In brief, the test consists of the participant pedaling an ergometer at a constant cadence (50 rpm’s), while exercise intensity is adjusted based heart rate responses for each 3 minute stage. VO2 peak is then extrapolated using heart rate measures obtained during the submaximal workloads, and the participant’s estimated heart rate max (e.g., 220-age), as previously described [30]. This test has been shown to provide a valid index of aerobic fitness [31-33].

Experimental Study Visit

The protocols for the experimental visit are summarized in a schematic in Figure 1B. Prior to experimental study visits, all participants were fasted and refrained from caffeine intake for 12 hours, and heavy exercise and alcohol for 24 hours. The participants were studied in a semi-recumbent position on an examination table, and instrumented with surface electrocardiography (ECG; GE Logiq 7), a blood pressure cuff on the upper right arm, and Doppler ultrasound (GE Logiq 7). The ECG was used to monitor the participant’s heart rate throughout the experimental procedures. The blood pressure cuff was used to determine systolic, diastolic and mean arterial pressure. The Doppler ultrasound machine was used to acquire brachial artery diameter and blood velocity signals. All measurements involving the use of an ultrasound for imaging and
analyzing arteries were performed according to published guidelines [4]. Importantly, all ultrasound measures and analyses were performed by the same study personnel.

*Flow-mediated Dilation (FMD)*

After instrumentation, the subjects rested quietly for 30 minutes, after which a 2 minute baseline ultrasound recording was performed on the brachial artery of the right arm. Next, a blood pressure cuff placed distal from the brachial artery on the forearm was inflated for 5 minutes to a supra-systolic pressure (220 mmHg). Ultrasound recordings commenced 30 seconds prior to, and until 3 minutes post-deflation of the occlusion cuff. The baseline diameter was defined as the average diameter of the two minutes measured following the resting period. The peak dilation was defined as the absolute change (mm) and percent change (%) between the largest diameter after cuff occlusion and baseline diameter [12].

*Low-Flow Mediated Constriction (L-FMC)*

The L-FMC diameter value was defined as the absolute (mm) and percent change (%) from the nadir diameter to baseline diameter in the last thirty seconds of the five-minute cuff occlusion period of the FMD measure [7].

*Cold Pressor Test (CPT)*

Following an additional ten minutes of rest after the FMD test, an additional 1 minute of baseline was recorded on the ultrasound machine prior to the subject’s opposite hand being placed in ice water for 2 minutes. Continuous diameter and blood velocity signals were obtained during the two minutes of CPT, and for 1 minute following removal of the hand from ice water. The nadir diameter obtained during the 2-minutes of
CPT was expressed as an absolute (mm) and percent change (%) from the pre-CPT baseline [12].

Maximal Vasodilation

Following the CPT test, an additional 10 minutes of rest was given to allow brachial artery diameter and blood flow to return to resting conditions, which was confirmed using the auto-calculation feature on the ultrasound machine. The subject’s hand (same limb as FMD) was then be placed into a warm bath (42-45 degrees Celsius), for 10 minutes. Ultrasound recordings were obtained for the last 2 minutes of this warm bath procedure, and averaged for the peak response to heating.

Data Analysis

All ultrasound data were acquired at 30-Hz using an analog video capture unit (Elgato software downloaded to Mac Book). These data were then analyzed off-line using an automated edge-detection software (QUIPU, Cardiovascular Suite) in accordance with guidelines [4]. Data were analyzed by playing the pre-recorded videos through the Cardiovascular Suite software. A region of interest (ROI) was selected on each video to ensure a clear portion for the edge-detection software to analyze brachial artery diameters. Blood velocity signals were also simultaneously analyzed using the software by obtaining the flow-envelope for both the antegrade (positive area) and retrograde velocity signals (negative area). Mean velocity was defined as the difference between antegrade and retrograde velocity signals. Shear rate (s\(^{-1}\)) was calculated as 8*mean velocity/diameter. Shear rate area-under-the-curve (AUC) up until peak diameter was calculated as the stimulus for FMD (FMD% / AUC), as previously described [4].
The vasoactive range indices were determined by examining the peak and nadir diameter values obtained from the aforementioned physiological stimuli. Both the traditional “vasoactive range [8]”, the more recent “vascular operating range [9, 12]”, and finally, an overall range (absolute peak – absolute nadir) were calculated as follows:

1. ‘Traditional’ Vasoactive Range: The peak value was defined as the highest diameter achieved during hyperemia of the FMD test, and nadir was defined as the minimum value obtained from L-FMC, both expressed as relative (%) and absolute changes (mm). Resting vascular Tone (%), which represents the baseline diameter expressed as a percentage on the vasoactive range was calculated as follows:

   \[ \text{Tone} \% = \frac{(D_{\text{peak}} - D_{\text{rest}})}{(D_{\text{peak}} - D_{\text{nadir}})} \times 100 \]

   …where \(D_{\text{peak}}\) represents the peak diameter achieved following cuff release; \(D_{\text{rest}}\) is resting diameter, and \(D_{\text{nadir}}\) is the minimum diameter achieved during cuff occlusion.

2. Vascular Operating Range: The peak value was defined as the highest diameter achieved during hyperemia of the FMD test, and nadir was defined as the minimum diameter response to CPT, both also expressed as relative (%) and absolute changes (mm).

3. ‘Absolute’ Vasoactive Range: The peak value was defined as the largest response achieved through either FMD or the warm bath, and the nadir was defined as minimum diameter response to either L-FMC or CPT, all expressed as relative (%) and absolute changes (mm).
Statistical Analysis

All statistical analyses were performed using S.P.S.S. software. To examine the sensitivity of the above described vasoactive range indices in detecting aerobic fitness, the following statistical tests were performed: independent samples T-tests were utilized to compare all vascular parameters between the high fit (HF) and low fit (LF) groups. Furthermore, correlational analyses were performed between L-FMC and aerobic fitness test results (i.e., HR recovery and VO₂ peak). Additional independent samples T-tests were utilized to compare other demographic variables between the HF and LF groups. Statistical significance was set at p<0.05.
Chapter 4: Results

Subjects

Demographical and aerobic fitness test results are summarized in Table 1.

Resting heart rate (HR), and diastolic blood pressure (DBP) were lower, while HR recovery, VO\textsubscript{2} peak, and physical activity scores were greater in HF as compared to LF (P<0.05).

