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Peripheral Vascular Function in Young Adults with Increased Risk of Cardiometabolic Disease

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PERIPHERAL VASCULAR FUNCTION IN YOUNG ADULTS WITH INCREASED RISK OF CARDIOMETABOLIC DISEASE

by

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ABSTRACT

Objective: To determine if young adults with elevated metabolic syndrome severity scores ($MetS_{index}$) suffer from peripheral vasculature dysfunction.

Methods: Mean arterial pressure (MAP), Femoral (FBF) and Brachial Blood flow (BBF), Femoral (FVC) and Brachial Vascular Conductance (BVC), and Tissue Saturation Index (TSI) were assessed in twenty-four age (19 + 2.25 years), Sex (male: n=18; female: n=6) and Race (White: n=4; Black/African American: n=4; Asian: n=16) matched individuals during post-occlusive reactive hyperemia (RHBF), passive limb movement (PLM) and Functional Sympatholysis (FS) trials. Carotid femoral pulse wave velocity (cfPWV) was also collected at baseline, and all values were compared between the Control (Con; negative $MetS_{index}$ score) and Elevated Risk (ER; positive $MetS_{index}$ score) groups using a combination of independent samples *t*-tests and repeated measures analyses of variance (RMANOVA).

Results: As expected, blood flow and vascular conductance significantly increased during all RHBF and PLM trials across both groups (all $p \leq 0.001$). However, no significant main effects of group, group by time interactions, or group by condition interactions were observed for femoral RHBF responses (all $p \geq 0.591$), PLM responses (all $p \geq 0.313$), brachial RHBF responses (all $p \geq 0.132$), or FS responses (all $p \geq 0.446$). Likewise, no group differences were observed for PWV (Con: mean= 5.14 $\pm$ 1.69m/s; ER: mean= 5.18 $\pm$ 1.84m/s; $p=0.953$).

Conclusions: The current findings suggest that peripheral vascular function appears to be unaffected in young adults presenting with elevated $MetS_{index}$ score. Our findings also suggest that $MetS_{index}$ may provide use as an “early warning” system for young adults at

risk of MetS-associated vascular dysfunction and highlights the need for further cardiovascular assessments in these individuals.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

AA	Arachidonic Acid
ACh	Acetylcholine
ADO	Adenosine
AUC	Area Under the Curve
AV	Atrioventricular
BBF	Brachial Blood Flow
BMI	Body Mass Index
BP	Blood Pressure
BVC	Brachial Vascular Conductance
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
CNS	Central Nervous System
CO	Cardiac Output
Con	Control
COX	Cyclooxygenase
CPP	Coronary Perfusion Pressure
CPT	Cold Pressor Test
CVD	Cardiovascular Disease
CVLM	Caudal Ventrolateral Medulla
DBP	Diastolic Blood Pressure
ECG	Electrocardiography
eNOS	Endothelial Nitric Oxide Synthase

EPI	Epinephrine
EPR	Exercise Pressor Reflex
ER	Elevated Risk
FBF	Femoral Blood Flow
FBG	Fasting Blood Glucose
FMD	Flow Mediated Dilation
FS	Functional Sympatholysis
FVC	Femoral Vascular Conductance
GABA	γ-aminobutyric acid
HDLC	High-Density Lipoprotein Cholesterol
HG	Handgrip
HTN	Hypertension
LDLC	Low Density Lipoprotein Cholesterol
MAP	Mean Arterial Pressure
MetS	Metabolic Syndrome
MSNA	Muscle Sympathetic Nerve Activity
MVC	Maximal Voluntary Contraction
NE	Norepinephrine
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NTS	Nucleus of the Solitary Tract
PG	Prostaglandins
PGIS	Prostacyclin Synthase

PHE	Phenylephrine
PLM	Passive Limb Movement
PSNS	Parasympathetic Nervous System
PWV	Pulse-Wave Velocity
RHBF	Reactive Hyperemia
ROS	Reactive Oxygen Species
RVLM	Rostral Ventrolateral Medulla
SA	Sinoatrial
SBP	Systolic Blood Pressure
sGC	Soluble Guanylate Cycase
SNA	Sympathetic Nerve Activity
SNP	Sodium Nitroprusside
TC	Total Cholesterol
TRG	Triglycerides
TSI	Tissue Saturation Index
USM	The University of Southern Mississippi
VLM	Ventrolateral Medulla
WC	Waist Circumference

CHAPTER I – INTRODUCTION

Autonomic regulation is understood as an unconscious process of maintaining homeostasis and occurs through a multitude of contributory mechanisms and pathways, one of which is the cardiovascular system. Though it is common to experience changes in this system through the aging process, factors, such as diet and exercise, can influence the regulatory processes, acting either to reverse/blunt or, if poor habits are developed and maintained, deleterious changes of the cardiovascular system. These factors contribute so heavily that, as of current, guidelines exist to aid in the course of healthy living, acting as a way to manage weight and maintain healthy cardiovascular function. However, though these guidelines exist, obesity is becoming increasingly more prevalent as a whole but more importantly is that it is becoming more prevalent in younger adult populations often leading to or associated with metabolic dysfunction and disease development, particularly metabolic syndrome (MetS). In fact, MetS prevalence increased by 35% from 1988-2012 in all socioeconomic groups, affecting ~30% of the adult population in the U.S of which 18% were young adults aged 18-39yrs. while 46% were aged over 60 yrs. (Moore et al, (2017); Wong et al (2015). Interestingly, from 2012-2016 MetS prevalence in young adults increased significantly from ~16% to 21% in men and from ~31% to 36% in women, indicating a potential increase in unhealthy lifestyles or at the least an earlier onset of unhealthy aging (Hirode and Wong, 2020). With an increasing prevalence of this condition in the younger populace, it is crucial to better understand changes associated with these developments as this knowledge could prove invaluable to identifying and potentially mitigating or reversing deleterious transformations in the regulatory systems. A major component of regulation, and one that is highly associated with structural and

functional changes as a result of disease development, is the vasculature with its contributing role being the adequate supply of oxygen and nutrients to both inactive and active tissues. It is well understood that with disease development, especially metabolic syndrome, the regulation of the vasculature is often impaired, resulting in inadequate blood supply and ischemic conditions that can lead to heart failure. In fact, it is well established that each individual risk factor that comprises this metabolic syndrome is associated with some dysfunction to the vasculature (as will be discussed later). However, though evidence exists regarding the associations between risk factors and dysfunction, this evidence is most prevalent in those that have aged past young adult stages and thus the influence of time and aging have also taken affect. So though these associations exist, this is not as understood or established in young adults. Furthermore, if each risk factor seems to have some independent association with vascular dysfunction, it is possible that dysfunction may begin to develop in young adults presenting with just one or two risk factors prior to any diagnosis of MetS. This is a crucial implication as vascular dysfunction at an early age can further increase the development of other risk factors and even cardiovascular diseases, further increasing mortality risk both at young and old adult ages. The following sections will serve as a brief overview of autonomic structures and processes, integrative cardiac physiology, and vascular function in healthy aging and in the presence of cardiometabolic dysfunction, of which will serve to inform and educate so that a broader understanding of how MetS may develop and affect vascular function can be established.

CHAPTER II – REVIEW OF LITERATURE

Autonomic Nervous System (structure and function)

CNS and Medulla

The Central Nervous System (CNS) is comprised of the brain and the spinal cord and includes a vast array of neural networks that serve to control everyday physiological functions. These include voluntary functions such as speaking, thinking, and locomotion, as well as involuntary functions, such as respiration and maintenance of cardiovascular homeostasis. While autonomic control within the CNS is highly complex, one brain region, known as the medulla, is considered particularly important for autonomic regulation of the cardiovascular system.

The medulla is a cone-shaped cluster of neurons situated at the base of the hindbrain which connects to the spinal cord, serving as an innervation site between the two. The medulla consists of two primary regions, the dorsal medulla and ventral medulla, the ventral medulla being further divided into the rostral ventrolateral medulla (RVLM) and the caudal ventrolateral medulla (CVLM). The RVLM is understood to be responsible for increases in sympathetic activity due to its influence on sympathetic preganglionic regions that integrate with the sympathetic trunk (as will be discussed later). The RVLM and its influence on sympathetic control has been demonstrated in numerous studies. In one particular by Ross et al (1984), electrical stimulation of the RVLM resulted in release of catecholamines (to be discussed) and a concurrent increase in cardioacceleration and arterial pressure. On the other hand, the CVLM is understood to act as an inhibitor to the RVLM, thus acting to its sympathetic influence. This relationship was confirmed in a study by Cravo and Morrison (1993) assessing the role of

the CVLM in tonic inhibition of sympathetic activity. Researchers assessed this through microinjections of kainic acid, a neurotoxic agent, in the CVLM of baroreceptor denervated cats and found steady increases of sympathetic activity and arterial pressure where, once peaked, arterial pressure remained elevated for ~30 minutes then declined. While it is apparent that both of these areas of the ventrolateral medulla demonstrate interplay, their influence on sympathetic activity is not mediated through direct synapses of tissues or organs but rather through a synapse with another structure within the medulla. These regions of the medulla are known to synapse with what is considered to be a neural integration hub, the nucleus of the solitary tract (NTS; located in the dorsal medulla), whereby afferent signals from the cardiovascular, respiratory, and musculoskeletal systems are received. The integration of the NTS and the ventrolateral medulla is complex and is partially mediated via a combination of inhibitory and excitatory pathways. For instance, the NTS contains a series of interneurons (responsible for relaying sensory information within the CNS), and these interneurons can either indirectly excite, or indirectly inhibit, sympathetic premotor neurons within the VLM. Thus, the NTS regulates sympathetic and parasympathetic activity from the medulla by integrating a series of afferent sensory projections and eliciting actions through these interneurons (Potts, 2002). As will be discussed later, this process is important for the regulation of autonomic and peripheral vascular responses during exercise.

To understand how the medulla aids in the regulation of these responses, it is equally important to understand the nervous system structures, namely the parasympathetic and sympathetic nervous systems, that comprise the CNS as well as how information is transmitted, and responses generated.

Parasympathetic Structure

The parasympathetic nervous system (PSNS) is a division of the autonomic nervous system that serves to regulate homeostasis through nerves extending from the cranium and sacral regions of the spine known as cranial and sacral nerves, respectively. Cranial nerves are nerves that originate either in the brain or brainstem and serve the head and neck regions through a variety of functions, such as transmitting sense, smell, and vision. There are 12 total cranial nerves, 4 of which, termed the oculomotor, glossopharyngeal, facial, and Vagus nerves, serve to control the activity of smooth and cardiac musculature. The primary cranial nerve responsible for parasympathetic influence is the Vagus Nerve (CN X) and like all cranial nerves, the Vagus nerve bypasses the spinal cord and branches to extend to multiple organs of the body. At the periphery, these fibers synapse with postganglionic neurons that innervate target organs. Such is the case with the heart where parasympathetic fibers synapse with neurons on the heart's surface that innervate the sinoatrial (SA) and atrioventricular (AV) nodes.

Sympathetic Structure

Sympathetic peripheral autonomic control is mediated via afferent and efferent nerves synapsing with the spinal tract that, together with integration of other structures, serve to compose the Sympathetic Nervous System (SNS). The spinal tract contains a column of gray matter that can be further divided into ventral, dorsal, lateral, and intermediate horns of which contain neuronal cell bodies that serve to transmit or receive information from the brain and periphery via ascending and descending fibers. It is widely accepted that ascending fibers are afferent in nature and synapse with the dorsal horn of the spinal cord, an afferent hub for the periphery, while the descending fibers are

efferent and synapse with the ventral horn (the portion dedicated to somatomotor functions) as well as the lateral horns (the portion involved in autonomic regulation of periphery). For sympathetic autonomic regulation, this lateral horn houses cell bodies of preganglionic neurons whose axons exit the spinal cord and synapse to the sympathetic trunk. The sympathetic trunk is a series of interconnected ganglionic bodies from which postganglionic neurons stem and synapse with tissues and organs, such as skeletal muscle and the heart.

Cardiac Conduction Pathway

The cardiac conduction pathway can be described as a pathway of electrical currents within the heart that results in both atrial and ventricular contractions. This pathway begins at the SA node where impulses are generated and travel across the atria and down to the AV node. At the AV node, there is a tenth-of-a-second delay which allows both atria to contract which forces blood into the ventricles. The impulses travel from the AV node through the Bundle of His which divides into left and right bundle branches that travel along the intraventricular septum. These branches extend to the apex of the heart where thin filaments known as Purkinje fibers travel up the ventricles of the heart (Padala et al, 2020). These Purkinje fibers are composed of electrically excitable cells that, when stimulated, serve to contract the ventricles. As mentioned, both the Vagus nerve of the PSNS and the SNS aid in the regulation of the heart, striking a balance between parasympathetic and sympathetic activity and maintaining homeostasis.

Neurotransmitters

The neural connections described above all rely on the transmission of information from a presynaptic neuron to a postsynaptic neuron or a target tissue. While

there are over 100 neurotransmitters acting within the human body, six neurotransmitters are particularly important for autonomic regulation of the cardiovascular system: Substance P, Acetylcholine (ACh), catecholamines Norepinephrine (NE) and Epinephrine (EPI), Glutamate, and γ-aminobutyric acid (GABA).

