# IMPACT OF ACUTE SUBMAXIMAL CYCLING EXERCISE ON SKELETAL MUSCLE MICROVASCULAR REACTIVITY INDUCED BY MENTAL ARITHMETIC

by

MELISSA J. MCGRANAHAN

(Under the Direction of Nathan Jenkins)

ABSTRACT

We investigated the impact of exercise prior to mental stress on muscle microvascular function. Near-infrared spectroscopy (NIRS) was used during arterial occlusion to assess muscle oxygen consumption ($mVO_2$), time to 50% of perfusion ($T_{1/2}$), post peak-hyperemic recovery slope, and basal muscle microvascular dilation. These parameters were assessed at baseline (BL), after rest (CON) or exercise (EX), and after mental stress. $mVO_2$ was significantly increased after EX compared to CON. $T_{1/2}$ decreased regardless of condition. Basal muscle microvascular dilation increased during mental stress in CON and EX, with mental stress and EX eliciting additive effects. Post peak-hyperemic recovery slopes indicated a transient microvascular dysfunction during CON that recovered after mental stress and was absent in EX. The study's primary finding was that the combination of exercise and mental stress produced additive effects on basal microvascular muscle oxygenation, suggesting that exercise and mental stress alter microcirculatory function through separate mechanisms.

INDEX WORDS: Exercise, Mental stress, Microvascular function, High trait anxiety

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# DEDICATION

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# CHAPTER 1

# INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death worldwide. In 2013, 32 percent of fatalities were related to CVD[1]. In the United States, 1 out of every 3 deaths are attributable to CVD diseases[1]. An early indicator of CVD is dysfunction of the endothelium which lines blood vessels. The endothelial cells mediate vasoconstriction and vasodilation, releasing factors in response to environmental stimuli. Several well established physiological factors for impairing endothelial function include obesity, impaired glucose control, and excess lipid levels. Over the past 25 years, there has been an increased interest in the role mental stress plays in dysfunction in blood vessels. Stress is a common complaint today; according to the American Psychological Association, 22 percent of individuals surveyed in 2011 reported extreme stress[2]. Of the 1,226 individuals surveyed, 44 percent said their stress had increased over the past five years[2]. Mental stressors have been associated with impaired vascular function[3-8], increased vascular inflammation[9], and activation of blood coagulation[10]. In addition, a recent review identifies stress as a modifier of metabolic function, and has implicated changes at the mitochondrial level[5]. These physiological responses to stress over time may lead to irreversible damage to the endothelium. Reactions to stressors may be of particular concern for those who are sensitive to stressors, such as individuals with higher trait anxiety.

Anxiety is characterized by a heightened sense of alarm when exposed to a stimulus perceived as threatening[11]. While transient states of anxiety in response to a perceived threat are natural, individuals with high trait anxiety are more likely to perceive a situation as threatening

and experience a greater intensity of state anxiety in a stressful situation. Repeated activation of the stress response due to a heightened interpretation of situations as stressful can, over time, lead to physiological and structural changes in the vascular system. Those with higher trait anxiety have dysfunctional autonomic activation[12], changes in vascular smooth muscle receptor types[13], increased inflammation[14], and impaired vascular smooth muscle response to nitric oxide [15]. Due to these factors, one would expect an increased risk of vascular dysfunction to be associated with higher trait anxiety. Indeed, individuals with anxiety symptoms and diagnosed with a clinical anxiety disorder had a 41 percent increased risk of developing cardiovascular disease[16]. Anxiety disorders are one of the most common mental disorders, with a lifetime prevalence rate of 28.8 percent[17]. It is somewhat surprising, then, that anxiety is studied less frequently compared to other mental disorders. With a substantial percentage of the population developing an anxiety disorder over their lifespan, it is critical to understand how stress may differentially affect the vascular system among those with higher trait anxiety levels, and find ways in which to mitigate the negative effects of stressors on this population.

Previous studies examining endothelial health in response to a mental stressor have used predominantly flow-mediated dilation, a method that assesses macrovascular responses[3, 18-20]. However, large arteries are primarily passive conduits to pressure-driven changes in blood flow, and are generally not involved in control of blood flow. The microvasculature, on the other hand, is the primary site of blood flow regulation, and may be a novel vascular bed to assess. Near-infrared spectroscopy is a non-invasive technology that has previously been used to assess resting oxygen levels in muscle and reactive hyperemic blood flow responses to an occlusion[21-23]. Additionally, NIRS correlates well with other techniques used to assess vascular function[24-26].

NIRS allows for microvascular responses to be continuously monitored and could potentially provide valuable insight as to how mental stress affects microvascular function.

The benefits of physical activity on cardiovascular disease risk factors are well established; however, common physiological risk factors such as lipid profile explain ~60 percent of the relationship by which physical activity decreases the risk of CVD[27]. The role of physical activity in enhancing psychological well-being is a potential mediator for lowering ones risk of CVD. Exercise has been shown to acutely improve both cardiovascular health markers (e.g., blood pressure[28], flow-mediated dilation[29], and inflammation[30]) and state anxiety levels in both clinical and non-clinical populations[31]. Therefore, it is plausible that acute exercise may enhance the vascular response to stressful situations.

Purpose:

The purpose of the proposed study was to evaluate the effects of an acute bout of sub-maximal exercise on microvascular function after a stressor. By comparing vascular responses to a mental stressor, we attempted to gain insight as to how stressors affect microvascular function. A secondary purpose was to explore the impact of mental stress on microvascular function in individuals with high vs. low trait anxiety.

*Primary Aims*

Aim 1: Determine the effect of a mental stressor on muscle microvascular function.

*Hypothesis 1*: Mental stress will impair microvascular function.

Aim 2: Determine if exercise before a mental stressor protects against stress-induced muscle microvascular dysfunction.

*Hypothesis 2*: Acute exercise prior to a mental stressor will attenuate stress-induced microvascular dysfunction.

*Secondary Aims*

Aim 3: Determine if individuals with high trait anxiety exhibit a greater mental stress-induced muscle microvascular dysfunction compared to individuals with low trait anxiety.

*Hypothesis 3*: Compared to low trait, those with high trait anxiety will have a greater decline in muscle microvascular function after a mental stressor.

Aim 4: Determine whether exercise has a larger effect on attenuating muscle microvascular dysfunction among those with high trait anxiety.

*Hypotheses 4*: Acute exercise will improve microvascular function after stress to a greater extent in individuals with high trait anxiety.

# CHAPTER 2

# LITERATURE REVIEW

## Stress

One challenge in studying the impact of stress on physiological outcomes is implementing a unified definition of "stress," and the physical manifestations which occur in response to a stressor. Walter Cannon, an early researcher in the physiological response to pain and emotional distress, focused on the role of neuroendocrine activation in response to perceived threat. His early work was instrumental in understanding the physiological changes occurring with stress[32]. Cannon coined the term "homeostasis," a concept whereby an optimum range of physiological parameters are tightly regulated and that when exposed to a perceived threat, systemic alteration from these ranges occur[33]. Cannon's work denoted the physiological response to a threat; the sympathetic nervous system primes the body to confront or flee from a threat, commonly referred to as the "fight or flight" response[32]. In the 1930's Hans Selye was one of the first to use the term "stress" in a physiology model, his work focused on emphasizing pathology due to stress. Some of his earliest work described the initial acute stress response and either the adaptation to the stress, or exhaustion as the animal was subjected to repeated stressors[34].

Sterling and Eyer developed the theory of allostasis, used to describe the body's adaptation in physiological responses to events or stressors in order to maintain physiological stability[35]. Previous theories considered homeostasis to be a static response irrespective of an individuals' characteristics, environment, and perceptions. These factors are all taken into consideration in allostasis theory. The internal and external factors help explain variation

observed across individual stress responses. An increase in allostatic load, characterized by the individual having an exaggerated response to stress or excessive stress, will cause pathological conditions[35]. Allostasis, as a theory, helps to explain the wide array of responses to a standardized stressor. The individual's perceived ability to overcome the stressful stimulus, as well as a sense of control, are factors which have been proposed to moderate stress perceptions. Early researchers provided insight into the physiological changes that occur when challenged with a stressor. The concept of stress is considered a response to a stimulus which causes a physiological shift away from homeostatic ranges, as well as behavioral changes[36]. These theories have helped to provide potential pathways by which individuals perceive situations differently. In particular, the theory of allostasis provides a theoretical lens for the evaluation of varying responses to stress.

**Stress Physiology**

The stress response is an integration of both peripheral and environmental factors. In response to a stressor, the autonomic and hypothalamus-pituitary-adrenal (HPA) axis mediate the stress response. A shift in the two branches of the autonomic nervous system occurs during a stressor; a withdrawal of the parasympathetic, and an increase in the sympathetic nervous system activity occurs during a stressor. Sympathetic nervous system activity increases the discharge of norepinephrine (NE) in the peripheral. Release of NE leads to increased heart rate, cardiac contractility, vasoconstriction in non-essential vascular beds, and vasodilation in vasculature supplying muscle. The locus ceruleus, a vital region in the brain involved in stress response, prompts the hypothalamus to release corticotrophin-releasing hormone (CRH). CRH then prompts the pituitary to release adrenocorticotropic hormone (ACTH). ACTH is secreted into the circulatory system, triggering the adrenal gland to release cortisol. Cortisol suppresses immune

responses, modulates energy balance, and increases blood pressure. Cortisol is released in a diurnal pattern, with highest values prior to waking followed by a sharp decline three hours after waking, and gradual decrease in afternoon and evening[37, 38]. Normal ranges used in medical testing for cortisol are 7-25 mg/dL in the morning to 2-9 mg/dL in the afternoon[39]. The time course and level of cortisol released due to stress varies based on the stressor used and time of day.

**Endothelial Health**

Endothelial health is considered an important indication of cardiovascular disease[40]. The endothelium, a layer of cells lining the vessels, is a key mediator in the maintenance of cardiovascular health. In particular, vascular tone is dependent on the balance between the release of vasodilators and vasoconstrictors from the endothelium. Two important molecules released from endothelial cells are nitric oxide and endothelin-1. Nitric oxide (NO) is released from the endothelial cell in response to shear stress, metabolites, and factors released from adjacent endothelial cells. NO released from the vascular endothelium initiates vasodilation in the vascular smooth muscle, increasing the blood flow to the dilated area. Blood flow is carefully controlled, the release of NO is regulated by vasoconstrictors and sympathetic nervous system control. Endothelin-1 (ET-1) is a potent vasoconstrictor secreted by the endothelial cells. ET-1 has been shown to increase reactive oxygen species and NAD(P)H oxidase, and is a key inhibitor of NO availability[41]. Exogenous administration of ET-1 impaired vascular dilation responses in healthy young individuals[42]. A common technique to assess endothelial function is to measure flow mediated dilation (FMD)[43]. This technique has been standardized to include the change in diameter of conduit arteries after a distal 5 minute occlusion. The change in the diameter relative to baseline is used as an indirect measure of endothelium NO production. In the literature, this is

the most widely used technique to assess vascular function. However, FMD measurements tend to have a large coefficient of variation, limiting the ability to detect small changes[44-46]. Techniques used to asses microvascular function, such as Near-infrared spectroscopy (NIRS), have reported smaller coefficient of variation compared to FMD[26] and have reported high reliability in assessment of blood flow kinetics[21-23] and muscle metabolism[23].

The microvascular network is made up of arterioles and capillaries. NIRS technique allows for a different aspects of microvascular function to be assessed. The oxygen saturation level as measured by NIRS, has been used to reflect the balance between oxygen delivery and oxygen utilization in muscle tissue while at rest[47]. $T_{1/2,}$ a common reperfusion based measure, is the time to reach half of the maximal reperfusion and is used as a measure of microvascular blood flow. Post peak-hyperemic vasodilation as measured by the change in slope of the oxygen saturation after reactive hyperemia is an indication of the balance between signals for vasodilation and vasoconstriction. Reperfusion kinetics of the microcirculation predictive of future cardiovascular events and provide insight into microvascular health[48]. While blood flow in the microvasculature at rest and in response to an ischemic state are valuable, the tissue specific metabolic rate is important to note. Microvascular responses are sensitive to metabolic demands and tissue specific oxygen consumption provide a better understand of how microvascular and metabolic demand are linked[49]. The rate of change of the oxygenated signal during cuff ischemia has been used to measure muscle metabolism[22, 23]. These measures together provide a broader understanding of microvascular health.