**Table 1.** Participant Demographics

<table>
<thead>
<tr>
<th>Metric</th>
<th>High Fit</th>
<th>Low Fit</th>
<th>P-value</th>
</tr>
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<tr>
<td>Age (years)</td>
<td>21±2</td>
<td>22±5</td>
<td>0.505</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>23±1</td>
<td>24±3</td>
<td>0.542</td>
</tr>
<tr>
<td>HR rest (bpm)</td>
<td>47±5</td>
<td>59±9*</td>
<td>0.011</td>
</tr>
<tr>
<td>SBP rest (mmHg)</td>
<td>111±5</td>
<td>117±8</td>
<td>0.134</td>
</tr>
<tr>
<td>DBP rest (mmHg)</td>
<td>63±6</td>
<td>72±4*</td>
<td>0.004</td>
</tr>
<tr>
<td>Lean Mass (pounds)</td>
<td>139±19</td>
<td>140±13</td>
<td>0.870</td>
</tr>
<tr>
<td>Fat Mass (pounds)</td>
<td>16±7</td>
<td>25±13</td>
<td>0.121</td>
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<td>Bodyfat (%)</td>
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<td>14±7</td>
<td>0.230</td>
</tr>
<tr>
<td>MVC (kg)</td>
<td>50±8</td>
<td>52±5</td>
<td>0.572</td>
</tr>
<tr>
<td>HR recovery (bpm)</td>
<td>38±12</td>
<td>24±9*</td>
<td>0.028</td>
</tr>
<tr>
<td>VO\textsubscript{2} peak (ml/kg/min)</td>
<td>55±10</td>
<td>38±6*</td>
<td>0.002</td>
</tr>
<tr>
<td>Physical Activity Score (AU)</td>
<td>78±22</td>
<td>37±15*</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Denotes significantly different from High Fit (P<0.05). BMI—body mass index, HR—heart rate, SBP—Systolic Blood Pressure, DBP—Diastolic Blood Pressure, MVC—Maximal Voluntary Contraction

Relationship between the Vasoactive Range and Aerobic Fitness

A comparison of absolute vascular parameters (e.g., mm) between the two fitness groups are presented in Table 2. No significant differences were observed between the HF and LF group for any vasoactive range measure. However, the HF group tended to have a greater L-FMC (mm) response (P=0.057), whereas, resting vascular Tone % tended to be greater in the LF group (HF=72±17 vs. LF=96±25%, P=0.055) (Figure 2). A correlational analysis revealed an inverse relationship between L-FMC% and HR.
recovery ($r=-0.631$, $P=0.016$), but not between L-FMC% and VO$_2$ peak ($r=-0.383$, $P=0.177$) (Figure 3).

### Table 2. Vascular Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High Fit</th>
<th>Low Fit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter Rest (mm)</td>
<td>3.91±0.44</td>
<td>3.76±0.45</td>
<td>0.534</td>
</tr>
<tr>
<td>Diameter Peak; FMD (mm)</td>
<td>4.16±0.52</td>
<td>4.05±0.47</td>
<td>0.670</td>
</tr>
<tr>
<td>Diameter Peak; Heating (mm)</td>
<td>4.11±0.46</td>
<td>4.01±0.50</td>
<td>0.710</td>
</tr>
<tr>
<td>Diameter Nadir; L-FMC (mm)</td>
<td>3.82±0.46</td>
<td>3.75±0.50</td>
<td>0.769</td>
</tr>
<tr>
<td>Diameter Nadir; CPT (mm)</td>
<td>3.83±0.44</td>
<td>3.79±0.58</td>
<td>0.888</td>
</tr>
<tr>
<td>FMDΔ; Peak - Rest (mm)</td>
<td>0.25±0.11</td>
<td>0.28±0.05</td>
<td>0.442</td>
</tr>
<tr>
<td>L-FMCAΔ; Rest - Nadir (mm)</td>
<td>0.095±0.06</td>
<td>0.02±0.07</td>
<td>0.057</td>
</tr>
<tr>
<td>Heating Δ; Peak - Rest (mm)</td>
<td>0.19±0.14</td>
<td>0.25±0.13</td>
<td>0.466</td>
</tr>
<tr>
<td>CPT Δ; Rest - Nadir (mm)</td>
<td>-0.09±0.08</td>
<td>0.03±0.18</td>
<td>0.148</td>
</tr>
<tr>
<td>FMD + L-FMC (mm)</td>
<td>0.34±0.11</td>
<td>0.30±0.05</td>
<td>0.422</td>
</tr>
<tr>
<td>FMD + CPT (mm)</td>
<td>0.34±0.14</td>
<td>0.26±0.15</td>
<td>0.34</td>
</tr>
<tr>
<td>Maximum - Minimum (mm)</td>
<td>0.39±0.13</td>
<td>0.37±0.06</td>
<td>0.62</td>
</tr>
<tr>
<td>Mean Shear Rate; Baseline (s$^{-1}$)</td>
<td>176±129</td>
<td>230±95</td>
<td>0.395</td>
</tr>
<tr>
<td>Shear Stimulus; FMD (AUC)</td>
<td>26325±11944</td>
<td>28784±6013</td>
<td>0.635</td>
</tr>
<tr>
<td>Peak Shear Rate; FMD (s$^{-1}$)</td>
<td>1095±305</td>
<td>1172±198</td>
<td>0.584</td>
</tr>
<tr>
<td>Shear Rate at Peak Heating Diameter (s$^{-1}$)</td>
<td>398±97</td>
<td>512±236</td>
<td>0.262</td>
</tr>
<tr>
<td>Shear Rate at Nadir CPT Diameter (s$^{-1}$)</td>
<td>104±43</td>
<td>124±105</td>
<td>0.648</td>
</tr>
</tbody>
</table>

FMD-flow-mediated dilation, L-FMC-low-flow mediated constriction, CPT-Cold Pressor Test.
Figure 2. Comparison of brachial artery flow-mediated dilation percent change [BAFMD] (Panel A), low-flow mediated constriction percent change [L-FMC] (Panel B), and resting vascular Tone % (Panel C) between High (black bars) and Low Fit (white bars) participants.
Figure 3. Scatter plots depicting results from Pearson Product Moment correlations between HR recovery and L-FMC% (Panel A), and VO2 Peak and L-FMC% (Panel B). *Denotes a significant inverse relationship between variables (P<0.05).
Chapter 5: Discussion

The primary purpose of this study was to evaluate different indices of the vasoactive range, and to compare them between individuals categorized as having high and low aerobic fitness. We hypothesized that the vasoactive range would be greater in high fit individuals; however, this was not the case. Thus, these measures do not appear to be sensitive in discriminating aerobic fitness in healthy young men. However, one unique finding was that the higher fit individuals tended to have a greater vasoconstrictor response to reductions in blood flow, whereas, vasodilatory function was similar between the groups. Furthermore, low-flow mediated constriction (L-FMC) was inversely correlated with aerobic fitness, as defined by HR-recovery from a 3-minute step-test, and estimated VO₂ peak from a YMCA cycle ergometry test. Collectively, these data demonstrate that L-FMC may be a more sensitive biomarker of aerobic fitness in healthy young men as compared to other vasoactive range indices.