Substance P is a neuropeptide that binds to the receptor NK-1 which has been suggested to lie within the NTS. In the NTS, these neuropeptides synapse with first order interneurons whose activation elicit inhibitory effects of the CVLM via GABA release from postsynaptic neurons (Halliwell et al, 2012). Thus, this neurotransmitter indirectly elicits increases in sympathetic activity from the RVLM through the action of NK1 receptors and the consequential inhibition of the CVLM. Furthermore, this excitation of the first order interneurons from substance P serves to also reset the baroreflex during bouts of exercise (this will be discussed in later sections).

Glutamate and GABA are among the primary neurotransmitters of the medulla where glutamate exerts excitatory influence and GABA exerts inhibitory influence. As the excitatory neurotransmitter, glutamate is required for activation of both the CVLM and RVLM. In support of this, a study by Zhou et al (2006) showed that glutamatergic neuron activation within the RVLM (via afferent stimulation) produced increases in arterial blood pressure. The authors also found that glutamate introduction into the RVLM via iontophoresis produced increased activity of sympathetic premotor cardiovascular neurons and were attenuated by glutamatergic receptor blockade. These findings together allude to glutamate's role as an excitatory neurotransmitter that contributes to increased sympathetic outflow from the RVLM. GABA, on the other hand, has been shown through multiple studies to have inhibitory effects upon the RVLM. One

such study by Lacerda et al (2003) showed decreases in mean arterial pressure (MAP) after direct GABA microinjection into the RVLM in rats. Another study by Dombrowski and Mueller (2017) found that microinjections of GABA into the RVLM of anesthetized rats created dose-dependent decreases in MAP as well as decreases in splanchnic sympathetic nerve activity.

The balance between excitatory and inhibitory inputs at the medulla regulates the release of catecholamines (hormones produced by brain, nerves, and adrenal glands) from sympathetic nerve terminals. There are two primary catecholamines involved in sympathetic activity, NE and EPI, and as will be discussed in later sections, both bind to adrenergic receptors on vascular smooth muscle to induce vasoconstriction and drive increases in blood pressure and cardiac output (Deuchers, 2015). In fact, NE spillover is used as an index of sympathetic nerve activity (SNA; Meredith et al (1993)). However, at the heart, these catecholamines induce chronotropic (i.e. tachycardia) and ionotropic (i.e., increased contractility) effects that drive increases in heart rate through a binding to β-adrenergic receptors.

Lastly, ACh is a neurotransmitter that functions in the SNS as well as the PSNS with its effects being dictated primarily by the acting receptors. In both systems, ACh is released from preganglionic neurons and binds to nicotinic receptors on postganglionic neuron terminals, essentially forming the process in which action potentials are generated and transmitted from neuron to neuron. However, the difference between the systems exists primarily at the postganglionic neurons that synapse with tissues and the receptors located on that tissue. The SNS, as mentioned, primarily releases catecholamines that bind to adrenergic receptors to elicit effects yet the PSNS, on the other hand, primarily

releases ACh from its postganglionic neurons which binds to muscarinic receptors on tissues. This ACh/muscarinic binding results in vasodilation (in peripheral vasculature) and/or bradycardic (at the heart) effects.

Integrative Autonomic Control

Central Command

Central Command is a feedforward mechanism thought to originate in higher brain regions, such as the motor cortex, that seemingly combines both somatic and autonomic systems together. Through this mechanism, voluntary motor control is believed to be accompanied by increases in muscle sympathetic nerve activity (MSNA; a measure of sympathetic outflow to skeletal muscle). Put simply, the attempt and/or initiation of muscle contraction elicits a near simultaneous increase in MSNA. In fact, Victor and colleagues demonstrated this association through use of a neuromuscular blockade in 1989. In this trial, researchers had participants perform isometric handgrip at 15% and 30% of maximal contractions while under the administration of tubocurarine chloride (Crurare) which acted to locally paralyze the working limb. Participants had reported that they used maximal effort in an attempt to achieve handgrip exercise, yet no force was ever produced. Interestingly though, MSNA increased by ~56% as well as did mean arterial pressure (MAP) by ~12 mmHg. These findings of central command contribution from were further supported in 1993 by Gandevia and colleagues who demonstrated that, even during fully body paralysis, the attempt to contract legs, arms, and trunk musculature evoked increases in both HR and BP where, interestingly, the attempt to perform handgrip contractions resulted in HR and BP values similar to pre-paralysis condition. These two studies seem to provide persuasive evidence regarding the

role of central command in eliciting the cardiovascular and sympathetic responses to exercise.

Baroreflex

The baroreflex is a homeostatic, physiological mechanism operating through negative feedback loops that functions to maintain blood pressure at near constant levels. This reflex is mediated through stimulation of stretch-sensitive afferent fibers located within the cardiovascular circuit, specifically the aortic arch and the carotid sinuses. These baroreceptors function as mechanoreceptors or stretch receptors that sense changes in pressure via arterial distention through which they become either loaded or unloaded (Thrasher, 2002). Baroreceptor loading occurs during increases in cardiac blood pressure and results in the generation of action potentials that transmit to and excite the second order neurons of the NTS which project to the parasympathetic cell bodies of the vagus nerve and the neurons synapsing to the CVLM (Michelini, 2007). The excitation of these second order neurons results in a glutamatergic influence on the CVLM and thus (as mentioned) an inhibitory signal to the RVLM thus decreasing sympathetic outflow while the simultaneous excitation of the parasympathetic cell bodies increases parasympathetic nerve traffic to the periphery. On the other hand, when these receptors are unloaded, such as when blood pressure is low, opposite effects occur (increased sympathetic outflow and decreased parasympathetic nerve traffic) (Michelini, 2007). During exercise, the increases in sympathetic outflow and vasoconstrictive responses would, if left to normal functioning parameters, load these baroreceptors and cause a competing effect, inhibiting sympathetic outflow and decreasing heart rate and consequential blood flow. This would eventually cause inadequate blood transport and oxygen perfusion within the active

tissues, leading to local hypoxia and ischemia. This does not occur during exercise, however, and rather the baroreflex is reset to a higher operating point so that heart rate and blood flow are able to scale with exercise intensity. This resetting of the baroreflex occurs, in part, via the exercise pressor reflex.

Exercise Pressor Reflex

The exercise pressor reflex (EPR) is a physiological feedback mechanism that is evoked from stimulation of skeletal muscle and regulates physiological parameters, such as blood pressure and heart rate, to support the increased demand for oxygen placed on active muscles during exercise (Teixeira and Vianna, 2022). This reflex depends upon a stimulation of muscle afferents located on and around skeletal muscle that send sensory information to the medulla (specifically the NTS). These muscle afferents primarily contributing to this mechanism are labeled as group III and group IV. Group III afferents are widely considered to be mechano-sensitive receptors that sense and are stimulated by tension within the muscle while Group IV afferents, on the other hand, are believed to be metabo-sensitive receptors that evoke a reflex known as the metaboreflex. Sensitization of these group IV afferents is thought to be mediated by chemosensitive receptor families, such as ASICs or TRP channels, by way of the accumulation of metabolic byproducts of exercise, such as lactic acid or bradykinin (Dubey et al, 2017). Unlike the baroreflex whose stimulation causes excitation of the CVLM, these muscle afferents serve to exert an inhibitory effect on the CVLM through the release of the aforementioned neurotransmitter, Substance P, within the NTS. The release of this neurotransmitter, as previously discussed, works through an excitation of first order interneurons which elicit GABAergic influence upon the second order interneurons.

Given that these second order interneurons synapse with three different sites of parasympathetic input (CVLM, baroreceptors, and parasympathetic cell bodies), their inhibition directly affects these pathways. For the baroreceptors, the inhibition of these interneurons raises the threshold by which these receptors are able to exert influence while at the CVLM their inhibition serves to decrease/eliminate GABAergic influence on the RVLM. Collectively, these factors collectively contribute to a robust increase in VLM activity in response to muscular contractions (Bauer et al, 1992).

Regulation of peripheral vascular tone

α- and β-adrenergic innervation of vascular smooth muscle: Peripheral vascular beds, like the heart, are comprised of smooth muscle that contain specialized adrenergic receptors that, when stimulated through the binding of the catecholamines NE and EPI, elicit vasoconstrictive and vasodilatory responses. These adrenergic receptors are divided into two groups termed alpha (α) and beta (β) adrenergic receptors which are further divided into subgroups 1 & 2 (α1 &2, β1 &2). As shown by Jie et al (1986) through infusion of known alpha agonists methoxamine and B-HT 933 as well as infusion of catecholamines, stimulation of α-adrenergic receptors results in a robust vasoconstrictor response, findings that have been replicated by others. However, other evidence suggests that β adrenergic stimulation elicits a vasodilatory response in the vascular smooth muscle. Such evidence presented by Schindler et al. (2004) showed that infusion of isoproterenol (a non-selective β agonist) into the constricted veins of the hand induced vasodilation to 67.4% without involvement of endothelium-derived epoprostenol (a prostaglandin shown to induce vasodilation). Notably, these effects occur separately from the aforementioned β-adrenergic mediated chronotropic and inotropic effects observed at

the SA node and atria. The competing influences of α- and β-adrenergic stimulation notwithstanding, the net result of EPI and NE release in the peripheral vascular beds during sympathoexcitation is typically defined by robust vasoconstriction, leading to an increase in vascular resistance.

The relationship between vascular tone (i.e., vasoconstriction and vasodilation), blood pressure, and blood flow can be explained using Poiseuille's equation.

$$Q = \Delta P \pi r 4 / 8 \eta l$$

Also known as the Hagan-Poiseuille equation, this equation explains that the rate of flow within a tube is directly proportional to the radius of that tube as well as the pressure at the two ends yet inversely proportional to a liquid's viscosity and the length of the tube length. More simply, this law states that as the radius decreases then resistance to flow increases and drives pressure upward. Thus, decreases in tube radius result in decreases in flow rate due to resistance. The opposite is true when radius increases (resistance is decreased, pressure drops, and flow rate is increased). Applied physiologically, during increases in sympathetic activity to periphery the resultant vasoconstriction at the vascular beds decreases vessel radius. This leads to increased resistance to flow which in turn increases pressure and decreases rate of flow. Vasodilation would have the opposite effect where the increase in radius of the vessel would ultimately result in increased rate of flow due to a decrease in resistance.

Endogenous Vasodilation of vascular beds: In addition to neurally mediated innervation, endogenous vasodilators also contribute to the maintenance of vascular tone.

Vasodilators are believed to be either endothelium-dependent or endothelium-independent which indicates, in part, the location of their production and release. One such endothelium-dependent vasodilator is Nitric Oxide (NO) and has been suggested to be the result of increased shear stress, a frictional force along the endothelial walls of vessels as a result of a combination of intraarterial pressure and blood flow. This interplay between endothelium, shear stress, and vessel dilation was highlighted in a study by Koller et al (1993), in which researchers removed the endothelium of isolated vessels and compared responses to vessels with in-tact endothelium. Removal of the endothelium corresponded with shear stress values of 470 dyne/cm^2, which resulted from stepwise increases in blood flow. However, when endothelium was present, the shear stress only reached 90 dyne/cm^2, demonstrating that the endothelium plays some substantial role in blunting shear stress. This study further demonstrated that, concomitant with those increases in shear stress, vessel diameter increased when the endothelium was present whereas diameter remained unchanged with the endothelium removed. Thus, Koller and colleagues supported that not only does endothelium seem to play a role in blunting the forces of shear stress but that this blunting is likely achieved through an increase in vessel diameters, potentially occurring through some compound, such as NO, or pathway dependent on the presence of the endothelium.

One proposed mechanism believed to contribute to this regulation of vessel diameter is known as the NO-sGC-cGMP pathway. NO is a molecule synthesized by arginine through NO synthase (NOS) enzymes of which there are three distinct isoforms (iNOS, eNOS, and nNOS) though only one is believed to play a role in vasodilation of vascular smooth muscle. eNOS, or endothelial NOS, is an enzyme within the endothelial

cells activated by aforementioned shear stress that, once activated, produces NO which is then released by the cells to bind with Soluble Guanylate Cycase (sGC). One of two versions of GC, sGC is an enzymatic hemoprotein that serves to catalyze the synthesis of Cyclic Guanosine Monophosphate (cGMP). The synthesis of cGMP has been suggested to mediate vasodilation within the smooth muscle of vascular beds as shown by Yu et al (2002) where researchers treated calcium-bathed cerebral arteries of rats with a cGMP derivative and found that cGMP reduced calcium sensitivity which led to a reduction in vasoconstrictor response by 60% compared to baseline.