The arterioles are involved in blood pressure control, the diameter changes in response to both sympathetic nerve activity and local metabolites. Constriction of the arterioles' luminal size have a large effect on total peripheral resistance and blood pressure. The microvascular

responses are related to the variation in receptor types and proportion of receptors in each vascular bed. When NE is released from a peripheral vascular nerve it will bind and cause either vasoconstriction via alpha1-adrenergic receptors or vasodilation via beta 1 and 2 adrenergic receptor types[50-52]. The microvascular network must be able to supply adequate blood flow to working muscle. Change in microvascular function can occur via several mechanisms, resulting in impaired microvascular function. Dysfunction of the microvasculature can, over time, play a role in the progression of cardiovascular diseases.

**Stress and Cardiovascular Disease**

Psychological stress is commonly considered a risk factor for the development of cardiovascular disease. Chronic levels of stress predispose individuals to atherosclerosis, coronary artery disease, and myocardial infarction[53-55]. Both acute and chronic stress are associated with an increased risk of CVD. After natural disasters such as earthquakes, the rates of both myocardial infarctions and sudden cardiac deaths increases[56]. The INTERHEART study compared stress levels among 11,119 diseased individuals and 13,648 healthy controls from over 50 countries. Both psychosocial and behavioral risk factors were measured, and after controlling for demographic and health-related confounders, those with higher work, home, and general stress levels had a 45% increased risk of myocardial infarction[57]. Heightened activation of the sympathetic nervous system is a potential mechanism for stress-induced atherosclerotic plaques, rupture of plaques, and clot formation. Using a cynomolgus monkey model, a pro-atherosclerotic diet, and social dominance, an indication of a stress response produced coronary artery narrowing twice that of their subordinate housemates. Administration of beta blocker propranolol in the socially dominant group attenuated plaque formation.

Cortisol, a key glucocorticoid released during activation of HPA-axis, is a potential pathway by which stress impairs vascular function. Cortisol production during stress is associated with an increased risk of hypertension, signaling of inflammatory cytokine release and the production of clotting factors. Hamar and Steptoe administer the Stroop word interference task, and a mirror tracing task to 446 subjects. At baseline, all subjects were normotensive. At the three-year follow-up, individuals with a cortisol response of 1 nmol or greater were 59% more likely to develop hypertension than those with lower cortisol responses[58], independent of BMI and age. Excessive blood pressure is a contributing factor to the development of atherosclerosis.

The role of cortisol may be best exhibited in conditions characterized by excess cortisol release, and the use of pharmacological agents to block vascular glucocorticoid receptors. Both case-control studies of Cushing's disease and pharmacological manipulations have implicated cortisol as playing a role in impairments in vascular function. Compared to matched controls, individuals with Cushing's disorder, characterized by excessive cortisol levels, had a significant decrease in flow-mediated dilation. Responses to nitroglycerine, a measure of smooth muscle vascular function, were not different between the groups. After medical treatment for hypersecretion of cortisol, both systolic blood pressure and FMD were comparable to healthy controls[59]. In healthy middle-aged individuals exposed to a public speaking task, FMD showed a decrease post-stressor. This was abrogated by administration of metyrapone, which inhibits the synthesis of cortisol[4]. Regarding mechanisms underlying the vascular effect of cortisol, experiments in isolated endothelial cells demonstrate that the administration of glucocorticoids decreases nitric oxide availability[60]. Overall, chronic exposure to cortisol can have deleterious effects on vascular function.

**Anxiety**

Genetic, physiological, and environmental factors all contribute to an individual's specific traits. It is the integration of all of these factors that result in high trait anxiety. Symptoms of anxiety include vigilance, avoidance, worry, and apprehension. Furthermore, somatic indicators of trait anxiety include changes in autonomic system activation patterns, which can lead to heart palpitations, muscular tension, increased galvanic responses, and trembling. The limbic system is involved in integrating environment and somatic information to initiate stress responses. The brain structures of the limbic system include the amygdala, hippocampus, and medial prefrontal cortex. The limbic system mediates threat appraisal, and can initiate or inhibit the branches of the autonomic systems. Individuals with high trait anxiety may have a hyperactive limbic system, causing them to perceive neutral and threatening stimuli as a greater threat than healthy controls. Among 65 middle-aged adults presented with a series of black and white facial expressions, those with high trait anxiety showed greater dysregulation of the limbic regions to neutral, happy and fearful faces, and a prolonged activation of the amygdala, as measured by functional magnetic resonance imaging[61]. The limbic system is a primary system involved in signaling the stress response, and can signal the area responsible for controlling the cardiovascular functions: the ventral lateral medulla. It receives information from various receptors in the periphery, and modulates cardiac and vascular tone in response. Sympathetic and parasympathetic branches of the autonomic nervous system initiate the stress response in the periphery; deviation in the function of these systems help to explain abnormal stress responses.

Changes in activation of the autonomic branches during stress account for physiological responses, individuals with high trait anxiety have been shown to have abnormal activation of

both branches. Sanchez-Gonsalez and colleagues found that among young individuals with higher anxiety levels, their cardiac responses to a speech task stressor were closer to those seen in middle aged participants than those of young low anxiety participants. High anxiety individuals showed a greater systolic blood pressure response and rate pressure product. Not only were these hemodynamic outcomes higher during the administration of the stressor, but they were elevated during the recovery period as well[62]. Abnormal autonomic response can effect heart rate variability (HRV). HRV is the fluctuation between cardiac cycles, it is measured by looking at the HRV high frequency (HF), which reflects the parasympathetic vagal output. A balance between sympathetic and parasympathetic nervous system activity controls heart rate, however individuals with anxiety have been shown to have decreased HRV, indicating a combination of excessive sympathetic activity and a decreased parasympathetic control. Perceived mental stress in both healthy individuals and those with high trait anxiety has been associated with decreased HRV, irrespective of fitness[63, 64]. Specifically, high trait anxiety has been associated with greater vagal withdrawal. Vagal withdrawal will lead to an increase in heart rate, this may exacerbate somatic feelings of anxiety.

Dysregulation of the HPA has been thought to play a role in anxiety. Initially, exaggerated responses to stress may occur, and after chronic activation of the HPA, this system's ability to respond to stress may be blunted. Children in foster care followed over six years were shown to have a lower cortisol awakening response, and higher cortisol reactivity to cognitive and speech tasks. These responses were associated with symptoms of general anxiety disorder and post-traumatic stress disorder[65]. The effects of cortisol response in adults with high trait anxiety varies across studies. In adults with high trait anxiety, blunted cortisol awakening responses, and decreased cortisol releases during and after a stressor have been reported[66-68]. A

limitation of these studies is that adverse events are not accounted for, the number and length of exposure to adverse events could explain the variations in cortisol response to stressors in studies. The earlier and more intense life events individuals experience may over time lead to exhaustion of the stress response. In a study of 354 heathy young adults those who reported adverse life events (mugged, sexual assault, threatened with a weapon, emotional adversity) predicted decreased heart rate variability and decreased cortisol response to speech and mental arithmetic stressors[69]. The role mental stress has on endocrine responses in individuals with high trait anxiety requires further research. Overall, there are irregular physiological responses to mental stress in individuals with high trait anxiety, which require further studies to better understand the endocrine responses.

**Anxiety and Cardiovascular Disease**

Anxiety symptoms have been correlated to atherosclerosis, hypertension, and coronary heart disease. Among highly anxious individuals, the risk for Coronary heart disease (CHD) is increased by 26%, such individuals also have a 46% increased risk of cardiac death[70]. A significant proportion of studies related to CVD looking at both clinically diagnosed individuals, and individuals with high trait anxiety have deleterious vascular outcomes. In males, higher trait anxiety scores were associated with a two-fold increase in atherosclerotic plaque formation and carotid intima thickness, compared to their low anxiety counterparts. Females with high trait anxiety also showed greater carotid intima thickness, however, anxiety was not significantly related to plaque formation[71]. Higher trait anxiety scores in older individuals were associated with a lower FMD response compared to low trait anxiety individuals[72]. Impairment of endothelial function and greater development of plaque in individuals with high trait anxiety may be related to the hormonal responses. Broadly and colleagues found that individuals clinically

diagnosed with depression had an abnormal vasoconstriction response during FMD. Administration of metyrapone, a cortisol antagonist, improved flow-mediated dilation by nearly 7 percent from baseline values[4]. While depression is not the same as anxiety, these two disorders are often comorbid and have some common symptoms. Decreased vascular responses related to anxiety could be due to a downregulation of beta adrenergic receptor types[73]. In healthy middle-age individuals, beta adrenergic sensitivity was decreased in those with higher anxiety irrespective of age, BMI, ethnicity, and gender[74]. While this was not specific to the vascular smooth muscle, impaired vascular function seen in high trait anxiety may be associated with abnormal receptor types. Higher trait anxiety has several physiological variations that in combination can increase the risk of cardiovascular disease; finding ways to mitigate and improve physiological response to stress are needed to decrease the risk of CVD.

**Exercise moderating effect of Stress and Anxiety on Endothelium**

Exercise itself is a stressor, and some of the physiological responses are similar to those seen when an individual is under psychological stress (i.e. increased heart rate, rise in blood pressure, sweating, autonomic activity, hormonal release). As stated previously, the stress response primes the body to fight or flee. These physiological responses aid in the individual's ability to perform physical activity. The physiological response to an exercise stressor can be considered beneficial, and potentially improve recovery of physiological responses to other stressors, in keeping with the cross-stressor adaptation hypothesis[75]. Exercise induces both psychological and physiological benefits that can help prevent the adverse effects of psychological stress. Improvements in both positive affect and state anxiety have been reported after 20 minutes of low to moderate intensity exercise[76,77]. While it is not well studied whether stress perception is affected after exercise, the hormonal physiological responses to stress have

been shown to be blunted after exercise. Zschucke and colleagues examined the effects of a 30 minute bout of moderate exercise in both sedentary and highly trained males on cortisol response to the Montreal imaging stress task (MIST); Compared to the placebo control condition, the aerobic condition showed a blunted cortisol increase to the MIST. No significant difference between trained and sedentary individuals was observed [78]. These results indicate exercise prior to a stressor may transiently decrease endocrine responses to stress. In the periphery, acute exercise may provide further protection to the vascular system by increasing the release of anti-inflammatory cytokines[79]. These effects in combination may provide improve endothelial function.

Exercise training improves endothelial function[80]. However, the role of acute exercise is less clear. The timing of measurements, as well as the intensity and duration of the exercise, are two factors that must be carefully considered. FMD has been shown to decrease immediately following acute moderate to high intensity exercise [81, 82]. However, improvements in FMD after moderate exercise intensity (50- 70% of maximal oxygen capacity) have been reported an hour after exercise[82-84]. A potential pathway for the improvement of vascular function is an increase in beta adrenergic receptors in the periphery. In healthy college age males and females, 25 minutes of cycling at 70% of peak oxygen uptake improved beta-adrenergic sensitivity in the vasculature for two hours post exercise[85]. Overall, acute exercise improves the physiological response to stress and provides a protective effect by modulating neural and endocrine pathways. The proposed study will provide insight as to whether acute exercise attenuates stress-induced microvascular dysfunction, and whether exercise-induced prevention of stress-evoked microvascular dysfunction occurs to a greater extent in those with high trait anxiety compared to low-anxiety individuals.

Chapter 3

# IMPACT OF ACUTE SUBMAXIMAL CYCLING EXERCISE ON SKELETAL MUSCLE MICROVASCULAR REACTIVITY INDUCED BY MENTAL ARITHMETIC[1]

[1] M. J. McGranahan, K. K. McCully, P. J. O'Connor, and N.T. Jenkins

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## Abstract

We investigated the impact of a single bout of exercise on mental stress-induced changes in skeletal muscle microvascular function in healthy males. Near-infrared spectroscopy (NIRS) was used to assess several aspects of microvascular function including muscle oxygen consumption ($mVO_2$), microvascular dilation, time to 50% reperfusion ($T_{1/2}$) and post peak-hyperemic recovery slope before (basal) and during a five-minute cuff occlusion. These parameters were assessed at baseline (BL), after rest (CON) or exercise (EX), and after mental stress. On two separate days, participants either rested for 25 minutes (CON), or completed 25 minutes of cycling exercise (EX) at a power output corresponding to 90% of their individual ventilatory threshold. Mental stress was evoked by a 10-minute serial subtraction test. Post peak-hyperemic recovery slope was significantly lowered after exercise compared to rest. The post peak-hyperemic recovery slope was maintained during EX and improved after mental stress during CON. $mVO_2$ was significantly increased after EX compared to CON. Mental arithmetic significantly decreased $T_{1/2}$ in both EX and CON, with no differences between conditions. Mental arithmetic caused an increase in basal muscle microvascular dilation in both CON and EX which was additive. Our finding that the combination of exercise and arithmetic produced an additive effect on basal microvascular muscle oxygenation suggests that acute exercise and mental stress induced by arithmetic alter muscle microcirculatory function through separate mechanisms.

