The Influence of Adjusting for Muscular Strength and Body Size on Sex Differences in Sympathetic Responses to Isometric Handgrip Exercise and Metaboreflex Isolation in Healthy Young Adults

by

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ABSTRACT

The exercise pressor reflex (EPR), composed of central command and the mechano- and metaboreflex, increases blood pressure (BP). Compared to males, healthy premenopausal females typically exhibit blunted increases in BP during exercise. However, recent evidence suggests that this sex difference may be attributed to differences in muscle strength and anthropometric measures of body size. Thus, statistical adjustments for these factors may attenuate the sex differences in BP responses during isometric handgrip (HG) exercise and post-exercise ischemia (PEI; metaboreflex isolation). Therefore, for the purposes of this thesis project, we sought to determine whether individual and combined adjustments for HG force and body size (height^2 and body surface area) would attenuate sex differences in EPR responses to HG and PEI in healthy young males and females.

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COMMONLY USED ABBREVIATIONS

- **ANS:** autonomic nervous system
- **SV:** stroke volume
- **HR:** heart rate
- **CO:** cardiac output
- **BP:** blood pressure
- **PNS:** parasympathetic nervous system
- **SNS:** sympathetic nervous system
- **ACh:** acetylcholine
- **cAMP:** cyclic adenosine monophosphate
- **SVR:** systemic vascular resistance
- **NE:** norepinephrine
- **E:** epinephrine
- **PKA:** protein kinase A
- **CVLM:** central ventrolateral medulla
- **RVLM:** rostral ventrolateral medulla
- **ADH:** antidiuretic hormone
- **RAAS:** renin-angiotensin-aldosterone system
- **EDV:** end diastolic volume
- **ESV:** end systolic volume

- **Ang II:** angiotensin II
- **ACE:** angiotensin converting enzyme
- **GFR:** glomerular filtration rate
- **CVD:** cardiovascular disease
- **PPG:** photoplethysmography
- **MSNA:** muscle sympathetic nerve activity
- **EPR:** exercise pressor reflex
- **RPE:** rate of perceived exertion
- **PEI:** post-exercise ischemia
- **HG:** handgrip
- **MVC:** maximal voluntary contraction
- **h^2:** height^2

CHAPTER ONE: AUTONOMIC CONTROL OF THE CARDIOVASCULAR SYSTEM

BACKGROUND: THE AUTONOMIC NERVOUS SYSTEM

I. Overview of the Autonomic Nervous System

The nervous system is the highly complex neural network responsible for the body's perception and reaction to the external world. (1) Consisting of the brain, spinal cord, and nerves, the nervous system works constantly to sense stimuli, process information, and generate feedback. (1) The autonomic nervous system (**ANS**), a branch of the peripheral nervous system, is responsible for unconscious and involuntary processes of the heart, organs, and glands. (1, 2) One of the most important aspects of the ANS is the regulation of cardiac muscle contractility, a key determinant of stroke volume (**SV**; how much blood is ejected per cardiac cycle) and heart rate (**HR**). (2) Together stroke volume and HR determine cardiac output **(CO)**. By regulating CO, and also peripheral vascular tone, the ANS modulates blood pressure (**BP**) which controls blood flow to organs throughout the body. (2, 3) While outside the scope of this review, the ANS also plays an integral role in the regulation of perspiration, respiration, peristalsis of the gastrointestinal tract, and other homeostatic activities. (2)

The ANS can be subdivided into two additional branches: the parasympathetic nervous system (**PNS)** and the sympathetic nervous system (**SNS**). (1, 2) Simply put, the PNS maintains resting homeostatic processes and is colloquially referred to as the "rest and digest" system. (1) Conversely, the SNS regulates the detection and reaction to excitatory stimuli and is colloquially known as the "fight or flight" system. (2) At rest, the PNS conserves energy by sustaining a comparatively low rate of respiration, constriction

of the pupils and bronchioles, regular digestion, and routine secretions from the pancreas, salivary glands, and lacrimal glands. (2, 4) When a stimulus is detected at rest, however, the PNS is downregulated and sympathetic activation occurs to prepare the body for physical activity or other forms of stress (e.g., conflict, demanding cognition, public speaking, etc.). (5) Sympathetic fibers innervate virtually every site of the body, and thus, the effects of SNS activation are broad and highly dependent on the stimulus. (5) For example, identification of a potential threat, emotional excitement, exercise, and illness including end-organ failure all trigger withdrawal of the PNS, largely by way of decreased vagal tone, and subsequent activation of the SNS. (2) The SNS generally opposes the resting effects of the PNS, and thus, these scenarios can elicit distinct and varying responses including but not limited to increased respiration, dilation of the pupils and bronchioles, inhibition of digestion, and stimulation of the reproductive organs. (2, 6)

Further, systemic circulatory mechanisms are arguably the most prominent aspect of sympathetic activation and will remain the central focus of the following sections. A series of cascading processes function to provide the body with adequate resources to effectively respond to a stimulus. Both the PNS and the SNS have their own specific fibers, target organs, neurotransmitters, and receptors that implement cardiovascular adjustments in line with each systems' role. Further, a number of reflexes (e.g., baroreflex and chemoreflex) and hormonal pathways (e.g., renin-angiotensin-aldosterone pathway) function to regulate BP changes upon sympathetic activation. Additionally, due to a number of adjustments within the cardiovascular, neural, and endocrine systems, increased HR and SV dramatically elevate CO (i.e., Frank-Starling mechanism) and

ultimately contribute to the elevated BP response. The following sections of this chapter aim to describe these autonomic adjustment mechanisms in detail.

i. Fibers, Neurotransmitters, and Receptors of the Autonomic Nervous System

Every organ system in the body is reliant on both the PNS and the SNS for regulation of their mechanisms, although the input of each system varies throughout the body. (2) For example, the vasculature depends more heavily on sympathetic drive for its net balance of vascular tone, as it is richly innervated with sympathetic neurons. (2, 3, 7) The heart, however, is subject to constant competition between sympathetic and parasympathetic control. (2, 7) At rest, the PNS is the dominant system, effectuating an individual's resting HR and contractility. (6) However, upon detection of an environmental stressor, the PNS is downregulated and the SNS assumes the role of the presiding overseer of cardiac activity. (3, 7) Despite the generally opposing actions of the SNS and PNS, both systems rely on the complex interactions between their respective fibers, neurotransmitters, and receptors. (2, 7)

a. The Parasympathetic Nervous System

Fibers of both the SNS and PNS innervate every organ and gland in the body including the cardiac tissue of the heart. (2) Parasympathetic fibers originate from the superior and inferior portions of the brain and spine, namely, in a craniosacral distribution. (7) Of the twelve cranial nerves, the oculomotor (III), facial (VII), glossopharyngeal (IX), and vagus (X) nerves rely on parasympathetic innervation. (2, 8, 9) These parasympathetic cranial fibers leave the brain and furcate to form large plexuses in the face and neck (e.g., ciliary, pterygopalatine, submandibular, and otic ganglia). (7, 10)

Similarly, the parasympathetic fibers of the lumbar and sacral spine also branch extensively into the visceral organs, where they modulate homeostatic processes. (10) Within the context of cardiac innervation, preganglionic neurons of the PNS specifically derive from the midbrain, pons, and medulla oblongata. (10) Importantly, these neurons synapse with postganglionic neurons in the heart. (2, 7) The vagus nerve, for instance, innervates cardiomyocytes in the sinoatrial and atrioventricular nodes, the two centers of electrical propagation within the heart. (7, 11) This nerve is considered to be the most important within the PNS as it contains 75% of all parasympathetic fibers that innervate the upper thorax, and because of this, it serves as the critical modulator of HR. (2-4, 7)

Moreover, all neurons within the nervous system rely on the use of neurotransmitters for the propagation of neural impulses. (12) Within the ANS, long series of preganglionic and postganglionic neurons, separated by small synaptic gaps, communicate via the transmission (preganglionic neurons) and reception (postganglionic neurons) of acetylcholine (**ACh**). (7) Specifically, the release of ACh from the preganglionic neuron triggers an electrochemical gradient across the postganglionic membrane, thus successfully continuing the nerve impulse. (2, 7) Both sympathetic and parasympathetic preganglionic neurons secrete ACh. (2) Postganglionic fibers of the PNS are also cholinergic (i.e., release ACh). (12) ACh has a relaxing, inhibitory effect on the surrounding viscera, therein summarizing the general state of all organ systems while under PNS control. (2, 6, 7) ACh is synthesized from acetyl coenzyme A and choline, a dietary nutrient, by the enzyme choline acetyltransferase. (13) In the nervous system, acetyltransferase primarily exists in the nerve terminal cytoplasm. (13)This reaction initially occurs within vesicles of the neuronal axon, and after calcium influx and the

consequential excretion and diffusion of ACh across the synaptic gap, detection of the neurotransmitter via cholinergic receptors on the surface of the postganglionic neuron initiates a neural impulse. (2, 13)

There are two types of cholinergic receptors in the body: nicotinic and muscarinic receptors. (2, 7) Nicotinic receptors are channel proteins that can be found at both the skeletal neuromuscular junction and at various points throughout the nervous system. (14) The primary objective of nicotinic receptors is to quickly mediate neural impulses via ion channels as a consequence of ACh detection. (14, 15) Muscarinic receptors, on the other hand, are G-protein-coupled membrane receptors, meaning receptor activity is coupled to a G-protein, often described as a “molecular switch” (16), to continue nerve transmission. (17) On this account, the signal transmission initiated by these muscarinic receptors is fairly long in comparison to the rapid propagation of nicotinic receptors. (15, 17) Briefly, ACh stimulation of muscarinic receptors decreases cyclic adenosine monophosphate (**cAMP**) synthesis via activation of inhibitory G-proteins which mitigate excitatory reactions within the cardiovascular system (e.g., reduced HR and contractility; vasodilation). (18-20) The cAMP mechanism is described in the next section. Muscarinic receptors are abundantly located in the brain, lungs, portions of the gastrointestinal tract, and most importantly in the scope of this review, the heart. (17) While there are a total of 5 subtypes of muscarinic receptors (M1-M5), subtypes M2 and M3 are the only varieties found in the cardiovascular system. (2, 17) M2 receptors can be found in the atrial and nodal tissue of the heart, while M3 receptors are predominantly found in the vascular endothelium. (2)

In summary, when a nerve impulse from a parasympathetic preganglionic nerve fiber reaches the cardiac tissue (e.g. vagal nerve synapsing at the sinoatrial or atrioventricular node) or endothelial tissue, ACh is released into the synapse and the postganglionic fiber receives and transmits the impulse. (2, 11) Upon impulse arrival at the postganglionic axon terminals within the heart, ACh is secreted into the surrounding viscera where M2 receptors detect its presence and elicit their responses. (9, 11) As stated previously, ACh has relaxing properties, and therefore, the primary consequence of ACh detection is decreased HR. (2, 9) Mechanistically, this is due to hyperpolarization of sinoatrial nodal cells, which extends the required time to return to threshold potential and slows the speed of depolarization from the sinoatrial node to the atrioventricular node. (2, 7, 11) This mechanism is predominantly controlled by the vagus nerve and thus, is commonly referred to as vagal tone. (21) ACh also decreases atrial contractility, thereby reducing SV, which alongside a reduction in HR via vagal tone, causes a drop in CO. (7, 11, 22) This cascade of events also reduces cardiac preload as per the Frank-Starling mechanism, as explained in a later section. Similarly, when M3 receptors within the vascular endothelium detect ACh, vasodilation occurs likely due to stimulation of local nitric oxide production leading to vascular smooth muscle cell relaxation. (23, 24) Decreased systemic vascular resistance (**SVR**) also results in subsequent reductions in BP through the Frank-Starling mechanism. (2, 7, 9)

b. The Sympathetic Nervous System

The majority of sympathetic fibers emerge from the thoracolumbar spine, specifically spinal nerves T1 – L2 (5, 18), although sympathetic fibers exist in each of the 31 spinal nerves. (18) Similar to the craniosacral parasympathetic nerves, these large

sympathetic nerves leave the spinal cord and furcate to form the celiac, aortic, mesenteric, hypogastric, and pelvic plexuses in the thorax which primarily function to regulate digestive, reproductive, and cardiac processes. (7, 18) In fact, there are approximately twenty times more postganglionic sympathetic fibers than preganglionic sympathetic fibers within the body, highlighting the vast influence of the SNS on the organ systems. (18)

As mentioned earlier, preganglionic neurons of the SNS are cholinergic, and thus depend on the release of ACh for their nerve impulse transmission. (5, 7) However, postganglionic sympathetic fibers excrete norepinephrine (**NE**), commonly referred to as "adrenaline," into the viscera they innervate, and are consequently called "adrenergic" fibers. (7, 9) Exceptions include sympathetic innervations of the sweat glands and the arrector pilli muscles of the epidermis. (5) Unlike ACh, NE has an excitatory, stimulating effect and its influence on the adrenergic receptors of the cardiovascular system is reflective of this. (6, 7) NE is produced within nerve terminals of various organs throughout the body. (25) Particularly, around 30% of circulating NE is produced by sympathetic innervations of the lungs, 25% from the kidneys, 20-25% from the skeletal muscle, 3% from the heart, and the remainder from the hepatic and mesenteric arteries, skin, brain, and adrenals. (26, 27) The first phase of NE biosynthesis begins in the cytosol with tyrosine, a dietary amino acid, that is converted into dihydroxyphenylalanine and then to dopamine via the enzyme tyrosine hydroxylase. (28) Dopamine is then taken up into vesicles within sympathetic neurons where it is converted into NE by β-hydroxylase. (28) This NE is stored and shielded from metabolism within the neuron and upon sympathetic stimulation, is excreted into the plasma, a phenomenon known as NE spillover. (25, 28)

NE can subsequently be converted into epinephrine (**E**), a neurotransmitter that has similar stimulating effects to NE, by way of phenylethanolamine N-methyltransferase. (28) E is primarily produced in the adrenal medulla. (6) Both E and NE contribute to the fight-or-flight response, which is mediated by adrenergic receptors throughout the body. (5, 7, 18)