While NO and its associated pathway play an important role in vasodilation, there are other mechanisms that contribute to the vasodilatory process. One such mechanism is through the lipids known as Prostaglandins (PG). Prostaglandins are a unique physiologically active group of lipids known as eicosanoids synthesized by the catalyzation of arachidonic acid (AA) via cyclooxygenase (COX) that can be found in nearly all tissues of the body (Langenbach et al, 1995). PG are unique because they can be both endothelium-dependent as well as endothelium-independent (produced regularly in skeletal muscles and other organs/tissues of the body). Cyclooxygenase is believed to have two isoforms, 1&2, of which COX-1 is expressed at rest and in normal functioning whereas COX-2 is primarily expressed during inflammatory processes and acts to increase production of PG. These cyclooxygenases, as mentioned, catalyze AA to form PGH2 which is the common precursor to the formation of other PG. One particular PG known as prostacyclin is considered to be a potent vasodilator and is formed through interaction of PGH2 and prostacyclin synthase (PGIS) located within the endothelium (Kirkby et al, 2013). This PG is believed to exert vasodilatory effects through the binding

of its IP receptor that stimulates the production of Cyclic Adenosine Monophosphate (cAMP) which acts through multiple pathways, such as further stimulation of NO or calcium inhibition. The interactions of arachidonic acid and cyclooxygenases and their formation of PGs have been shown in numerous studies across the decade, such as by Carroll et al (2013) who found that, during exercise, transient increases in COX-1 activity correlated to increased PG synthesis. It was also shown by Carroll et al (2013), through muscle biopsies, that after exercise at 70% intensity, COX-2 activity increased significantly, indicating that during activity, PG production is increased by activation of both isoforms. To further this, the contributions of AA and COX to the regulation of vascular tone through vasodilation have been supported in studies such as one by Bhagat et al (1995) whereby infusion of AA into constricted veins of participants resulted in dose-dependent vasodilatory responses, the greatest vasodilatory response occurring at the highest dose, which were inhibited through the oral ingestion of 1g of aspirin (a COX inhibitor). The importance of these pathways is multifaceted as they contribute to overall vascular regulation of which extends to activities, such as exercise, that demand increased oxygen supply to allow the continuation of performance through mitigation of ischemia. Though exercise, or general activities of high intensity, result in increased sympathetic activity and concomitant vasoconstriction, these pathways allow for a mechanism known as functional sympatholysis to occur which serves to offset the initial vasoconstriction.

Functional Sympatholysis

Functional Sympatholysis is a physiological mechanism by which vasoconstriction of active tissues induced by sympathetic outflow is attenuated (Thomas, 2014). As discussed, exercise increases sympathetic activity and the vasoconstrictive response elicited by the release of EPI and NE at the periphery occurs systemically, and as such, occurs even at the vessels of the active tissues. Thus, the sympathetically mediated increase in CO that improves blood transport to active tissues is placed in competition with the vasoconstriction occurring at the vascular beds of the active muscles which limits oxygen perfusion. Left unabated, this vasoconstriction at the active periphery would eventually lead to local hypoxia and ischemia. However, functional sympatholysis serves to offset this dilemma by blunting the vasoconstrictive responses in the vascular beds of active tissues, resulting in an increase in local blood flow and thus improving oxygen perfusion. This mechanism seems to be a conjunction of the vasodilators NO and PG as their release is largely mediated by increased sympathetic activity and the consequential increases in shear stress along endothelium. The supporting research for these vasodilators and their independent contributions have been shown by numerous researchers, such as Chavoshan et al (2002) who found that after pharmacologically inhibiting NO synthase the participants demonstrated an inability to attenuate sympathetic vasoconstriction while research by Wilson and Kapoor (1993) showed that infusion of indomethacin (a COX inhibitor) into the exercising forearm of participants decreased blood flow compared to control trial. However, literature by Dinenno and Joyner (2004) suggests that these vasodilators are incapable of offsetting vasoconstrictive responses independently. This was supported through the measure of

FVC (forearm vascular conductance; blood flow ÷ mean arterial pressure) and the assessment of effects of independent inhibition as well as combined inhibition of NO and PG on dynamic handgrip exercise in conjunction with infused alpha receptor agonists (Phenylephrine and Clonidine) and a norepinephrine agonist (Tyramine). They found that neither of the endogenous vasodilators were capable of significantly impacting vasoconstrictive responses. However, researchers did find that the combined inhibition of both PG and NO resulted in FVC decreases (indicative of greater vasoconstriction) of 15 and 20%, indicating that, though they are incapable of significantly impacting vasoconstriction independent of one another, together these vasodilators play a significant role in vascular tone during activity. Another purported contributor to functional sympatholysis involves the adrenergic receptors at the vascular beds. It has been suggested that during exercise, these adrenergic receptors decrease responsiveness and thereby allow for greater vasodilation to occur due to a decrease in binding capabilities. The decrease in responsiveness of the receptors was studied by Buckwalter et al (2001) whereby phenylephrine (α1 agonist) and clonidine (α2 agonist) were administered (during separate trials) into the hindlegs of dogs at rest and during exercise. While both agonists produced similar vasoconstriction at rest, it was found that alpha receptors decrease responsiveness in an exercise-intensive manner. During both mild (3mph @ 0% treadmill incline) and heavy exercise (6mph @ 10% treadmill incline), α2 receptors showed decreased responsiveness whereas α1 receptor responsiveness decreased only at heavier exercise. To further validate these findings, the researchers assessed responses in a group of sympathectomized dogs with the intent of reducing the influences of norepinephrine and potentially confounding the effects of clonidine. In this

group, researchers found similar responses indicating no confounding effects of prejunctional α2 receptors.

As is apparent, autonomic control is an integrative process of many components and separate physiological systems, each of which individually dictate and determine the effectiveness of the autonomic system both at rest and at exercise. With that said, it is now important to understand and determine how these autonomic responses are altered in individuals demonstrating unhealthy aging, such as those with hypertension and/or hyperglycemia, and how this differs from the healthy aging process.

Vascular Function in Healthy Individuals vs with Cardiometabolic Disease

Influence of Healthy Aging on Vascular Function

Vascular function refers to the health of an individual's vasculature and its ability to maintain and regulate tone to meet oxygen demands essential for homeostasis. This is a crucial aspect to cardiovascular health as its dysfunction can often result in inadequacies in blood transport and oxygen perfusion, leading to the onset and development of numerous health conditions, such as peripheral artery diseases, stroke, and even heart attack. In healthy individuals, vessels are elastic, and vascular tone is more easily regulated by the contributions of numerous endogenous vasodilatory mechanisms. However, the vessels and their ability to sufficiently meet oxygen demands seem to be impaired in unhealthy individuals presenting with one or more unhealthy characteristics, such as overweight/obesity, hypertension, hyperlipidemia, etc. Some evidence suggests this may be mediated, in part, by decreased elasticity of the vessels. A study by Gujral et al (2020) examined pulse wave velocities (PWV; a measure of arterial stiffness) across individuals of different ethnicities (South Asians, White, African

Americans), and found that, when divided into sub-cohorts of "healthy" and "unhealthy" (identified as absence or presence of diabetes, hypertension, overweight/obesity, dyslipidemia, and smoking), the healthy cohort demonstrated lower PWV than the opposing cohort (indicating that the "healthy" cohort maintained elasticity of vessels).

Health status plays a major role in vascular function, but it is equally important to understand that vascular function is further affected through advancing age and that these age-related changes in function closely associate with the health status of the individual. Aging, even among individuals absent of disease or conditions, has been associated with systemic cell senescence, or cell death, of which extends to the endothelium and leads to alterations in vascular tone and function. The presence of endothelial cell deterioration with aging has been shown through the expression of certain proteins that serve as senescence markers. In fact, Rossman et al (2017) compared venous endothelial cell protein expression (p53, p21, and p16) of healthy, sedentary older adults (absent of diseases or conditions, non-smokers) to healthy, sedentary young adults and found that the expression of these proteins was 116%, 119%, and 128% higher in the older group, respectively. Following this trend, the authors also found that arterial protein expression of p21 and p53 were 21% and 26% greater in older adults. This further translated to decreases in flow-mediated dilation (FMD; measure of endothelium-dependent dilation) within the older sedentary group, indicating that endothelial cell senescence is not only prevalent as aging occurs but that its presence impairs vasodilatory capabilities. In further support of changes in vascular function, Königstein et al (2021) found that in healthy adults (non-smoking, absent of diseases and medications, normal heart function) ranging from ages 20 to 91, FMD decreased by 63.6% and 47.1% in women and men,

respectively. However, it appears that, though occurring progressively with age, these changes in markers of cell senescence are blunted in exercising healthy adults. For example, the study by Rossman et al (2017) also measured protein expression in healthy, exercising older adults and found that, compared to the sedentary older adults, exercise-trained adults presented with 68%, 32%, and 71% lower venous expression of p52, p21, and p16, respectively, and 35% and 39% lower arterial expression of p53 and p21. They further found that exercise was also shown to affect FMD whereby the trained adults had higher brachial artery FMD compared to the healthy sedentary adults but was no different than the healthy young adults. Furthermore, in a cross-sectional study by DeSouza et al (2000) consisting of sedentary and exercise-trained men (N=68; aged 22-76 years) researchers found, through forearm blood flow assessments after intra-arterial infusion of acetylcholine (a vasodilator), a 25% decrease in responsiveness in the sedentary older adults but no age-related differences in vascular function in exercise-trained men and that, when compared to the young adults, no difference between exercise and young groups existed. These findings may be explained through the multiple mechanisms and pathways, such as NO, PGs, and adrenergic receptors, that exist within the body which function synergistically to contribute to and dictate vasodilatory responses and regulate vascular tone. One such mechanism that influences vascular function in aging is the bioavailability of NO. As NO is released from endothelial cells, the senescence of these cells would be expected, in consequence, to decrease the production, release, availability, and the effectiveness of this important vasodilator. In fact, Matsushita et al (2001), through *in vitro* assessment of human aortic endothelial cells, found that senescent cells presented with a 50-75% decrease in eNOS expression which persisted in both static and

shear stress introduced conditions and correlated to a decrease in NO production. In further support, Yoon and colleagues (2010) found that *in vitro* human endothelial cells demonstrated an age-dependent decrease in NO production correlating to decreased eNOS activity and expression. However, Taddei et al (2000) contrasted these findings by demonstrating that NO availability is seemingly preserved in healthy elderly adults who participate in physical activity. This study, comparing young and elderly athletes to young and elderly sedentary adults (all deemed healthy by BP assessment), utilized measurements of forearm blood flow to assess the effectiveness of cannulated infusions of L-NMMA (a NOS inhibitor) against acetylcholine (shown to activate eNOS). They found that in the young athletes, elderly athletes, and young sedentary participants the vasodilatory response significantly blunted by L-NMMA but in the elderly sedentary group the L-NMMA was ineffective. This study suggests that healthy aging reduces NO bioavailability and its role in vasodilation but that this reduction is blunted or reversed by maintaining a healthy, active lifestyle.

Though NO is a potent vasodilator and a major contributor to vascular tone, other vasodilators, such as the aforementioned PGs and specifically prostacyclin, play an equally vital role in vascular function. During aging, similar to NO, there seems to be evidence of a decrease in PG-mediated vasodilatory responses, though it has been unclear whether these age-related decreases are due to the decrease in PG production or simply by reduced responsiveness to these particular lipids. Nevertheless, the evidence suggests a correlation between age, PG, and decreased vascular function. For instance, Singh and colleagues (2002) found that, through the administration L-NMMA, aspirin, and measure of forearm blood flow, that vascular function was altered in older participants compared

to young participants (both groups deemed healthy as absence of CVD or conditions), and that both inhibitors produced dose-dependent constrictive responses (the highest dose-response being similar between both inhibitors) independently of one another. Nicholson et al (2009) further supported this by demonstrating smaller reductions in % FVC (forearm vascular conductance) in healthy older participants compared to young healthy adults (compared to baseline) when administered Epoprostenol, indicating an impairment in vascular function and ability to regulate vascular tone. Researchers further concluded that these findings, taken together with assessments of NO, suggest that responsiveness to PGs decreases with healthy aging.

As vascular function is multi-faceted, due in large part to its numerous contributors, it is important to remember that vasodilators are but only one contributing factor and that the adrenergic receptors further contribute to the process. It is understood that aging is commonly associated with increases in sympathetic activity that do not necessarily translate to increases in vasomotor output nor increases in vascular tone. Such paradoxical findings are suggested to be, among many factors, related to a decreased responsiveness in these receptors, as increased sympathetic activity should certainly increase adrenergic actions. In fact, this decrease in responsiveness has been demonstrated by Dinenno and Joyner (2002) through the use of alpha-adrenergic agonists (phenylephrine and clonidine) and the comparison of their effects on forearm blood flow between healthy sedentary older men (aged~ 65 years, free of CVD, normotensive) and young healthy sedentary men. At baseline, forearm blood flow was similar between groups but when administered phenylephrine, the older group displayed a blunted vasoconstrictor response. The administration of clonidine, in contrast, resulted in no

significant between-group differences. They further showed differences in vascular function through administration of Phentolamine (a non-selective alpha-adrenergic blockade), finding significant vasodilation in both groups but significantly lower increases in blood flow in the older cohort. Taken together, these studies suggest that age-related decreases in adrenergic responsiveness may be specific to α1 receptors whereas the function of α2 receptors remained preserved.