## Introduction

The health consequences of excessive mental stress are severe, and chronic stress is becoming increasingly prevalent in modern society. According to the American Psychological Association, 22 percent of individuals surveyed in 2011 reported extreme stress[2]. Furthermore, chronic stress has been implicated in cardiovascular impairment[86]. A major stress hormone such as cortisol is involved in the stress response, chronic elevations in cortisol can adversely affect endothelial function and impair nitric oxide bioavailability[, 60]. Acutely, stress can lead to decreased function of coronary vessels in coronary artery disease patients[87] and impaired endothelial function in large vessels such the brachial and femoral arteries[4, 6-8]. However, stress responses in the microcirculation are not as well characterized. The microvasculature network plays a vital role in regulation and control of blood pressure, and the remodeling of microvascular networks is thought to play a role in the development of hypertension and atherosclerosis [88, 89]. A recent study examining coronary and peripheral microvascular function and mental stress-induced ischemia found that peripheral microvascular function impairment was strongly correlated to coronary microvascular responses[90].

Skeletal muscle microvascular function can be assessed by evaluating the ability of the vascular bed to dilate in response to an increase in shear stress after an occlusion. The reactive hyperemic blood flow causes the release of nitric oxide (NO) from the endothelial cells causing vasodilation[91]. Continuous Wave Near-Infrared Spectroscopy (CW-NIRS) is a non-invasive technique used to assess microvascular reactive hyperemia and correlates well with current standards for measuring endothelial health[21, 24-26, 92, 93]. Studies assessing muscle microvascular responses to stress have shown mixed results. While some have indicated vasodilation[94-96] in response to mental stress, others have reported no changes[97, 98]. The previous studies were

limited in that reactive hyperemic measurements were only taken at various intervals, and microvascular responses were not measured continuously. CW-NIRS can be used throughout a mental stressor to provide insight into the muscle microvascular responses in real time. As well, measures of reactive hyperemia and sustained vascular tone prior to and after a mental stressor can be used to provide a better understanding how a mental stressor affects microvascular function. The microcirculatory response in muscle is significantly moderated by metabolic demand,[49] and mental stress has previously been reported to increase whole body oxygen consumption[99]. However, muscle specific oxygen consumption after mental stress has previously not been studied. Assessment of muscle oxygen consumption using CW-NIRS potentially can provide novel insight into mental stress-induced changes specifically in skeletal muscle.

Stress is a complex psychobiological process related to how organisms respond to actual or perceived physical or psychological stressors. Transient increases in state anxiety are common responses to stressors and the frequency and magnitude of state anxiety responses are influenced by an individual's predisposition toward anxiety; that is, the level of trait anxiety[100]. Anxiety has been associated with elevated risk of CVD. For example, diagnosis of a clinical anxiety disorder is associated with a 41% increased risk of CVD[16]. Elevated trait anxiety repeatedly has been associated with multiple physiological characteristics potentially related to cardiovascular health, including dysfunctional autonomic activation[12], changes in vascular smooth muscle receptor types[13], low serum acetylcholinesterase activity[101], increased inflammation[14], and impaired vascular smooth muscle response to nitric oxide[15]. Variations in physiological responses to mental stress may be influenced by state or trait anxiety.

The benefits of aerobic exercise for cardiovascular health are well established, and some evidence indicates that even acute exercise can modify certain cardiovascular responses to

mental stress. For example, a single session of exercise prior to a mental stressor has been shown to lower blood pressure responses to the stressor, and improve endothelial function measured by flow-mediated dilation[19 102]. Acute moderate exercise has also been shown to attenuate the cortisol response to mental stress[78]. However, the impact of acute exercise prior to a mental stressor on microvascular function is not known.

The purpose of this study was to determine how a mental stressor affects skeletal muscle microvascular function, and determine the impact of moderate exercise prior to mental stress on both psychological and microvascular stress responses. We hypothesized that a widely used, standardized mental stressor would impair microvascular dilation, and that moderate aerobic exercise prior to the stressor would improve microvascular function. Moreover, we tested whether the microvascular responses were associated with participants' level of trait anxiety or changes in state anxiety.

## Methods

### *Study Participants*

Healthy male subjects between the ages of 18-35 were recruited to participate in the current study (n=15). Approval for the study was received from the University of Georgia institutional review board, and all participants provided written informed consent prior to data collection. Exclusion criteria were as follows: a BMI ≥ 30; current regular smoker or any cigarette smoking during the prior 2 yr; involved in collegiate sports or training to place as a top performer for a race or competition; presence or history of medical illness, including bipolar disorder, schizophrenia, depression, cardiovascular disease, metabolic disease, a musculoskeletal injury, or a respiratory disease; currently taking any medications for hypertension, a metabolic

disorder, a mental health condition; or taking 3 or more prescription or non-prescription drugs in any class.

***Experimental Procedures***

Participants made 3 visits to the Non-invasive Muscle Physiology Laboratory. To minimize the potential influence of diurnal variation in cortisol, participants were asked to report to the lab within 4-6 hours of normal waking on three separate occasions. They were asked to refrain from ingestion of alcohol or performing any exercise for 24 hours prior the visits. During the initial visit, a detailed explanation of the experiment protocol and the associated risks were provided. Participants were blinded to the primary purpose of the study and told the study was measuring cognitive function and vascular health. Once consented, a health history, Spielberger State-Trait anxiety inventory (STAI), and food log form were administered. Height and weight were measured, and body composition was assessed via Dual X-Ray absorptiometry (DXA). A reactive hyperemic response measurement using NIRS was performed to familiarize participants with the procedure. A maximal oxygen uptake test ($VO_2$max) was performed on a cycle ergometer. The participants provided a food log and were asked to eat similarly the day of their second and third visit. Participants were asked to report back to the laboratory within 3-7 days of the initial visit. The order of CON and EX visits was randomized and counter-balanced among study participants such that on the first visit 7 completed the control condition and 8 completed the exercise condition. For each participant, all three visits were performed across 2-6 weeks. After the final visit, participants were debriefed as to the true purpose of the study.

***Control Visit (CON):*** During the control visit, participants completed the STAI and food log form which took ~10 minutes. Then an initial saliva sample was collected which took ~5 minutes. Next, the participant assumed a supine position and heart rate and blood pressure were

measured continuously via a small finger blood pressure cuff placed on the index finger of their right hand (Ohmeda 2300 Finapres). The baseline vascular assessment was performed using NIRS, a procedure which took ~30 minutes. The participant sat up and a second saliva sample was obtained, then the participant sat alone quietly on the cycle ergometer for 25 minutes. After this, another saliva sample was collected then the STAI was completed. The participants then rested in a supine position on a padded table. After resting for 5 minutes, a second vascular assessment via NIRS was performed, followed by an additional 10 minutes of supine rest. Two research team members dressed in white lab coats with clip boards then entered the room (1 male, 1 female), administered a serial arithmetic subtraction test, and then left the room. Next a saliva sample was collected and the STAI was completed. The final NIRS-based vascular assessment was administered within 15 minutes of completion of the stressor. Lastly, the individual sat up and a final STAI was filled out.

***Exercise Visit (EX):*** For the EX condition, all procedures were identical to the CON condition except that instead of sitting quietly on the cycle ergometer for 25 minutes, participants completed a 25-min submaximal exercise bout that consisted of a 5-min warm up followed immediately by 20 min of cycling at 90% of their ventilatory threshold. This intensity and duration of exercise was chosen based off previous studies indicating improvement in vascular function outcomes at this intensity[103]. Figure 3.1 depicts the layout of measurements during the control and exercise conditions.

***Maximal Exercise and Submaximal Exercise bouts***

Maximal oxygen consumption ($VO_2$max) was measured via a ramped bicycle protocol[104]. Power output was increased by 30 watts every two minutes until volitional exhaustion. Expired gases were continuously measured via indirect calorimetry (Parvo Medics TrueOne 2400; Parvo

Medics, Salt Lake City, UT), and heart rate was continuously monitored throughout the exercise test via a chest heart monitor (Polar; Polar Electro Inc., Lake Success, NY). A maximal test was completed by all participants. The test was considered maximal if the oxygen difference between the last two stages of the test was less than 250 mL/min. In the absence of a plateau, if two of the following criteria were met the participant was considered to have reached maximal oxygen consumption: Respiratory exchange ratio >1.10, rate of perceived exertion >17, maximal heart rate within 10 beats per minute of age predicted maximum.

The power output for the submaximal exercise bout was determined from individual's ventilatory threshold obtained from the maximal exercise session. Two members of the research team independently identified the ventilatory threshold using the v-slope method, and a workload that elicited 90% of the ventilatory threshold was calculated[105]. During the exercise visit, all participants performed a five-minute warm-up, prior to performing 20 minutes of exercise at 90% of their ventilatory threshold. Participants' rating of perceived exertion was obtained every five minutes and an average rate of perceived exertion is reported.

***Near-Infrared Spectroscopy Assessment of Microvascular Function***

All vascular measures were taken in a temperature-controlled room (22-24℃). Reactive hyperemia was measured using Continuous-Wave Near-Infrared Spectroscopy (CW-NIRS). This non-invasive technique indirectly measures oxygenated, deoxygenated, and total hemoglobin in the muscle microvasculature and has been shown to correlate well with other techniques of microvascular functions[106-108]. This measure is highly reproducible and the standard error of the measurement for the outcome used in this study has ranged from ~7- 27%[22]. Baseline reactive hyperemic responses were measured in the left gastrocnemius muscle via CW-NIRS (PortaMon, Artinis Medical Systems) this site was chosen because of the lower coefficients of variation[21]

and it being moderately activated during cycling[109]. The adipose tissue thickness (ATT) was measured via ultrasound (LOGIQ, GE HealthCare). The CW-NIRS device was secured to the calf, and the leg was elevated to the level of the heart [21]. A blood pressure cuff was placed proximal to the knee and was rapidly inflated (Hokanson) to 250-300 mm Hg for ~5 minutes. The cuff was rapidly deflated, and the blood flow responses were measured until values returned to baseline. As shown in figure 3.2, we examined the following parameters from NIRS data:(i) the time course for the oxygen signal to reach 50% peak reperfusion blood flow after the occlusion and is a measure of perfusion rate ($T_{1/2}$)[21]; (ii) Muscle metabolic rate was measured using the slope of the decline in oxygen levels during the occlusion (expressed as muscle oxygen uptake or $mVO_2$)[22, 23]; (iii) The $HbO_2$ Range was used to normalize the NIRS signal[110]; with just prior to cuff release being 0% saturation and 100% being the peak hyperemic response (Range of the $HbO_2$ from the cuff release to peak response are expressed in optical density units) and (iv) the slope of $HbO_2$ after peak blood flow during the recovery period (post peak-hyperemic recovery slope,), taken as an index of sustained microvascular dilation evoked by the increase blood flow in response to deflating the cuff; (v) percent changes in basal muscle microvascular dilation examines mental stress-induced changes in microvascular flow[111] (see figure 3.3).

***Mental Stress Task***

Serial subtraction was used as the mental stressor and was administered by two research team members dressed in white lab coats, each with a clip board and pen for making notes (1 male, 1 female). These individuals corrected participants every time an incorrect answer was given and prompted participants to increase their pace every 15 seconds. The first serial subtraction involved sequentially subtracting 13 from a 4-digit number, and if an incorrect response was given, they were asked to start over. This continued for 5 minutes. Then then

participants serially subtracted the number 7 from a 3-digit number. This task lasted 5 minutes. Thus, the stressors lasted 10 minutes in total. Participants were told that a video recording would be taken during the tasks to increase the magnitude of the stress stimulus. Although no video was actually recorded, a member of the research term held a recording device with the camera lens pointing in the participants' direction, and the light was turned on to create the illusion that responses were being recorded. Previous studies have shown greater responses to stressor tasks when social evaluation and video recordings occur[112].

***Psychometrics***

The State and Trait Anxiety Inventory (STAI) was used to assess individuals' stable (trait) and transient (state) anxiety. A large body of correlational and experimental evidence supports that state and trait scores provide valid measures of state and trait anxiety[113, 114]

***Blood Pressure and Pulse Rate***

Blood pressure was measured using Ohmeda 2300 Finapres. A small finger cuff was placed on the middle finger and data was continuously measured at 200 Hz. Acquisition of blood pressure data was carried out using a Biopac MP150 physiological data acquisition system (Biopac Systems Inc., Goleta, CA) and AcqKnowledge software (Biopac Systems, Inc.). Two-minute averages were taken at baseline, after rest or exercise, and during the mental stress test at 0-2 min, 5-7 min, and 8-10 min. Due to technical problems, blood pressure data pulse rate were only available from a subset of n=7 and n=6 participants.