Similar to muscarinic receptors, all adrenergic receptor subtypes are G-protein-coupled receptors. (18, 20) However, unlike the inhibitory G-proteins of muscarinic G-coupled receptors, adrenergic receptors typically activate stimulatory G-proteins upon detection of NE or E. (2, 18, 20) This variety of G-proteins dissociate when activated, and in turn activate adenylyl cyclase, the enzyme that mediates the conversion of adenosine triphosphate to cAMP. (2, 20, 29) Increased cAMP initiates a number of physiological responses including downstream signaling pathways in the endocrine, muscular, immune, and central nervous systems. (20, 30, 31) Most importantly, cAMP activates protein kinase A (**PKA**), an enzyme which directly influences ion flux, genetic transcription, and metabolism (20, 32) and ultimately regulates HR (i.e., chronotropic control) and contractility (i.e., inotropic control). (20, 33) Therefore, detection of NE or E via adrenergic receptors upregulates cAMP production, PKA activation, and results in altered cardiovascular activity. (2) Whether this activity is inhibitory or stimulatory depends on the classification of the target cell (18), however, generally, PKA activation enhances cardiac contractility by way of increased calcium influx. (34)

There are two main types of adrenergic receptors: α and β receptors. Subtypes α_1, α_2, β_1, and β_2 exist in the cardiovascular system. (2, 5, 18) Of α and β receptors, α receptors are more abundant. (18) α_1 receptors are found within the smooth muscle cells

that lie close to the sympathetic innervations of the vasculature, and to a lesser extent, within the heart. (2) Like α_1 receptors, α_2 receptors are also expressed in the vascular smooth muscle, however, they often reside on the neuronal membrane of the postganglionic cell. (2, 18, 35) α_2 receptors are the only adrenergic receptors to cause a decrease in cAMP, and therefore, these receptors stand alone in their effect of decreased contractility of the heart. (18, 20, 34) β_1 receptors are the main adrenergic receptor type of the heart. (2, 18) Specifically, they can be located in the innervations of the sinoatrial and atrioventricular nodes and on the surface of cardiomyocytes within the atria and ventricles. (2, 18) β_2 receptors are primarily found in the smooth muscle of the vasculature and the skeletal muscle. (2) There are also a small amount of β_2 receptors within the coronary circulation of the heart, making up around 30% of total β receptors in the atria and 20% in the ventricles. (36)

Briefly, sympathetic neural impulses are transmitted from preganglionic fibers to postganglionic fibers via ACh. (2, 6) The impulse is propagated down the postganglionic neuron, and upon arrival at the axon terminal, calcium influx results in NE or E excretion into the surrounding tissue. (2, 6) As explained previously, α_1, β_1, and β_2 receptors are expressed within the heart, although β_1 receptors are the most common. (2, 18) When these receptors detect NE, they activate their coupled G-protein, causing its subsequent dissociation, and stimulate cAMP production. (18, 20, 29, 34) In turn, PKA is activated and as a result, increased calcium influx into the heart has a positive inotropic effect. (20, 29, 31, 34) Additionally, α_1, β_1, and β_2 adrenergic receptors all elicit increases in HR and conduction velocity between the sinoatrial and atrioventricular nodes. (2, 18, 29)

The vasculature experiences simultaneous alterations upon catecholamine detection via adrenergic receptors dispersed throughout the vascular smooth muscle. It is important to note several nuances here. Because the vasculature is more dependent on the SNS than the PNS for its resting state, detection of NE by α_1 and β_2 receptors determines basal vascular tone. (2, 7) However, because the number of α_1 receptors within the vasculature outweighs that of β_2 receptors, vasoconstriction and increased SVR are primary outcomes of SNS activation. (2) Additionally, similar to the cAMP-PKA mechanism in the heart, α_1 receptors upregulate PKA, thereby increasing calcium influx in the smooth muscle cells and triggering vasoconstriction. (18, 31, 37) At the same time, modified sympathetic neurons within the adrenal medulla release E into the circulation to also bind with α_1 and β_2 receptors. (2) At low concentrations, β_2 receptors have a higher affinity for E than α_1 receptors, and thus, increases in CO and vasodilation can be observed upon SNS activation. (38, 39) At higher concentrations, E acts on α_1, β_1,and β_2 receptors in the heart and vasculature and induce vasoconstriction and increased CO through heightened HR and SV, all resulting in increased BP. (38-41) The effects of α_2 receptors are more complex, as they can initiate vasodilation or vasoconstriction due to the differing qualities of the α_2 subtypes (2A, 2B, 2C). (18, 35, 42) Additionally, α_2 receptors are unique in the fact that they inhibit cAMP production via G-protein activation and, because of their location within the postganglionic membrane, regulate a NE-dependent negative feedback loop in which they directly control the amount of NE released there. (18, 35)

ii. Autonomic Regulation of Blood Pressure

The ANS utilizes a number of different mechanisms to regulate BP. These mechanisms begin with sensory neurons throughout the body eliciting an overall afferent (i.e., bottom-up) signal that is transmitted to the central nervous system. (3, 6, 7) This impulse is then integrated and propagated as an efferent (i.e., top-down) response to the skeletal muscle and visceral organs in order to make adjustments for the afferent signal. (3, 6, 7) The following reflexes play a key role in modulation of BP.

a. The Baroreceptor Reflex

The baroreceptor reflex, or the baroreflex, is an essential aspect of cardiovascular control. (43) Baroreceptors are sensory afferent mechanoreceptors, located throughout the heart, that utilize stretch mechanisms to respond to changes in BP in a negative-feedback manner. (7, 44, 45) For example, when BP is elevated via sympathetic outflow, baroreceptors within the aortic arch and carotid sinuses are activated due to a consequential heightened pressure in these arteries. (2, 7, 44) These receptors then send afferent signals to certain areas of the brain responsible for consolidating and propagating efferent impulses to the rest of the body. (2, 44, 45) Namely, the nucleus tractus solitarius of the in the medulla oblongata of the brainstem processes the afferent signals of the baroreflex and then stimulates the central ventrolateral medulla (**CVLM**), which subsequently activates the rostral ventrolateral medulla (**RVLM**), the area that oversees the propagation of efferent SNS outflow. (44, 46) These efferent impulses then elicit sympathetic inhibitory responses such as systemic vasodilation and reduced CO via negative chronotropic and inotropic effects. (2, 7, 44) There are additional baroreceptors in the atria, venae cavae, and pulmonary veins, however these receptors are fewer in

number and more sensitive to changes in volume than to changes in pressure. (2, 44) The latter two baroreceptors, sometimes referred to as the cardiopulmonary baroreceptors, detect decreases in BP, and initiate antidiuretic hormone (**ADH**) release from the posterior pituitary as well as activate the renin-angiotensin-aldosterone system (**RAAS**) to increase BP to a resting level via increased arterial vasoconstriction and water retention. (2, 7, 44) Regardless, all baroreceptors work in unison to induce autonomic responses and prevent dramatic fluctuations in BP. (6, 45)

b. The Chemoreceptor Reflex

Chemoreceptors are another important physiological mechanism that functions to preserve homeostatic cardiovascular activity. (47) Chemoreceptors can be found in two distinct locations within the body and are classified accordingly. (2, 7) Peripheral chemoreceptors are located within the carotid and aortic bodies, small organs dispersed in the bifurcation of the common carotid arteries (48), similar to that of the more common arterial baroreceptors. (49, 50) Central receptors, alternatively, lie within the RVLM. (2, 50) Both types of chemoreceptors oversee changes in partial pressures of oxygen and carbon dioxide as well as pH levels of circulating blood. (2, 50, 51) For instance, during exercise, a buildup of lactate, carbon dioxide, and hydrogen ions significantly decreases blood-oxygen content and pH, ultimately producing rapid ventilation in order to meet the heightened oxygen demand. (52, 53) As these chemical changes are detected by peripheral and central chemoreceptors, afferent nerve impulses stimulate the nucleus tractus solitarius, ultimately causing the RVLM to increase SV and thus, CO and BP via elevated SNS efferent discharge. (2, 49-51) Meanwhile PNS activity is downregulated in order to increase HR, respiration, and gas exchange. (2, 49-51)

c. The Frank-Starling Mechanism

In the 19th century, Otto Frank's work on frog hearts produced the realization that ventricular stretching prior to contraction resulted in increased ventricular contractility due to the innate contractile components of cardiomyocytes. (54) Starling et al. in the early 20th century expanded on this early discovery and found that increased venous return to the heart seemingly increased filling pressure (like that of Frank's ventricular stretching), which produced an increased SV. (54) Starling also tested the opposite mechanism and found that decreasing venous return lowered SV, and therefore, concluded that venous return directly affects SV. (54) This mechanism, defined as the heart's ability to change contractility and adjust SV in response to changes in venous return, is now known as the Frank-Starling Law. (55-57)

SV (L/beat) is defined as the volume of blood pumped through the heart in one contraction. (6, 56, 58) SV has a direct relationship with HR (beats/min) to determine CO (L/min), or the volume of blood pumped through the heart every minute (3, 7):

$$\textbf{CO (L/min) = HR (beats/min) x SV (L/beat)}$$

CO (L/min) and changes in SVR ($mmHg \cdot min \cdot L^{-1}$), or the overall balance between systemic vasoconstriction and vasodilation, determine BP (mmHg), or the ultimate pressure of circulating blood on the arterial walls (3, 6, 7). Therefore, BP is determined by HR, SV, and SVR:

$$\textbf{BP (mmHg) = CO (L/min) x SVR (mmHg·min·L}^{\textbf{-1}}\textbf{)}$$

$$\textbf{BP (mmHg) = [HR (beats/min) x SV (L/beat)] x SVR (mmHg·min·L}^{\textbf{-1}}\textbf{)}$$

Further, the adjustment in SV seen in the Frank-Starling mechanism is reliant on changes in two cardiac factors: elasticity and contractility. (55, 58, 59) Elasticity reflects

changes in preload (end diastolic volume; **EDV**), or the volume of blood that fills the heart before contraction. End systolic volume (**ESV**), or leftover blood in the heart after contraction is controlled by both cardiac contractility, the intrinsic strength of cardiomyocytes, and afterload, the force resisting the ejection of blood by the heart. (3, 6) These two concepts relate to SV as follows (3, 7):

SV = EDV (preload) – ESV (afterload)

Increased EDV, caused by increased venous return, increases the amount of pressure against the walls of the heart. (57, 60) Additional recruitment of the SNS and its influence on the heart enhances cardiac contractility via α_1, β_1, and β_2 adrenoreceptors, as previously explained. (2, 18) The increased stretch of the cardiomyocytes, due to increased volume, optimizes the length-tension curve, and results in myocytes contracting harder with greater stretch. (56, 59, 60) Increased afterload also increases the intraventricular pressure following a contraction. (58, 59) All this to say, increased arterial pressure (i.e., peripheral resistance) tends to diminish ESV (i.e., increased ventricular afterload). (58, 59)

Although the Frank-Starling Mechanism gives an idea as to how the heart intrinsically adjusts to changes in ESV and EDV, it doesn't reveal much about what causes these changes. (56, 59, 60) The five primary underlying mechanisms of changes in EDV and ESV are complex and entangled. Therefore, for the purposes of this review, only a rudimentary explanation of each is provided.

The first factor that affects SV is venous return. (56) As previously discussed, increased venous return leads to enhanced contractility, thereby raising SV. (56, 59) Decreased venous return produces the opposite effect. (57, 58) Prolonged ventricular

filling time, the second factor, is closely related to increased venous return as it allows more time for the ventricles to fill with blood, resulting in increased SV. (61) Typically, shorter ventricular filling time decreases SV. (61) However, in the context of exercise ventricular filling time greatly decreases but SV still increases due to increases in HR, EDV, and ESV. (62) Filling time is primarily a consequence of changes in HR and conduction velocity due to cardiac autonomic innervations, the third determinant. (61, 63, 64) Sympathetic and parasympathetic innervations affect SV via the impact of their neurotransmitters (NE and E; ACh) and receptors (α_1, α_2 β_1,and β_2; M2, M3) on the heart and vasculature, as explained in the previous section. (38-41, 65) Innervations also play an important role in regulating HR and conduction velocity via the stimulation and withdrawal of the vagus nerve (22, 45), which along with SV, dictates CO. (3, 7) Additionally, hormones have a major effect on the changes in contractility and SV. (2, 7) The following section will describe the RAAS in detail. Besides the hormones associated with the RAAS, key players in SV modulation include E and NE, which as previously explained affect vascular tone via corresponding receptors throughout the cardiovascular system (40, 41), hormones created by the heart like atrial-natriuretic and brain-natriuretic peptides (66-68), hormones produced in the vascular endothelium such as endothelin-1 (69, 70), and the thyroid hormone, thyroxine (71, 72). These hormones have varying and integrative effects on BP regulation, primarily by way of changes in vessel diameter, the last determining factor. (2, 7) Vascular compliance within the arteries and veins have contradicting effects on SV. (73, 74) Arterial vasoconstriction produces resistance against the heart walls to pump out blood after a contraction. (73, 74) This results in increased afterload and concomitant decreases in SV and CO. (73, 74) Additionally, arterial

vasoconstriction leads to increased mean pressure which in turn, activates the arterial baroreflex and the PNS to lower HR, ultimately contributing to the decrease in CO. (44, 45) Arterial vasodilation has opposing effects. (73, 74) Within the veins, however, increased vasoconstriction results in decreased preload, and therefore, decreased contractility and SV, while venous vasodilation would produce the opposite effect. (73, 74) Evidently, changes in venous return, ventricular filling time, cardiac innervations, hormonal fluctuations, and venous and arterial diameter have complex autonomic interactions in order to regulate BP via the Frank-Starling mechanism.

d. The Renin-Aldosterone-Angiotensin System

The RAAS is a hormonal pathway that also contributes to the equilibrium of acute and chronic BP as well as fluid balance by way of alterations in vascular tone and blood volume. (73, 75, 76) Three primary mechanisms are known to activate the RAAS pathway: increased SNS outflow via renal β_1 receptors, low sodium concentrations in the distal tubules of the kidney, and/or detection of decreased blood flow through macula densa cells. (2, 73, 75-77) All of these physiological signals indicate systemic hypovolemia, which eventually induces decreased blood pressure. (2, 73, 77) Therefore, the goal of the RAAS is to increase blood volume, and therein venous return, SV, and CO, which together raise BP. (73, 75)

Upon detection of one or all of these mechanisms, renin is released into the circulation by the juxtaglomerular cells of the kidney, which line the afferent arterioles of the renal glomerulus. (2, 73, 76) Renin then cleaves a hepatic enzyme, known as angiotensinogen, to form the decapeptide angiotensin I. (2, 73, 75, 76) Angiotensin I is further cleaved into the octapeptide Angiotensin II (**Ang II**) by angiotensin-converting-

enzyme (**ACE**) which is produced in the vascular endothelium of the lungs. (2, 73, 75, 76) Ang II is the key effector hormone of the RAAS and its systemic effects are numerous and multifaceted. (76)