As the evidence suggests, age is often heavily associated with deleterious changes in vascular function of which can often be blunted or eliminated entirely through healthy, active living. However, as of current, the presence of cardiometabolic conditions is ever-increasing, often as the result of unhealthy dietary eating habits and sedentary activity levels which lead to the development of obesity, hypertension, hyperglycemia, and/or dyslipidemia. This is an important implication as each of these conditions have been suggested to exert influence on vascular function, often correlating to impairments in vascular tone regulation and consequently leading to cardiovascular stresses and increased risk of mortality (Recall the impaired vascular function in unhealthy individuals mentioned in the above section). These conditions serve as primary risk factors for what is termed as Metabolic Syndrome (MetS), a condition that is defined by having three or more of these cardiometabolic diseases, of which is estimated to affect ~25% of the worldwide population (Macías et al. 2021). In the following section, this condition, its risk factors, and their reported independent effects on vascular function will be discussed in more detail.

Cardiovascular Conditions Affecting Vascular Function

As mentioned previously, the prevalence of MetS has been on an upward trend over the past few years which is an important implication as MetS has become heavily associated with increased mortality risk and cardiovascular diseases. In fact, Isomaa and colleagues in 2001 found that MetS increased risk of coronary heart disease and stroke by threefold and significantly increased cardiovascular mortality. As vascular function is a key factor in regulating and maintaining homeostasis, it is feasible to assume or expect that MetS may exert its deleterious influences through the vasculature. Limberg et al (2013) demonstrated that though endothelium-dependent vasodilation is preserved in young, MetS adults, these individuals present with impairments in prostacyclin-mediated dilation and thus indicates some level of vascular dysfunction as PGs are understood to aid in regulating vascular tone (recall PGs in above sections). Harrell and colleagues in 2012 found that young adults with MetS present blunted hypoxia-mediated cerebrovascular dilation, demonstrated by lower cerebrovascular conductance and flow velocity in the middle cerebral artery. Furthermore, as this condition is a combination of multiple other conditions, it is plausible to assume that the risk factors comprising this condition may also exert some deleterious influence on the vasculature independently, thus implying that the vascular dysfunction and increased mortality risk associated with MetS is merely a consequence of an accumulation of deleterious influences. If this is the case, which seems highly likely, then vascular dysfunction may begin to occur with one or two of these risk factors prior to the diagnosis of MetS and may contribute further to the development of other risk factors. Thus, understanding the associations between

vascular function and each independent MetS risk factor is crucial in understanding the development of peripheral vascular dysfunction during the progression of MetS.

Obesity is commonly understood to be characterized as the accumulation of excessive adipose tissue to a point that a Body Mass Index (BMI) of $\geq$30kg/m^2 or a waist circumference (WC) of $\geq$88cm for women and $\geq$102cm for men (an indicator of abdominal obesity) is achieved and is estimated to affect nearly 50% of the adult population as of 2018 (NIDDK, n.d.). While obesity is a complex condition due in large by its development being the result of a multitude of contributing factors, such as environment, genetics, family history, etc, its presence is understood to have deleterious associations of which extend to the vasculature, impeding a crucial factor of autonomic regulation. For instance, obesity has been shown by Furukawa and colleagues (2004) to correlate to increases in reactive oxygen species (ROS) and inflammation of which affect regulation of vascular tone (to be discussed in more detail later). To demonstrate the association between ROS and obesity, these researchers utilized various methods across both human and animal subjects. Firstly, Furukawa and colleagues measured lipid peroxidation (a marker for oxidative injury), via blood Thio-barbituric acid reactive substance and urinary 8-epi-prostaglandin-F2α, in nondiabetic human participants and found that as BMI increased so too did this marker for oxidative injury. Furthermore, researchers demonstrated an inverse relationship between BMI and plasma adiponectin (adipocytokine released to regulate insulin sensitivity and inflammation) and that this inverse relationship also existed between measures of lipid peroxidation and plasma adiponectin. Interestingly, this study demonstrated that obesity may influence oxidative stress independent of associated conditions, such as hyperglycemia. Thus, to further

expand on this idea, researchers assessed two groups of obese mice, diabetic and nondiabetic, and compared lipid peroxidation levels as well as markers for ROS to a control group of nonobese mice. They found that the nondiabetic, obese group presented with marginal levels of hyperglycemia while the diabetic, obese group had significant levels of hyperglycemia but that both demonstrated similar exaggerated levels of plasma lipid peroxidation compared to control. These exaggerated levels of peroxidation correlated to increases in ROS (H_2O_2, specifically) in both obese groups. Interestingly, these researchers further demonstrated that peroxidation and H_2O_2 was significantly elevated in white adipose tissue (the tissue most associated with increases in BMI and obesity) and that adiponectin was significantly decreased in both obese groups, together indicating that adipocytokine dysregulation may play a role in oxidative stress. Interestingly, this study suggests that obesity may exert some deleterious cardiovascular influence independent of pre-existing conditions like hyperglycemia.

Hyperglycemia and associations with impaired vascular function have been demonstrated through numerous animal studies assessing vasodilation and vessel diameter. For example, Bohlen and Lash in 1993, through topical application of glucose concentrate on rat arterioles, found immediate impairments in vasodilation when in the presence of hyperglycemia. For this, researchers applied varying concentrates of glucose (200mg, 300mg, 500mg) to rat arterioles and assessed responses across three time points (20min, 40min, 60min) then assessed responses between independent administrations of differing doses (20, 50, 100, 200, and 500 nAmp) of ACh and Nitroprusside after 1h. During glucose administrations, there was an observed, transient vasoconstriction across all concentrates but was only significant for 300mg and 500mg up to 20min with 500mg

extending to 40min. When administering ACh, vasodilation in the 300 and 500mg groups was impaired across all doses but was significant at doses of 100nA (more than 50% impairment) and 200nA (30-40% impairment) as well as at 500nA in the 500mg group (25-30% impairment). However, when applying nitroprusside (an agent known to increase cGMP production) at doses of 500nA (considered as severe hyperglycemia) vascular function or vasodilation was not significantly altered. Mayhan and colleagues (1991) assessed responses of ACh, nitroglycerin, ADP, and a thromboxane analogue between nondiabetic and diabetic (blood glucose >300mg/dL) rat cerebral arterioles. Interestingly, at baseline, MAP and diameters were similar between groups, though body weight was lower in diabetic group. Regardless, administration of ACh at 1 and 10μM dilated arterioles by 8 and 14%, respectively, in nondiabetic rats but constricted by 3 and 1% in diabetic rats. ADP at doses of 10 and 100μM dilated arterioles in nondiabetics by 11% and 22%, respectively, yet minimally in diabetic rats (1% and 2%) while nitroglycerin and the thromboxane analogue showed similar responses between groups. This study further investigated the effects of L-NMMA, indomethacin, and SQ-29548 (a TxA_2-PGH_2 receptor agonist; a thromboxane known to cause vasoconstriction) against ACh and ADP responses and found that indomethacin and SQ-29548 restored responses in diabetics to those similar of the nondiabetic group, leading researchers to suggest that diabetes and the presence of hyperglycemia may impair vasodilation through production of a cyclooxygenase constrictor substance, such as TxA_2-PGH_2. Interestingly, Tesfamariam and colleagues supported these findings just years prior in 1989 through assessments of isolated aortas taken from diabetic rats (blood glucose >300mg/dL). Researchers induced contraction of these aorta via administration of phenylephrine then

exposed them to ACh in increasing concentrations, finding that dilation was significantly decreased in the diabetic aorta compared to nondiabetic and that at higher concentrations the diabetic aorta began to constrict which did not occur in nondiabetic aorta. However, upon introduction of indomethacin the diabetic aorta restored ACh-induced relaxation to similar values of the nondiabetic aorta. It is worth noting that the diabetic aorta of this study also presented with elevated triglycerides and cholesterol which may have their own influence on these findings, as will be discussed shortly.

Dyslipidemia has been suggested to be present in ~70% of those with obesity and is characterized by elevated levels of triglycerides and/or LDL (Low Density Lipoprotein), AKA hyperlipidemia, or low levels of HDLs (High Density Lipoproteins). Hyperlipidemia is a multifaceted condition but is characterized by elevations in blood levels of triglycerides and/or LDLs typically as a result of excessive adipose tissue and impaired lipid metabolism. The associations between hyperlipidemia and vascular dysfunction have been demonstrated on numerous occasions, one of such by Preik et al (1996) who assessed the relationship between hypercholesteremia (excess LDL) and vasodilation. In this study, it was found that separate administrations of both ACh and bradykinins elicited increases in forearm blood flow in both control and hypercholesteremic groups but that the control group demonstrated greater blood flow increases than their cohorts (ACH: 13.3ml/min vs 10.7ml/min; bradykinin: 13.2ml/min vs 9.4ml/min). Though hyperlipidemia, much like other conditions, can contribute to vascular dysfunction in a plethora of manners, one particular contributing pathway is believed to be mediated by reactive oxygen species (ROS). ROS are reactive oxygen containing molecules that are produced normally as a result of metabolism, such as lipid

metabolism, and purportedly regulated by or at least associated with, NADPH oxidases (NOX). In fact, Haddad et al (2011) demonstrated a reduction in ROS after exposing apocynin-mediated, NOX-inhibited HUVECs to ox-LDL. These findings are further supported by Beswick and colleagues (2001) who similarly demonstrated ROS reductions in rat aorta after treatment with apocynin. Furthermore, evidence suggests that NOX is up regulated in those presenting with hyperlipidemia and is concomitant with intracellular ROS levels, of which correlates to impairments in endothelial cells (Li et al, 2017). The link between ROS and vascular function lies within the endothelium-dependent vasodilator NO, whereby the increases in ROS result in a decrease in NO bioavailability. Supporting this, Ghosh et al (2004) demonstrated that NO inactivation resulted from increased ROS (NOX-derived O_2^-) causing increased vascular tone. Interestingly, the study by Beswick et al (2001) found similar demonstrations in ROS-mediated vascular tone contributions by administering apocynin long-term (28 days) to hypertensive rats which resulted in decreases in blood pressures, lending some credence to an association between ROS and hypertension as well.

Lastly, hypertension and its effects are multifaceted and while it is not fully understood whether it is a primary or secondary cause, vascular dysfunction and its correlation to hypertension cannot be overlooked. This condition, whether essential or secondary, is widely understood to be characterized by the chronic presence of systolic blood pressures (SBP) $\geq$130 mmHg and/or diastolic blood pressures (DBP) $\geq$80 mmHg with diagnosis ranges varying among healthcare professionals (CDC, 2021). The correlation between hypertension and vascular dysfunction has been well documented through assessments, such as FMD and the aforementioned PWV, through which

commonly demonstrate blunted flow responses and indicate that a central mechanism for peripheral autonomic regulation is compromised in those presenting with the condition. In fact, Gokce et al (2001) assessed and compared FMD (through cuff occlusion of the brachial artery) between hypertensive (SBP >140mmHg, DBP >90, or hypertensive history) (n=109) and normotensive (age and sex matched) participants (n=119) and demonstrated that hypertensive participants had lower FMD compared to their normotensive cohorts (8.5 $\pm$ 5.3% vs. 11.7 $\pm$ 6.3%, respectively). Another study by Plavnik et al (2007) further demonstrated that FMD was significantly lower in those with hypertension (n=28) compared to their normotensive cohorts (n=33) (9.8 $\pm$ 7.0% vs. 13.9 $\pm$ 8.2%, respectively) after assessment of 5-minute brachial occlusions. As FMD is heavily associated with NO, these findings appear to allude to the idea that hypertension and its relationship with vascular dysfunction may be due to altered NO-mediated dilation (potentially through pathway disruption) thus seemingly lending to an association between NO and hypertension itself. In fact, Huang et al (1995) did well to demonstrate this association through the disruption of the eNOS-encoded gene of mice whereby, through this genetic disruption, the mice elicited greater basal blood pressures compared to their cohorts (110mmHG vs 81mmHG, respectively). However, while NO plays a major role in vascular regulation, it appears that hypertensives display altered responses outside of this endothelium-dependent contributor. In particular, Panza et al (1990) assessed the effects of ACh on vascular tonality of the forearm between 18 hypertensive and 18 normotensive participants. Through the infusions of ACh, researchers demonstrated that the hypertensive group, at the highest dose of infusion, elicited blunted increases in blood flow and greater vascular resistance compared to normotensive

counterparts (9.1 ± 5 ml/min/100ml vs 20.0 ± 8 ml/min/100ml, respectively). However, researchers also assessed the effects of an α-adrenergic blockade (phentolamine) in combination with ACh, attempting to eliminate the possibility that ACH-mediated vasodilatory effects could potentially be caused by presynaptic binding and thus catecholamine inhibition. The administration of the phentolamine resulted in a significantly increased vasodilatory response in both groups but was substantially more blunted in the hypertensives, indicating that not only is vascular dysfunction in hypertensives extended to endothelium-dependent vasodilators, such as NO and ACh, but that this dysfunction may be further explained by a greater activation of these α-receptors through a potential consequence of a basal increase in sympathetic activity, a concept supported through findings of increased SNA by Vongpatanasin et al (2011) and Delaney et al (2010). As these findings demonstrate clear peripheral vascular dysfunction in hypertensives, it is important to conceptualize to what extent this dysfunction exerts influence, particularly during exercise or general events demanding strenuous work to be performed. Per previous mention, the combinative mechanisms that function to regulate vascular tone, in part, form the process known as functional sympatholysis. Given that current evidence alludes to potential alterations of contributing pathways, one could deduce that this would also reflect upon the effects of this sympatholysis. In fact, findings of Delaney et al (2010) demonstrate greater increases in MAP during 30% and 40% of isometric handgrip exercise of which remained significantly more elevated than their normotensive cohorts during post exercise ischemia and follows the findings of Vongpatanasin et al (2011) who demonstrated similar findings in MAP during application of lower body negative pressure (LBNP; method that unloads baroreceptors

and increases SNA) and handgrip (HG) exercise. Furthermore, Vongpatanasin et al (2011) demonstrated that hypertensives, when compared to their normotensive cohorts, display greater decreases in both FBF (ml/min) and FVC during LBNP, HG, and their combination (FBF= 76 ± 11 mL/min, 447 ± 56 mL/min, and 384 ± 50 mL/min **vs**. 85 ± 10 mL/min, 474 ± 41 mL/min, and 477 ± 39 mL/min, respectively; FVC= 73 ± 11 mL/min/mmHg, 397 ± 49 mL/min/mmHg, and 343 ± 41 mL/min/mmHg **vs** 103 ± 12 mL/min/mmHg, 513 ± 50 mL/min/mmHg, and 506 ± 44 mL/min/mmHg, respectively) of which also correlated to reductions in muscle oxygenation, another finding supported by Dipla et al (2017).