***Cortisol***

Prior to each saliva sample, participants rinsed their mouth with water, and samples were collected 10 minutes later. Saliva samples were collected via passive drool. The samples were taken after the NIRS assessment, after rest/exercise, after mental stress, and at the end of the

visit. Samples were frozen immediately after collection and stored at -80℃ until analyzed. A subset of participants (n=10) were used to evaluate free salivary cortisol levels during these conditions. Cortisol levels were quantified in duplicate using an ELISA kit (Alpco., Salem NH).

***Data Analysis***

A large effect size of 4.33 SD has been reported for stress-induced impairments in large conduit artery FMD[115]. Because the stressor length and type varied and FMD in large conduit arteries for this study are not evaluated, a conservative effect size of 1.0 SD was used for power analysis. An a priori power analysis indicated a sample of 15 participants would provide > 80% power to detect a .50 standard deviation change in microvascular function post-stress compared to baseline in the exercise condition compared to no change in the control condition. The calculation assumed a two-tailed alpha value of 0.05 and a correlation between repeated measures of r = 0.60[116].

The oxygenated hemoglobin signal ($HbO_2$) was selected for the analysis of stress induced changes of microvascular function. The raw data collected from the NIRS device were exported and analyzed by two individuals. Individuals analyzing the NIRS data were blinded to the Psychometric scores of participants. A custom-written routine in MATLAB® R2014b (MathWorks Inc.) was used to analyze wash-in kinetics. Intraclass correlations were calculated for each of the NIRS outcomes.

Statistical Analysis was conducted using IBM SPSS, version 24 (Amronk, New York, USA). To test whether order of the visits affected any of the outcomes, an ANOVA with Condition (2) and Time (3) with visit order as a between-subjects factor was performed. NIRS data were analyzed using a repeated measures ANOVA with Condition (2) and Time (3). When the interaction was significant, the potential influence of trait anxiety was tested using trait

anxiety as a covariate in an ANCOVA model. Similarly, the potential influence of changes in state anxiety throughout the experiment was tested using state anxiety scores as time varying covariates. State anxiety data also were analyzed using a Condition (2) by Time (5) repeated measures ANOVA. Blood pressure and heart rate data were analyzed using a Condition (2) by Time (6). The Greenhouse-Geisser adjustment was used when the assumption of sphericity was violated. Fisher's least significant difference was used for post hoc pairwise comparisons. Statistical significance was accepted at $P \leq 0.05$.

## Results

### *Subject Characteristics*

Nineteen individuals volunteered for the study. Four were excluded from analysis (One was excluded due to poor data quality, two dropped out from the stud; one for scheduling issues and one did not want to perform the mental stressor again, and one disclosed on the initial visit that they had been diagnosed with an exclusion criteria). A total of 15 individual were used for data analysis. Participant characteristics are presented in table 3.1. Participants identified as Caucasian (n=13), Asian (n=2), and other (n=1). Participants were in the low end of the normal range for Vo2peak and trait anxiety.

### *Psychometrics*

State-anxiety scores are presented in figure 3.4. No Condition x Time interaction or main effect of condition were observed. There was a main effect of time on state-anxiety scores ($F_{(2,\ 1.3)} = 8.0, p < .05, \eta 2 = .36$). In both conditions, state anxiety scores were low (compared to norms) at baseline, and post-hoc tests showed that state anxiety decreased after the NIRS measurements, did not change after the rest/exercise conditions, increased after the mental stress and returned to baseline levels at the end.

***Blood Pressure and Pulse Rate***

A main effect of time on systolic blood pressure was observed $\left(F_{(2,\ 2.3)} = 22.4, p < .001, \eta 2 = .79\right)$. Systolic blood pressure was higher during mental stress at all time points during the stressor (138.6±2.93, 144.0±3.22, and 146.8±3.55) for both conditions compared to baseline (121.12±4.30) and the end of the testing session (128.05±2.33). Diastolic blood pressures had main effects for Condition which approached statistical significance ($F_{(2,\ 2.2)} = 5.2, p = .063, \eta 2 = .464$) and a main effect of Time which was significant ($F_{(2,5)} = 20.5, p < .001, \eta 2 = .77$). Diastolic blood pressure was significantly elevated during the mental stressor (83.62±1.79, 84.00±2.14, and 84.65±2.08) compared to baseline (72.22±2.57) and end of visit (73.90±1.87). Blood pressure responses are shown in figure 3.5. A main effect of Time on pulse rate was observed ($F_{(2,5)} = 6.5, p < .05, \eta 2 = .56$) as shown in figure 3.6.

***Near-Infrared Spectroscopy Assessment of Microvascular Function***

Intraclass correlation coefficients for all NIRS outcomes ranged from .83 to .99, values > .70 are considered adequate. No order effect was observed for any of the NIRS outcome. NIRS outcome measures are illustrated in figure 3.2. A representative tracing of the $O_2HB$ signal is presented in figure 3.3. For $T_{1/2}$ a main effect of time was significant ($F_{(1.4,13)} = 11.5\ p < 0.05, \eta 2 = 0.45$). Post hoc pairwise comparisons indicated a significant decrease in $T_{1/2}$ after mental stress compared to baseline ($9.16 \pm 0.53$ vs $8.04 \pm 0.46, p < 0.05$) and after rest/exercise ($8.68 \pm 0.439$ vs $8.04 \pm 0.46 p < 0.001$)(Figure 3.7).

For O2range, a main effect of time on $O_2$Range was observed($F_{(1.4,13)} = 11.9\ p < 0.05, \eta 2 = 0.46$) and is illustrated in Figure 3.8. Oxygen range was higher after mental stress compared to either baseline($34.41 \pm 2.94$ vs $31.61 \pm 2.48, p < 0.05$) or after rest/exercise($34.41 \pm 2.94$ vs $31.20 \pm 2.62, p < 0.001$).

An interaction effect of Condition x Time was observed for basal muscle microvascular dilation ($F_{(1.52,49)} = 6.8\ \mathrm{p} < 0.05, \eta 2 = 0.33$) and the data are presented in Figure 3.9. Compared to baseline, basal muscle microvascular dilation was significantly reduced after rest in the control condition( $76.64 \pm 1.14$ vs $72.21 \pm 1.21, p < .001$), and non-significantly increased after exercise ($77.98.41 \pm 1.71$ vs $80.85 \pm 1.38, \mathrm{p} = .069$). Compared to post-rest/exercise, basal muscle microvascular dilation was increased significantly during mental stress both CON and EX conditions($\mathrm{p} < 0.001$). The mental stress-induced basal muscle microvascular dilation was greater in EX compared to CON ($90.70 \pm 1.50$ vs $85.01 \pm 1.98$, high $\mathrm{p} < 0.05$). However, the magnitude of the increase during mental stress was larger after rest (~13%) compared to the increase from after exercise (~10%). After mental stress, basal muscle microvascular dilation was significantly reduced in both conditions and the value returned to baseline (75.87±1.49 vs 76.64±1.13, p=0.516) in the control condition but was elevated above baseline in the exercise condition (83.16±1.12 vs. 77.97±1.71, p<0.05). When trait anxiety scores were included in the model as a covariate, the interaction was no longer significant $\left(F_{(1.51,39)} = 0.38\ \mathrm{p} < 0.77, \eta 2 = 0.028\right)$. When state- anxiety levels during the visit were include the Condition x Time interaction was no longer significant ($F_{(1,111)} = 2.33, \mathrm{p} = 0.08$), indicating that trait anxiety and state anxiety partially mediates the muscle microvascular response.

Post peak-hyperemic recovery slope responses showed an interaction of Condition x Time for the Recovery slope ($\mathrm{F}(2,13) = 5.5, \mathrm{p} < 0.05, \eta 2 = 0.28$). As shown in figure 3.10 the post peak-hyperemic recovery slope significantly increased from BL to rest in the control condition ($0.17 \pm .01\ vs.\ 0\ .21 \pm 0.1\ p < 0.05$). After mental stress there was a decrease in Post peak-hyperemic recovery slope, compared to after rest ($0\ .21 \pm 0.1\ vs.\ 0.18 \pm 0.01, p < 0.05$). Between the control and exercise condition at the rest vs. exercise time point there was a

significant increase in sustained vasodilation after exercise $(0.21 \pm 0.01\ vs.\ 0.15 \pm 0.01 p < 0.05)$. During the EX condition, there were no differences in the post peak-hyperemic recovery slopes across time. When trait anxiety was included as a covariate the Condition x Time interaction was no longer significant $(F(2,26) = 1.22, p < 0.311, \eta 2 = 0.07)$. When state anxiety level was included as a time-varying covariate the Condition x Time interaction was no longer significant $(F(2,13) = 2.66, p = 0.08,)$.

***Muscle Metabolism***

A significant interaction effect of Condition x Time on $mVO_2$ was observed $(F(1.1, 28) = 6.5\ p < 0.05, \eta 2 = 0.319)$, as seen in figure 3.11. Post hoc comparisons indicated $mVO_2$ was significantly increased after EX compared to rest$(0.18 \pm 0.00\ \text{vs.}\ 0.015 \pm 0.00, p < 0.05)$. Within the control condition, mVO2 was increased after mental stressor compared to rest$(0.015 \pm 0.00\ \text{vs.}\ 0.016 \pm 0.00, p < 0.001)$. In EX condition, there was an increase in $mVO_2$ from BL to after EX by $(0.016 \pm 0.00\ \text{vs.}\ 0.018 \pm 0.00, p < 0.05)$ and from BL to after MS approached statistical significance $(0.016 \pm 0.00\ \text{vs.}\ 0.019 \pm 0.00, p = 0.065)$. When trait anxiety was included as a covariate the Condition x Time interaction was no longer significant $(F(1.1,26) = 1.20, p < 0.317, \eta 2 = 0.09)$. When state anxiety level were included as a time-varying covariate the Condition x Time interaction was no longer significant $(F(2,13) = 2.10, p = 0.13,)$.

***Cortisol***

Salivary cortisol concentrations are presented in figure 3.12. No effects of condition x time $(F_{(3,27)} = .52, p = .59, \eta 2 = .06)$ Condition$(F_{(1,1)} = .09, p = .77, \eta 2 = .01)$ or time $(F_{(1,3)} = .66, p = .58, \eta 2 = .07)$ were observed. The intra-assay variability was 2.9% and 2.4 %. The inter-assay variability was 4.6%.

## Discussion

The effects of acute exercise on microvascular function and hemodynamic responses during and after mental stress were investigated in healthy males. The main finding of our study was that the combination of exercise and mental stress produced an additive effect on basal microvascular muscle dilation, suggesting that the acute exercise and mental stress increase muscle microvascular flow through largely separate mechanisms. If mental stress and exercise were working through the same mechanisms it could be hypothesized that the control and exercise to have similar basal muscle dilation during mental stress and both would return to pre-mental stress values. However, in the exercise condition a further increase and sustained elevation in basal muscle microvascular dilation was observed during mental stress and after mental stress. Control values during mental stress increased and returned to baseline values in the control condition after mental stress. Together, these data suggest somewhat separate mechanisms governing the acute vasodilator responses to mental stress and the sustained basal muscle microvascular vasodilation which persists following aerobic exercise.

### *Impact of Exercise, Mental Stress, and the Combination on Basal Microvascular Flow*

Many of the previous studies evaluating mental stress and microvascular responses have had primary hyperemic responses as the vascular outcome[94, 98, 118, 119]. These responses are largely mediated by shear stress placed on the vessel and are an indicator of total vessel dilation in response to hyper-physiological stress. The basal muscle microvascular dilation data presented in this study provides information as to the change in microvasculature tone under normal conditions; the mental stress induced basal muscle microvascular dilation in both conditions and both conditions had similar magnitudes of increase. This in part may be explained, by the increase in blood pressure leading to a great shear stress placed on the vessels during mental

stress. Shear-stress is an important stimulus for NO production. Studies using hyperemic response have indicated that a large portion of stress induced dilation is blunt with blockage of NO-dependent[120, 121]. The basal muscle microvascular dilation observed in the rest condition, during mental stress, may have been mediated by changes in shear stress.