To begin, Ang II stimulates AT1 receptors, another type of G protein-coupled receptor within the arteries and adrenal cortex, to constrict renal arteries, as well as afferent and efferent arterioles, and stimulate aldosterone secretion from the adrenal cortex. (2, 75) Aldosterone primarily functions to increase sodium and water retention and potassium excretion at multiple tubular sites within the kidney. (75) This, in turn, increases blood volume while maintaining the filtering capacity of the glomerulus, otherwise known as the glomerular filtration rate (**GFR**). (73, 75) Increased GFR raises arteriole pressure and decreases sodium concentration. (73) Within the context of the cardiovascular system, Ang II is a potent vasoconstrictor and thus elicits systemic vasoconstriction. (73, 75, 76) AT1 receptor stimulation also initiates NE release and inhibits NE reuptake from sympathetic nerve endings throughout the body, thereby amplifying sympathetic discharge. (73) Moreover, Ang II stimulates the release of vasopressin, or ADH, from the posterior pituitary. (73, 75) ADH release prompts thirst centers in the hypothalamus to promote water intake and continue water retention in the kidneys. (73, 75) Another hormone, adrenocorticotropic hormone, which is the primary regulator of cortisol production in the adrenals, is also stimulated by Ang II and released by the anterior pituitary gland. (75) Once released, cortisol then magnifies the already-amplified cardiovascular response to SNS stimulation. (73, 75)

iii. Clinical Measures of Autonomic Function

Assessment of sympathoadrenal activity during exercise offers important insight into the underlying autonomic mechanisms of the cardiovascular system. Analyses of changes in HR, skin and core body temperature, skin conductance, regional vascular conductance, and circulating E and NE have been used previously to reflect total SNS discharge during states of stress. While all of these methods reliably indicate sympathoexcitation, two additional techniques are more frequently used: BP evaluation and microneurography. The following section will outline the precise protocols, advantages, and shortcomings of using these techniques in a laboratory setting.

a. Blood Pressure Reactivity

One technique for indirectly examining autonomic activity during exercise is the recording of beat-to-beat BP. In the early 20th century, researchers realized that sympathetic activation during exercise produced increased variability in BP waveforms. (78) This increased BP variability was soon after considered predictive of general cardiac health. (79, 80) As it is now referred to, BP reactivity is defined as the net change from resting BP to peak BP during a stressor, and although it differs greatly between individuals, BP reactivity has continually been proven diagnostically valuable. (81, 82) For example, similar to the association between low cardiorespiratory fitness and future cardiovascular disease (**CVD**) development (83), heightened BP reactivity during exercise has been linked to future (both short- and long-term) development of hypertension, a major risk factor for atherosclerosis, and other CVDs. (82, 84-88) Hypotension during exercise, although extremely rare (affecting only 6% of the population), has also been linked to CVD diagnosis. (87) Further, increased exercising BP reactivity has been established as a determinant of CVD endpoints such as cardiac

events, structural and functional changes of the myocardium, and death. (89, 90) Therefore, BP assessment during exercise offers both diagnostic and prognostic insight and is thus a warranted approach to uncover individual autonomic regulation. (87)

Previously, BP was evaluated by using mercury manometers (91) and later, manual sphygmomanometers. (92) However, more recent developments in medical technology have produced automatic instruments like 24-hour ambulatory BP monitors and finger photoplethysmography (**PPG**) sensors which allow for the continual assessment of BP. (93-95) Unlike 24-hour ambulatory BP monitors, which are used to periodically track waking and sleeping BP changes (96), PPG continually tracks changes in blood volume in a specific vascular bed. (97) PPG utilizes optical measurement, or the illumination of infrared light onto the surrounding tissue to detect the amount of light absorption or reflection from vessels, ultimately determined by the amount of blood present within the vessel walls. (97, 98) This measurement results in a pulsatile beat-to-beat BP waveform recording (i.e., pulsatile factor, “ac”), which can subsequently be superimposed with lower frequency baseline changes in respiration, SNS activity, and thermoregulation (non-pulsatile factor, “dc”). (97, 99)

When placed, PPG sensors cover veins, capillaries, and arteries. (97) Additionally, multiple complex processes contribute to detectable changes in BP, such as modulation of the respiratory, cardiac, and vascular systems, immunological and endothelial molecular discharges, and modifications due to pathological developments and genetic predispositions. (100) Therefore, PPG readings must be interpreted as a comprehensive representation of the modulation of the cardiovascular system. (100) PPG is a relatively simple, inexpensive, and non-invasive approach to measure BP reactivity. (97, 101) It

documents each BP oscillation within a given amount of time, allowing future analysis of variation in these oscillations during a bout of exercise. (97, 101) Further, PPG is a convenient and dependable technique that is capable of recording stable readings during large-scale body movements like exercise. (97)

Despite the reliable and user-oriented nature of PPG, the number of necessary adjustments and validations such as noise elimination, multi-site measurements, and event detections and visualizations present a shortcoming of this technique. (97) Individual calibrations must be conducted for each patient, which can be time consuming and often highly dependent on skin color and anthropometric measurements. (97) Regardless of these limitations, PPG devices have been repeatedly validated as reliable and accurate in analyzing autonomic outflow in the form of BP reactivity. (101, 102)

b. Microneurography

The only direct approach to determine sympathetic traffic to peripheral nerves during exercise is with microneurography, a technique that records potentials emitted by postganglionic sympathetic nerve endings in the skin or muscle. (103) This approach was developed in 1965 by Dr. Karl-Erik Hagbarth and Dr. Åke Vallbo at the Uppsala University Hospital in Uppsala, Sweden. (104) Neural impulse analysis of afferent or efferent fibers were initially used to identify sensory receptors and their corresponding effector organs in order to improve treatment options for neurological patients. (105) However, in the decades since, microneurography has been popularized and standardized, and is now predominantly used to examine the neural changes that ensue from various pathologies and research interventions. (104)

Microneurography involves insertion of a tungsten microelectrode into the peroneal nerve, adjacent to the fibular head on the anterior side of the lower leg. (103)

Commonly, percutaneous electrical stimulation is performed in order to locate and visualize the nerve relative to the anatomical landmarks of the leg. (103) A recording electrode is then inserted into the nerve and a ground electrode outside of the nerve to subsequently record local electrical signals. (103) Alternatively, some groups elect to use ultrasound sonography to visualize the nerve bundle prior to insertion. (106-108) Both approaches require patience and attention to precise detail. (103, 106-108) Finetuning via small adjustments of the electrode position is typically required to ensure a sufficient recording. (103) MSNA is confirmed by the presence of a pulse-synchronous response to Valsalva maneuver and tapping of a nearby tendon, but absence of the same response to stroking of the skin or startling. (109-111) Considering all of this, microneurography is a complex and difficult-to-learn technique that requires years of practice on the part of the executant. (103)

The neural activity that is recorded from skeletal muscle innervations is classified as muscle sympathetic nerve activity (**MSNA**). Resultant MSNA voltages manifest on the neurogram as pulse-synchronous “bursts” of sympathetic discharges separated by short periods of neural silence. (103) The two most common ways to quantify the rate of sympathetic discharge are burst frequency (bursts/minute) and burst incidence (bursts/100 heart beats). (103) Supplementary electrocardiogram data is displayed in tandem with MSNA recordings to verify the alignment of cardiac cycles and bursts. (103) This quantification of MSNA, in the form of neurograms, has proven to be highly reflective of the neural changes experienced with autonomic dysregulation resulting from numerous neurologic conditions. (103) For example, patients with hypertension, obesity, and heart failure consistently demonstrate significantly greater MSNA than healthy adult controls

and these observations have been corroborated by a number of studies. (112, 113) Further, Cui et al. concluded that MSNA has additional seasonal fluctuations, with greater MSNA counts in the winter. (114)

However, studies investigating the reproducibility of MSNA have found mixed results, with some suggesting better short- and medium-term reproducibility than plasma NE concentrations (115) and others finding poor mid-term reproducibility within subjects, one limitation of microneurography, especially during metaboreflex isolation. (115, 116) Additionally, MSNA is generally interpreted as a constituent of the sympathetic activation to the entire body, although it is acknowledged that sympathetic nerve activity within the heart and other effector organs likely differs greatly from MSNA. (103) The impracticability of obtaining SNS from the internal organs of human subjects poses another major limitation of microneurography. (6) An additional limitation lies in the difficulty of performing microneurography during full body or large-muscle dynamic exercise, as excessive movement can shift or displace the electrode from the nerve. (6) Minor discomfort during the procedure has also been reported in a small portion of subjects, however, when performed correctly, the likelihood of permanent nerve damage is extremely low. (6, 103)

i. Autonomic Regulation during Exercise

The ANS plays a particularly important role in regulating circulatory adjustments to ensure adequate blood flow for the heightened metabolic demands of active skeletal muscle during exercise. The elevated provision of blood flow during exercise is largely initiated by the exercise pressor reflex (**EPR**). (117) The EPR is a peripheral feedback

mechanism that is made up of three secondary reflexes: central command, the mechanoreflex, and the metaboreflex. (117, 118)

a. Central Command

Central command is a feedforward mechanism that functions to brace the body for physical activity by evoking a number of anticipatory cardiovascular responses. (119) For example, anticipatory mental stimulation (i.e., explaining an upcoming exercise to a participant) activates higher brain centers such as the motor cortex and the hypothalamic and mesencephalic motor regions, with the latter two controlling activity of the cardiovascular and ventilatory systems. (119-122) The body then initializes a small increase in sympathetic outflow in preparation for exercise. (119-123) Consequently, HR rises slightly due to inhibition of vagal tone via PNS withdrawal, in part due to the "resetting" of the baroreflex stimulus-response curve. (124-126) This increases the arterial pressure setpoint at which the baroreflex is triggered to suppress further increases in HR and contractility. (127) Additionally, central command appears to lower the operating BP point (i.e., the pre-exercising BP) to prevent the exaggerated surges in BP upon the initiation of exercise. (125, 128, 129)

Beyond central command, matching blood flow to the physiological demands of acute exercise require later adjustments by the EPR once exercise has started. (119) This failure of the central process produces a latency period in which the exercise-induced demands within the muscle are not appropriately met. (119) Separately, while some argue that central command may be associated with muscle mass and exercise intensity, evidence agrees that it is a force-independent mechanism and instead, may be better represented as an effort-related process. (130-132) Hence, directly examining brain activity associated with motor planning before exercise can provide important information

as central command is influenced by anticipation of a perceived effort. (119) However, there are currently no techniques that immediately and precisely assess this parameter. (119) Therefore, subjective evaluation of effort, or rate of perceived exertion (**RPE**), is often used to grade central command activity, despite the fact that the relationship between central command and RPE remains vaguely defined. (119, 133) Further obfuscating the use of RPE to semi-quantitatively assess the central command contribution to exercise hemodynamic response. (119) For example, prior evidence suggests that RPE is affected by somatosensory mechanisms from the internal organs (134); cognitive processes related to intelligence, environmental surroundings, exercise experience, and familiarity with one's exertional and exhaustive cues (135); depression and neuroticism (136); and additional signaling pathways related to pain, discomfort, temperature, and thirst. (137) Hence, assessing RPE should not be regarded as infallible in the discernment of central command activation and its ensuing cardiovascular alterations.

b. The Mechanoreflex

Skeletal muscle mechanoreceptors, similar to arterial baroreflex mechanoreceptors, detect changes in pressure within the intramuscular vasculature that come about from mechanical distortion during muscle contraction. (138-140) Once stimulation of these mechanoreceptors has occurred, thinly-myelinated group III (mechanically-sensitive) nerve fibers send afferent impulses to cardiovascular centers, such as the RVLM, in the brain to initiate a series of efferent responses. (141, 142) The sensory-feedback pressor mechanism occurs rapidly, usually within 200 milliseconds of contraction. (118)

c. The Metaboreflex

Specific skeletal muscle chemoreceptors, known as metaboreceptors, similarly respond to metabolic changes within the exercising muscle also due to repeated contractions. (117, 143) Muscle contraction specifically produces a number of potent vasodilatory compounds. (144, 145) These metabolites include: deprotonated phosphate and lactic acid (146), potassium ions (147), bradykinins (148), arachidonic acid (149), adenosine triphosphate (150), prostaglandins (148), and nitric oxide (151), as well as decreased pH (152) and oxygen (153) levels. (117, 143, 145, 154, 155) These compounds are formed locally in the skeletal muscle and are secreted from endothelial cells, erythrocytes, and the sarcolemma upon contraction. (145) Once metaboreceptors detect an accumulation of these vasodilators, afferent impulses are transmitted to the brainstem via group IV (metabolically-sensitive) fibers, which unlike group III afferents are unmyelinated but respond much more slowly, between 5-30 seconds after contraction. (118, 139) The metaboreflex can be isolated with a technique called post-exercise ischemia (**PEI**). (156) PEI successfully traps metabolites in the exercising limb via arterial cuff occlusion following exercise, thus isolating the cardiovascular and circulatory effects of the metaboreflex. (156) Hence, common observations during PEI include elevated BP and HR due to continued metabolic stimulation of group IV fibers, although these responses are highly dependent on a number of factors including exercise intensity and duration, cardiorespiratory fitness, and muscle mass. (156-159)

d. The Exercise Pressor Reflex

The metaboreflex and mechanoreflex work in unison to stimulate the RVLM, via mechano- and chemoreceptors and group III and IV afferents, and in turn, increase SNS

activity throughout the body. (160, 161) As a consequence, heightened SNS outflow evokes a number of physical reactions during exercise. First and most obviously, due to their inherently opposing nature, SNS activation results in PNS withdrawal, primarily inhibiting vagal tone and effectuating a HR that is elevated past the increases of central command. (162) Second, sympathetic nerve activity in the kidneys stimulates renin release, and thereby activates the RAAS, ultimately increasing sodium and water retention and further raising BP. (75, 76) On some occasions, up to a five-fold increase from resting plasma renin concentrations can be observed during exercise, further corroborating the importance of the RAAS as a powerful BP regulation mechanism. (163) Additionally, sympathetic activity in the musculature, or MSNA, which as previously discussed is quantifiable with microneurography, increases. (164, 165) This heightened MSNA coincides with increased E and NE production and secretion (i.e., NE spillover) from nerve terminals which typically results in vasoconstriction via α_1 adrenergic receptors in the vasculature. (142, 165) However, in the active skeletal muscle, the rate of production and concentration of local vasodilatory compounds overrides this vasoconstriction, and vasodilation ensues. (44, 145) Concomitantly, NE spillover in the resting muscles and viscera results in dramatic vasoconstriction, hence the inhibition of digestion and glandular secretions during SNS activation. (118, 161) This systemic balance between vasodilation of the active muscles and vasoconstriction of the resting tissue during exercise is known as “functional sympatholysis” (145, 166) which is described further below.