Purpose of Study

Considering that metabolic syndrome is defined as the presence of three or more comorbidities, each of which independently affect vascular function, and considering evidence suggesting that vascular dysfunction may precede the development of these comorbidities, it seems likely that peripheral vascular dysfunction would precede the development of metabolic syndrome. However, to the author's knowledge, no studies have directly examined this using a quantifiable measure of metabolic syndrome risk. Thus, the purpose of this study is to determine if vascular dysfunction develops prior to metabolic syndrome, specifically in young adults presenting with greater risk of cardiometabolic disease. Given the current literature, we propose that young adults at risk of cardiometabolic disease will present with vascular dysfunction absent of metabolic syndrome. If this is supported by our findings, this would have crucial implications as cardiometabolic conditions are increasing in prevalence within younger adult populations.

CHAPTER III – METHODS

Participants and Study Design

An *a-priori* power analysis (presented in the *Statistical Analyses* section) indicated that 66 young adults (18-39 yrs.) would be required to achieve statistically significant ($p<0.05$) differences in the hemodynamic responses to functional sympatholysis testing (described below) using a 3 (group) by 2 (condition) by 2 (time) repeated measures design. MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP)_III guidelines, defined as having three or more of the following risk factors: 1) resting systolic blood pressure (SBP) $\geq$130 mmHg or a diastolic blood pressure (DBP) $\geq$85 mmHg, 2) fasting triglycerides (TRG) $\geq$150 mg/dL, 3) fasting blood glucose (FBG) $\geq$100 mg/dL or HbA1C $\geq$ 5.7%, 4) fasting HDL cholesterol (HDL-C) <50 mg/dL in females or <40 mg/dL in males, 5) waist circumference (WC) $\geq$88 cm for females ($\geq$80 cm for Asian females) or $\geq$102 cm for males. Individuals with prescribed medications for control of a risk factor were understood as having that risk factor. Exclusion from study was assessed through age (<18 years OR > 39 years of age), metal implantation (complete joint replacements and/or significant presence of metal plates), limb amputation (limbs or parts of limbs missing), presence/reliance on implants such as pacemakers, history of heart failure or valvular or neurodegenerative diseases, kidney or liver disease, cardiomyopathy (dilated or hypertrophic), diabetes (gestational or type 1), traumatic brain injury within past two years, blood/plasma donations in recent 20 days based on procedural blood collections, impairments or injuries of the physical nature that prevented performance of any aspect/trial of the study, consumed supplements or medications that may have interfered

with study results, pregnant or breastfeeding, or was prescribed and followed a medical diet. If able, participants were matched to a control participant by age (within 5 yrs.), biological sex, ethnicity, and race. Participants attended two visits: 1) a cardiometabolic prescreening, and 2) cardiovascular assessments. Both visits required that the subjects arrived at least 8 hours postprandial (including abstention from caffeine or prescription/over-the-counter medication/supplements for 12 hours) and had avoided intense physical activity (i.e. exercise) for 24-hours prior. The first visit served as an assessment of cardiovascular risk factors and determined the grouping that the participants were placed in based on MetS criteria. The second visit served as a cardiovascular assessment, particularly peripheral vascular function of the participants through assessments of PWV, reactive hyperemia (RHBF), and functional sympatholysis.

Cardiometabolic Prescreening

Anthropometrics and Body Composition

Upon arrival on the first visit, participants signed the informed consent (online) as well as completed a questionnaire for health history. Anthropometrics included height and weight, and body circumferences (via a traditional tape measure). Body composition was assessed using Dual X-Ray Absorptiometry (DXA) and was used as descriptor variables for participants. During DXA, participants were asked to remove any metal or accessory items as well as to tie hair (if long) into a bun. Participants were then asked/assisted to lie in supine position and in the center of the platform with their feet together and hands placed by sides. Participants were requested to face forward (toward the ceiling) and to refrain from movement or to lie as still as possible for the duration of the scan (~7 minutes depending on body proportions). Blood pressure was collected in a

seated position via an automated non-invasive sphygmomanometer (Omron Medical Systems, Muko, Kyoto, JP).

Blood Lipids and Glucose

Lipids and FBG were assessed using a cholesterol analyzer (Cholestech LDX, Abott, Abbott Park, IL), which were calibrated prior to each new batch of cassettes using two (high/low) multianalyte control solutions, per manufacturer recommendations. Blood samples (~40µL of capillary blood) were collected via fingerstick via lithium heparin-lined capillary pipette and then applied to a pre-packaged cartridge which measured LDL-C, HDL-C, total serum cholesterol, TRG, and BG. LDL-C was automatically calculated as follows:

$$\text{LDL} - C = \text{TC} - \text{HDL} - C - (\text{TRG}/5)$$

HbA1C was recorded using an automated HbA1C analyzer (A1CNow+, pts diagnostics, Whitestown, IN), whereby ~5 µL of capillary blood was collected from the finger via extraction tube then inserted into a pre-packaged sample dilution tube, mixed, and then placed into a single use cartridge that was inserted into the analyzer. During this process, the analyzer performed over 50 internal quality assurance checks that assessed errors in hard- and software as well as reagents and did produce HbA1C estimates if errors were detected.

MetSindex Calculation

MetS severity scores were calculated using equations posed by Gurka et al. in 2014. This method utilized the criteria most commonly used to classify MetS (criteria mentioned above) and assigned values based on sex and race/ethnicity. The final score was represented as a Z-score where the more positive scores represented greater risk of disease (Gurka et al, 2014). The equations utilized were as follows:

Non-Hispanic White and Asian Males: $-5.4559 + 0.0125 * WC - 0.0251 * HDL + 0.0047 * SBP + 0.8244 * \ln(Tri) + 0.0106 * Glu$

Non-Hispanic White and Asian Females: $-7.2591 + 0.0254 * WC - 0.0120 * HDL + 0.0075 * SBP + 0.5800 * \ln(Tri) + 0.0203 * Glu$

Non-Hispanic Black Males: $-6.3767 + 0.0232 * WC - 0.0175 * HDL + 0.0040 * SBP + 0.5400 * \ln(Tri) + 0.0203 * Glu$

Non-Hispanic Black Females: $-7.1913 + 0.0304 * WC - 0.0095 * HDL + 0.0054 * SBP + 0.4455 * \ln(Tri) + 0.0225 * Glu$

Hispanic Males: $-5.5541 + 0.0135 * WC - 0.0278 * HDL + 0.0054 * SBP + 0.8340 * \ln(Tri) + 0.0105 * Glu$

Hispanic Females: $-7.7641 + 0.0162 * WC - 0.0157 * HDL + 0.0084 * SBP + 0.8872 * \ln(Tri) + 0.0206 * Glu$.

These equations will then be used to aid in the grouping of participants of which there are three: no presence of MetS risk factors and negative MetS score(-/-), presence of MetS risk factors but negative MetS score(+/-), and presence of MetS risk factors AND positive MetS score (+/+).

Assessment of Peripheral Vascular Function

Visit 2 consisted of four experiments comprised of a multitude of individual trials that assessed PWV, RHBF, and Functional Sympatholysis (FS). This visit began with a brief explanation of procedures to participants, followed by participants positioning themselves into supine position on a padded table whereby they were instrumented and remained for the entirety of the study (unless verbalizing withdrawal/discontinuation). Instrumentation was as follows: automatic non-invasive sphygmomanometer (SunTech; Omron) positioned on upper segment of ipsilateral arm for blood pressure and heart rate, non-invasive photoplethysmographic BP device (Finapres Nano, FMS, Netherlands) placed on middle finger and strapped to lower segment of ipsilateral arm for continuous BP assessment, three-lead ECG (PowerLab, AD Instruments) for assessment of heart rate, and a pneumography belt positioned around abdomen for respiration assessment. Once instrumented, participants were asked to perform three trials of 5-second maximum voluntary contractions (MVC) using a handgrip dynamometer of which the highest achieved contraction was used for calculation of 25% MVC, a guideline used during functional sympatholysis assessments.

cfPWV

The first trial assessed PWV between the carotid and femoral arteries. For this procedure, the participant was additionally instrumented with a pneumatic cuff around the upper thigh located ~20cm from the common femoral artery. Once in place, the carotid artery was palpated and marked by a researcher who then collected two additional measurements (via tape measure) as follows: Carotid artery to sternal notch, sternal notch to center cuff. These measurements were added to the software (SphygmoCor XCEL,

ATCOR medical, Naperville, IL) utilized for PWV to ensure proper assessments. After entry of measurements, the researcher placed a small, non-invasive probe over the carotid marker, and maintained a sufficient signal (dictated by equipment) for ~5 seconds at which time the upper thigh cuff inflated. After this inflation, the researcher maintained this signal until a measurement was recorded. This process was repeated twice with a third measurement being recorded only if a difference of $\geq$0.5 m/s existed between the first two measures.

Reactive Hyperemia

The next trial consisted of femoral and brachial RHBF assessments. These were assessed by having the participant rest for 2 minutes (baseline) after which a pneumatic cuff was activated for 5 minutes, occluding 100% blood flow (BF) to the area located distally. This was then followed by a 5-minute period of cuff deflation, the first three of which acted as a RHBF assessment while the following two acted as recovery. For femoral RHBF assessment, a pneumatic cuff was positioned just below the knee while BF was recorded at the superficial femoral artery above the cuff. Brachial RHBF was assessed after PLM (to be discussed) and consisted of the pneumatic cuff being situated below the elbow with flow assessed at the brachial above the cuff. RHBF was quantified as the relative increase in the total area under the curve (AUC) for BF and VC across the first two minutes of the post-occlusion period.

Passive Limb Movement

Passive limb movement was conducted in which the participant's lower leg was moved through flexion and extension by a fellow researcher while blood flow through the common femoral artery was assessed. During this trial, the participants rested for 2 minutes after which the lower limb was passively moved for 5 minutes. Following this passive limb movement, a 2-minute recovery period was provided, and blood flow continued to be assessed. Similar to the RHBF protocol, passive limb movement responses were quantified as the AUC for BF and conductance, as well as the peak change in VC.

Functional Sympatholysis

This assessment consisted of two control trials and one final experimental trial. The first control trial required the participant to perform rhythmic handgrip exercise at 25% MVC in sync to a metronome set at 60bpm (one contraction per second). Brachial blood flow was recorded for 2 minutes at baseline, for 2 minutes of exercise, and again for 2 minutes of recovery. Participants rested for ~10 minutes, or until blood pressure stabilized at which point the next control trial, the cold pressor test, was conducted. During this trial, the participant submerged his/her foot into an ice bath for 2 minutes (submersion to the ankle) while BBF is collected. BF was recorded similar to the rhythmic handgrip trial (2-minute baseline, 2-minute foot submersion, 2-minute recovery). Participants were again given up to 10 minutes to return to a baseline blood pressure value, at which point the final trial commenced and consisted of a combination of the cold pressor and rhythmic handgrip tests. During this test, participants performed the protocol mentioned prior for the cold pressor test (foot submerged in ice water) while

simultaneously performing the rhythmic handgrip exercise. BF was recorded at the brachial artery, and the trial consisted of a 2-minute baseline, 2-minute HG/CPT, and 2-minute recovery. The degree to which sympatholysis occurred in participants was calculated as the difference in maximum decrease in VC between CPT and combined trials (CPT %ΔVC – (HG +CPT %Δ VC)) where conductance is calculated as BF/MAP.