The increase in basal muscle microvascular dilation observed after exercise may be due to increases in muscle temperature, metabolic by-product, modulation of receptor type such as beta receptors, and reduced post-exercise muscle sympathetic nerve activity. Previous studies of acute exercise have indicated substances such as endothelin-1, prostaglandins, and NO are involved in regulation of microvascular function and are increased during exercise[122,91, 123, 124]. According to Pearson and colleagues temperature increases of ~ 1℃ account for approximately of half of calf microvascular dilation response observed with exercise[125]. Additionally, exercise at ~70% of $VO_2$max have previously been report to improve beta receptor sensitivity [85] and a pharmacological study indicated a 21% decrease of blood flow in responses to mental stress with the administration of propranolol a beta agonist[126]. Although not measured during the present study, these factors may have contributed to our observation of elevated basal muscle microvascular dilation during mental stress after exercise in the present study.

***Peak Hyperemia and Post Peak-Hyperemic Slope Response***

Contrary to previous studies indicating improvements of hyperemic response with exercise[29] and impairments with rest[127] hyperemic responses as measured by $T_{1/2}$ were not increased after exercise or decreased after rest. However, the post-peak hyperemic slope did indicate a reduction in shear-induced microvascular dilation in CON. These data could be explained by a decrease in shear stress during the 25 minutes of rest. The post-peak hyperemic slope after mental stress, in the control condition, indicated that stress induced a greater dilation.

Interestingly, after mental stress there was a significant decrease in the $T_{1/2}$ indicating a greater hyperemic response. The magnitude of the hyperemic response was also greater after mental stress as indicated by a larger $O_2$ range. Vranish and colleagues found that 10 minutes of sitting produced a decrease in shear stress[127]. The increases in $T_{1/2}$, $O_2$ Range, and post-peak hyperemia slope after mental stress may be explained by an increase in shear stress during mental stress.

The hyperemic response as measured by $T_{1/2}$ and $O_2$ range, however are indicators of microvascular ability to response to a physiological stress (i.e. 5-minute ischemia). While not measured in the study, vasodilation responses observed during stress may be due to local NO production. Blockade of NO synthase activity via the administration of L-NMMA blunted the hyperemia during mental stress by ~47%,[120, 121] indicating that approximately half of the mental stress-induced changes in microvascular flow are NO-mediated. Alternatively, mental stress has previously been reported to increase pulse wave velocity a measure of atrial stiffness. Three minutes of mental arithmetic increased pulse wave velocity for ~ 30 minutes[128]. All of our measurements were taken ~15 minutes after the mental stress, thus it is possible the increase in hyperemic response observed in the control condition may be reflective of increased arterial stiffness. This hypothesis should be examined in future studies.

***Muscle Metabolism in Response to Rest or Exercise and Mental Stress***

While mental stress has been implicated in metabolic dysfunction and prolonged stress is thought to modify mitochondria [129], to our knowledge, these data are the first to suggest that acute mental stress increases the metabolic rate of resting skeletal muscle. Interestingly, the combination of prior exercise and mental stress did not further augment metabolic activity beyond exercise alone. The lack of effect of mental stress on $mVO_2$ in EX suggests that the metabolic effects of exercise were sufficient to overpower any metabolic effects of mental stress,

which are likely to be more subtle. Due to the variability in our results, it is possible that our study was underpowered to observe a meaningful change. Nevertheless, it is plausible that exercise and mental stress may increase muscle oxygen consumption in resting muscle. Acute exercise increases resting metabolic rate for up to three hours post exercise[130]. The increase in $mVO_2$ observed in this study may have been the result of sustained post-exercise increases in muscle oxygen consumption.

***Blood Pressure Responses***

A lower diastolic blood pressure was observed in the exercise condition at all-time points which approached statistical significance. Moderate exercise has previously been shown to improve blood pressure responses to a Stroop stressor[131]. Due to the smaller sample size for these data, there was insufficient power to detect the effects of exercise on blood pressure responses to mental stress. A large effect was observed with a $\eta 2 > .25$ for diastolic blood pressure and a small to medium effect ($\eta 2 = .059$) was observed for systolic pressure. Conversely, the lower diastolic pressure observed in conjunction with the basal muscle microvascular dilation data indicate the peripheral microvascular dilation may help to explain the post-exercise induced hypotension. Future studies of both microvascular and macrovascular function are needed to determine the role of exercise has on the different vascular branches in terms of hypotension.

***Cortisol Responses across Conditions***

Although previous studies have indicated that cortisol decreases endothelial function [4, 132], saliva cortisol responses to stress were not significantly elevated in the present study, suggesting that cortisol is unlikely to have mediated the effects of mental stress in our experiment. The stressor used in this study consistently induced an elevation in state anxiety and tension. However, we did not observe the increase in cortisol production that has previously

been reported with stressors [4, 133]. The cortisol values at baseline were elevated in this study were compared to other studies, but this may be due to ingestion of caffeinated beverages[134], insufficient sleep[134, 135], and timing of previous meal[136]. To the best of our abilities we tried to control for these factors by asking subjects to stop caffeine consumption 4 hours prior to their visits, and eating a snack 2 hours prior to their visits.

***Trait and State Anxiety a Mediator of Microvascular Response***

Both trait and state anxiety levels were found to moderate of the responses observed in the basal muscle microvascular dilation, post peak-hyperemic response, and the $mVO_2$. To the authors' knowledge, this is the first study indicating anxiety levels moderate peripheral microvascular responses to stress with and without prior exercise. Previous studies evaluating brain microvascular responses in the lateral prefrontal cortex to a stressor have found that state anxiety levels were positively correlate with oxygen saturation levels[137]. However, the relationship between brain blood flow responses and peripheral microvascular responses has yet to be explored. How anxiety moderates peripheral vascular function is not well understood. A potential mechanism is via increases in sympathetic outflow. A recent study evaluating muscle sympathetic nerve activity found that individuals with high trait anxiety have greater muscle sympathetic nerve amplitude responses to mental stressors[138]. Previous studies have reported a decreased muscle sympathetic nerve activity is concurrent with increased vasodilation after exercise[139, 140]. In light of this study, exercise performed may have blunted the muscle sympathetic nerve activity to a greater extent in those with high anxiety levels and enabled a greater microvascular dilation response. Future studies are need to better understand how state and trait anxiety levels impact microvascular function.

The initial interaction observed with the muscle oxygen consumption indicated that muscle metabolic rate is moderated by both trait and state anxiety levels. A previous study examining whole body resting metabolic rate found that individuals with higher state and trait anxiety levels also have higher resting metabolic rates[141]. The muscle oxygen consumption increases from after rest to after the mental stress in the control condition may be explained by the state anxiety induced by the mental stressor.

***Limitations***

While the vascular responses and muscle oxygen consumption were different with the addition of trait and state anxiety levels, the participants included in our group would primarily be classified as low trait anxiety according to the STAI Manual cut point (i.e. less than 1 SD above the population average). Future studies are needed to determine whether microvascular function is affected in individuals with true high trait anxiety. There was no significant effect of mental stress on cortisol levels; a potential reason for this may be that we did not provide a control snack at the beginning of the visits. Experimental visits were 4 hours in length, and by the end of the visits participants had fasted for 6-8 hours. Finally, whether exercise would provide similar benefits to physically inactive individuals is not clear.

***Conclusions***

In conclusion, we found mental stress and acute endurance exercise altered muscle microvascular and metabolic function in the calf muscle. Our data suggest separate mechanisms are responsible for the exercise- and stress-induced changes in basal muscle microvascular dilation responses observed. Mental stress increased muscle oxygen consumption under resting conditions but not after exercise, suggesting that the metabolic responses to exercise may override those of mental stress. However, future studies are needed to determine if this was truly

significant or due to a stabilization of metabolism based on the elimination of external factors. Overall, our study provides evidence that the mechanisms involved in mental stress-induced changes in microvascular regulation are separate from those associated with exercise.

**Table 3.1. Participant Characteristics. Values are Mean ± SD, BMI, body mass index; $VO_{2peak}$, highest oxygen uptake values obtained.**

| **Baseline Characteristic** | |
|---|---|
| Age (yr) | 22.27± 2.31 |
| Weight (kg) | 80.62± 8.53 |
| Height (m) | 1.79 ± 0.06 |
| Calf Adipose Tissue (cm) | 0.53 ± 0.37 |
| Systolic Blood Pressure (mmHg) | 123.12 ± 37.33 |
| Diastolic Blood Pressure (mmHg) | 72.29 ± 22.33 |
| BMI ($kg/m^2$) | 24.32 ± 2.17 |
| Body Fat (%) | 18.33 ± 3.93 |
| $Vo_{2peak}$ (ml/kg/min) | 40.82 ± 5.67 |
| $VO_{2Peak}$ RER | 1.35±0.06 |
| $VO_{2Peak}$ RPE | 19.23±0.83 |
| Exercise RPE | 12.8±1.50 |
| Trait anxiety (STAI-Y2) | 32.84 ± 9.28 |
| State anxiety (STAI-Y1) | 26.93±5.97 |

**Figure 3.1: Sequence of Measurements during Control and Exercise Conditions.**

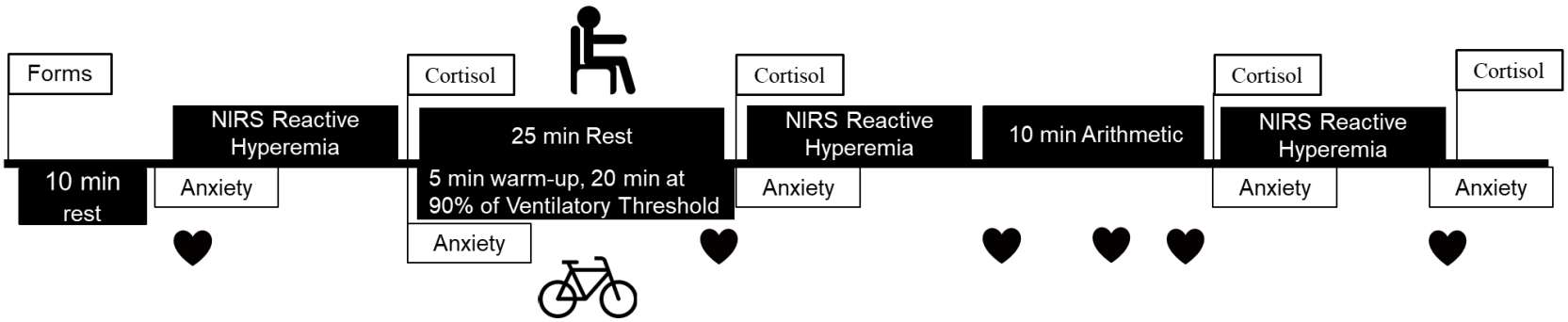


**Figure 3.1** Anxiety boxes indicate time points that state anxiety were taken. Cortisol boxes denotes the points in the study when saliva was collected. Hearts indicate the points at which blood pressure and heart rate were analyzed.

**Figure 3.2 Representative Tracing of Occlusion and Hyperemic Response.**

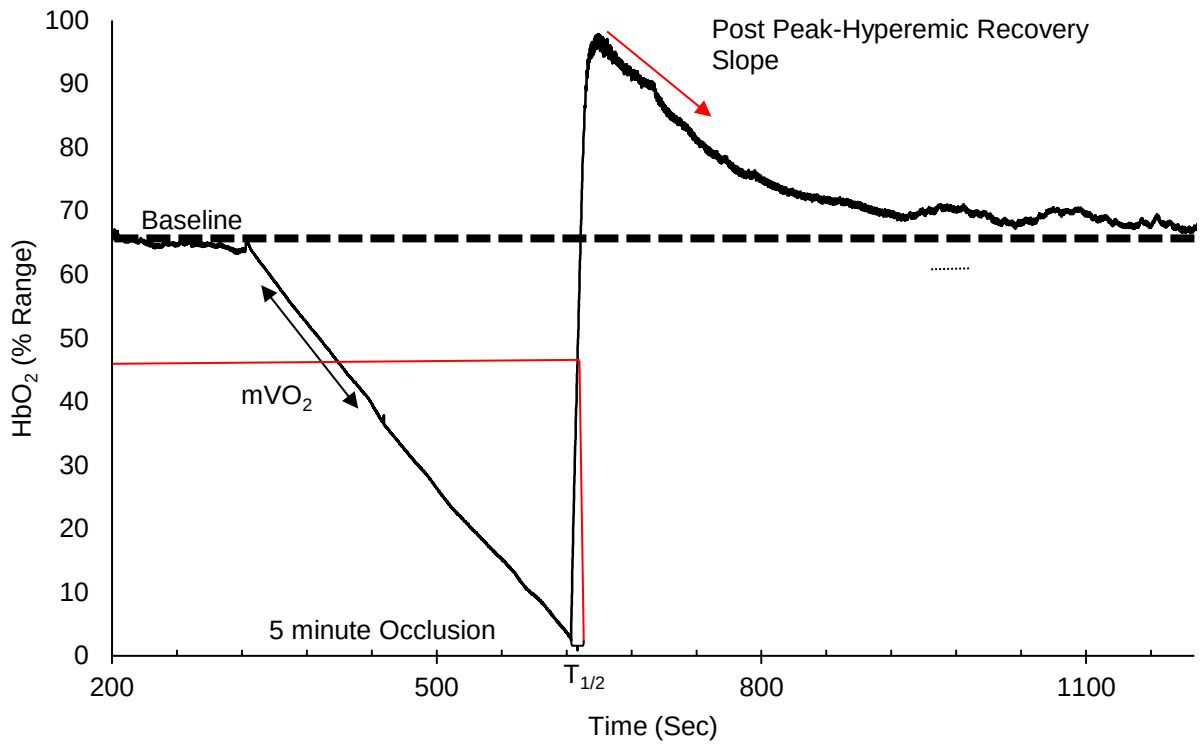


**Figure 3.2** Broken lines indication basal muscle microvascular dilation. Black arrow represents slope of muscle oxygen consumption ($mVO_2$). Red line intersection indicates time of reprofusion: time to reach 50% of reprofusion ($T_{1/2}$). Red arrow is the post peak-hyperemic recovery slope. Oxygen Range values are obtain from the absolute $HbO_2$ values and are not presented.