Vasodilatory Function and Aerobic Fitness

Previous work has evaluated endothelial-dependent vasodilator (i.e., FMD) function among individuals of varying fitness status and age. In line with a recent meta-analysis, our results demonstrate that vasodilator function is not sensitive to fitness status in healthy, young men [34]. For example, Montero and colleagues [34] reported that differences in artery vasodilator function between athletes and age-matched controls were only found in the older subjects. The authors attributed this finding to be the result of the vasculature not being adaptable to exercise training, unless impairment already exists (i.e., aging or disease). Therefore, in the present study, vascular function may have
already been normal in these healthy young men, despite the differences in their aerobic fitness.

Our data also demonstrate that maximal vasodilation to heating was similar between aerobic fitness groups in healthy young men. On the contrary, previous work has demonstrated that skin vasodilator responses to local forearm heating is greater in aerobically trained individuals [24]. In that study, local heating was shown to induce an initial rapid increase in forearm skin vasodilation (i.e., first 10 minutes of heating; ~42°C), followed by an incremental increase up until a plateau around 35 minutes, an effect that was more pronounced in aerobically trained individuals. Based on this evidence, we would hypothesize that a greater skin vasodilation would subsequently induce a greater up-stream brachial artery dilation.

An alternative explanation for the lack of difference noted for heating induced vasodilation between groups in the present study could be that endothelial-independent vasodilatory function (i.e., smooth muscle function) was similar. For instance, a recent meta-analysis demonstrated that maximal vasodilation to endothelial-independent vasodilation (i.e., sodium nitroprusside iontophoresis) was not associated with VO2 max in healthy young individuals [35]. Therefore, despite any differences in skin blood flow responses to heating between fitness groups [24], maximal brachial artery vasodilation to heating would be similar.

Vasoconstrictor Function and Aerobic Fitness

Low-flow mediated Contraction (L-FMC) has recently emerged as an important marker of cardiovascular health in humans, because it provides supplemental information to vasodilator responses induced through FMD [7]. To our knowledge, our study was the
first to directly compare differences in L-FMC between high and low aerobically fit young men. Although no statistical differences were noted, the higher fit individuals demonstrated a trend for a greater L-FMC response (P=0.057). A correlational analysis revealed an inverse relationship between L-FMC and heart rate recovery, suggesting a potential influence of fitness on vasoreactivity in healthy young men. In agreement with our data, one recent study showed that heavy and moderate intensity interval training leads to a greater L-FMC response [28]. The authors suggested that the increased L-FMC may have been due to either increased production of vasoconstrictor substances, or a decrease in vasodilator substances; in other words, the authors suggest that the interval training altered the balance between vasoactive molecules [28], thus, promoting greater vasodilation at rest. This finding may explain why resting Tone% was greater in the low fit group of the present study. Tone% has been shown to reflect balance between vasodilatory and vasoconstrictor influences [8], and this balance seems to favor more vasodilation in high fit (i.e., less tone) individuals.

In regards to vascular responses to sympatho-excitation (i.e., CPT), limited evidence exists discerning the influence of aerobic fitness status. Our data show that brachial artery diameter changes to CPT were not different between the two groups. Indeed, previous evidence demonstrates that cardiac responses to CPT are not different between athletes and healthy controls [36]. Therefore, the stimulus for vasoconstriction (i.e., sympatho-excitation) to a localized cold stimulus may not have been different between the two groups in the present study.

*Relationship between Vasoactive Range Indices and Aerobic Fitness*
Conflicting evidence exists regarding the association between the vasoactive range and exercise training status in healthy young individuals. In the present study, it was hypothesized that higher fit individuals would exhibit greater vasodilatory, and vasoconstrictor responsiveness, thus a larger vasoactive range, which was not the case. On the contrary, previous work examining the “vascular operating range”[12] demonstrates that power athletes have a larger degree of vasoreactivity. The present study assessed recreationally active individuals, and not competitive athletes. Thus, there may be unique vascular adaptations to heavy weightlifting that would promote a larger vasoactive range following training [37]. Furthermore, a similar study comparing the vascular operating range between heart failure patients, and age-matched controls found a blunted range in the patient group. Interestingly, four weeks of localized handgrip training improved vasoreactivity in both patients and healthy controls [9]. Thus, the vascular operating range does appear to be sensitive to training status [9].

For the present study, a potential rationale for the similarity in overall vasoreactivity between the high and low fit individuals, could simply be that there was not a large enough separation in fitness levels for the groups. Future studies should further examine the vasoactive range indices between competitive athletes (e.g., distance runners) and age matched healthy controls, in order to further discern potential aerobic fitness differences in overall vascular responsiveness.

Limitations

There are some limitations in the present study that should be discussed. Firstly, all participants were recreationally active, which means that athletic and non-athletic populations were not directly evaluated. Additionally, the aerobic tests used to
characterize fitness were sub-maximal, and direct measures of oxygen consumption were not performed. Finally, the sample size for this study design may have been limited. Indeed, a larger sample size may have provided greater statistical power for examining differences between the fitness groups.
Chapter 6: Conclusion

In the present study, we found that the vasoactive range was not a sensitive measure for discriminating aerobic fitness levels in healthy young men. Furthermore, vasodilatory function was similar between high and low fit individuals. However, one unique finding was that higher fit individuals tended to have a greater vasoconstrictor response to reductions in blood flow. Finally, low-flow mediated constriction was inversely related to aerobic fitness levels, as defined by HR-recovery from a 3-minute step-test. Future studies should further evaluate the clinical utility of vasoactive range indices in discriminating cardio-metabolic risk among patient populations.
References


## Medical History Form

All of the information provided in this form is voluntary.