The redistribution of blood flow during exercise is driven by two key factors: a whole body increase in SVR except to active muscle and increased CO. Increased SVR assists

in supporting an elevated arterial BP, which is needed to redirect blood flow and increase perfusion to the active muscles during acute exercise. (118, 145, 167) On the other hand, the other primary contributor to increased BP during acute exercise is CO, which also increases blood flow to active skeletal muscle sites. (123, 168, 169) Increased concentrations in E and NE also elicit positive inotropic effects in the heart, resulting in greater SV and thus supporting the necessary alterations in CO and blood flow. (167) Resetting of the baroreflex causes desensitization of the baroreceptors and allows for increased pressure within the heart without stimulating baroreflex-induced modulation. (142, 170) In sum, the EPR is essential for eliciting the adequate circulatory and hormonal alterations to meet the needs of the active skeletal muscle during exercise.

Although any form of exercise will elicit the EPR in humans, one of the most commonly used methods is isometric handgrip (**HG**) exercise. (171) This technique involves performing a sustained, constant-force HG contraction on a dynamometer without bending or tensing the rest of the arm. (172) Because of the minimally involved muscle mass and negligible movement, an important advantage of isometric HG exercise is the availability of the rest of the body for other simultaneous tests such as BPR and MSNA. Hence, why many studies evaluating autonomic responses to exercise pair these techniques together. (110, 173-177) Typically, the executed HG force is 30-40% of the subject's maximal voluntary contraction (**MVC**), averaged from three maximal squeezes. (172) If the metaboreflex is also being analyzed, PEI is immediately implemented following the end of exercise. (178) Isometric HG has been repeatedly validated as predictive of future CVDs like hypertension (81, 117) and coronary artery disease (179, 180), as well as CVD-associated mortality (181), and thus, may have diagnostic

properties. (182) Sensitivity to metaboreflex isolation also seems to be exaggerated in those with hypertension, a leading risk factor for CVD development. (183, 184) In other words, individuals with exacerbated autonomic responses (e.g., increased BPR and MSNA) during HG and PEI seem to be at a heightened risk of future CVD development. (84, 179) The next chapter will analyze sex differences in EPR responses to isometric HG exercise and PEI.

iv. Functional Sympatholysis

As previously explained, the typical vascular outcome of SNS activation is vasoconstriction due to heightened sensitivity of α_1, α_2, and β_2 adrenergic receptors and increased catecholamine release. (38-41) And indeed, increases in NE, E, and SVR along with a concomitant elevation in arterial BP is observed with EPR activation. (118, 154) However, the aforementioned accumulation of vasodilators within working skeletal muscle, accounted for by the metaboreflex, simultaneously attenuates the sympathetically-mediated vasoconstriction, a phenomenon known as “functional sympatholysis”. (142, 145, 166, 185) The vasculature is constantly under the influence of this competition between SNS control and local vasodilators, although no single compound is sufficient enough to entirely overcome the effects of the SNS. (145, 167) Functional sympatholysis typically redistributes blood flow away from the resting muscles, skin, kidneys, and gastrointestinal tract and into the vascular beds of the active muscles. (166, 169) Localized vasodilation increases intramuscular perfusion and allows for the ample provision of oxygen and other nutrients during periods of increased metabolic demand, such as acute bouts of intense exercise. (118, 145, 167) This increased blood

flow is typically rapid at the onset of exercise, although a steady state is generally established within 10-150 seconds dependent on exercise duration and intensity. (150) Further, perfusion is more often directed to fast-twitch glycolytic fibers than to fast- or slow-twitch oxidative fibers, and thus, the extent of blood flow seems to be related to the peak aerobic function of the specific fibers within the contracting muscle. (186) Increases in blood flow (i.e., relative increases) are also more pronounced in small distal arterioles than in large proximal arteries, which permits the demands of most metabolically active fibers to be supplied while still supporting the increased systemic BP via vasoconstriction of more proximal vessels. (187-189) It is additionally important to note that functional sympatholysis does not qualify as an “all-or-none” physiological response and is better defined as a continuum of vascular responses that are highly dependent on a number of factors including SNS outflow and exercise intensity. (189)

CHAPTER TWO: SEX DIFFERENCES IN AUTONOMIC FUNCTION DURING STATIC EXERCISE

I. Sex Differences in Cardiovascular Disease Prevalence

CVDs are the leading cause of mortality in the United States, with the CDC recently reporting 659,000 CVD-related deaths per year, or one death every 36 seconds. (190) Moreover, as of 2018, nearly half (49.2%, 126.9 million) of all adults (≥ 20 years of age) in the United States are living with a CVD. (190) Hypertension is a primary risk factor for all CVDs and affects over 100 million adults in the United States. (190-193) There are large sex differences in CVD and hypertension prevalence, such that males are at a much higher risk of diagnosis and mortality during young adulthood than young females. (190, 194) Regarding the sex differences in CVDs, the 2015-2018 National Health and Nutrition Examination Survey (NHANES) demonstrated that the prevalence of CVDs among males aged 20-39 years was 33.4%, while only 17.5% of age-matched females had been diagnosed. (190) Hypertension makes up the majority of these cases. (190) Similar to the well-documented sex differences in resting BP, females typically exhibit blunted BP responses during small and large muscle mass exercise testing. (195, 196) Importantly, heightened BP responses to stressors predict future hypertension development, although the underlying mechanisms are still unknown. (81, 82, 84-86, 179, 197, 198) Therefore, examination of sex differences in exercise-related mechanisms, like the EPR, may hold clinical significance for the prevention of hypertension and CVDs in healthy young adults. The following chapter will assess the existing literature and hypotheses related to sex differences in BP responses to exercise and metaboreflex isolation.

i. Sex Differences in the EPR and Metaboreflex Isolation

At rest, males have higher BP than females (199) and therefore, it is no surprise that males also typically reach higher maximal BP during exercise. (174, 178, 195, 200-204) However, several studies also indicate that males exhibit exaggerated BP reactivity and MSNA to stressors. (174, 178, 195, 200-204) That is to say, males not only display greater absolute maximal BP than females, but they also demonstrate larger changes from resting BP to maximal BP (i.e., BP reactivity). The underlying mechanisms of this sex difference in BP reactivity, if better understood, may help to uncover the source of the sex disparity in the risk of hypertension and CVDs throughout the lifespan. Nonetheless, this research topic has been a source of contention amongst experts for decades and even today, there are several plausible explanations for the heightened BP reactivity demonstrated by males during physical stressors.

The first hypothesis for the sex difference in BP reactivity is that upon afferent stimulation of the SNS (i.e., metaboreflex and mechanoreflex) via exercise, males may have greater efferent responses in the form of heightened MSNA and NE spillover. However, the evidence for this concept is limited and inconclusive. For instance, although resting MSNA tends to be lower in females (205-207), several studies have found no sex differences in MSNA responsiveness, measured with microneurography, during isometric HG exercise (175, 202, 207) or during HG with PEI. (175) Others have identified females as having blunted increases in MSNA during HG and PEI and have concluded that this finding is the source of the sex difference in BP reactivity. (174, 195) However, there may also be some specificity regarding the stimulus. For example, Ettinger et al. found attenuated MSNA increases in females only during a normal HG exercise but not during

an ischemic rhythmic HG exercise protocol with cuff occlusion, suggesting that abundant blood flow is required for manifestation of the sex difference. (174) This discovery implies that the difference in sympathetic outflow may rest on dissimilarities in metabolite clearance or accumulation during exercise. In contrast, there is limited research indicating no sex difference in catecholamine production during endurance (167) and resistance exercise. (208) Sanchez et al. did find an earlier spike in E production in males, although this is likely due to increased muscle strength and thus, accelerated sympathetic recruitment. (208) In theory, higher MSNA and greater catecholamine concentrations would raise BP via increased cardiac output and increased TPR via vasoconstriction (i.e., sympathetic transduction). Thus, some suspect that sex differences in sympathetic transduction, not SNS outflow is the root cause of the sex differences observed in BP regulation.

The second hypothesis is that premenopausal females have diminished vascular responsiveness to sympathetic activation than males. (209-212) Sympathetic transduction is a mechanism that induces systemic vasoconstriction and vasodilation for a given amount of sympathetic discharge. (213) Evidence suggests that sex differences in BP reactivity may be due to differences in sympathetic transduction secondary to differences in adrenergic receptor expression and/or activity. (209-212) For example, Hogarth et al. concluded that premenopausal females exhibited less peripheral vascular resistance for the same increase in MSNA during stressors. (211) It has since been inferred that the primary mechanism for this phenomena is a heightened sensitivity of β_2-adrenergic receptors in females. (176, 210, 212) Kneale et al. found that for similar administration of β_2-adrenergic agonists, premenopausal females exhibit less vascular

resistance than age-matched males, presumably due to β_2-adrenergic mediated vasodilation. (212) Additionally, females display similar responses (i.e., blunted vasoconstriction) upon infusion of NE. (210, 212, 214) Further, Samora et al. examined the effects of HG exercise with a supplementary β_2-blockade, by way of the commonly-prescribed β-blocker, propranolol, and found that this combination mitigated the preexisting sex difference although the mechanisms differed. (176) When compared to a placebo protocol, β_2-blockade greatly reduced the BP reactivity in males due to a decreased CO. (176) Females also experienced a decrease in CO with β_2-blockade, although their BP did not significantly change from placebo, due to a significant increase in TPR. (176) These findings suggest that under normal conditions, males increase BP via increased CO while females avoid this drastic elevation in BP despite increased CO, due to β_2-adrenergic mediated vasodilation. (176) That is to say that in females, detection of NE leads to blunted sympathetic transduction, as the vasodilatory effects of β_2-adrenergic receptors override that of α-receptors. (176, 210, 211, 214) The sex difference in adrenergic receptor activity could thus explain the sex difference observed in BP reactivity to exercise. It has also been suggested that this mechanism may enhance the washout of metabolites during exercise in females, further contributing to the blunted pressor response (175), as supported by Ettinger et al. mentioned above. (174) However, an important consideration for the discussion of this hypothesis is the undisputed fact that in general, males are significantly stronger than females. (175) Hence, there should be substantial sex differences in the intramuscular pressure and vascular occlusion (i.e., the mechanoreflex) during muscle contraction. (175) The next hypothesis argues that greater muscle strength in males explains, in part, the sex in BP reactivity discussed herein.

The third hypothesis relates HG force and muscle mass to the sex differences observed in BP reactivity during exercise. For instance, in 1989, Seals et al. study that demonstrated larger BP increases in response to exercise with greater muscle mass (two-handed HG at 30% MVC) compared to exercise with a smaller muscle mass (one-handed HG at 30% MVC). (215) Because males tend to have greater skeletal muscle mass and fat-free mass than females (216) and significantly stronger maximal squeezing forces (174, 195, 200, 203, 217, 218) some propose this to be the origin of the sex difference in BP reactivity during exercise. (204) In fact, research suggests that females do indeed have a blunted mechanoreflex when using a passive limb movement technique. (219) The passive limb movement method isolates the mechanoreflex, as it does not actively induce muscle contraction and therefore, avoids metabolite accumulation. (220) On average, females demonstrate a MVC between 40-60% that of males. (221) According to this hypothesis, significantly weaker muscle contraction would lead to less vasoconstriction and stimulation of the intramuscular group III afferents and ultimately, lower overall SNS outflow. (203, 204) Therefore, some studies have examined the possibility of adjusting for muscle strength to attenuate the mechanoreflex-dependent difference in BP. (174, 175, 204, 222) The results of these studies have been conflicting. For example, some studies have found that after adjusting for MVC, the sex difference in BP and/or MSNA during HG and metaboreflex isolation persist, with females continuing to exhibit blunted increases when compared to males. (174, 214) Others have concluded that this statistical adjustment successfully abolishes the sex difference. (175, 204, 217, 222) Interestingly, Lee et al. found that within a strength-matched cohort, consisting of the strongest females and weakest males, similar BP responses to HG and PEI were

apparent. (175) This further suggests that irrespective of sex, strength is the driving factor behind sex differences in EPR activation and BP response to exercise. (203) Anthropometric measures have also been used to normalize HG strength between the sexes, and recent findings suggest that height^2 (**h^2**) is the best statistical adjustment of body size. (223) However, those who have not found significant results by adjusting for HG strength or body size insist that sex differences in BP reactivity to exercise is strength-independent and rather, is related to biological distinctions in skeletal muscle composition, the final hypothesis. (174)

There are over 3000 genetic differences between the sexes in the makeup of the skeletal muscle. (224) Namely, females generally have a significantly greater proportion of type I slow-twitch fibers and lower proportion of type IIa or IIb fast-twitch fibers compared to males. (225) Moreover, Staron and colleagues found that males have a greater cross sectional area of all three major fiber types (I, IIa, IIb) than in females (18.6%, 59.2%, and 65.5% larger, respectively). (226) That said, the fourth hypothesis proposes that these intrinsic sex differences in skeletal muscle composition results in altered metabolite production and metaboreflex activation, thereby resulting in alterations in BP-regulating processes and an overall greater BP-raising stimulus. (175) For example, Saito et al. examined MSNA increases between muscles with different predominant fiber types (forearm v. soleus; fast-twitch, high glycolytic v. slow-twitch, oxidative) and found that MSNA increased significantly more with forearm exercise than with exercise primarily performed by the soleus, in part because the fiber type there allowed for faster production and accumulation of metabolites via glycolysis. (227) Other studies have substantiated this claim in animal models, with electrical stimulation of

oxidative fibers eliciting smaller increases in BP than glycolytic fibers. (228) Taken together, these data suggest that because females are significantly more reliant on oxidative fibers during exercise than males, metabolite buildup may be delayed and ultimately diminish the cumulative effects of the metaboreflex (i.e., heightened BP reactivity and MSNA). Even more, evidence suggests that females do indeed express lower pH and concentrations of enzymes associated with glycolysis (e.g., dihydrogenphosophate, pyruvate kinase, phosphofructokinase, and lactate dehydrogenase) during exercise than in males. (174, 229) There is the added factor that females, due to their greater proportion of oxidative fibers, may also be protected against rapid muscle fatigue during exercise. Multiple studies have assessed this possibility although the results are conflicting with some (217) finding significantly longer endurance times in females and others (195, 208) concluding no sex difference. Regardless, the dissimilarities between the sexes in skeletal muscle expression poses an important problem that is undoubtedly related to the difference in autonomic response during exercise.