**Figure 3.3 Representative Tracing of Mental Stress and Hyperemic Response.**

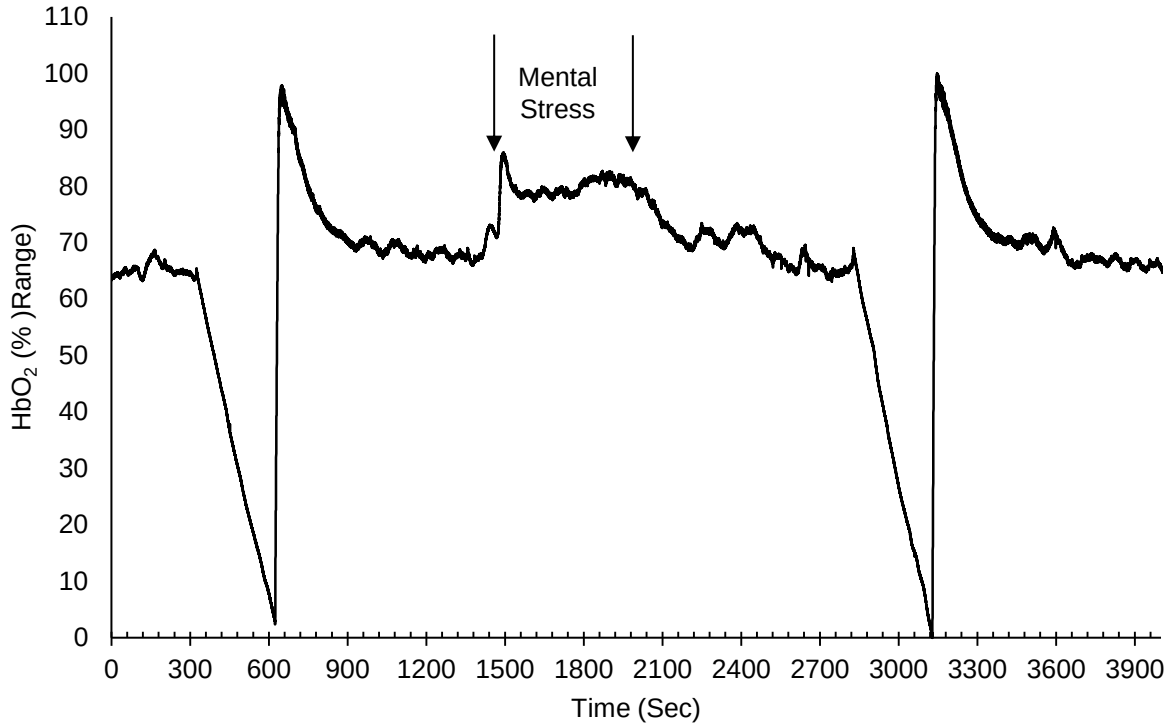


**Figure 3.3** Arrows indicate the points the mental stress started and stopped.

**Figure 3.4 State Anxiety Scores during Control and Exercise Conditions.**

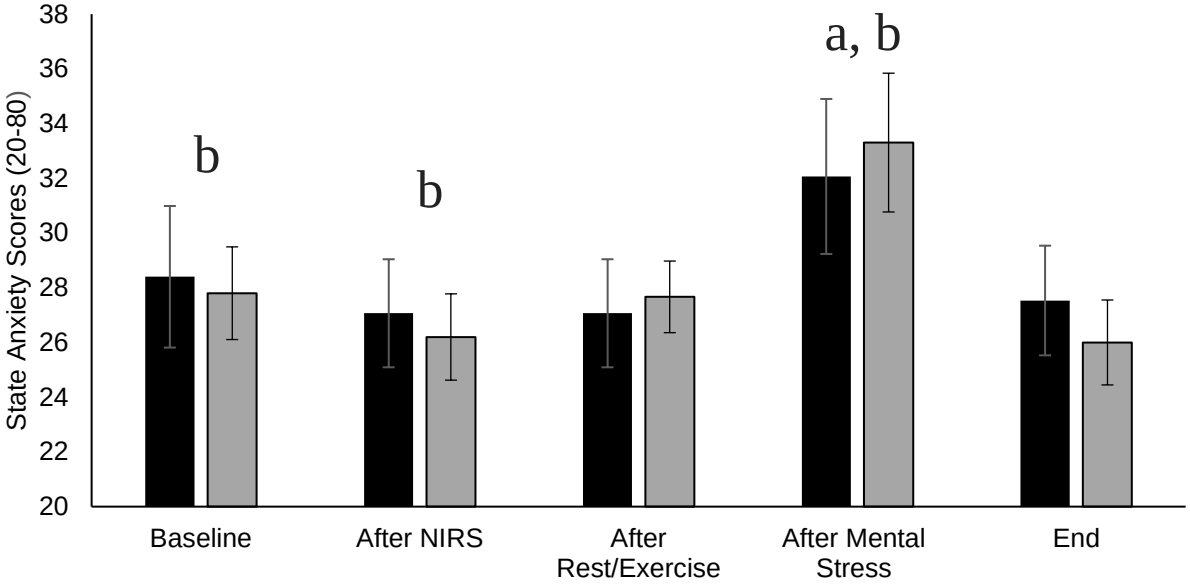


**Figure 3.4** A main effect of time was observed for state anxiety levels. “a” signifies significantly different from all other time points. “b” signifies significant differences between time points with the same letter. Means with different letters denote a significantly different from other time points across time ($p < 0.05$). n=15

**Figure 3.5 Two Minute Average Blood Pressure Responses during Control and Exercise Conditions.**

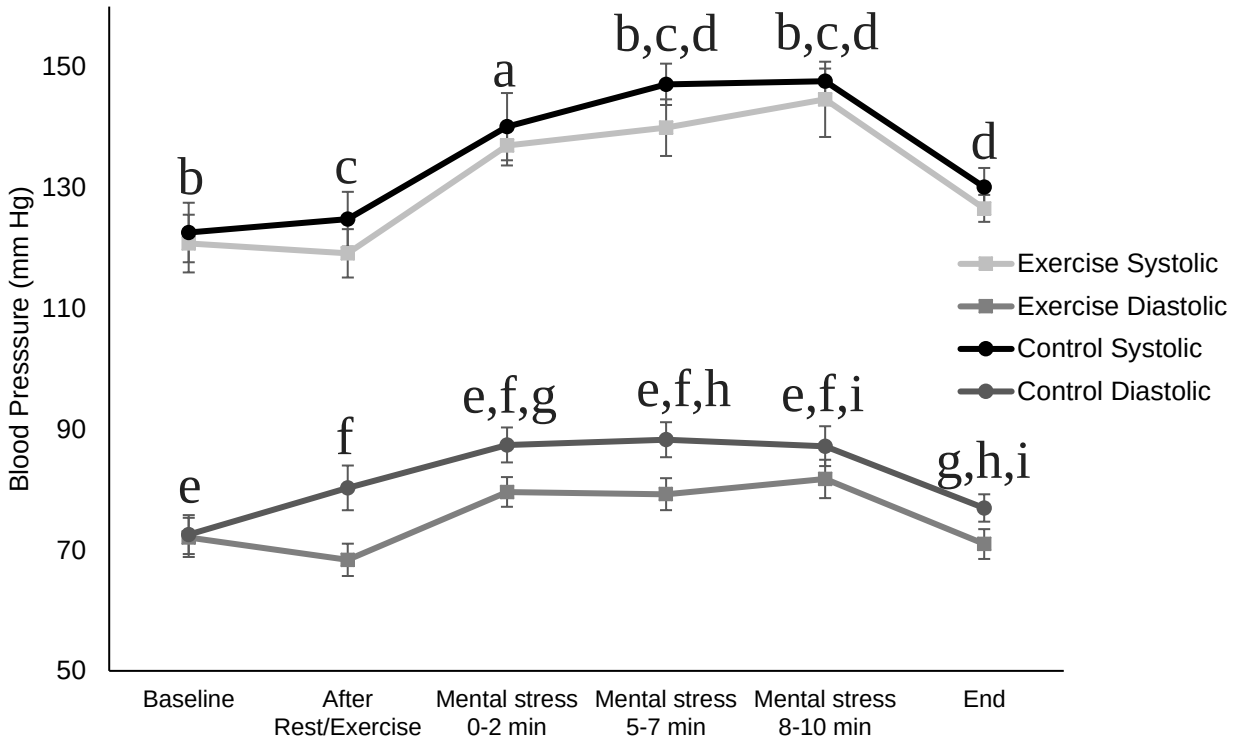


**Figure 3.5** A main effect of time was found for both systolic and diastolic blood pressure. "a" signifies significantly different from all other time points. Letters "b" through "i" signify significant differences from time points with same the letters ($p < 0.05$). Data are based on a subset of N=7 participants and are presented as means ± SE.

**Figure 3.6 Pulse Rate Responses during Control and Exercise Conditions.**

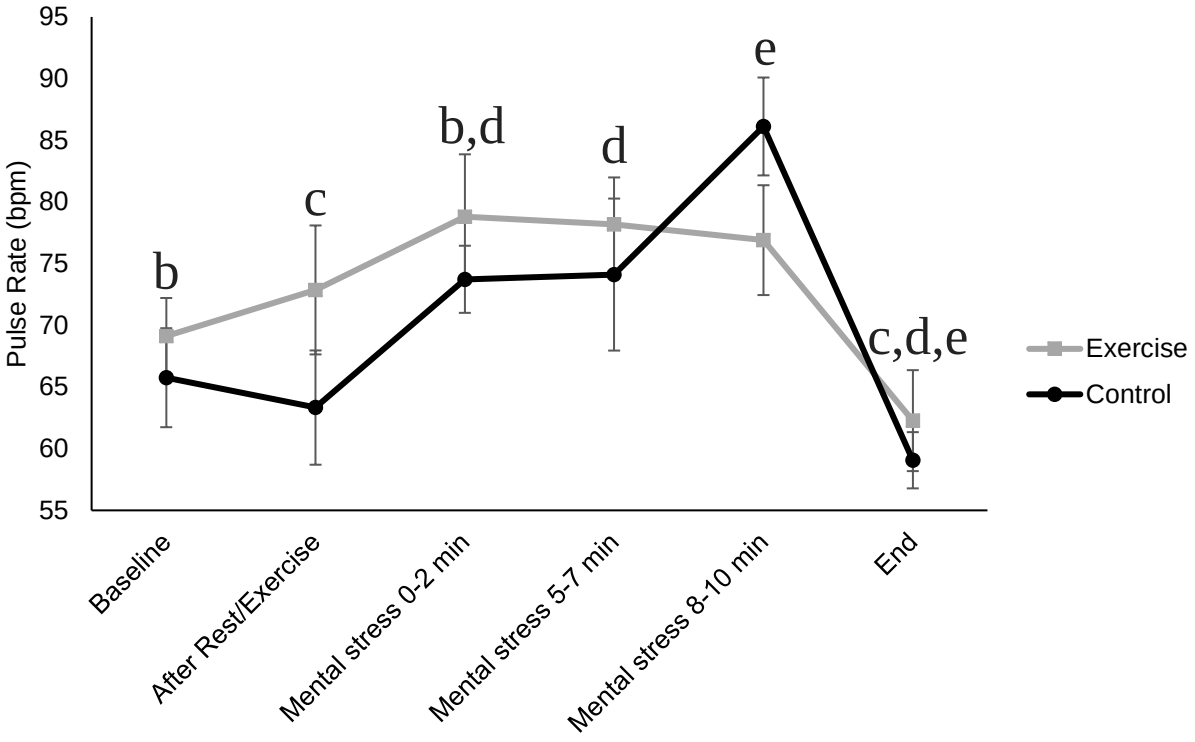


**Figure 3.6** Pulse rate data are presented as means and SD N=6. Letters "b" through "e" signify significant differences betweeen time points with same letter. Data are presented as means ± SE. Means with different letters denote a significantly different from other time points ($p < 0.05$).