**Date:**

**Last Name** | **First** | **MI**
--- | --- | ---

**Occupation:**

**Home Phone:**

**Work:**

**Cell/Pager:**

**Address:**

**DOB:** / /  

**Age:**  

**Gender M / F**  

**Height:**  

**Weight:**

**Highest Education Achieved:**

**Race:**
- [ ] **Hispanic or Latino** - A person of Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin," can be used in addition to "Hispanic or Latino."  
- [ ] **American Indian or Alaska Native** - A person having origins in any of the original peoples of North, South, or Central America, and who maintains a tribal affiliation or community attachment.  
- [ ] **Asian** - A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in the previous data collection strategies.)  
- [ ] **Black or African American** - A person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black" or "African American."  
- [ ] **Native Hawaiian or Pacific Islander** - A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific islands.  
- [ ] **White** - A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

**Primary Care Physician:**

**Name:**

**Address:**

**Emergency Contact:**

**Name:**

**Relationship:**

**Phone #:**

**Medications:** Include over the counter, drugs, oral contraceptives, dietary supplements.

**Name/Dosage/How often taken:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Dosage</th>
<th>How often taken</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Allergies:**

| Allergies | |
|-----------|
|           |

**Smoking History:**

- Do you smoke?  
  - [ ] Cigarettes?  
  - [ ] Pipe / Cigar?  
  - [ ] Other?  
  - If you quit, what year did you quit?  

**# of packs smoked per day:**  

**For how many years:**
Appendix B

Godin Leisure-Time Exercise Questionnaire

INSTRUCTIONS

In this excerpt from the Godin Leisure Time Exercise Questionnaire, the individual is asked to complete a self-explanatory, brief four-item query of usual leisure-time exercise habits.

CALCULATIONS

For the first question, weekly frequencies of strenuous, moderate, and light activities are multiplied by nine, five, and three, respectively. Total weekly leisure activity is calculated in arbitrary units by summing the products of the separate components, as shown in the following formula:

Weekly leisure activity score = (9 \cdot \text{Strenuous}) + (5 \cdot \text{Moderate}) + (3 \cdot \text{Light})

The second question is used to calculate the frequency of weekly leisure-time activities pursued "long enough to work up a sweat" (see questionnaire).

EXAMPLE

Strenuous = 3 times/wk
Moderate = 6 times/wk
Light = 14 times/wk

Total leisure activity score = (9 \cdot 3) + (5 \cdot 6) + (3 \cdot 14) = 27 + 30 + 42 = 99

Godin Leisure-Time Exercise Questionnaire

1. During a typical 7-Day period (a week), how many times on the average do you do the following kinds of exercise for more than 15 minutes during your free time (write on each line the appropriate number):

   Times Per Week

   a) STRENUOUS EXERCISE
      (HEART BEATS RAPIDLY)
      (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

   b) MODERATE EXERCISE
      (NOT EXHAUSTING)
      (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

   c) MILD EXERCISE
      (MINIMAL EFFORT)
      (e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snowmobiling, easy walking)

2. During a typical 7-Day period (a week), in your leisure time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

   OFTEN          SOMETIMES          NEVER/RARELY
   1.                2.                        3.
Appendix C

NOTICE OF COMMITTEE ACTION

The project has been reviewed by the University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the “Adverse Event Report Form.”
- If approved, the maximum period of approval is limited to twelve months. Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: 14102905
PROJECT TITLE: Reliability of the Vascular Operating Range and Predictive Value in Determining Cardiovascular Fitness
PROJECT TYPE: New Project
RESEARCHER(S): Daniel P. Croceur, Ph.D.
COLLEGE/DIVISION: College of Health
DEPARTMENT: Human Performance and Recreation
FUNDING AGENCY/SPONSOR: HPR Exercise Science
IRB COMMITTEE ACTION: Expedited Review Approval
PERIOD OF APPROVAL: 11/24/2014 to 11/23/2015
Lawrence A. Holman, Ph.D.
Institutional Review Board
### HUMAN RESEARCH APPLICATION PROCEDURES

Use this form to apply for IRB review. IRB approval is required before human subjects research can begin.

- In order to complete this form you will need both CITI certificates for all investigators, completed consent forms, and any survey instruments and funding permission letters. All of these documents must be attached as icons (see instructions at the bottom of this form) in order for this application to be accepted.
- Before completing this form, review the information included on the sample consent forms and FAQ section of the IRB website: [http://www.usm.edu/research/institutional-review-board](http://www.usm.edu/research/institutional-review-board).
- Student researchers must send the completed form with all attachments to Research Advisors (Honor's Thesis Advisor, Master's Thesis Director, or Dissertation Director) for review. Faculty researchers should send the form directly to department chairs.
- Student Research Advisors must review the form, attach their own CITI certifications, and provide authorization where indicated before sending the application to departmental chairs.
- Department chairs must review the completed form, provide authorization where indicated, and submit completed versions of this form and all necessary authorizations to irb@usm.edu using their Southern Miss email address.

### SECTION 1: INVESTIGATOR INFORMATION

<table>
<thead>
<tr>
<th>Project Title</th>
<th>Reliability of the Vascular Operating Range and Predictive Value in Determining Cardiovascular Fitness</th>
</tr>
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<tbody>
<tr>
<td>Principal Investigator</td>
<td>Daniel P. Credeur, Ph.D.</td>
</tr>
</tbody>
</table>

| Campus ID | 944855 |
| College | Health |
| Department | HPR |
| Department Phone | |

**Research Purpose (check one):**

- [ ] Undergraduate project
- [ ] Honors Thesis Project
- [ ] Graduate project
- [ ] Faculty or staff research

<table>
<thead>
<tr>
<th>Student Research Advisor (if applicable)</th>
<th>Funding Agency/Sponsor (if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name: Daniel Credeur</td>
<td>Organization: HPR Exercise Science</td>
</tr>
<tr>
<td>Phone: 266-6303</td>
<td></td>
</tr>
</tbody>
</table>

**USM Email**: daniel.credeur@usm.edu

**Grant #:**

Describe your expertise and qualifications related to this research:

Daniel Credeur (PI): My research focuses on understanding the physiological mechanisms governing blood flow distribution at rest and during varying modalities of exercise in human health and disease. To do this, my laboratory utilizes Doppler-Ultrasonography and application tonometry to non-invasively assess blood flow and peripheral vascular function and structure. I am proficient in vascular ultrasound scanning and have conducted numerous studies in various human subject populations (See Attached Articles). In addition, I have performed exercise related research in a variety of human subject populations including the elderly, healthy young, competitive athletes, metabolic disease and chronic heart failure patients. Finally, I am certified in CPR and AED through the American Red Cross.
Cardiovascular disease (CVD) is considered the number one cause of morbidity and mortality in the United States. Disruption within the vascular endothelium is believed to be an initiating event for CVD development, including atherosclerosis. The technique known as flow-mediated dilation (FMD) is considered the non-invasive ‘gold standard’ for assessing vascular endothelial function in humans, and is typically performed by examining an artery’s vasodilatory responsiveness to increases in blood flow and shear stress. This hyperemic response is mediated through temporary circulatory arrest (5 mins) of blood flow through a limb using a pneumatic blood pressure cuff inflated to a supra-systolic pressure (220 mmHg), and measuring the diameter and blood velocity, via Doppler-ultrasound of an adjacent artery (e.g. brachial artery during forearm occlusion: Figure 1). The concept of FMD implies that increases in arterial shear stress induce endothelial cell release of various vasodilators, which result in smooth muscle relaxation with subsequent vasodilation of an artery. The response is presented as a maximal percent increase in artery diameter from resting values along with shear-rate, which is calculated using blood velocity data. FMD has high prognostic value such that for every 1% increase in the measurement translates to a 13% decrease in CVD related events, such as myocardial ischemia and stroke. However, novel assessment strategies beyond the traditional FMD approach have the potential to provide added mechanistic insight into cardiovascular health and fitness.