Statistical Approach

Assessment of normal distribution was evaluated via Shapiro-Wilks tests and visual inspection of box and Q-Q plots. Demographics were compared between participant groups (listed above) using one-way analyses of variance (ANOVA). One-way ANOVAs were also used to examine group differences in PWV, RHBF, PLM, and FS responses. Any significant differences were further examined using a post-hoc comparison employing a Tukey correction for multiple comparisons.

CHAPTER IV – RESULTS

Demographics

One-hundred thirty-eight individuals completed this two-visit study, thirty of whom completed all peripheral vascular assessments within the timeframe of this thesis project and could be included in the analysis. These thirty individuals were separated into three initial groups: control (Con; n=12), elevated risk (ER; n=12), and MetS (n=6). Due to the inability to effectively match all participants in the MetS group by age, sex, and race, and because the primary focus of this study was to compare cardiovascular responses between individuals with elevated risk vs. healthy controls, the six individuals in the MetS group were excluded from our final analysis. This resulted in a final sample of twenty-four participants with a mean age of 19 $\pm$ 2 years, an average height of 168.3 $\pm$ 7.7 cm, an average weight of 68.0 $\pm$ 14.1 kg, and an average BMI of 20.5 $\pm$ 4.4 kg/m^2. The final sample was 25% female (n=6), 75% male (n=18), 100% non-Hispanic (n=24), 16.7% Black/African American (n=4), 16.7% White (n=4), and 66.6% Asian (n=16). Demographics and group comparisons for the final sample can be found in **Table D1**.

Health Markers/PWV

Surprisingly, only HDLC (34 $\pm$ 9 mg/dL vs. 50 $\pm$ 11 mg/ dL, p=0.001) was significantly different between the MetS vs. control groups, respectively. In contrast, the ER group did not present with statistically significant differences in BMI compared to controls (21.3 $\pm$ 5.6 kg/m^2 vs. 19.7 $\pm$ 2.72 kg/m^2, p=0.228, respectively) nor were there significant differences in WC (84.7 $\pm$ 11.1 cm vs. 78.3 $\pm$ 3.1 cm, respectively, p=0.07), SBP (119 $\pm$ 8 mmHg vs. 118 $\pm$ 13 mmHg, p=0.819, respectively), DBP (77 $\pm$ 11 mmHg vs. 79 $\pm$ 6 mmHg, p= 0.601, respectively**)**, LDLC (80 $\pm$ 20 mg/dL vs. 79 $\pm$ 23 mg/dL,

p=0.923, respectively), TRG (122 ± 44 mg/dL vs. 99 ± 66 mg/dL, p=0.331, respectively), TC (139 ± 19 mg/dL vs. 150 ± 23 mg/dL, p=0.209, respectively), FBG (90 ± 6 mg/dL vs. 87 ± 2 mg/dL, p= 0.131, respectively), or HBA1C (4.66 ± 0.43% vs. 4.71 ± 0.43 %, p=0.781, respectively). Likewise, mean cfPWV was also not significantly different between groups (mean diff= -0.043 ± 0.721 [95% confidence interval (CI): -1.538/1.45], p=0.953; **Table 1**), indicating that no significant differences seem to exist in arterial tonality between healthy and individuals at elevated risk for cardiometabolic disease.

RHBF

As expected, repeated-measures ANOVA revealed significant main effects of time for peak MAP (F=66.59, p<0.001; **Fig 1-A**), peak FBF (F=78.41, p<0.001; **Fig 1-B**), peak FVC (F=66.86, p<0.001; **Fig 1-C**), and peak TSI (F=35.67, p= <0.001; **Fig 1-D**) during femoral RHBF, as well as for peak MAP (F= 34.69, p<0.001; **Fig 3-A**), peak BBF (F=69.27, p<0.001; **Fig 3-B**), peak BVC (F=69.67, p<0.001; **Fig 3-C**), and peak TSI (F=89.83, p<0.001; **Fig 3-D**) during brachial RHBF. However, when assessing effects between groups, we found no significant main effects of group or group by time interactions during femoral (MAP: F≤1.35, p≥0.258; FBF: F≤0.298, p≥0.591; FVC: F≤0.230, p≥0.636; TSI: F≥0.00228, p≥0.962; **Fig 1**) nor brachial (MAP: F≤2.44, p≥0.132; FBF: F≤0.343, p≥0.564; FVC: F≤1.82, p≥0.891; TSI: F≤1.58, p≥0.222; **Fig 3**) trials. Similarly, no significant between-group differences were observed for MAP AUC (mean diff= 398.0 ± 255.4 mmHg*sec [95% CI: 927.5 /-131.6], p=0.133; **Fig 2-A**), FBF AUC (mean diff= 3245.8 ± 2058.5 mL/min*sec [95% CI: 7514.9/-1023.4], p=0.129; **Fig 2-B**), FVC AUC (mean diff= 32.8 ± 26.9 mL/min/mmHg*sec [95% CI: 88.5/-23.0], p=0.236; **Fig 2-C**), and TSI AUC (mean diff= -57.7 ± 148.4 %*sec [95% CI: 250.2/-

365.6], p=0.701; **Fig 2-D**) during femoral FMD nor in MAP AUC (mean diff= -75.04 ± 265.0 mmHg*sec [95% CI: 474.6/ -624.7], p=0.780; **Fig 4-A**), BBF AUC (mean diff= -883.04 ± 897.6 mL/min*sec [95% CI: 978.6/-2744.6], p=0.336; **Fig 4-B**), BVC AUC (mean diff= -7.94 ± 11.3 mL/min/mmHg*sec [95% CI: 15.4/-31.3], p=0.488; **Fig 4-C**), and TSI AUC (mean diff= -195.04 ± 113.1 %*sec [95% CI: 39.5/-429.6], p=0.099; **Fig 4-D**) during brachial RHBF.

PLM

Results indicated significant main effects of time for peak MAP (F= 64.77, p<0.001; **Fig 5-A**), peak FBF (F= 94.11, p<0.001; **Fig 5-B**), peak FVC (F= 67.87, p<0.001; **Fig 5-C**), and peak TSI (F= 28.46, p<0.001; **Fig 5-D**) during PLM trials. However, similar to the RHBF trials, no significant between-group effects nor group by time interactions were observed for peak MAP (F≤0.708, p≥0.409; **Fig 5-A**), peak FBF (F≤ 0.907, p≥0.351; **Fig 6-B**), peak FVC (F≤ 0.530, p≥0.474; **Fig 5-C**), and peak TSI (F≤ 2.88, p≥0.104; **Fig 5-D**). Similarly, independent sample T-tests found no significant differences between groups for MAP AUC (mean diff= -106.28 ± 102.8 mmHg*sec [95% CI: -319.5/107.0], p=0.313; **Fig 6-A**), FBF AUC (mean diff= -336.22 ± 1820.7 mL/min*sec [95% CI: -3439.6/4112.0], p=0.855; **Fig 6-B**), FVC AUC (mean diff= 5.65 ± 24.4 mL/min/mmHg*sec [95% CI: -44.9/56.2], p=0.819; **Fig 6-C**), and TSI AUC (mean diff= -280.24 ± 168.7 %*sec [95% CI: -630.2/69.7], p=0.111; **Fig 6-D**).

Functional Sympatholysis

When responses to the CPT and CPT+HG trails were compared between groups, significant condition by time interactions were observed for mean BBF (F=77.91, p<0.001; **Fig 7-B**), mean BVC (F=94.34, p<0.001; **Fig 7-C**), and mean TSI (F=63.37,

p<0.001; **Fig 7-D**). These differences were explained by significant increases in BBF (mean diff= 64.40 ± 7.04 mL/min [95% CI; 50.60 /78.19], p<0.001; **Fig 7-B**) and BVC (mean diff= 0.629 ± 0.067 mL/min/mmHg [95% CI; 0.511 /0.746], p≤0.001; **Fig 7-C**), and decreases in TSI (mean diff= -7.17 ± 1.12 % [95% CI; -4.81/-9.522], p<0.001; **Fig 7-D**) during the CPT+HG trial, which were not observed in the CPT trial. Instead, BVC (mean diff= 0.008 ± 0.02 mL/min/mmHg [95% CI; -0.381 /1.091], p=0.983; **Fig 7-C**), BBF (mean diff= 1.35 ± 1.51 mL/min [95% CI; -8.133/8.173], p=0.807; **Fig 7-B**) and TSI (mean diff= 0.355 ± 0.376 % [95% CI; -0.381 /1.091], p=0.781; **Fig 7-D**) remained unchanged during CPT (all effects of group: F≤0.602, p≥0.446; all interaction effects: F≤1.23, p≥0.279). When assessing AUC, we found significant effects by condition for BBF (mean diff= 7402 ± 812mL/min*sec [95% CI; 5810.48/8993.52], p<0.001; **Fig 8-B**) and condition effects for TSI (mean diff= 899 ± 126%*sec [95% CI; 652.04/1145.96], p<0.001; **Fig 8-D**). In contrast, no significance was found between groups in BVC (p= 0.673; Fig 8C) or MAP (p= 0.296; **Fig 8-A**) nor were there significant condition by group interactions in BVC (p= 0.939; **Fig 8-C**), MAP (p= 0.975; **Fig 8-A**), TSI (p= 0.255; **Fig 8-D**).

CHAPTER V – DISCUSSION

This study tested the hypothesis that peripheral vascular function would be impaired in those presenting with an elevated risk for MetS. This hypothesis was based on the expectation that individuals with an elevated $MetS_{index}$ would be susceptible to dysfunction at the endothelial cells as certain MetS risk factors have been found to be associated with deleterious changes in the regulatory abilities of the vasculature. However, our findings do not currently support this hypothesis. Instead, we found no significant differences between groups nor any significant group by condition interactions across implemented trials. These findings suggest that young adults with an elevated $MetS_{index}$ do not exhibit significant peripheral vascular dysfunction compared to healthy controls. These findings may be explained by several potential factors, each of which is explained in more detail below, beginning with the time course of risk factor development.

Time Course of Risk Factor Development

As noted above, our study evaluated vascular dysfunction in young adults with positive $MetS_{index}$, and our results indicate that vascular function is not impaired in these individuals. One possible explanation for not finding significance between groups is the timing of risk factor development. Specifically, it could be possible that some participants within this study may have recently developed such risk factors and, and although these individuals present as unhealthy given this criteria, peripheral vascular function may have still been very much intact. For example, hypertension, and more specifically resting blood pressure, is heavily correlated to vascular dysfunction (Bruno et al, 2017; Gokce et al, 2001). Therefore, the magnitude of vascular dysfunction would presumably be greater

in an individual who has a five-year history of hypertension compared to an individual who developed hypertension within the past year. Supporting evidence for this can be found in literature assessing the onset age of hypertension and mortality risk. A study published by Wang et al. in 2020 assessing mortality risk in over 100,000 individuals found that when hypertension formed earlier in adulthood, mortality risk increased compared to individuals who formed the condition late in life. Hyperglycemia is another risk factor known to be heavily associated with vascular dysfunction. However, an animal study conducted by Klabunde et al. (2007), which consisted of assessments of coronary artery function of isolated Sprague-Dawley rats found no differences between normoglycemic and acute hyperglycemic conditions. In this, researchers assessed the coronary vascular responses to NO, Sodium Nitroprusside (SNP), Adenosine (ADO), Phenylephrine (PHE), and L-NAME after exposure to normoglycemic (100mg/dL D-glucose) and hyperglycemic (500mg/dL D-glucose) environments. Researchers found that coronary perfusion pressure (CPP) increased similarly among groups (20-25%) after PHE administration with no significant differences between groups. This trend followed with administration of L-NAME where CPP increased by 40-60% with no significant differences between groups. When administering ADO and SNP independently, they found that ADO at 1μM did not elicit vasodilation in any of the groups nor did SNP at 0.01μM. However, both ADO and SNP elicited dose-dependent responses with ADO at 10 and 100μM causing vasodilation in both conditions but attenuating in normoglycemic group after 60 minutes and SNP eliciting vasodilation at 0.1 and 1.0μM with no difference in attenuation rates between groups. Therefore, simply inducing hyperglycemia does not seem to impair vascular function acutely, indicating that

individuals with recent onset of hyperglycemia may not have developed the vascular impairments typically associated with type 2 diabetes (Klabunde et al, 2007; Henry et al, 2004). Moreover, it is intriguing that LDLC and triglycerides were not significantly elevated within the risk group despite this group presenting with a significantly lower HDLC. It is equally interesting that, regardless of this low HDLC, the ER group did not demonstrate significant decreases in PWV when compared to controls. These findings may also be explained by duration of low HDLC levels, given that HDLC functions to transport excess cholesterols, such as LDLC, from the bloodstream to the liver which can accumulate over time. Thus, provided a persistent state of low HDLC, this transportation process can become insufficient, leading to elevations in oxidized LDLC in the vascular wall, which can lead to the development of arteriosclerosis and atherosclerosis. Evidence suggests that, if left unmanaged, this condition worsens over time, leading to greater reductions in arterial diameter and arterial stiffness (Mohammad et al, 2022). Thus, though the elevated risk group had significantly lower HDLC, its effect upon the vasculature may be delayed.