**Figure 3.7 Time to 50% of Reperfusion Response during Control and Exercise Conditions.**

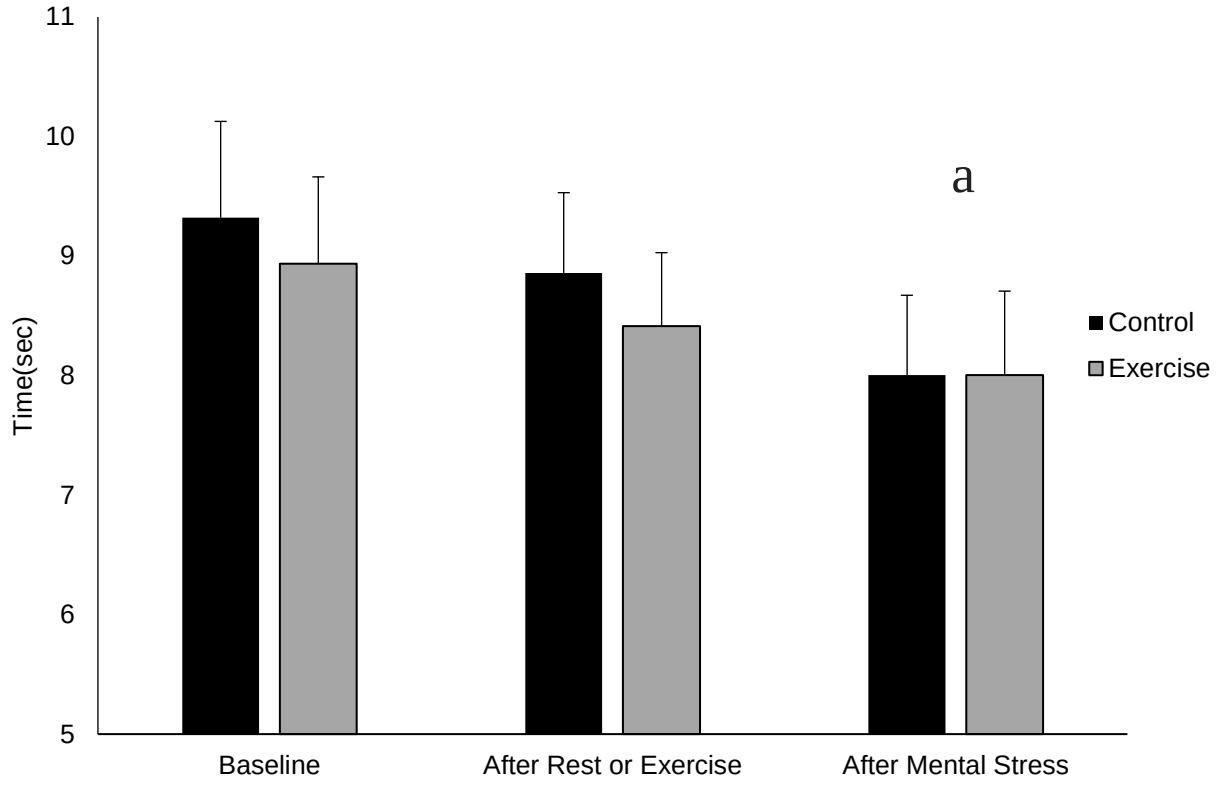


**Figure 3.7** Letter "a" denotes that time to 50% reperfusion was significantly decreased after mental stress compared to all time points ($p < 0.05$). n=15

**Figure 3.8 Oxygen Ranges during Control and Exercise Conditions.**

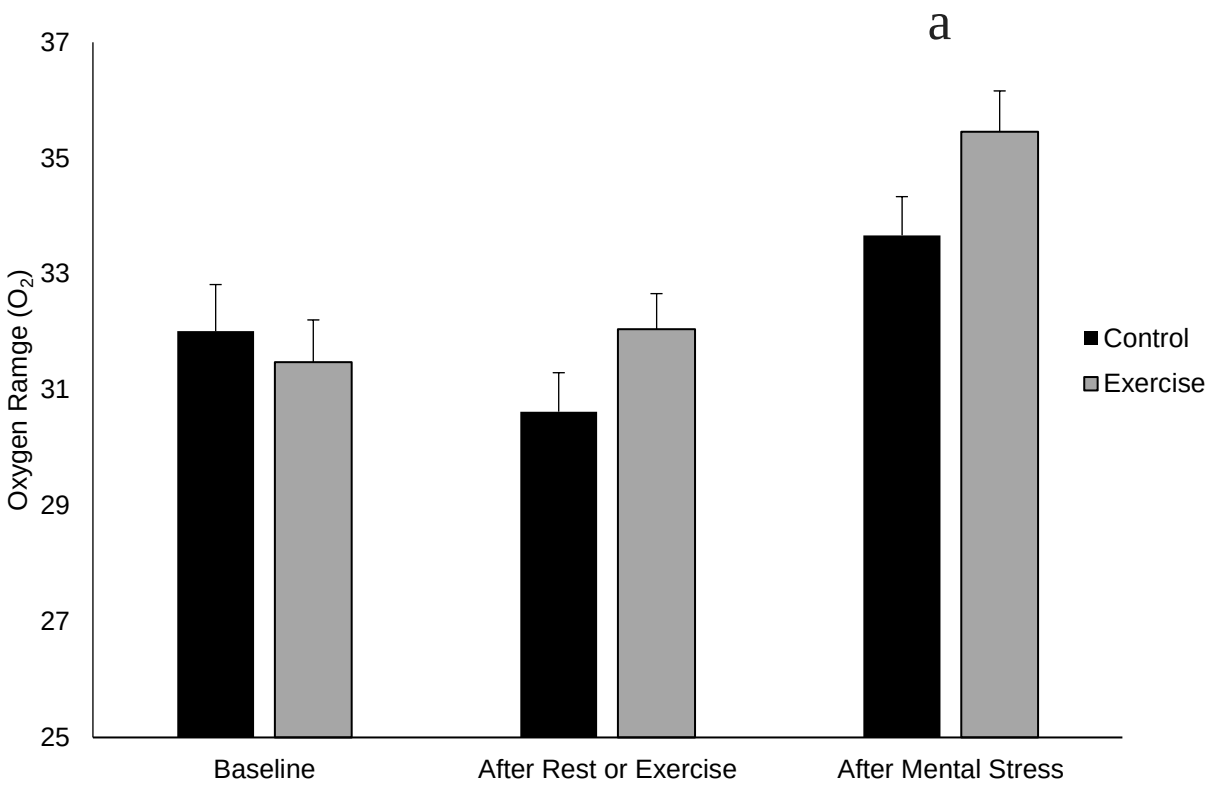


**Figure 3.8** Letter "a" denotes that oxygen range was significantly increased after mental stress compared to all time points ($p < 0.05$). n=15

**Figure 3.9 Basal Muscle Microvascular Dilation Responses during Control and Exercise Conditions.**

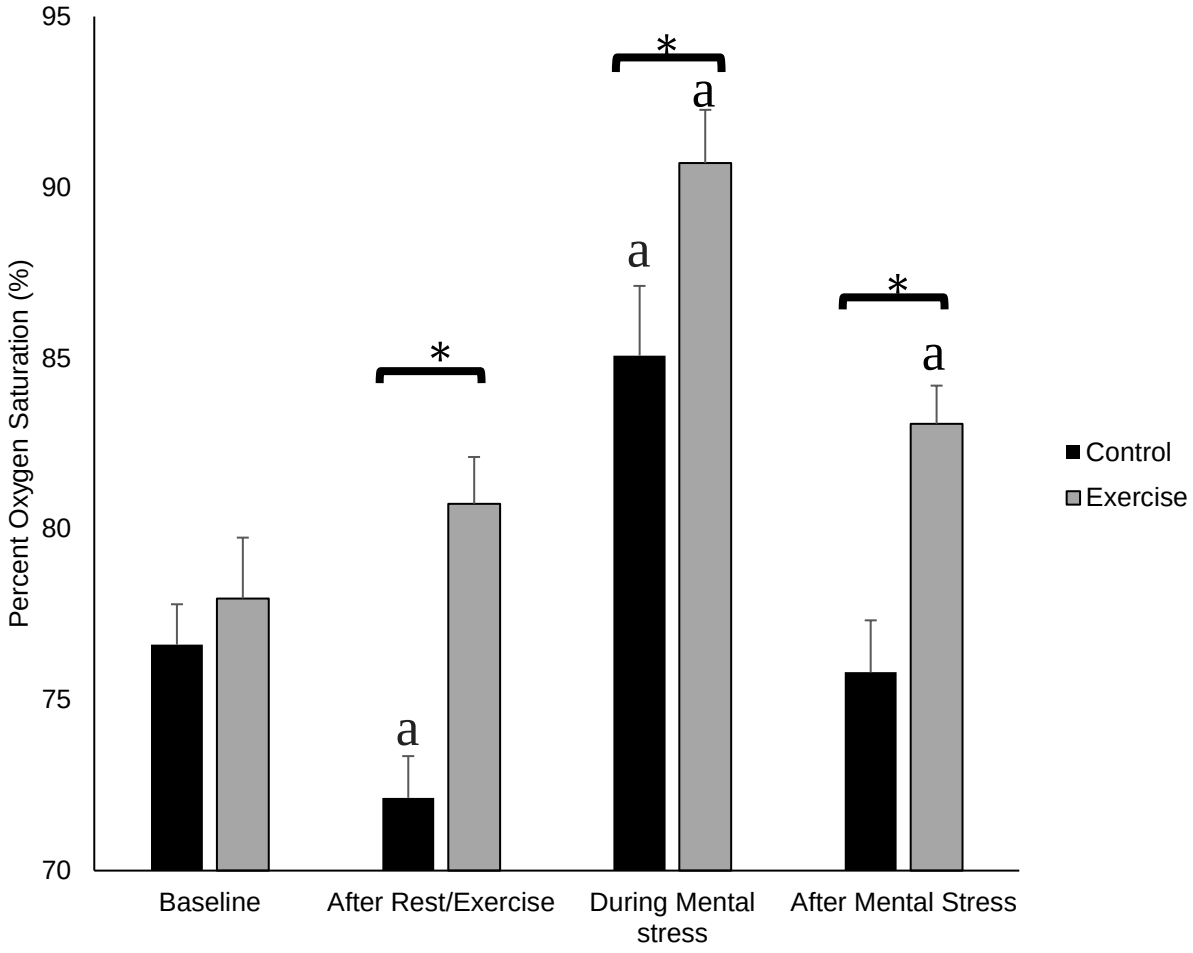


**Figure 3.9** Percent Basal Muscle Microvascular Dilationwas significantly increased in the exercise condtion compared to rest at after exercise, during mental stress, and after stress (*$p < 0.05$). Letter "a" signifies significant differences at all other time points within the condition ($p < 0.05$). n=15