An artery’s ability to vasoconstrict is also an important physiological response, and can be assessed non-invasively using a procedure known as the cold pressor test (CPT). This test activates the sympathetic nervous system to induce a vasoconstrictor response of an artery. The CPT consists of submersion of a person’s hand in cold water for 2 mins while measuring artery diameter in the opposite limb with Doppler-ultrasound. While the vasoconstrictor and vasodilator responses measured independently can provide valuable information regarding the health of the vessel, the combination of the two responses may provide a more expansive ‘barometer’ of vascular health. This vascular operating range (VOR), originally proposed by Credeur et al 2012 accomplishes this. However, the degree of reliability and sensitivity of the VOR in determining cardiovascular health and fitness is currently unknown.

The primary goal of the proposed study will be to first examine the reproducibility of the VOR assessment in humans. A secondary goal of this study will be to examine this vascular reactivity assessment among a group of healthy young participants categorized into high vs. low cardiovascular fitness as defined by standard physical fitness assessments. Collectively, these data will expand upon an important clinical assessment of vascular function, and may improve upon the sensitivity by which these traditional vascular assessments (vasodilation and vasoconstriction) can categorize cardiovascular fitness and disease risk in human subjects.

SECTION 2: RESEARCH PROCEDURES

Briefly describe the project and its goal(s) in two to three paragraphs.
Are any of the subjects under 18 years of age?
☐ Yes  ☐ No

Note: Parental consent is required for participants under the age of 18.

Describe subject population, number of subjects to be included, and criteria for selection.

The participants for this study will be recruited from USM and the surrounding Hattiesburg, MS area. The proposed studies will utilize a multiple tests approach for Protocol 1 to determine the inter-day reproducibility of the vascular assessment strategy described below. A cross-sectional design will be implemented for Protocol 2. A total of 30 healthy male subjects between the ages of 18 and 35 years of age will be recruited for this study, and 10 randomly selected subjects from this cohort will be studied for Protocol 1.

How will participants be recruited?

☐ Class announcement  ☐ Oral Announcement  ☐ Email announcement
☐ Poster campus advertisement  ☐ Television, Radio or Newspaper ad  ☐ Advertising Agency
☐ Other (explain): In addition to posted advertisement, we plan to recruit by word of mouth on campus and within the Hattiesburg, MS community. Attached is a document containing the verbiage we will use for oral recruitment and email announcements.

For adult subjects, how will you verify that individuals are over 18?

☐ Survey or interview
☐ No adults will be participating in this research
☐ Other (explain):

Indicate consent procedures (check all that apply)

☐ Information letter
☐ Oral presentation & Short Consent Form
☐ Long Consent Form
☐ Assent form (children or subjects with disabilities)
☐ Request for waiver of consent
☐ Not applicable

Detail procedures for obtaining participants’ consent or justify request for waiver.

Participants will fill out a University approved Informed Consent form.

How many interactions will be required with each subject?

1  ☐ 2 - 3  ☐ 4 - 9  ☐ 10 or more

Maximum length of each interaction

☐ Less than 10 minutes
☐ Less than an hour
☐ Less than three hours
☐ Three hours or more
☐ No direct interaction with subjects

Where will interactions take place?

☐ On campus
☐ Off campus
☐ Online

Indicate means of data collection (check all that apply)

☐ Personal Interview
☐ Questionnaire or survey
☐ Audio or video recording
☐ Behavioral Observation
☐ Focus Group Inquiry
☐ Other (explain below):

Do any of the following apply to your study?

☐ Use of human biological samples  ☐ Yes  ☐ No
☐ Use of physical exercise  ☐ Yes  ☐ No
☐ Medical examinations or procedures  ☐ Yes  ☐ No
☐ Use of drugs or biological products  ☐ Yes  ☐ No

Numerical data gained from instrumentation

Give a step by step explanation of human subjects data collection procedures.

Instrumentation

Surface Electrocardiography (ECG): After cleaning the skin with an alcohol swab, adhesive electrodes will be secured onto the skin of the shoulder, chest and oblique abdominal regions, and connected to ECG leads for the determination of heart rate and rhythm.

Blood Pressure Measurement: Blood pressure will be obtained non-invasively via automated sphygmomanometry which will consist of wrapping a blood pressure cuff around the participant’s left or right upper arm and inflated briefly to obtain arterial blood pressure values. This procedure will be repeated several times throughout the duration of the experiment.

Doppler Ultrasound: Arterial dimensions and blood velocity will be recorded non-invasively using a duplex...
Doppler ultrasound system (GE, PS) (Figure 2). This device consists of a probe placed over the skin of the upper and lower extremities in conjunction with a water-based gel to improve conduction of the ultrasound signal.

Research Procedures: A schedule of the procedures to be performed is depicted in Table 1.

Screening: Participants will report to the Laboratory of Applied Physiology located in the School of Human Performance and Recreation at their scheduled time. After providing informed consent, participants will complete a medical health history and physical activity questionnaire (Forms Attached: Appendix 1 and 2) followed by an in-lab screening and familiarization session to: 1) determine whether quality ultrasound images can be obtained, and 2) to familiarize the participant with the research laboratory and experimental procedures. In addition, physical fitness will be determined by the following assessments:

Height and Weight: Participant height and weight will be determined by standard methods including a scale and stadiometer.

Body Composition: The participant's percentage of body fat and lean body mass will be determined by a non-invasive process called bioelectrical impedance amplitude (BIA). During this test the subject will stand barefoot on a specialized scale for ~2 minutes while a very low level, imperceptible, electrical current is passed through their body. The magnitude of electrical impedance is used to estimate percentage of body fat and lean body mass (e.g., muscle). This is a widely used, and safe, commercial assessment of body composition (Figure 3).

Handgrip Strength: Handgrip strength will be measured using a handgrip dynamometer. The subject will be asked to squeeze as hard as possible on the device for 1 sec and a value will be recorded from the face gauge on the device (Figure 4). Three to five trials will be performed and the three highest values obtained will be averaged and considered maximal handgrip strength.