Protective Effects of Age

Our study focused on determining the presence of vascular dysfunction in young adults with positive $MetS_{index}$ scores, yet it is possible that the age of participants may have provided some protective benefit even when an individual presented with known risk factors or positive $MetS_{index}$ scores. As mentioned previously, age is often associated with deleterious changes in the body which extend to the vasculature. It is possible that had this study focused on a middle or older aged population, significant differences between groups may have been observed, indicating that risk factors exert more influence

as we age. For example, Bruno and colleagues in 2014 found that the media-to-lumen ratio (MLR) (a parameter for assessing structural changes in arteries) was relatively similar between hypertensive and normotensive young adults. In contrast, when assessing hypertensive vs normotensive older aged adults, the hypertensive groups demonstrated greater MLR independent of hypertension duration. This may be due to a host of reasons such as age-associated increases in cell senescence. As mentioned previously, cell senescence is a common feature of aging and tends to occur throughout the body, including within the peripheral vasculature. With this, older age tends to be associated with senesced endothelial cells which are suspected to diminish the availability of NO and increase the production of ROS. Recall the findings of Rossman and colleagues in 2014 which demonstrated significant decreases in endothelial dependent dilation in older adults compared to young adults. They found that this decrease in dilation correlated heavily to the increased expression of proteins indicative of endothelial cell senescence. Young adults, on the other hand, maintain the ability to regenerate endothelial cells and therefore maintain the production and release of endothelial dependent vasodilators, such as NO. With that, given that our mean age for this study was 19 ± 2 years, it is possible that individuals within the young age range do not experience significant deleterious effects of MetS risk factors as any damage or dysfunction mediated by the presence of risk factors is mitigated by the body's ability to regenerate cells.

Risk Factor Phenotypes

It is important to reiterate that our study assessed those presenting with positive a $MetS_{index}$ score but not those meeting the criteria for a full MetS diagnosis. Therefore, not all participants in the ER group presented with the risk factors most commonly associated

with known dysfunction, such as hypertension or hyperglycemia. In fact, as outlined in **Table 2**, only two individuals in the ER group presented with hypertension, and HDLC was the predominant risk factor in the ER group (*n*=10). Because of this, vascular dysfunction may be more noticeable with specific metabolic syndrome phenotypes (combinations of specific risk factors) compared to other phenotypes. Considering prior evidence that hypertension and hyperglycemia are both highly associated with vascular dysfunction (Xiang et al, 2007; Bruno et al, 2017), individuals with a hypertensive-hyperglycemic phenotype would likely demonstrate impaired vascular function. Yet, within our study, the means of the elevated risk group suggested largely normotensive and normoglycemic participants, potentially explaining why no differences in vascular function were observed between groups. Furthermore, more participants in the control group (*n*= 4) presented with hypertension compared to the ER group. While seemingly paradoxical, it is important to recall that the equations used to assign an individual as high (>0.00) or low (<0.00) risk account for the weight of several risk factors; not only hypertension. Therefore, the accumulation of several biomarkers that approximate the threshold for inclusion in a MetS diagnosis may result in a positive $MetS_{index}$, whereas hypertension alone oftentimes will not. As such, a participant may still be considered low risk, despite meeting the ATP III criteria for hypertension. Ultimately, this presentation of hypertension within the control group likely masked any hypertension-related decreases in vascular function in the ER group, and therefore, more research is necessary to better understand vascular dysfunction in individuals with a hypertensive metabolic risk phenotype.

Even though we cannot draw inferences regarding potential vascular dysfunction in individuals with a hypertensive or hyperglycemic metabolic risk phenotype, our findings still provide important information related to other phenotypes. Given that the ER group included in this study did present with a predominantly low HDLC phenotype, we can conclude that individuals with an elevated risk of cardiometabolic disease associated with a low HDLC MetS phenotype do not suffer from pronounced vascular dysfunction compared to individuals considered to be low-risk. This information has important clinical implications, which will be discussed further in later sections.

CHAPTER VI – LIMITATIONS

Firstly, given that our sample size was only 24 participants, it is possible that the study was underpowered for the group by time by condition interactions evaluated during the functional sympatholysis testing, and that more significant findings may have occurred with a larger body of participants. A larger sample size would have also likely allowed us to not only examine more individuals presenting with known dysfunction-associated risk factors, such as hypertension and hyperglycemia, but also more effectively match participants across groups, particularly in the MetS group. Another consideration for this study is the lack of an "ideal health" control group. As discussed previously, participants within our control group presented with certain MetS risk factors, such as hypertension, elevated waist circumference, FBG, TRG, and low HDLC. This occurred because the calculations utilized for grouping, which accounted for age, sex, and ethnicity, returned negative $MetS_{index}$ scores though these individuals presented with risk factors. While this demographic reflects the general population of the U.S. (who would also present with 1-2 risk factors), future studies may consider including an additional control group in which participants do not present with any risk factors.

It is also worthy of note that we did not have the capability of drug infusions. Traditionally, functional sympatholysis has been tested using infusions of alpha agonists, such as PHE or tyramine (Dinenno and Joyner, 2004). Since we did not have the ability to infuse these vasoactive drugs, we used the CPT as a non-pharmacological method of evoking sympathetic vasoconstriction. While the CPT is a well-documented sympathetic stimulus (Elias and Ajayi, 2019; Okada et al, 2016) it is likely that the infusion of pharmaceuticals, such as phenylephrine or SNP, may alter our current results as these are

known to induce dose-dependent degrees of vasoconstriction. Finally, we lacked the technical ability to measure beat-by-beat diameter changes and thus we were only capable of calculating FBF and FVC for RHBF and PLM trials using mean vascular diameters for each 1-minute period. Unfortunately, this method only allows inferences to be drawn rather than formulating direct conclusions about the state of endothelial function in this population.

CHAPTER VII – APPLICATION AND FUTURE DIRECTIONS

Our research currently suggests that young adults with an elevated $MetS_{index}$ do not present with peripheral vascular dysfunction, a finding that contradicts the expectations provided through other risk factor research. These current findings provide 2 primary implications. Firstly, given that this population does not seem to exhibit suspected deleterious impacts of MetS risk factors, there may be an opportunity to prevent any potential cardiovascular dysfunction expected to occur with these risk factors over time. In other words, while the $MetS_{index}$ is not able to detect overt vascular dysfunction, it does provide an "early warning" system to alter individuals to the risk of vascular dysfunction previously observed in individuals with MetS (Sprung et al, 2020; Czernichow et al, 2010). Secondly, these findings also highlight the need for more specific assessments for the detection of emerging cardiovascular dysfunction in young adults with poor cardiometabolic health.

CHAPTER VIII – CONCLUSIONS

In conclusion, our current study suggests that young adult individuals presenting with elevated $MetS_{index}$ scores do not exhibit significant peripheral vascular dysfunction compared to healthy cohorts. Furthermore, our evidence suggests that the $MetS_{index}$ may provide as "early warning" for individuals at risk of MetS-associated vascular dysfunction. Lastly, our findings highlight the importance of the need for further specific cardiovascular health assessments in young adults who are predisposed to poor cardiometabolic health. In closing, it seems that more research needs to be conducted using $MetS_{index}$, particularly in middle and older aged adults for further determination of its usefulness in detecting vascular dysfunction.