**Figure 3.10 Post Peak- Hyperemic Recovery Slope Responses during Control and Exercise Conditions.**

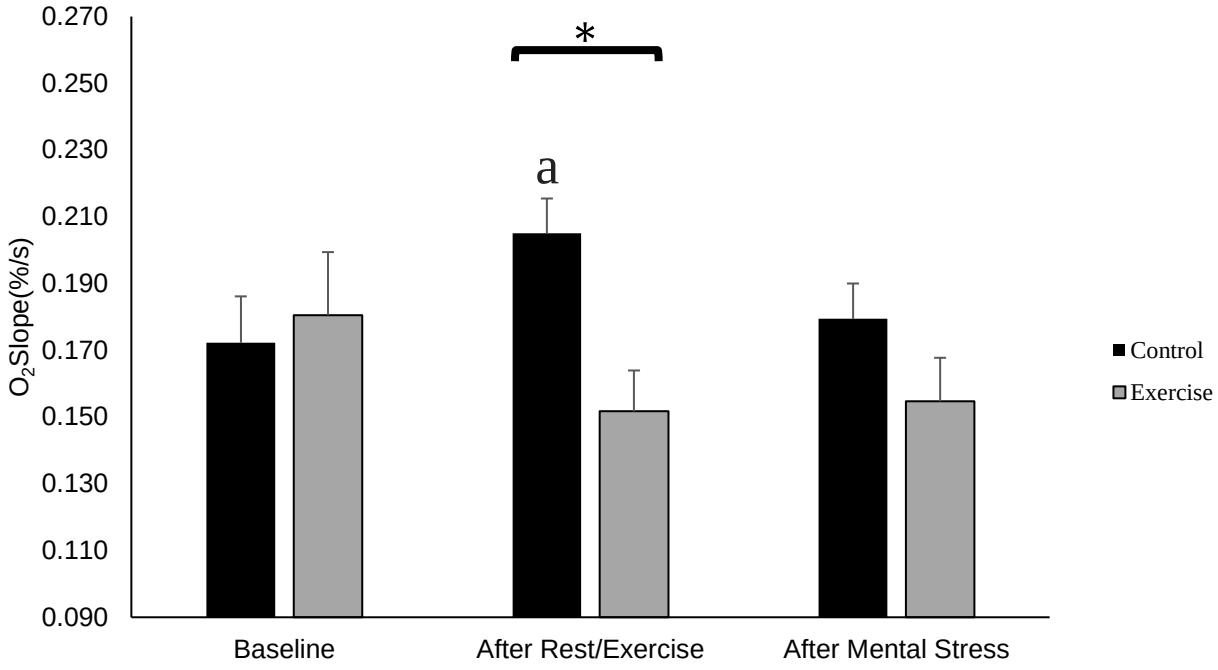


**Figure 3.10** Significant difference between after rest and exercise were observed. In the control visit, "a" denotes a significant improvement in the recovery slope after mental stress compared to all other time points ($p < 0.05$). Data are presented as absolute means ± SE. n=15

**Figure 3.11 Muscle Oxygen Consumption ($mVO_2$) Responses during Control and Exercise Conditions.**

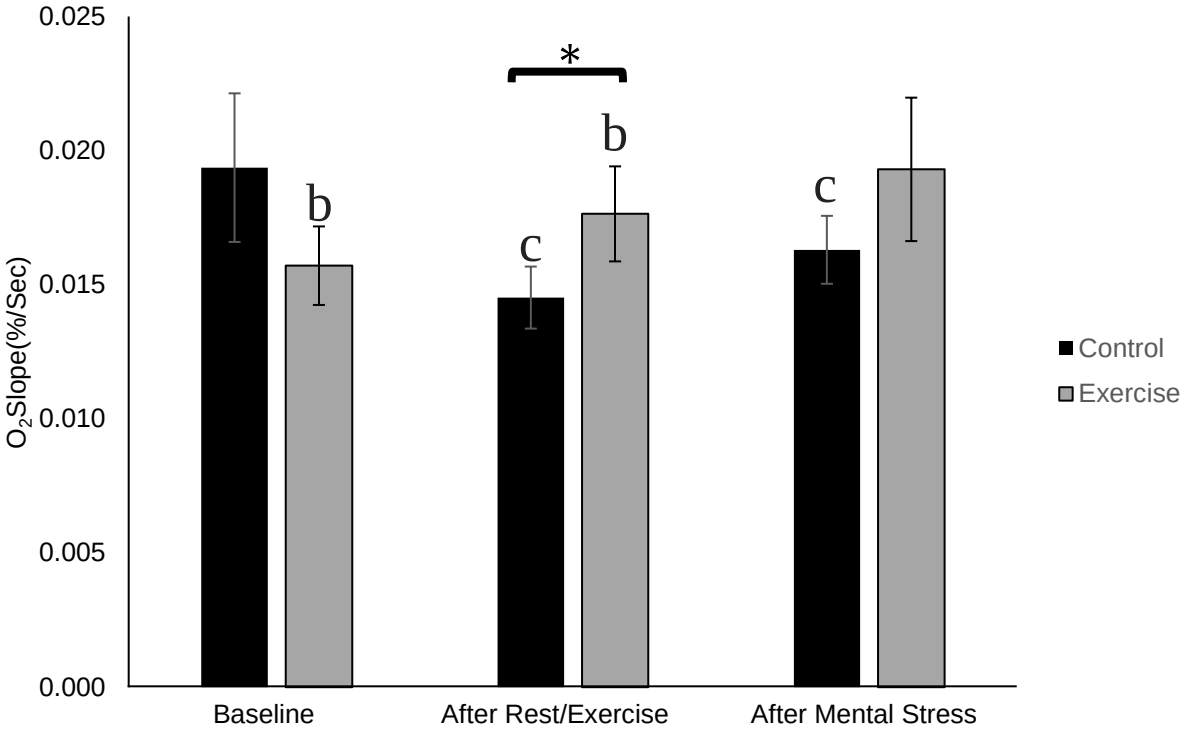


**Figure 3.11** $mVO_2$ was significantly increased after exercise compared to after rest (*$p < 0.05$).. Letters "b" and "c" denote significant differences between time points with the same letter ($p < 0.05$). Data are presented as absolute means ± SE. n=15

**Figure 3.12 Cortisol Responses during Control and Exercise Conditions.**

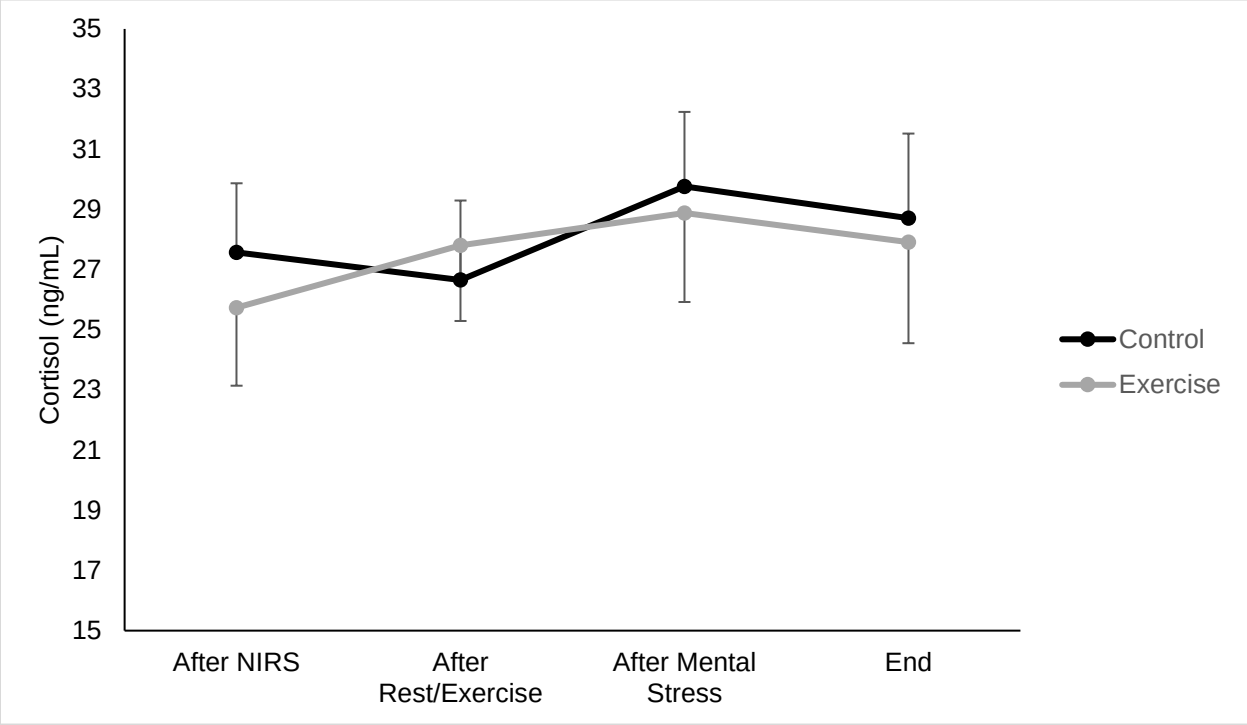


**Figure 3.12** No differences were observed at any on the time points. Data are presented as means ± SE. n=10

# CHAPTER 4

## SUMMARY AND CONCLUSION

The effects of acute aerobic exercise on muscle microvascular responses to mental stress were examined in healthy males. Consistent with our hypothesis, exercise increased muscle microvascular dilation as indicated by both an increase in basal muscle microvascular dilation and sustained dilation in the recovery phase of the post-peak hyperemic slope. Interestingly, mental stress increased basal muscle microvascular dilation and exercise prior to mental stress increased the vasodilatory response observed. Furthermore, the statistical analysis indicated that resting $mVO_2$ was increased after exercise and was greater after mental stress in the control condition.

Had $mVO_2$ been the main outcome of this study, longer rest periods at baseline or exercise prior to the baseline may have helped to stabilize the variability observed. However, this would increase the time burden on the participants. An alternative option could also be to decrease the length of cuffs and increasing the number of cuffs at each time points may have provided a more accurate measure of metabolism without an increase of visit length. Adding a controlled meal as well as monitoring blood glucose levels throughout the visit may have helped better to understand and control the variability observed. Future studies are needed to determine factors that influence the variability associated with baseline measures.

Future studies are also needed to better understand the role of shear stress, NO production, and receptor changes in muscle microvascular responses to stress and exercise. Shear stress is a potent stimulus for NO production and the data presented in this paper suggest that

changes in shear stress may relate to the decreases observed after rest in basal muscle microvascular dilation and post-peak hyperemic recovery slope. To determine the extent to which shear stress is involved could be examined by placing a shear stress stimulus (i.e. heat) on the lower extremity for the duration of the rest period and comparing microvascular responses to groups which had exercised or rested but did not receive heat. However, this would only provide data to explain the exercise responses. To investigate the role of shear stress on the increases in basal muscle microvascular dilation, $T_{1/2,}$ $O_2$ range, and post-peak hyperemic recovery slope due to exercise during mental stress and after mental stress a manipulation of shear stress could be performed. By placing a cuff above diastolic blood pressure but below systolic blood pressure, shear stress could be decreased during exercise and mental stress. This would allow for a better understanding of the extent to which shear stress changes are related to the microvascular responses observed in this study.

The role of shear stress could also be examined through administration of pharmacological agents such as L-NMMA, a NO synthesis blocker. Administrating the NO blocker prior to exercise or prior to mental stress in the rest condition would provide insight into the NO contribution to both the exercise-induced and stress-induced responses. Based on previous, studies other pharmacological blocking agents could be administered to determine how other metabolites or receptors are involved in regulating microvascular flow changes. For example, a local beta receptor blocker, prostaglandins inhibitor, or histamine inhibitor, could be administered prior to exercise. These experiments would provide a comprehensive understanding of changes in vasodilators which occur during exercise.

While we found that exercise improved microvascular function response to mental stress, all of our subjects were regularly physically active males. Sedentary individuals may not have

the same benefit of enhanced microvascular function after exercise. A previous study found that sedentary overweight individuals had impairments in flow-mediated responses after exercise compared to active controls[29]. Therefore, it seems plausible that the acute exercise-induced changes observed in this study may not have been observed in sedentary males. Future studies are needed to see if sedentary individuals have a similar microvascular response to exercise and mental stress as active individuals.

While our results from young, healthy, physically active males did not show a decrease in muscle microvascular response to stress, studies in other populations have implicated impaired vascular function with chronic stress as a potential link between stress and cardiovascular disease[86]. A recent study examining coronary blood flow in response to stress found that peripheral vasculature had similar responses to mental stress as coronary microvasculature. The microvascular responses in older, diabetic patients, or individuals with atherosclerotic cardiovascular disease may have abnormal reactions to stress and future studies are need to better understand the role chronic stress on vascular health. However, if acute exercise induced similar responses in at-risk groups, such findings could have significant implications for exercise recommendations for these groups.

This study was unable to obtain a sufficient sample to evaluate the role of high trait anxiety on microvascular responses to exercise and stress. This area needs further exploration, in particular finding individuals who are diagnosed with anxiety disorders would be the best sample to gain insight as to the role anxiety in microvascular function. Due to the high prevalence of anxiety disorders across the lifespan, a better understanding of the interaction between cardiovascular changes and anxiety are needed. However, it can be challenging to recruit individuals with anxiety disorders for research studies.

In conclusion, mental stress and acute exercise altered muscle microvascular dilation. Our data suggest separate mechanism are responsible for the changes observed. By understanding the precise mechanisms involved in the microvascular dilation observed after exercise, we may gain insight as to how exercise increases cardiovascular health. This may be of particular important for at-risk groups and future studies are needed to help determine if exercise-induced stress responses are similar in those with anxiety disorders and/or at risk for cardiovascular disease.

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