3-min Step Test: After resting quietly for 5 mins in a chair, a resting heart rate will be manually palpated from the subject's wrist over the radial artery. This will be determined by palpating the pulse at the wrist while using a stethoscope with a small cone on the radial artery. The step test requires that the individual step up and down, alternating legs on a standardized step height (12-20 inches) for 3 minutes at a cadence of 24 steps per minute. After 3 minutes of stepping are completed the participant will stop and another heart rate will be palpated immediately while the subject continues to stand. A third heart rate will be palpated 20 seconds into recovery from the 3 minute step test (Figure 5).

YMCA Cycle Test: The YMCA cycle test is a 6-12 minute sub maximal, steady state exercise test performed on a cycle ergometer (Figure 6) to determine cardiopulmonary function. The subject will be properly positioned on the ergometer with a slight bend in the knee. The exercise test will begin at a 2 minute warm-up to acquaint the participant with the ergometer and prepare for the exercise intensity for the first stage of the test. The specific protocol consists of 3-minute stages performed at 50 rpm's with appropriate increments in workload (See YMCA Cycle Test Protocol Attached). Heart rate will be monitored at least twice during each stage, as well as blood pressure and rate of perceived exertion (RPE Chart Attached) once per stage. In addition, the participant will be monitored continuously for any signs or symptoms suggestive of intolerance to the exercise (e.g., abnormal blood pressure response). Importantly, prior to this test all participants will be screened using a medical health history questionnaire in order to exclude anyone with a chronic metabolic, neurologic, or cardiovascular complication. The cycle test will be terminated upon the subject reaching 70% of their age predicted (220-age) maximum heart rate (equating to 60% of VO2 max for young men as determined via Swain Method in ACSM guidelines). If the participant fails to conform with test protocol, experiences any signs or symptoms, voluntarily stops or requests to stop, or experiences an emergency situation following completion of cycling, the participant will continue pedaling with no resistance for a 2-5 minute cool down. All physiologic observations will be continued for at least 5 minutes post cool down unless abnormal responses occur, which would warrant a longer post-test surveillance.

Study Visit 1 and 2: Vascular Reactivity Protocol

Prior to experimental study visits, all participants will be fasted and refrained from caffeine intake for 12 hours, and heavy exercise and alcohol for 24 hours. The participants will be studied in the supine position on an examination table. The Doppler ultrasound machine (See Figure 2) will be used to acquire brachial artery diameter and blood velocity following the protocols described below. All measurements involving the use of an ultrasound for imaging will be performed using published guidelines. All ultrasound data will be acquired and analyzed using a computer imaging software (QUIPU - FMD studio). The protocol to assess the VOR will consist of 3 phases to determine the artery's responsiveness to various non-invasive physiological stimuli.

Phase 1: After the attainment of a quality ultrasound signal, two minutes of baseline ultrasound data will be
recorded. Next, a blood pressure cuff positioned around the subject's forearm proximal to the ultrasound probe will be inflated to a supra-systolic pressure (220 mmHg) for 5 mins (See Figure 1). Data will be recorded continuously for 30 sec prior to and for 3 mins post-release of the occluding cuff.

Phase 2: Will commence 5 minutes following Phase 1. The subjects opposing hand or foot (opposite limb from ultrasound scanning) will be placed into an ice bath (c1 degree Celsius) up to the level of the wrist for 2 mins. Ultrasound data will be recorded for 1 min prior to, during and for 1 min following removal of the hand/foot from the ice bath.

Phase 3: Will commence 10 mins following completion of Phase 2. The hand from the subject's ultrasound scanned limb will be placed into a warm bath (38-42 degrees Celsius) for 10 mins. Ultrasound data will be recorded during the last 2 mins of the 10 min warm bath. Importantly, if there is evidence of an open cut or wound on the hand or foot prior to submersion in ice water or warm bath, then this procedure will not be performed.

From these data, a vascular physiological operating range will be determined.

<table>
<thead>
<tr>
<th>Does your research involve only the collection of anonymous data?</th>
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<td>Yes ☐ No ☐</td>
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<th>Does your research involve sensitive information?</th>
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<td>Yes ☐ No ☐</td>
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<th>Does your research involve hidden video or audio recordings or deception?</th>
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<td>Yes ☐ No ☐</td>
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**SECTION 3: RISKS AND BENEFITS**

Indicate all potentially vulnerable subjects involved in the study.

- ☐ Children
- ☐ Nursing home patients
- ☐ Mentally ill patients
- ☐ Pregnant females
- ☐ Prisoners
- ☐ HIV positive individuals
- ☐ Other: Not applicable

If your research involves prisoners, explain how it is directly relevant to prisoners or the prison system (check all that apply):

- ☐ the causes and/or effects of incarceration
- ☐ the process of incarceration
- ☐ prisons as institutional structures
- ☐ the conditions of prisoners or prisoners
- ☐ procedures for improving the wellbeing of prisoners
- ☐ other (explain):

Detail the methods that will be employed to protect vulnerable subjects.

Note: All research involving prisoners requires compliance with federal regulations pertaining to biomedical and behavioral research involving prisoners as listed in CFR 6359 Subpart C. Research must be directly relevant to prisoners or prisoners (e.g., the effects of incarceration, criminal behavior, prison infrastructures, etc.). Completion of the CITI Research with Prisoners Module is also required.

**How will you maintain confidentiality?**

- ☐ Anonymous data
- ☐ Electronic data will be password protected
- ☐ Physical data will be locked in a file drawer
- ☐ Publication-confidential data
- ☐ Other (explain):

Describe final disposition of data:

All participant information and data will be handled according to university policies and will be kept by the principal investigator (Dr. Daniel Creede) in a locked filing cabinet in HPR 223, to which only the principal investigator and Co-investigator will have access. Individual subject codes will be used for each participant during data collection and analysis. The investigators are aware of the confidentiality requirements and the proper conduct of such a study. All participant data will be de-identified when transferred electronically to an external hard-drive for data back-up, as well to spreadsheets for analysis, which will be performed on a password protected computer. Only aggregate, de-identified, group data will be presented at conferences or published in peer reviewed journals. Following completion of the study, all hard copies of participant data...
<table>
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<tr>
<th>Risks, inconveniences, or discomforts subjects are likely to experience (check all that apply):</th>
<th>Detail potential risks, inconveniences and discomforts subjects are likely to experience, if any.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>Legal</td>
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<td>Psychological</td>
<td>Social</td>
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<td>Financial</td>
<td>Other</td>
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<tr>
<td>Occupational</td>
<td>None</td>
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</tbody>
</table>

Participants will be exposed to sub-maximal exercise workloads as part of the physical fitness assessment during the screening visit. These protocols are commonly taught in exercise physiology classes and do not pose a great threat to the participants.