Although these four hypotheses address why the immediate sex difference in BPR and MSNA may exist during exercise, they do not confront where and why these sex differences initially emerge during development. The next two sections attempt to tackle this topic.

ii. Female Hormones and Contraception

The most commonly suggested mechanism for the previously discussed sex differences in sympathetic outflow, vascular responsiveness, and skeletal muscle

strength and composition is the ever-changing hormonal profile throughout the female lifespan.

While a number of different hormones (e.g., estrogen, progesterone, luteinizing hormone, follicle stimulating hormone, testosterone, etc.) fluctuate cyclically and collaborate to maintain fertility and overall health in females, estrogen is perhaps the most fundamental. Throughout a normal 28-day menstrual cycle, estradiol fluctuates, peaking during the late follicular phase (days 12-14) right before ovulation (day 14), and declining to reach a nadir during menstruation (days 1-7). (230) There is a smaller second peak during the mid-luteal phase (days 20-22), although at this point progesterone is at its own peak, rising rapidly after ovulation before quickly dropping prior to menstruation. (230) Estrogen is a C_{18} sex steroid produced in the granulosa cells of the ovaries in premenopausal females. (231) Besides maintenance of ovulation and development of secondary female sex characteristics, estrogen has a sweeping range of effects on the general function of most other physiological systems including bone mass preservation, insulin regulation, and protection of cognition. (231) Because of the decline of estrogen levels and the steep rise in CVD risk after menopause (discussed below), estrogen has been deemed a cardioprotective hormone, with even synthetic estrogen replacement therapy demonstrating a wide range of improvements in cardiac, autonomic, and vascular function, as discussed in detail below. (232) For example, research has suggested that β_2-receptor sensitivity is directly related to estrogen levels and that estrogen supplementation in ovariectomized rats can improve β_2-mediated vasodilation in the mesenteric vasculature. (233) Estrogen has also been used to increase nitric oxide production in the endothelium (234, 235) and protect against cardiac injury (236), and has

been further linked to enhanced resting limb blood flow (237), lower α_1-adrenoreceptor concentration, and decreased systemic sympathetic innervation. (238) Thus, the literature on sex differences in autonomic function often focus heavily on the role of estrogen as a potential mediator for the pressor response in females.

Estrogen has been consistently and directly linked to the EPR in animal models. (239-241) Interestingly, Hayes et al. found attenuated pressor responses with estrogen administration in decerebrate male cats. (241) Additionally, increases in BP were blunted during the high estrogen phase (i.e., ovulation) as opposed to the low estrogen phase (i.e., menstruation) in rats. (239) These results in tandem support the possibility that estrogen may be a major underlying factor between male and female EPR responses.

However, human studies have come up with contrasting results between and within female participants, most of which suggest a cardioprotective effect of estradiol supplementation, but no effect of menstruation status, on cardiovascular adjustments to the EPR. (195, 242-245) One study that has directly examined the relationship between estrogen administration and HG exercise is Wenner et al., who concluded that estradiol administration attenuated increased sympathetic responses (i.e., BP reactivity and MSNA) in postmenopausal women, a population with relatively low endogenous plasma estrogen concentrations. (246) However, this attenuation did not reach the levels of premenopausal women. (246) Thus, this study brings up the fact that acute estrogen administration may not drastically affect postmenopausal females due to the prolonged period of estrogen depletion prior to administration. Therefore, the discrepancy between pre- and post-menopausal females even after estradiol administration may be a result of this possibility. That being the case, perhaps the effects of estrogen are exposure-

dependent and produce the most potent sympathetic responses when the hormone is accumulated in the female body over time. In correspondence with the findings from Wenner et al., Ettinger et al. detected heightened MSNA, but not BP, response to HG exercise and PEI during menstruation (i.e., early follicular phase, low estrogen) when compared to the late follicular phase (high estrogen) in premenopausal females. (243) This same study concluded that MVC and metabolite accumulation, however, did not differ across the ovarian cycle. (243) These findings suggest that despite similar stimuli, normal estrogen fluctuations in young females may offer some protection against exaggerated MSNA responses during exercise. Alternatively, Minson et al. found that high estrogen phases were associated with increased resting sympathetic discharge, such that MSNA and NE concentration were higher during the mid-luteal phase (244) and Jarvis et al. determined that increases in BP and MSNA during HG and PEI were blunted in females regardless of menstrual phase. (195) Although there is limited support for these latter two findings, if resting and exercising SNS activity is indeed higher when circulating estrogen is high, the prior cardio-protection conclusion may be more nuanced than originally anticipated. For instance, despite the acute fluctuations in estrogen throughout the menstrual cycle, estrogen always remains elevated in healthy young females when compared to males and older females. Therefore, research comparing premenopausal females throughout the menstrual cycle do not typically produce significant differences like those investigating sex differences.

These contrasting results amongst human models and between animal and human studies make the effects of hormonal fluctuations in the female EPR hard to interpret. There are a few reasons for this. First, the use of synthetic hormones in the

aforementioned animal models poses a major complication when comparing the human findings. (247) Exogenous and endogenous estrogen have differing release schedules, concentrations, and origins. (247) Secondly, the minimal human research we have examining different phases of the ovarian cycle for their comparisons, do so differently, with some (243) comparing the menstruation phase against the late follicular phase (i.e., high estrogen) and some (195, 244) against the mid-luteal phase (i.e., high estrogen and higher progesterone). However, because of the selected phases and results from Jarvis et al. (195), it is possible that the elevation in progesterone may conceal any estrogen-dependent changes in autonomic function, as progesterone has been previously demonstrated to influence cellular metabolism in animal models. (247, 248) Finally, the small fluctuations in hormones that premenopausal females experience throughout the menstrual cycle are likely not substantial enough to produce noticeable effects like those observed between males and females. (195, 249) However, females who take oral contraception, and therefore, reduce the acute fluctuations of hormones during each cycle exhibit increases in BP during HG similar to that of men and significantly higher than that of non-contraceptive users. (177, 249) More research is needed in this area, although it appears that the menstrual cycle should be controlled for by only evaluating female subjects during one cyclic phase, as important differences throughout the cycle are plausible (250), and oral contraceptive users may need separate consideration when examining autonomic function during exercise. (195) To date, only one study to our knowledge has assessed sex differences in the EPR response during HG and PEI while controlling for the ovarian cycle and oral contraception. (175)

iii. Menopause

As previously explained, the sex difference in CVD prevalence between young males and age-matched (premenopausal) females is drastic. (190) However, with increased age, this dissimilarity between CVD diagnoses lessen. (190) For example, the NHANES review reported that approximately 77.5% of males aged 60-79 are living with a CVD, and around 75.4% of age-matched females are. (190) Even further, females aged 80 years and older had an even more similar prevalence (90.8%) of CVD than age-matched males (89.4%). (190) When compared to the statistics mentioned above (33.4% males and 17.5% females, ages 20-39), not only is it obvious that CVD risk greatly increases with age for both sexes, but the previously apparent sex difference seems to dissipate after approximately 45 years of age. (190, 193) Menopause, on average, occurs at 50 years of age in females (251) and is associated with many significant physiological changes which are primarily due to the drastic reduction in estrogen production that coincides with menopause. (252) Among these alterations are changes in muscle mass as well as cardiovascular and neural adjustments during exercise like increased BPR, enhanced peripheral vascular resistance, and heightened MSNA. (196, 210, 237, 246, 253, 254) Thus, age is an important consideration when examining the autonomic responses to exercise testing.

First, estrogen appears to have significant effects on muscle morphology. The influence of estrogen on body composition has been supported in several animal studies which concluded that loss of estrogen, via ovariectomy in mice, results in increased body weight and individual muscle weight (255) and increased muscle fiber diameter (types I, IIa, and IIb). (256, 257) Further, reintroduction of estrogen following ovariectomy causes

an overall reduction in body weight (255) and decreases diameter of all fiber types to below baseline width. (256) Estrogen also affects contraction and relaxation time in mice, as ovariectomy appears to decrease time to peak tension and increase time to relaxation while subsequent estrogen supplementation reverses the decrease to peak time. (258) Additionally, Suzuki et al. demonstrated that normal female mice increase their twitch tension significantly following ovariectomy while subsequent estrogen supplementation reduces this change to below baseline tension. (256) All of these findings confirm that estrogen plays a role in maintaining the female skeletal muscle response to exercise pre-menopause and upon the start of menopause, substantial changes to muscle morphology ensue.

Additionally, menopause appears to bring about changes in vascular physiology and function, especially in the realm of β_2-adrenergic receptor sensitivity. Prior work suggests that postmenopausal females exhibit exaggerated vasoconstriction and sympathetic transduction to the peripheral vasculature. (210, 259) More specifically, Fadel et al. found that NE infusion in postmenopausal females results in similar increases in vasoconstriction to age-matched males and seems additionally unaltered by β-blockade, suggesting that β-adrenergic vasodilation does not seem to override α-adrenergic vasoconstriction during exercise in postmenopausal females like observed in premenopausal females. (259) Further, one month of estrogen supplementation in postmenopausal females attenuated the vasoconstrictor response to HG exercise. (259) Hart et al. corroborated these findings and found that the ability for β_2 vasodilation to offset α vasoconstriction significantly decreases in postmenopausal females, whereas with β-blockade, forearm vascular conductance did not change in postmenopausal females but

increased in young females. (210) This suggests that the decline in estrogen during menopause may change the sensitivity of β_2-adrenergic receptors in their ability to ameliorate the typical vasoconstrictor effect of MSNA on the vasculature. (210) The combination of reduced muscle mass and strength and augmented transduction ultimately coalesce into obvious changes in the management of BP during exercise in postmenopausal females.

Aging in both sexes brings about steeper increases in MSNA during exercise and a more positive relationship between sympathetic outflow and BP, whereas in younger adults MSNA is not associated with changes in mean BP (44, 109), but this phenomenon is particularly pronounced in older, postmenopausal females. (210) Notably, BP reactivity to exercise and PEI is elevated in postmenopausal females when compared to premenopausal females. (214, 260-263) Minimal research has been done to directly examine this relationship between autonomic function, estrogen, and menopause. Wenner et al. found that while resting BP was similar between pre- and post-menopausal females, the increases in both BP and MSNA during isometric HG exercise and PEI was significantly higher in postmenopausal females. (246) However, both BP reactivity and MSNA were attenuated during both timepoints after one month of estrogen administration in postmenopausal females (246), a finding that had been exhibited previously. (264) One study further examined this age difference in female adults and found that the mechanisms underlying the increases in BP between pre- and post-menopausal females differed significantly. (260) Choi et al. demonstrated that the BP reactivity in premenopausal females was due to significantly greater increases in CO and SV, while postmenopausal females experienced significantly altered increases in peripheral

vascular resistance likely due to decreased sympathetic transduction often seen with age. (260)

On the other hand, mixed-sex cohorts have not found such distinct responses to HG exercise or metaboreflex isolation. For example, Lalande et al. found that changes in MSNA and BP were similar between younger and older groups, although HR and CO increased more drastically in younger subjects. (173) Similarly, Ettinger et al. did not find significant age increases in MSNA responses to HG exercise, despite higher absolute burst frequencies in older subjects. (174) However, unlike Lalande's findings, the older cohort displayed higher absolute MAP values during exercise. (174) Cauwenberghs et al. also found an increase in BP reactivity in response to exercise in older age groups, but no sex differences. (218) However, changes in left ventricular volume, ESV, EDV, SV, and CO all decreased with age. (218) The results of these studies suggest that the inclusion of males may attenuate the age differences otherwise observable in all-female cohorts.

Although incomplete, the current literature suggests that aging brings about significant alterations in autonomic function during exercise and metaboreflex isolation in females, and therefore, attentiveness should be employed when analyzing the sex differences in these areas.

CHAPTER THREE: THE INFLUENCE OF ADJUSTING FOR MUSCULAR STRENGTH AND BODY SIZE ON SEX DIFFERENCES IN SYMPATHETIC RESPONSES TO ISOMETRIC HANDGRIP EXERCISE AND METABOREFLEX ISOLATION IN HEALTHY YOUNG ADULTS

I. Introduction

Nearly half of all adults in the United States ≥20 years of age are living with a cardiovascular disease (**CVD**) and young males are at a significantly higher risk of CVD diagnosis and mortality than premenopausal females. (190) Although there is an abundance of research investigating sex differences in CVD risk, the underlying mechanisms are still not fully understood. Exercise testing is an effective tool for assessing the presence of CVD risk. For example, exaggerated blood pressure (**BP**) reactivity to exercise has been associated with increased risk of future hypertension development. (81, 84, 86, 117, 197, 198) Acute exercise increases BP and muscle sympathetic nerve activity (**MSNA**) via the exercise pressor reflex (**EPR**). (118, 161, 265) Consisting of the mechano- and metaboreflex, the EPR functions to increase cardiac output and, along with sympatholysis, increases blood flow to the skeletal muscle in order to meet the heightened metabolic demands of exercise. (161, 265) Vascular occlusion during muscle contraction stimulates mechanically-sensitive group III nerve afferents that send impulses to specific areas of the brain, namely the rostral ventrolateral medulla in the brainstem, to upregulate efferent sympathetic activity reflected by increased MSNA and ultimately produce the mechanoreflex. (160, 161) Similarly, metabolite production and accumulation in the skeletal muscle during exercise stimulate metabolically-sensitive group IV nerve afferents, which also provoke elevated MSNA via the metaboreflex. (160, 161) In conjunction with the EPR, local mediated sympatholysis leads to increases in

skeletal muscle blood flow through smooth muscle vasodilation to meet the heightened metabolic demands of exercise.