APPENDIX A – RHBF

Figure 1. Femoral RHBF

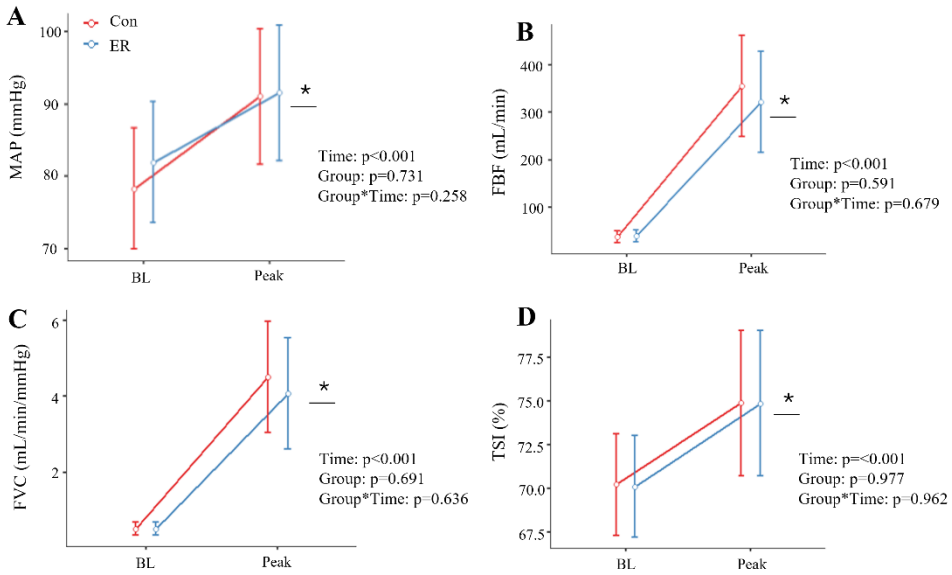


Figure 2. Femoral RHBF AUC

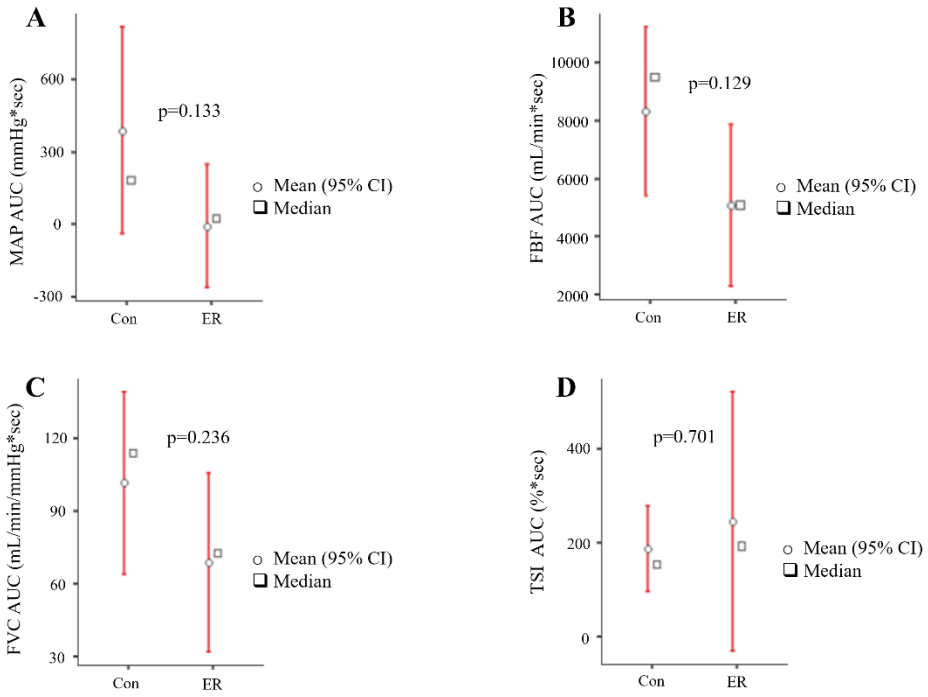


Figure 3. Brachial RHBF

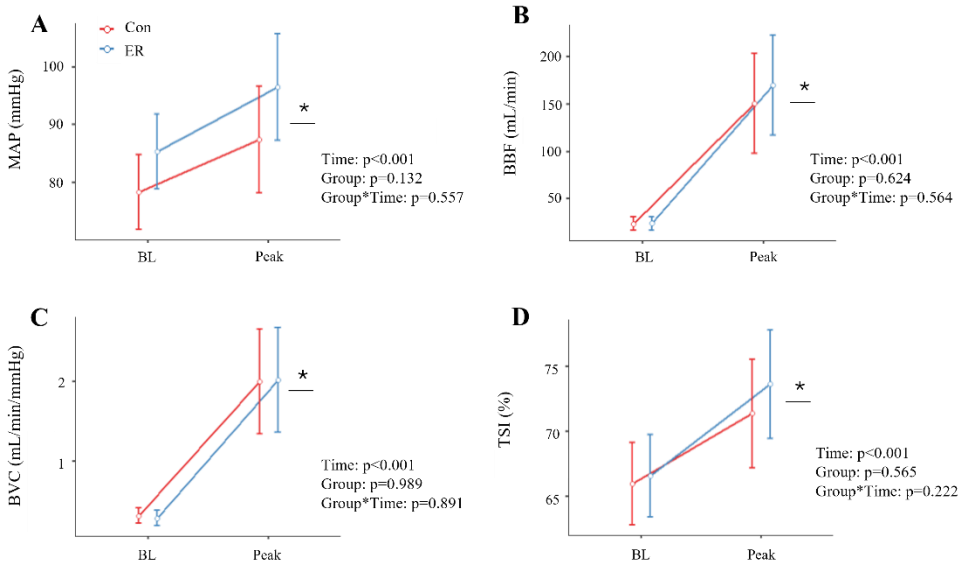
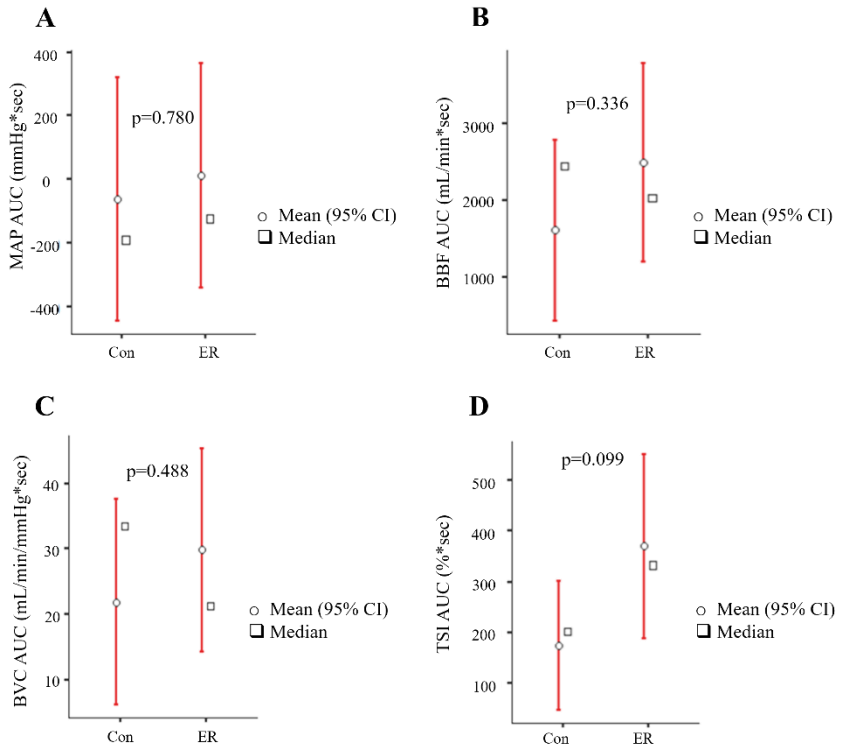


Figure 4. Brachial RHBF AUC



APPENDIX B – PLM

Figure 5. Passive Limb Movement

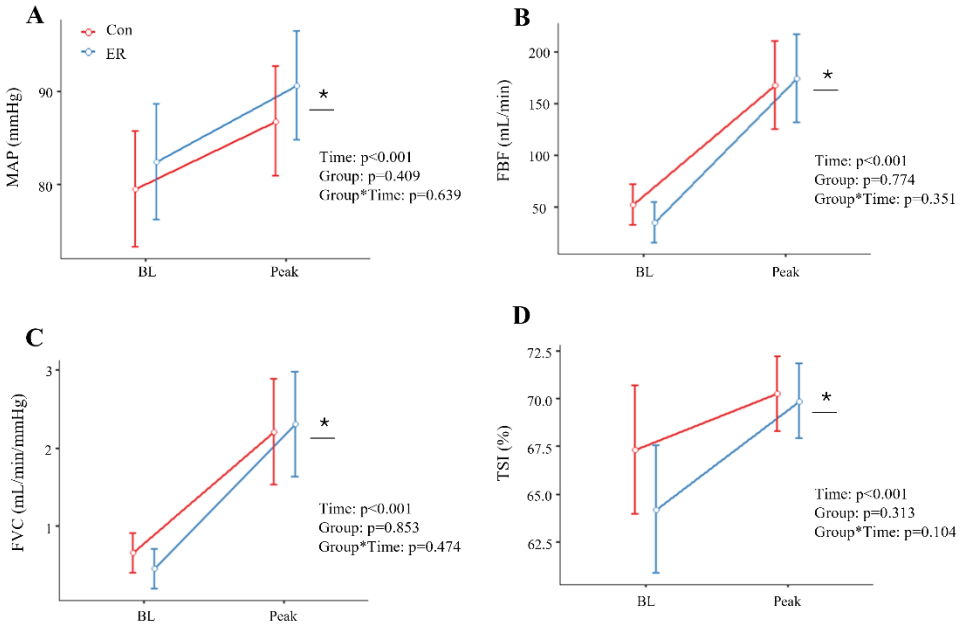
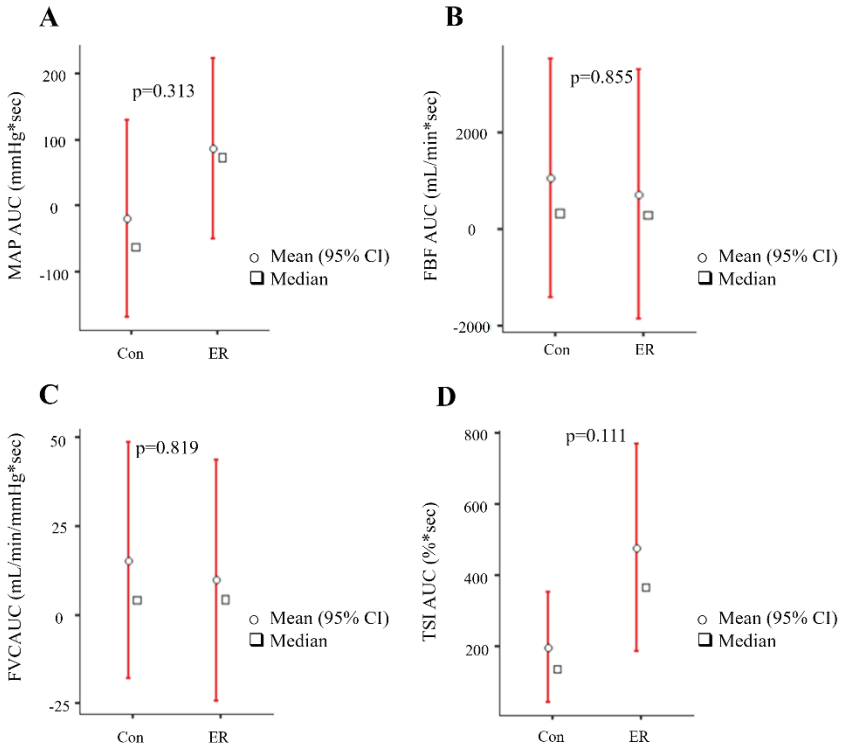


Figure 6. Passive Limb Movement AUC



APPENDIX C – FS

Figure 7. Functional Sympatholysis

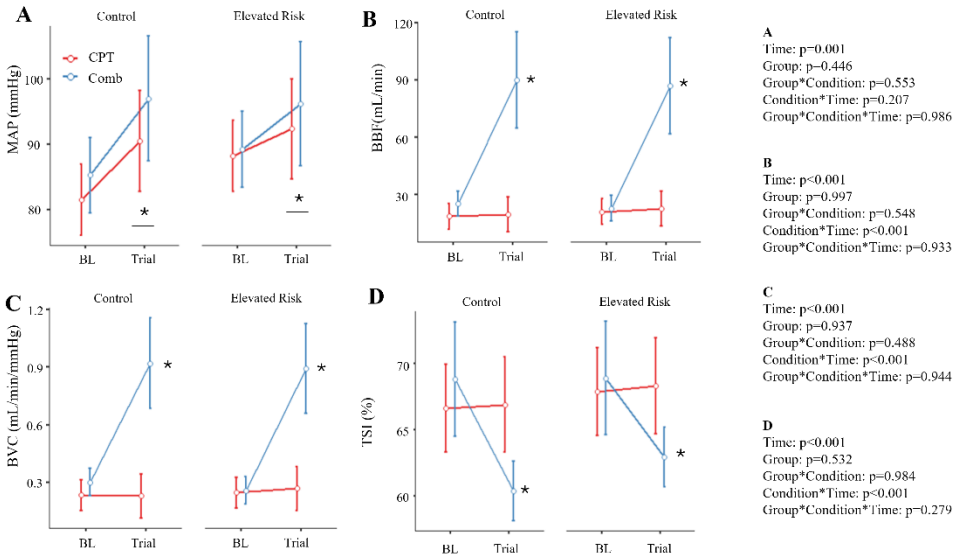
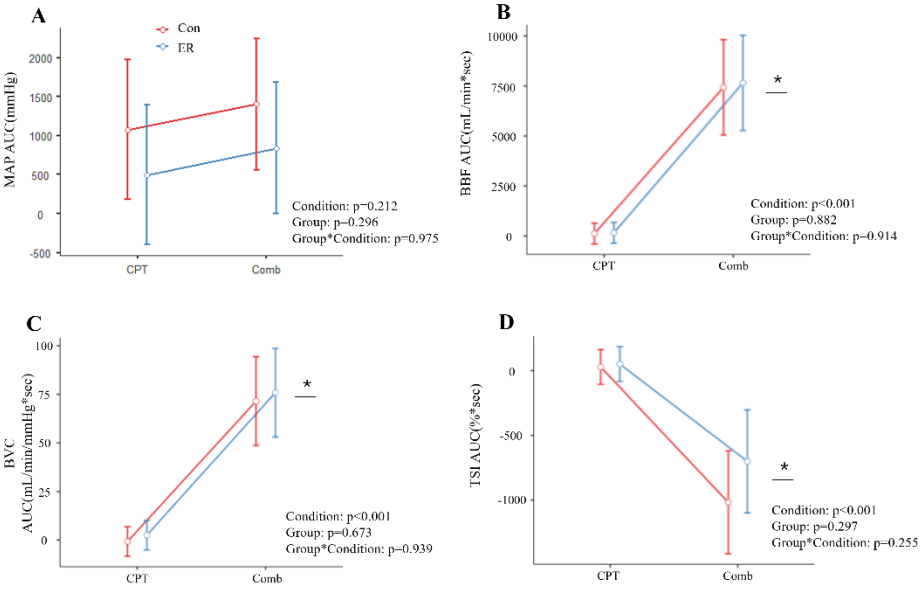


Figure 8. Functional Sympatholysis AUC



APPENDIX D – TABLES

Table 1 *Demographics*

	Group	Mean	SD	p-value
Age	Con	19.917	1.165	1.00
	ER	19.917	3.029	
Height (CM)	Con	167.625	7.805	0.67
	ER	169.042	8.065	
Weight (KG)	Con	64.7	5.966	0.26
	ER	71.4	18.948	
BMI	Con	19.725	2.72	0.37
	ER	21.374	5.637	
WC_Iliac	Con	78.333	3.114	0.07
	ER	84.7	11.169	
SBP	Con	118.167	13.684	0.82
	ER	119.25	8.625	
DBP	Con	79.583	6.895	0.60
	ER	77.583	11.098	
HDL	Con	50.833	11.48	0.001*
	ER	34.833	9.163	
LDL	Con	79.5	23.095	0.92
	ER	80.417	20.8	
TRG	Con	99	66.824	0.33
	ER	122	44.335	
TC	Con	150.917	23.88	0.13
	ER	139.417	19.388	
FBG	Con	87.167	2.29	0.78
	ER	90.417	6.802	
HBA1C	Con	4.708	0.436	0.78
	ER	4.658	0.434	
MetSindex	Con	-0.663	0.4	<0.001*
	ER	0.302	0.289	
Mean cfPWV	Con	5.14	1.69	0.953
	ER	5.18	1.84	

Table 2 *Risk Factor Count*

	Group	N
HTN SBP	Con	3
	ER	2
HTN DBP	Con	2
	ER	1
HTN	Con	4
	ER	2
Low HDLC	Con	3
	ER	10
Elevated WC	Con	1
	ER	1
Elevated TRG	Con	1
	ER	3
Elevated FBG	Con	0
	ER	0

APPENDIX E – IRB Approval Letter

Office *of*
Research Integrity

THE UNIVERSITY OF SOUTHERN MISSISSIPPI

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NOTICE OF INSTITUTIONAL REVIEW BOARD ACTION

The project below 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 regulations (45 CFR Part 46), and University Policy to ensure:

- The risks to subjects are minimized and 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 involving risks to subjects must be reported immediately. Problems should be reported to ORI using the Incident form available in InfoEd.
- The period of approval is twelve months. If a project will exceed twelve months, a request should be submitted to ORI using the Renewal form available in InfoEd prior to the expiration date.

PROTOCOL NUMBER:	23-0446
PROJECT TITLE:	A Multi-Modal Approach to Improving Early Detection of Cardiometabolic Risk in Young Adults in Mississippi
SCHOOL/PROGRAM	Kinesiology
RESEARCHERS:	PI: Jonathon Stavres Investigators: Stavres, Jonathon Ray~Graybeal, Austin J~Thorsen, Tanner Austin~Aultman, Ryan~Newsome, Taquoris Ashuad~Vallecillo Bustos, Anabelle~Henderson, Alex~McCoy, Stephanie M~Renna, Megan Elizabeth~Bonfils, Kelsey A~Behringer, Kylee Faith~
IRB COMMITTEE ACTION:	Approved
CATEGORY:	Expedited Category
PERIOD OF APPROVAL:	05-Sep-2023 to 04-Sep-2024

Donald Sacco, Ph.D.
Institutional Review Board Chairperson

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