All other potential risks are minimal and are outlined below:

**Surface ECG** - Some people may have a skin irritation from the patches that connect the wires on the chest to the computer. Skin and hair are pulled slightly when the patches are removed after the test. Research personnel will attach and remove the patches as carefully as possible.

**Blood Pressure Cuff Inflation** - The blood pressure cuff will squeeze the arm tightly, however, any discomfort will be alleviated as soon as the pressure in the cuff is released.

Describe the methods that will be employed to mitigate any potential risks, inconveniences or discomforts.

Each participant will be closely monitored for any signs or symptoms suggestive of intolerance to the exercise or any emergency situation. Once again, the subjects will be screened prior to participating in any facet of the study to exclude individuals with any known pathology. Nonetheless, the PI is CPR/ED certified, will be present during all exercise tests, and will be able to respond quickly to any emergency situation.

Describe any potential benefits subjects may gain as a result of participation.

While there is no direct benefit to the subject for participating, the knowledge gained from this research will benefit the scientific community and will potentially lead to the development of future recommendations used for the detection and the prevention and/or treatment of cardiovascular disease.

List all incentives subjects will receive for their participation.

No incentives other than knowledge regarding their physical fitness level will be given to the participants

If individuals are unwilling or unable to complete their participation, how will their incentives be distributed?

- Not Applicable (no incentives will be offered);
- They will still receive all incentives;
- They will be informed that they will receive no incentives;
- They will receive partial incentives (explain);

SECTION 4: CHECKLIST AND AUTHORIZATION

The following documents must be attached to this form:

- CITI Common Course Certificate (mandatory for all investigators and student advisor)
- CITI HS Course Certificate (mandatory for all investigators and student advisor)
- Research proposal approval from dissertation or master's thesis committee (if applicable)
- Study recruitment documents (if applicable)
Survey questions (if applicable)
Researchers letter from external organization participating in the project (if applicable) on official letterhead
Assent form for minors (if applicable)
Consent forms (long or short if applicable) and any related documents (mandatory)
Letter to parents (if applicable)

Instructions for Attaching Documents:

1) Place the cursor where you want the attachment to appear.
2) Select the "Insert" tab at the top of MS Word.
3) Select "Object," located on the far right of the tool bar (PC) or the bottom of the list (MAC).
4) Select the "Create from File" tab and check the box that states "Display as Icon." Note: Do not check the box that says "link to file."
5) Browse to the location of your document, and double click on it.
6) Repeat these steps for each document to be attached.

Note for Mac Users: Word for MAC is unable to attach .pdf files, so you will have to first save the Citi certificates or any other .pdf files you intend to attach as a .doc or .rtf file before attaching them. There are several ways to accomplish this. You may use Adobe to open the file and then select "File" and "Save as" and change the file type to an .rtf or .doc format. Alternatively, you may also download or create your own .pdf to .doc application or simply save the application and then open the file on a PC to attach them as instructed above.

Instructions for Authorization:

1) Type your name and date in the appropriate box.
2) Students should email the form to their advisors, who should add their name and then send it to department chairs for review. Department chairs should add their name and send the finalized form to info@usm.edu.

By typing my name below, I acknowledge that I have read, understood, and approve of the information contained herein.

Daniel P. Credouer
Principal Investigator
11.19.14

Preston Bell
Student Research Advisor (if applicable)
11.19.14

Department Chair

Date
Appendix D

INSTITUTIONAL REVIEW BOARD
LONG FORM CONSENT

LONG FORM CONSENT PROCEDURES

This completed document must be signed by each consenting research participant.
- The Project Information and Research Description sections of this form should be completed by the Principal Investigator before submitting this form for IRB approval.
- Signed copies of the long form consent should be provided to all participants.

Today's date: 11-24-2014

PROJECT INFORMATION

Project Title: Reliability of the Vascular Operating Range and Predictive Value in Determining Cardiovascular Fitness

Principal Investigator: Daniel P. Credeur, PhD
Phone: 601-266-6303
Email: daniel.credeur@um.edu

College: College of Health
Department: Human Performance and Recreation

RESEARCH DESCRIPTION

1. Purpose:

   The purpose of this study is to evaluate the day-to-day reproducibility of a novel cardiovascular assessment used to examine the health of your arteries. In addition, we will be determining how this novel assessment relates to your physical fitness. Collectively, these data will expand upon an important clinical assessment of vascular health, and may improve upon the sensitivity by which traditional vascular assessments can categorize cardiovascular health in human subjects.

2. Description of Study:

   You will be in the study for 2-3 study visits; one for consenting, artery screening, and the physical fitness assessments which will last about 90 minutes, and the second and third visits lasting about 2 hours. You can stop participating at any time. Your decision to withdraw from the study will not affect in any way your medical care and/or benefits.

   Below is a detailed description of the procedures to be performed during each visit to the laboratory:

VISIT 1: You will make a trip to the Human Performance and Recreation building to Dr. Daniel Credeur's Lab for a review of the inclusion/exclusion criteria and to discuss the informed consent form including an oral explanation of the study purpose, protocol, and potential risks and benefits. After signing the informed consent, a Doppler ultrasound will be performed in both arms (brachial artery) and to establish that a good quality image can be obtained. In addition, we will perform a series of physical assessments such as determining your height and weight, and also your body composition by having you stand on a specialized scale (BIA). You will also be asked perform a step test and a moderate intensity exercise bout on a specialized bicycle to determine your cardiorespiratory fitness. Finally, you will be asked to squeeze on a gripper 3-5 times to determine your handgrip strength. Following these assessments, you will be scheduled for Visit 2.

VISIT 2: Upon arrival to the laboratory, you will be asked to use the bathroom and change into a scrub top and bottom that will be provided to you. Your height and weight measurements will be taken again. Next, you will then be asked to lie down and you will be set up for baseline assessment of ECG, blood pressure, and flow-
mediated dilation (described below in detail) in your right or left arm. After baseline assessments, a series of
other vascular tests will be performed to determine how quickly your arteries respond to either a cold or warm
sensation, induced by temporarily placing your hand or foot in ice water and warm water.

VISIT 3: You may be asked to come back for a third visit to repeat all procedures performed on the second study
visit, if you are randomized into this particular study group.