Prior evidence suggests that sex differences exist in the EPR, wherein females demonstrate consistently blunted BP reactivity to isometric handgrip (**HG**) exercise and metaboreflex isolation. (174, 178, 195, 200-204, 218) However, the existing research on similar MSNA responses between males and females is inconclusive. For instance, Ettinger et al. found that males exhibited significantly greater BP and MSNA responses to HG exercise and metaboreflex isolation than age-matched females. (174) Conversely, other studies have demonstrated sex differences in BP reactivity without concomitant sex differences in MSNA responsiveness to exercise. (175, 202)

Although several hypotheses have been proposed to explain why the sex difference in exercising BP reactivity exists, a leading theory is that differences in absolute muscle strength between males and females may be influencing BP responses. (174, 175, 204) For example, Seals et al. demonstrated that BP reactivity and MSNA increased more during two-handed isometric HG exercise as opposed to one-handed exercise, suggesting that the resultant sympathetic response is dependent on the amount of exercising muscle mass. (215) Subsequent studies that statistically adjusted for muscle strength have found attenuated differences in exercising BP in males and females. (174, 175, 204, 222) Notably, Lee et al. concluded that statistical adjustment and employing a strength-matched cohort (i.e., relatively strong females and weak males) attenuated sex differences in BP reactivity during HG exercise and metaboreflex isolation. (175) Further, some studies have investigated the relationship between role of anthropometric measurements of body size and HG strength and have established that height^2 (**h^2**) and

body surface area (**BSA**) may be the promising methods for normalizing HG strength. (223, 266) However, to the best of our knowledge, the separate and combined influence of statistically adjusting for muscle strength and body size on sex differences in BP and MSNA responses to exercise and metaboreflex isolation has not yet been explored.

Therefore, the purpose of the present study was to determine whether adjusting for absolute HG force and measures of body size (h^2 and BSA) on BP and MSNA responses to isometric HG exercise and metaboreflex isolation. We hypothesized that 1) females would exhibit blunted responses in BP to isometric HG exercise and post-exercise ischemia (**PEI**); and 2) adjustments for HG force and body size would attenuate the initial sex differences with HG force producing the largest effect. We also aimed to investigate whether utilizing a strength-matched cohort would successfully attenuate the sex differences in BP and MSNA similar to a recent study. (203)

II. Methods

We analyzed and actively collected data from six different current and recent studies conducted in two separate laboratories at the University of Delaware and Auburn University to achieve a large sample size. Each of these studies (NCT04334135, NCT04244604, NCT04576338, NCT02881515, NCT03560869, one unlisted) had their own separate purposes and hypotheses and data from some of these protocols have been previously published. (110, 267-271) All participants provided their verbal and written consent. Procedures and protocols were approved by the Institutional Review Boards of the University of Delaware and Auburn University and are in accordance with the Declaration of Helsinki.

a. Participants

Participants provided a complete medical history during an initial screening visit. This visit also included collection of height and weight for calculation of body mass index (**BMI**, kg/m^2) and resting BP, measured in triplicate following 5 minutes of seated, quiet rest. Exclusion criteria included a BMI ≥30 kg/m^2, high BP (>140/90 mmHg), any overt or uncontrolled chronic diseases (e.g., CVD, diabetes mellitus, cancer, kidney disease, etc.), current pregnancy or breastfeeding, or any tobacco use. Due to the hormonal decline following menopause, we also only included participants that fit the National Institute of Health's (NIH) definition of a young adult (i.e., 19-39 years). All female participants were studied during the self-reported early follicular phase (days 1-5) of the menstrual cycle to avoid any hormonal interaction.

b. Experimental Visit

Prior to the experimental visits, participants abstained for ≥12 hours from alcohol, caffeine, and exercise and fasted from food for ≥4 hours. After resting BP and anthropometric measures were collected, we obtained each participant's maximal voluntary contraction (**MVC**), defined as the average of at least three maximal squeezes using a HG force dynamometer (ADInstruments MLT004/ST; ADInstruments Inc., Colorado Springs, CO). Afterwards, subjects were instrumented with three electrodes for single-lead electrocardiogram assessment and oscillometric BP cuff on the upper arm. Beat-to-beat BP was collected by placing a finger cuff on the middle finger and determined via photoplethysmography (Finapres NOVA/Pro; Finapres Medical Systems, Enschede, Netherlands). We also assessed each participant's rate of perceived exertion (**RPE**)

because differences in RPE may influence BP response to stressors and could confound any potential sex differences. (130, 132, 272)

Participants underwent a 10-minute quiet rest period in a dim, temperature-controlled room while we recorded their resting beat-to-beat BP values (ADInstruments, LabChart 8; ADInstruments Inc., Colorado Springs, CO). Following the 10-minute rest period was a 1-minute instructional period in which participants received final instructions and guidance on the upcoming HG and metaboreflex isolation protocols. Participants then performed isometric HG exercise at 40% of their calculated MVC for two minutes followed by three minutes and 15 seconds of metaboreflex isolation via PEI, and 2 minutes of recovery. RPE was recorded once each minute of exercise. PEI was executed by inflating a rapid inflation brachial cuff to ~225 mmHg around the exercising arm 5 seconds prior to the end of exercise.

c. Microneurography

We obtained MSNA data in a subset (n=29) of our participants. For these participants, microneurography was performed during the 10-min rest period by placing a tungsten microelectrode into the peroneal nerve using standard microneurography protocols. (109, 111) Use of a nerve traffic analyzer (model 662c-4, Nerve Traffic Analyzer, Univ. of Iowa Bioengineering) allowed raw signals to be amplified (80-90,000X), band-pass filtered (0.7-2.0 kHz), rectified, and integrated (time constant, 0.1s). MSNA was confirmed by a pulse-synchronous response to end-expiratory breath-hold and tendon tapping but not to gentle skin stroking or startle stimulus (i.e., skin afferents). Microneurography was conducted by experienced laboratory members (AT Robinson,

MM Wenner, WB Farquhar). MSNA data was continuously collected throughout the HG and PEI techniques.

d. Statistical Analyses

Data from the multiple trials were organized and consolidated (by MA Tharpe, oversight by AT Robinson, assistance by BA Linder). We then compared anthropometric and baseline measures between sexes with unpaired parametric and Mann-Whitney t-tests. Additionally, changes in BP and MSNA from baseline to our two timepoints (HG and PEI) were analyzed using two-way mixed model ANOVAs (time x sex interactions). Timepoints were defined as the last minute of HG (minute 2) and PEI (minute 3), and changes in BP and MSNA were calculated from baseline (**BSL**) to HG and from BSL to PEI. We determined sex differences in BP and MSNA changes using Bonferroni post-hoc testing. We then adjusted for the influence of average 40% HG strength, h^2, and BSA with ANCOVAs. A strength-matched cohort was compiled (described below), and the hemodynamic responses of these participants were compared using unpaired t-tests. Statistical significance was defined as $p<0.05$ and all data are presented as means ± SD. All statistical analyses were performed using Jamovi version 2.2.5 and GraphPad (Prism version 9).

III. Results

In total, we analyzed data from 112 participants (n=36 females, 76 males). Our participants were generally young, healthy, and normotensive (BSL demographics presented in **Table 1**). Height, body mass, and MVC (**Figure 1d**), but not BMI (**Figure 1c**), were lower in females than in males. Additionally, females had significantly lower

resting brachial systolic BP than males (**Table 1**) although resting BP was within the normal range for both sexes. There were no apparent sex differences in resting brachial diastolic BP (**Table 1**), mean BP (**Table 1**), or RPE (**Table 1**).

Our covariates are presented in **Table 2**. h^2 (**Figure 1a**), BSA (**Figure 1b**), and average 40% HG strength (**Figure 1e**) were all significantly different between the sexes. However, differences in RPE between males and females were not statistically significant (**Figure 1f**). There was a correlation between average RPE and the increase in systolic BP during HG (r=0.230, p=0.015) but not during PEI (r=0.176, p=0.064) although there was a trend for a correlation.

a. Blood Pressure Reactivity

As anticipated, males and females demonstrated statistically significant increases in systolic, diastolic, and mean BP during both HG and PEI (**Figure 2**). Average 40% HG force was correlated to systolic and diastolic BP during HG (**Figure 3a**). There were also correlations during PEI for both systolic and diastolic BP (**Figure 3b**). There was a significant sex x time interaction for changes in systolic BP (**Figure 2a**). Additionally, post-hoc analyses revealed that males exhibited significantly greater systolic BP changes compared to females from BSL to HG (minute 2: females: 21 ± 12 mmHg, males: 27 ± 15 mmHg, p=0.005) and to PEI (females: 19 ± 14 mmHg, males: 28 ± 16 mmHg, p<0.001). There was also a significant sex x time interaction for diastolic BP (**Figure 2b**). Changes in diastolic BP were significantly greater in males than in females from BSL to HG (females: 19 ± 9 mmHg, males: 22 ± 11 mmHg, p=0.050) and to PEI (females: 12 ± 9 mmHg, males: 18 ± 10 mmHg, p=0.003). Similarly, there was a significant sex x time interaction for mean BP (**Figure 2c**). Post-hoc testing suggested changes in mean BP

were trending towards a sex difference from BSL to HG (p=0.051) and revealed a significant sex difference from BSL to PEI (females: 15 ± 11 mmHg, males: 23 ± 13 mmHg, p=0.003).

b. Muscle Sympathetic Nerve Activity

We obtained MSNA recordings from 29 participants (n=11 females, 18 males). Similar to the full cohort, MSNA males had significantly greater height, body mass, MVC, and resting brachial systolic BP, but not BMI when compared to MSNA females (BSL demographics presented in **Table 3**). However, unlike our full cohort, males were slightly older than females and also had slightly more elevated resting brachial diastolic and mean BP (**Table 3**). Additionally, there were significant sex differences in h^2, BSA, and average 40% HG force (**Table 3**). As expected, all MSNA measures increased with HG exercise and PEI (**Figure 4**). However, there was no significant sex x time interaction in burst frequency (**Figure 4a**) and post-hoc testing revealed no significant sex differences from BSL to HG (p=0.688) or to PEI (p>0.999). Similarly, there was no significant sex x time interaction in burst incidence (**Figure 4b**) or in total MSNA (**Figure 4c**). Changes in burst incidence (HG: p=0.723, PEI: p>0.999) and total MSNA (HG: p>0.999, PEI: p>0.999) did not differ between males and females from BSL to either timepoint, as indicated by post-hoc testing. Therefore, no statistical adjustment with covariates was performed on initial MSNA analyses.

c. Adjustment for Covariates

As shown in **Table 4**, adjustment for average 40% HG force revealed attenuated sex differences in systolic, diastolic, and mean BP during HG. Comparably, adjustment for h^2 abolished pre-existing sex differences in systolic, diastolic, and mean BP during

HG. Adjusting for BSA also attenuated BSL sex differences in systolic, diastolic, and mean BP. During PEI, adjustment for average 40% HG force attenuated systolic, diastolic, and mean BP. Additionally, adjusting for h^2 attenuated the initial sex differences in systolic, diastolic, and mean BP. Adjustment for BSA produced similar attenuations in systolic, diastolic, and mean BP.

d. Strength-matched Cohort

Our strength-matched cohort was composed of the 20 weakest males (average 40% HG force = 81-138 ± 18N) and 20 strongest females (average 40% HG force = 96-156 ± 18N). Similar to the full cohort and MSNA cohort, the strength-matched cohort had significant sex differences in anthropometric measures wherein males had greater h^2, BSA, and body mass, but not BMI, when compared to females (**Table 5**). Additionally, males and females had similar resting systolic, diastolic, and mean BP values (**Table 5**). As intended, the strength-matched cohort exhibited no significant sex differences in MVC (**Figure 5a**) or average 40% HG force (**Figure 5b**). There were no significant sex x time interactions in systolic (**Figure 6a**), diastolic (**Figure 6b**), or mean (**Figure 6c**) BP. Post-hoc testing suggested that increases in systolic BP (**Figure 6a**) did not differ between the sexes from BSL to HG ($p>0.999$) or to PEI ($p>0.999$). Similarly, no sex differences in diastolic BP (**Figure 6b**) were apparent during HG ($p>0.999$) or PEI ($p>0.999$), or in mean BP (HG: $p>0.999$, PEI: $p>0.999$, **Figure 6c**).

IV. Discussion

The present study aimed to assess the influence of muscle strength and anthropometric measures on sex differences in BP and MSNA during isometric HG

exercise and metaboreflex isolation. The primary novel finding of this study was that covariate adjustment for 40% HG force, h^2, and BSA successfully attenuated initial sex differences in BP responses, such that males had greater BP reactivity during HG and PEI. We also found that when we isolated the 20 strongest females and 20 weakest males to create a strength-matched cohort, there were no sex differences in BP responses to exercise. Lastly, there were no significant sex differences in MSNA during HG exercise or PEI, irrespective of differences in 40% HG force, h^2, and BSA between sexes. These findings contribute to the growing body of literature seeking to uncover the mechanisms that underlie sex differences observed in hemodynamic and autonomic responses to exercise.

Previous studies have demonstrated that females have blunted BP responses to isometric HG exercise and metaboreflex isolation when compared to age-matched males. (174, 178, 195, 200, 203, 204) However, the underlying mechanisms behind this sex difference remain uncertain. Because there is a drastic sex difference in fat-free muscle mass (216) and muscle strength (174, 175, 195, 200, 217, 218) some studies have investigated the effect of statistically adjusting for MVC. (174, 175, 204, 222) For example, Ettinger et al. concluded that during static adductor pollicus exercise at 60% MVC adjusting for absolute force did not attenuate the initial sex differences in BP reactivity. (174) However, multiple recent studies have found contrasting results, with statistical adjustment for muscle force successfully attenuating the sex differences in hemodynamic and autonomic responses to stressors between males and females. (203, 204, 217, 222) Similarly, we found that statistical adjustment for average 40% maximal HG force attenuated the pre-existing sex differences in exercise BP reactivity in healthy young

adults. One possible explanation for the differences between the findings of Ettinger et al. and the present study is that while we only evaluated females during days 1-5 of the menstrual cycle, Ettinger et al. did not control for the menstrual cycle in premenopausal females which could have introduced more variability among female participants. Specifically, cyclical fluctuations in female sex hormones throughout the menstrual cycle affect the EPR (195, 243), whereby BP and MSNA responses are heightened during low hormone phases (i.e., early follicular) compared to high hormone phases (i.e., late follicular). (243)

One of the reasons for the initial sex differences in exercising BP include dissimilarities in skeletal muscle fiber distribution and subsequent metabolic accumulation, which may be influenced by sex hormones. (229, 273) Specifically, females have a higher proportion of type I oxidative fibers than males who have greater percentages of type IIa glycolytic fibers. (225) This discrepancy may explain why females also exhibit blunted metabolite production and accumulation during isometric HG exercise and PEI when compared to males. (174) For example, Lee et al. recently concluded that muscle strength accounts for approximately 8-18% of total variance in BP responses to HG exercise and PEI. (175) Therefore, it is possible that the remaining effect is attributable to sex differences that may include afferent sensitivity or metabolite production or clearance differences in the end organ responses (i.e., transduction of the sympathetic nerve signal to constriction) between the sexes. (175) One factor that we considered was RPE, a subjective evaluation of effort, during exercise. Prior evidence has suggested no sex differences in RPE during HG exercise (200, 217) and our results

substantiate these findings. We did not find a difference in RPE between males and females during exercise.