Procedures to be performed:

Body Composition - Your percentage of body fat and lean body mass will be determined by a non-invasive
process called bioelectrical impedance amplitude (BIA). During this test you will stand barefoot on a
specialized scale for ~2 minutes while a very low level, electrical current is passed through your body. The
magnitude of electrical current is used to estimate percentage of body fat and also lean body mass (e.g.,
muscle). This is a widely used, and very safe, commercial assessment of body composition.

Handgrip Strength - Your handgrip strength will be measured using a specialized grip testing device. You will be
asked to hold the device by your side and squeeze as hard as you can for about 1 sec. This may be repeated
3-5 times to determine your grip strength.

3-minute Step Test - After resting quietly for 5 mins in a chair, your resting heart rate will be determined manually by
the investigator by placing 2 fingers over your wrist and feeling for a heartbeat. The step test requires that you
step up and down, alternating legs on a standardized box for 3 minutes at a cadence of 24 steps per minute.
After 3 minutes of stepping are completed you will stop and another heart rate will be measured immediately.
A third heart rate will be measured 20 sec into recovery from step test.

YMCA Cycle Test - The YMCA cycle test is a 6-12 minute sub maximal, steady state exercise test performed on a
cycle ergometer to determine your cardiorespiratory fitness. You will first be properly positioned on the
ergometer with a slight bend in your knee. The exercise test will begin with a 2 minute warm-up, followed by
3-minute stages performed at 50 rpm’s with appropriate increments in work rate adjusted depending on how
your body is responding to the exercise. You heart rate will be monitored at least twice during each stage, in
addition your blood pressure and rate of perceived exertion (RPE Chart) will be determined once per stage.
This test will end upon you reaching a certain percentage of your predicted max heart rate, or if you desire to
stop. Following completion of cycling, you will continue pedaling with no resistance for a 2-3 minute cool
down. We will continue to monitor your heart rate and blood pressure for about 5 minutes post cool down.

Blood Pressure (BP) - A blood pressure cuff will be wrapped around the upper arm to periodically measure blood
pressure.

Heart Rate (HR) - Electrodes (patches) will be placed on the surface of the chest for heart rate measurements per
ECC.

Flow mediated dilation (FMD) - A blood pressure cuff will be placed around your forearm. This cuff will be inflated,
as is done when your blood pressure is being measured, but instead of deflating the cuff immediately it will
remain inflated for 5 minutes. We will measure the blood flow to your arm by placing a probe over the brachial
artery (upper arm) of your arm, during and after inflating the cuff. The probe will provide a measure of the
speed at which your blood is traveling through your artery.

Cold Pressor Test (CPT) - Your left hand or foot will be placed into an ice bath (~34 degrees Fahrenheit) up to the
level of your wrist for 2 mins. Ultrasound data will be recorded for 1 min prior to, during and for 1 min following
removal of your hand/foot from the ice bath.

Warm Hand Bath - Your right hand will be placed into a warm bath (100-108 degrees Fahrenheit) for 10 mins.
Ultrasound data will be recorded during the last 2 mins of the 10 min warm bath.

3. Benefits:

If you agree to take part in this study, there may or may not be a direct medical benefit to you. You may
expect to benefit from taking part in this research to the extent that you are contributing to medical knowledge.
Our hope is that the information gained from this study will develop future recommendations for the
prevention and/or treatment of vascular disease.
4. Risks:

While in the study, you are at risk for the side effects described below. You should discuss these with the investigator and/or your doctor. There may also be other side effects that we cannot predict:

ECG: Some people may have a skin irritation from the patches that connect the wires on the chest to the computer. Skin and hair will be subjected to slight pressure when the patches are removed after the test. Research personnel will attach and remove the patches as carefully as possible.

Blood pressure cuff inflation: The blood pressure cuff will squeeze the arm tightly; however, any discomfort will be alleviated as soon as the pressure in the cuff is released.

Moderate Intensity Exercise: Engaging in moderate intensity physical activity will cause your heart rate and blood pressure to increase. This is a normal physiological response. In addition, your breathing rate will also increase. You may also experience some slight discomfort in your leg muscles due to fatigue. However, these sensations will subside upon stopping the exercise. The exercise protocols used in this study are commonly taught in exercise physiology classes and do not pose a great threat to your health.

For the reasons stated above the investigator will observe you closely while giving the treatment described and, if you have any worrisome symptoms or you feel that the investigator has described to you, notify the investigator immediately.

5. Confidentiality:

Information produced by this study will be stored in the investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law. It is possible that your medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the study sponsor and/or federal or state government agencies in the course of carrying out their duties. If your record is inspected or copied by the study sponsor (and/or its agents), or by any of these agencies, the University of Southern Mississippi will use reasonable efforts to protect your privacy and the confidentiality of your medical information. The results of this study may be published in a medical book or journal or used for teaching purposes. However, your name or other identifying information will not be used in any publication or teaching materials without your specific permission.

6. Alternative Procedures:

An alternative is to not participate in this research study.

7. Participant's Assurance:

This project has been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations.

Any questions or concerns about rights as a research participant should be directed to the Chair of the IRB at 601-266-5997. Participation in this project is completely voluntary, and participants may withdraw from this study at any time without penalty, prejudice, or loss of benefits.

Any questions about the research should be directed to the Principal Investigator using the contact information provided in Project Information Section above.

CONSENT TO PARTICIPATE IN RESEARCH

Participant's Name:
Consent is hereby given to participate in this research project. All procedures and/or investigations to be followed and their purpose, including any experimental procedures, were explained to me. Information was given about all benefits, risks, inconveniences, or discomforts that might be expected.

The opportunity to ask questions regarding the research and procedures was given. Participation in the project is completely voluntary, and participants may withdraw at any time without penalty, prejudice, or loss of benefits. All personal information is strictly confidential, and no names will be disclosed. Any new information that develops during the project will be provided if that information may affect the willingness to continue participation in the project.

Questions concerning the research, at any time during or after the project, should be directed to the Principal Investigator with the contact information provided above. This project and this consent form have been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations. Any questions or concerns about rights as a research participant should be directed to the Chair of the Institutional Review Board, The University of Southern Mississippi, 118 College Drive #5147, Hattiesburg, MS 39406-0514, (601) 266-5367.

Include the following information only if applicable. Otherwise delete this entire paragraph before submitting for IRB approval: The University of Southern Mississippi has no mechanism to provide compensation for participants who may incur injuries as a result of participation in research projects. However, efforts will be made to make available the facilities and professional skills at the University. Participants may incur charges as a result of treatment related to research injuries. Information regarding treatment or the absence of treatment has been given above.

________________________________________  __________________________________________
Research Participant                          Person Explaining the Study

________________________________________  __________________________________________
Date                                          Date