Additional indicators of body size, such as height, waist circumference, and body mass, have been used to normalize HG strength between the sexes. (223) Nevill et al. concluded via allometric scaling that h^2 was the body size parameter most closely associated with HG force. (223) We further sought to examine the effect of controlling for BSA, since prior evidence has illustrated a close association between HG strength and BSA. (266) Our findings support these previous results, as statistical adjustments for h^2 and BSA during HG and PEI successfully attenuated the initial sex differences in BP reactivity. In addition to statistically adjusting for muscle strength, some have proposed that controlling for absolute HG force during exercise may be a more accurate method of attenuating sex differences in BP responses. As a result, Lee et al. were the first to utilize a strength-matched cohort, wherein the BP responses from the 20 strongest females and 20 weakest males were compared. (175) Upon analysis, no sex differences were observed from the Lee et al. study and our results from the current investigation corroborate this finding.

Aside from BP, sex differences in MSNA responses during exercise have been attributed to the fact that upon afferent stimulation of the sympathetic nervous system via the mechano- and metaboreflex, males have elevated efferent sympathetic discharge, although the evidence for this hypothesis is limited. It is well-accepted in the literature that males have heightened resting MSNA levels (205-207) although several studies have found no such sex difference at rest. (174, 175) Our findings support this similarity in resting MSNA between males and females. Further, evidence of MSNA responses to

exercise is inconclusive, with some studies suggesting females have smaller increases in MSNA than males during HG and/or PEI (174, 195) and others concluding no such distinction. (175, 202, 207) Additionally, Sanchez et al. found no significant sex differences in norepinephrine or epinephrine concentrations during exercise. (208) In this study, we found no significant sex difference in MSNA responses to HG exercise or PEI. There is a possibility that when sex differences in MSNA do occur within a specific cohort that they may be partially strength dependent. In such a scenario, physical or statistical adjustments for HG force may attenuate the initial effect like that of sex differences in BP reactivity. However, in addition to two others (174, 203), our study is one of only three to examine the impact of such adjustments on MSNA. Ettinger et al. observed blunted increases in female MSNA when compared to males but concluded no effect of physically adjusting for MVC during an adductor pollicus protocol. In contrast, both Lee et al. and the present study found no sex difference in MSNA response to HG exercise or PEI, and therefore did not attempt to further amplify this result via statistical or physical adjustment for muscle strength or anthropometric measures. (203) Consequently, further work is needed to conclude that the attenuation of MSNA responses between males and females is attributed to external factors.

We acknowledge that our design is not without its limitations. First, we did not obtain plasma norepinephrine or epinephrine concentrations during exercise or metaboreflex isolation, which if collected, may have been a supplemental indicator of full-body sympathetic activation during these timepoints. Additionally, MSNA recordings were only obtained in a subset of our sample (n=29) because of resource availability. Our limited sample size may have contributed to the lack of sex differences in MSNA

responses, although previous literature supports this finding. (175, 202, 207) Because we controlled for age (i.e., menopause) and menstrual status, our female sample size (n=36) was significantly smaller than our male sample (n=76). In future studies, a more balanced sample would be ideal. Lastly, because of our already minimal female sample size, we did not control for estrogen concentration, prior pregnancy status, or contraceptive use. These three considerations have been shown to influence autonomic responses to EPR activation (210, 241, 242, 244, 246, 274) Future studies should take action to control for these factors in hopes of greater generalization of the findings here. Regardless, one novel strength of our study was the consideration of multiple covariates on BP and MSNA responses to HG exercise and PEI.

In closing, we conclude that there are sex differences in BP, but not MSNA, responses during exercise and metaboreflex isolation and that this sex difference is partially due to differences in muscle strength and anthropometric measures. This study furthers our understanding of the physiological processes that underlie the sex differences in BP and MSNA responses during exercise in healthy young males and females.

TABLES & FIGURES

Table 1

	Participant Descriptives		
	Females	**Males**	**P-value**
N	36	76	
Race	20W, 9B, 2L, 3O, 2U	52W, 18B, 2L, 4O	
Age	24 ± 4	24 ± 3	0.760
Mass (kg)	65 ± 13	81 ± 12	<0.001*
Height (cm)	162 ± 8	179 ± 7	<0.001*
Body mass index (kg/m²)	24 ± 4	25 ± 3	0.406
Resting systolic BP (mmHg)	106 ± 12	112 ± 11	0.018*
Resting diastolic BP (mmHg)	65 ± 8	66 ± 9	0.565
Resting mean BP (mmHg)	79 ± 9	81 ± 9	0.227
MVC (N)	271 ± 82	449 ± 116	<0.001*
Rate of perceived exertion (6-20)	13 ± 2	14 ± 2	0.257

Table 1. Baseline demographics for all participants. BP, blood pressure; maximal voluntary contraction, MVC. Race: W, White; B, Black; L, LatinX; O, other; U, unspecified. Data presented as mean ± SD. Significant sex differences (p≤0.05) indicated by *.

Table 2

	Covariates		
	Females	**Males**	**P-value**
Average 40% HG force (N)	104 ± 29	165 ± 49	<0.001*
Height² (cm²)	26236 ± 2468	32194 ± 2334	<0.001*
Body surface area (m²)	1.7 ± 0.2	2.0 ± 0.2	<0.001*

Table 2. Baseline covariates for all participants (n=112). HG, handgrip. Data presented as mean ± SD. Significant sex differences (p≤0.05) indicated by *.

Table 3

	MSNA Cohort		
	Females	**Males**	**P-value**
N	11	18	
Age	23 ± 2	25 ± 4	0.036*
Mass (kg)	62 ± 8	80 ± 11	<0.001*
Height (cm)	164 ± 7	178 ± 9	<0.001*
Body mass index (kg/m²)	23 ± 3	25 ± 3	0.085
Resting systolic BP (mmHg)	101 ± 9	119 ± 11	<0.001*
Resting diastolic BP (mmHg)	59 ± 7	71 ± 11	<0.001*
Resting mean BP (mmHg)	73 ± 7	86 ± 13	<0.001*
MVC (N)	278 ± 93	431 ± 76	<0.001*
Rate of perceived exertion (6-20)	12 ± 2	13 ± 6	0.276
Average 40% HG force (N)	109 ± 24	151 ± 33	0.001*
Height² (cm²)	27025 ± 2262	31753 ± 2947	<0.001*
Body surface area (m²)	1.7 ± 0.1	2.0 ± 0.2	<0.001*

Table 3. Baseline demographics (top panel) and covariates (bottom panel) for muscle sympathetic nerve activity (MSNA) cohort. Data presented as mean ± SD. Significant sex differences (p≤0.05) indicated by *.

Table 4

		Before Adjustment	After Adjustment		
			40% HG	**H²**	**BSA**
HG	**Δ Systolic BP**	0.005*	0.377	0.863	0.850
	Δ Diastolic BP	0.050*	0.416	0.216	0.977
	Δ Mean BP	0.051	0.424	0.300	0.830
PEI	**Δ Systolic BP**	<0.001*	0.215	0.826	0.219
	Δ Diastolic BP	0.003*	0.280	0.744	0.209
	Δ Mean BP	0.003*	0.233	0.790	0.259

Table 4. P-values of changes in systolic, diastolic, and mean blood pressure (BP) during handgrip (HG) and post-exercise ischemia (PEI) before and after statistical adjustments via ANCOVAs. N=112. Data presented as mean ± SD. Significant sex differences (p≤0.05) indicated by *.

Table 5

	Strength-matched Cohort		
	Females	**Males**	**P-value**
N	20	20	
Age	24 ± 5	25 ± 4	0.759
Mass (kg)	65 ± 12	77 ± 13	0.005*
Height (cm)	162 ± 8	176 ± 8	<0.001*
Body mass index (kg/m²)	25 ± 5	24 ± 4	0.878
Resting systolic BP (mmHg)	108 ± 14	111 ± 11	0.506
Resting diastolic BP (mmHg)	66 ± 10	66 ± 10	0.907
Resting mean BP (mmHg)	80 ± 11	81 ± 10	0.730
MVC (N)	328 ± 49	318 ± 55	0.740
Rate of perceived exertion (6-20)	13 ± 2	14 ± 2	0.303
Average 40% HG force (N)	124 ± 18	115 ± 18	0.143
Height² (cm²)	26338 ± 2363	31118 ± 2761	<0.001*
Body surface area (m²)	1.7 ± 0.2	1.9 ± 0.2	<0.001*

Table 5. Baseline demographics of strength-matched cohort (n=40). Cohort organized by grouping 20 strongest females (relative to average 40% average handgrip force) and 20 weakest males. Data presented as mean ± SD. Significant sex differences (p≤0.05) indicated by *.

Figure 1

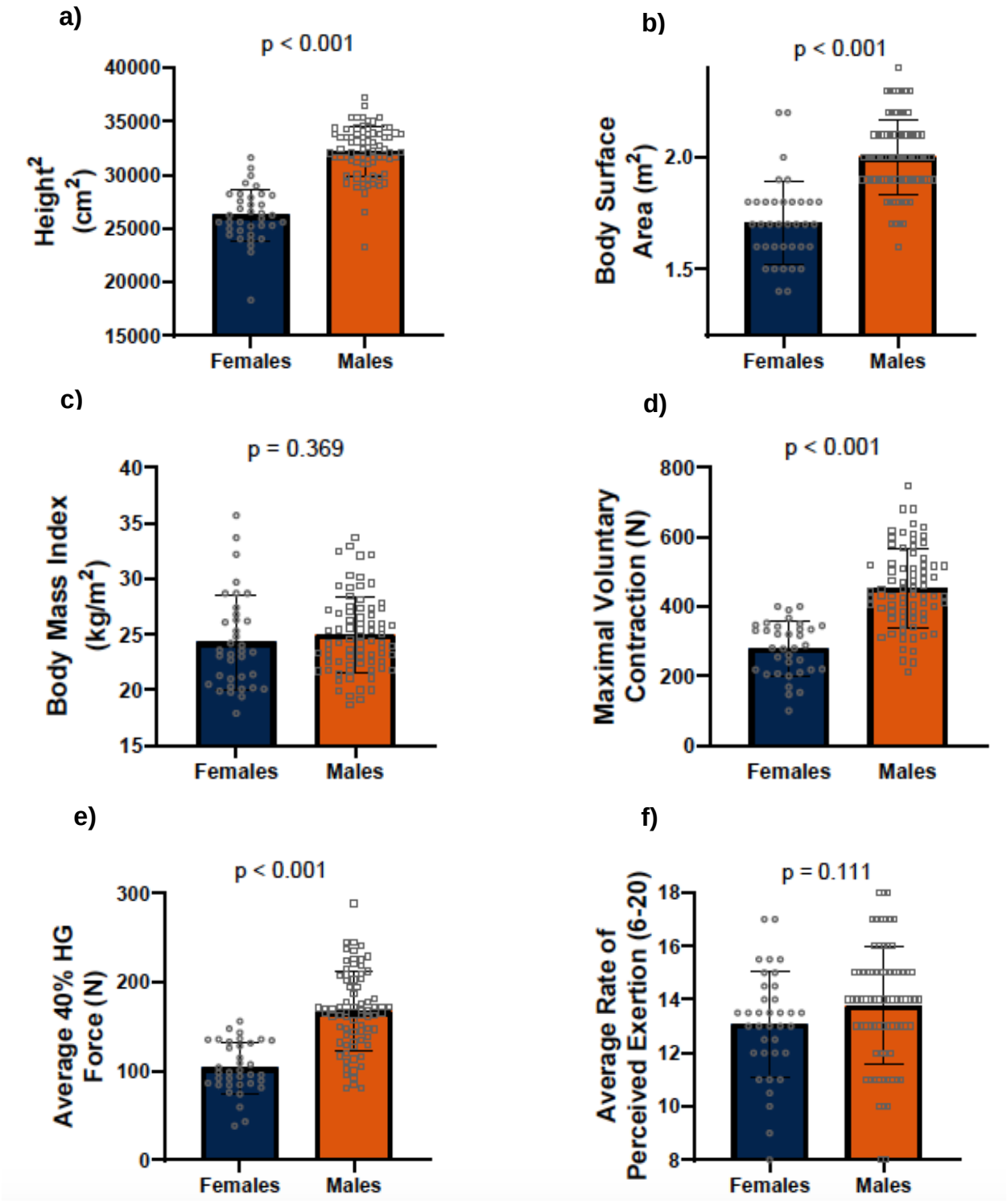


Figure 1. Comparison of height^2 (graph a), body surface area (graph b), body mass index (graph c), maximal voluntary contraction (graph d), average 40% handgrip (HG) force (graph e), and average RPE (graph f) between the sexes in all participants. Data obtained from 112 participants and represented as mean ± SD.

Figure 2

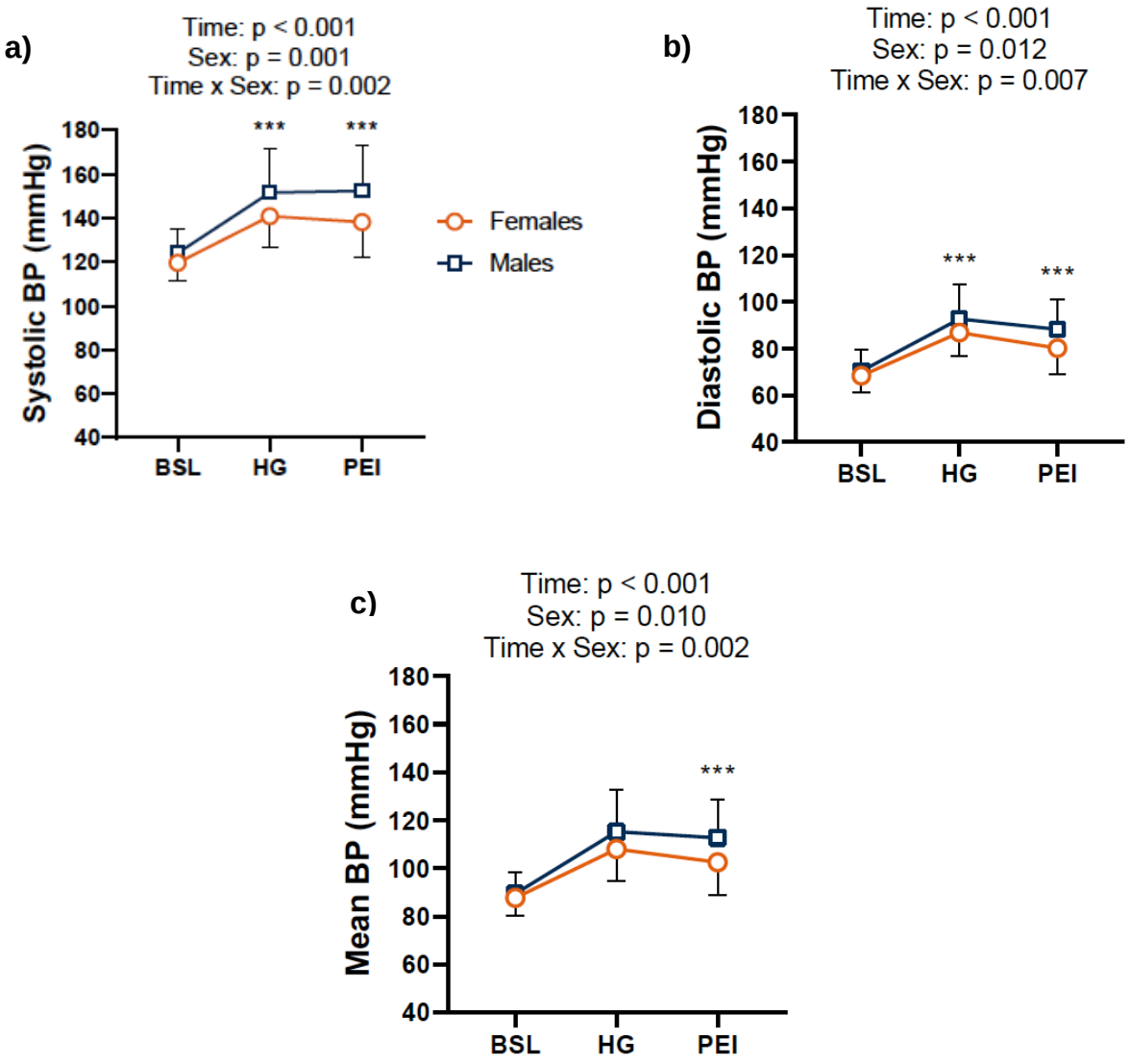
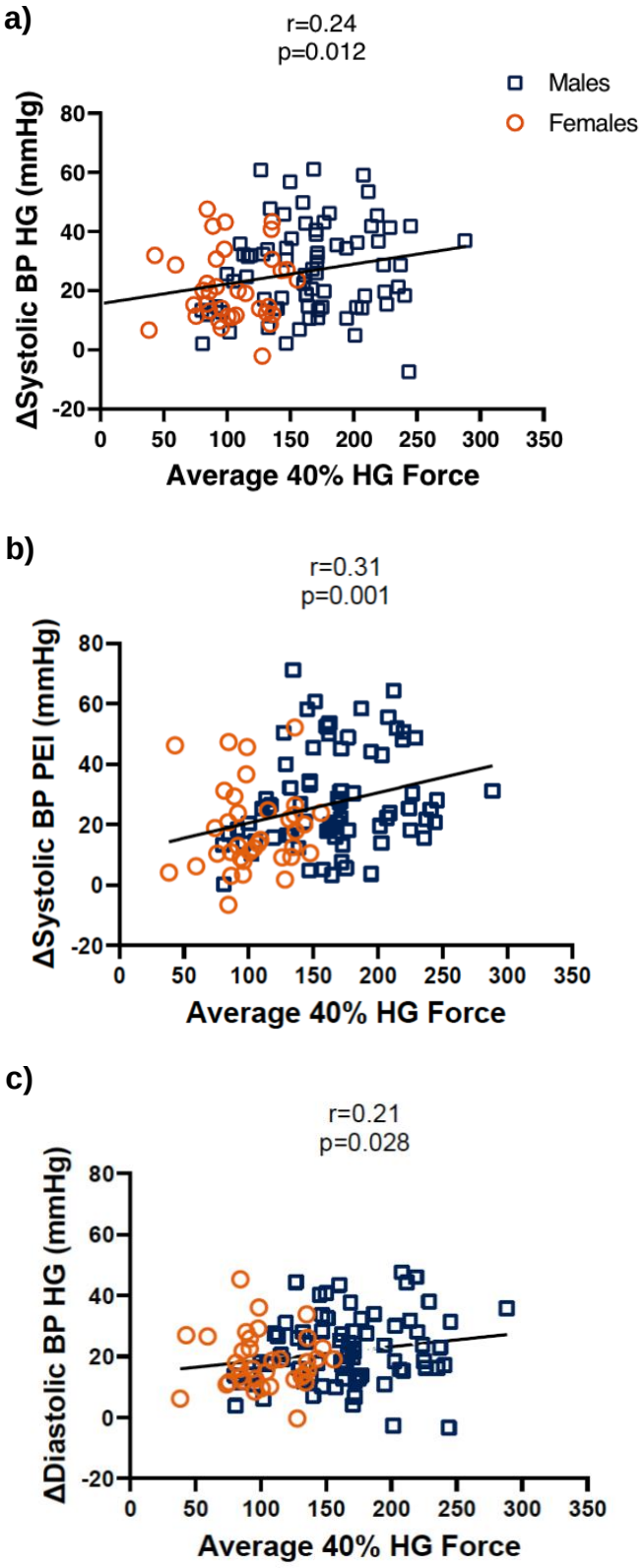


Figure 2. Systolic (graph a), diastolic (graph b), and mean blood pressure (BP) (graph c) responses to isometric 40% handgrip (HG) exercise and post-exercise ischemia (PEI). Data collected from 112 participants and represented as mean ± SD. Significant sex differences ($p \leq 0.05$) via Bonferroni post-hoc testing is indicated by ***.

Figure 3


a)
r=0.24
p=0.012
Males
Females
ΔSystolic BP HG (mmHg)
Average 40% HG Force
b)
r=0.31
p=0.001
ΔSystolic BP PEI (mmHg)
Average 40% HG Force
c)
r=0.21
p=0.028
ΔDiastolic BP HG (mmHg)
Average 40% HG Force

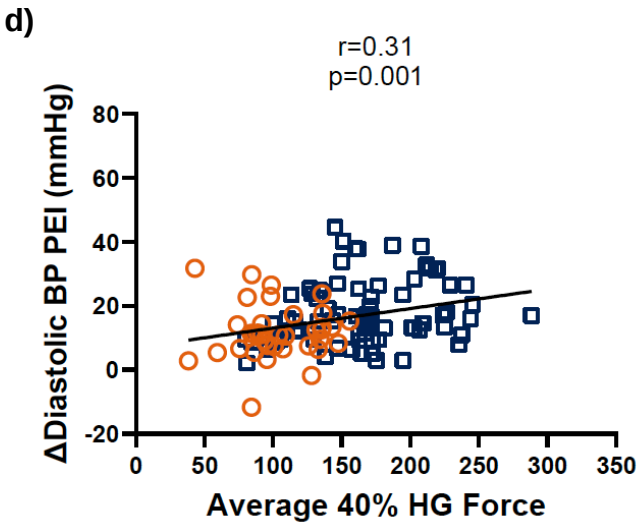


Figure 3. Relationships between average 40% handgrip (HG) force and changes in systolic blood pressure (BP) during HG (graph a) and PEI (graph b). Changes in diastolic BP during HG (graph c) and PEI (graph d).

Figure 4

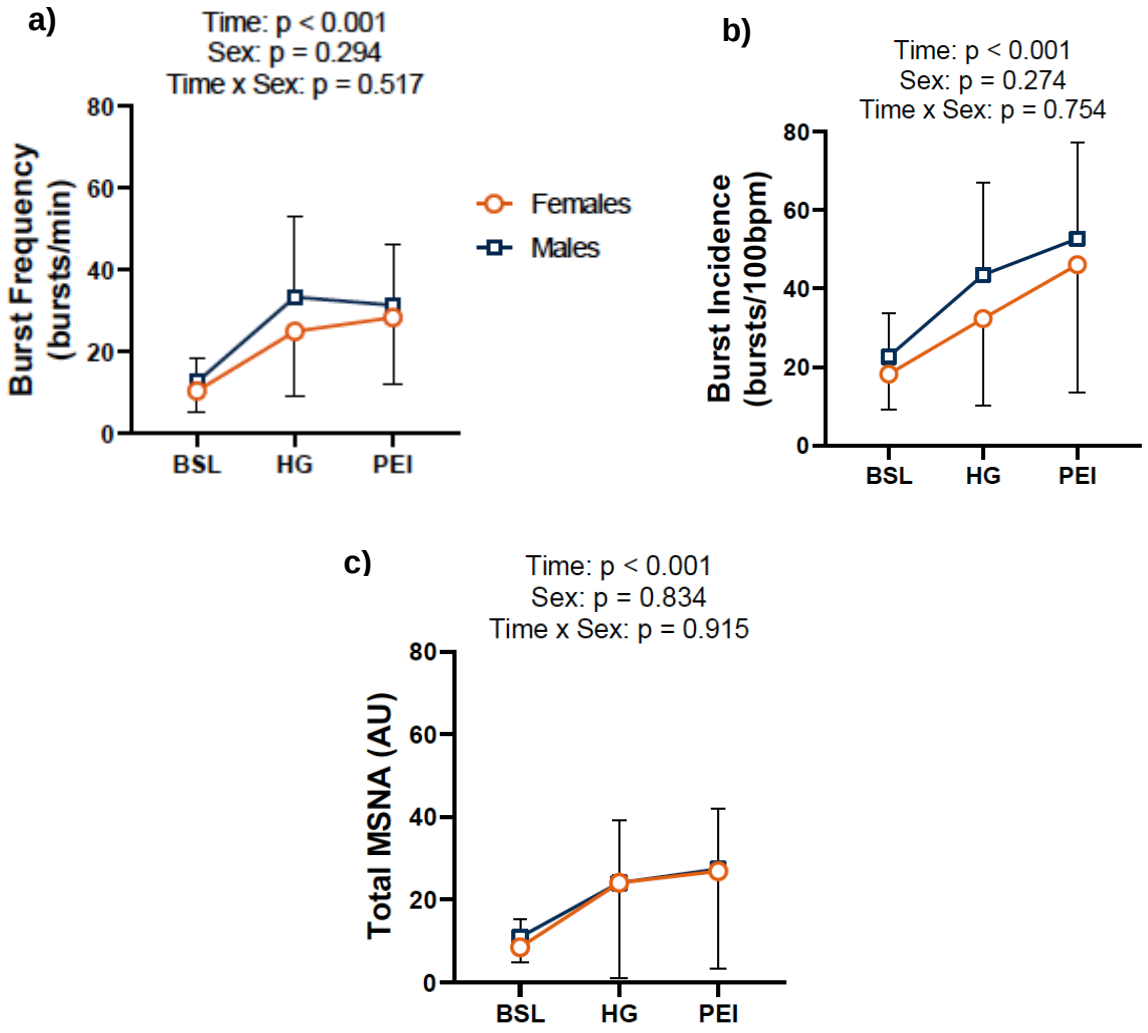


Figure 4. Burst frequency (graph a), burst incidence (graph b), and total muscle sympathetic nerve activity (MSNA) (graph c) responses to isometric 40% handgrip (HG) exercise and post-exercise ischemia (PEI). Data collected from 29 participants and represented as mean ± SD. Significant sex differences ($p \leq 0.05$) via Bonferroni post-hoc testing is indicated by ***.

Figure 5

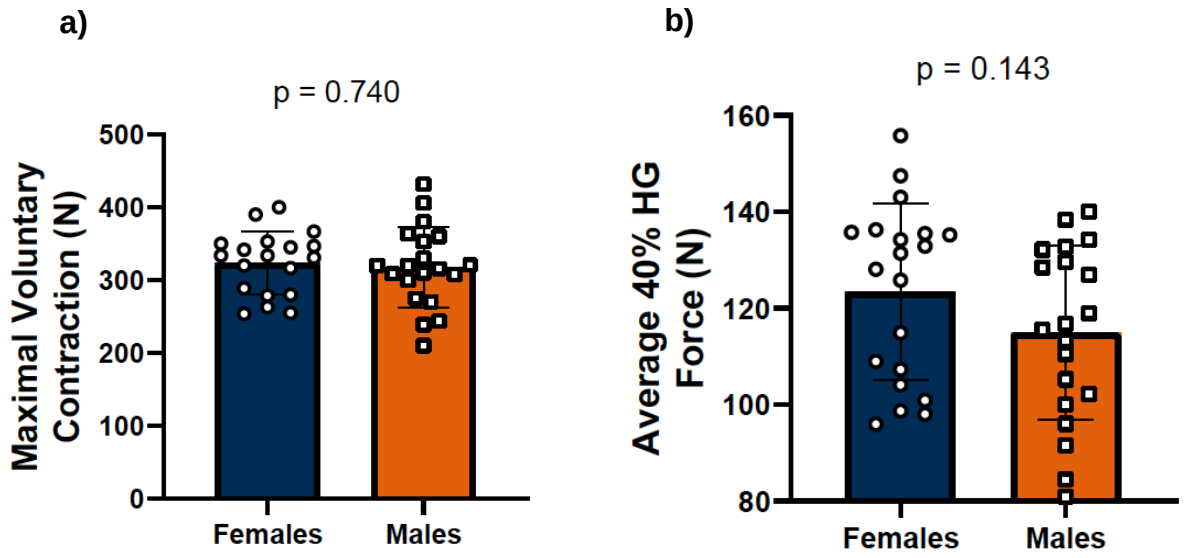


Figure 5. Comparison of maximal voluntary contraction (graph d) and average 40% handgrip (HG) force (graph e) between the sexes in the strength-matched cohort (n=40). Data represented as mean ± SD.

Figure 6

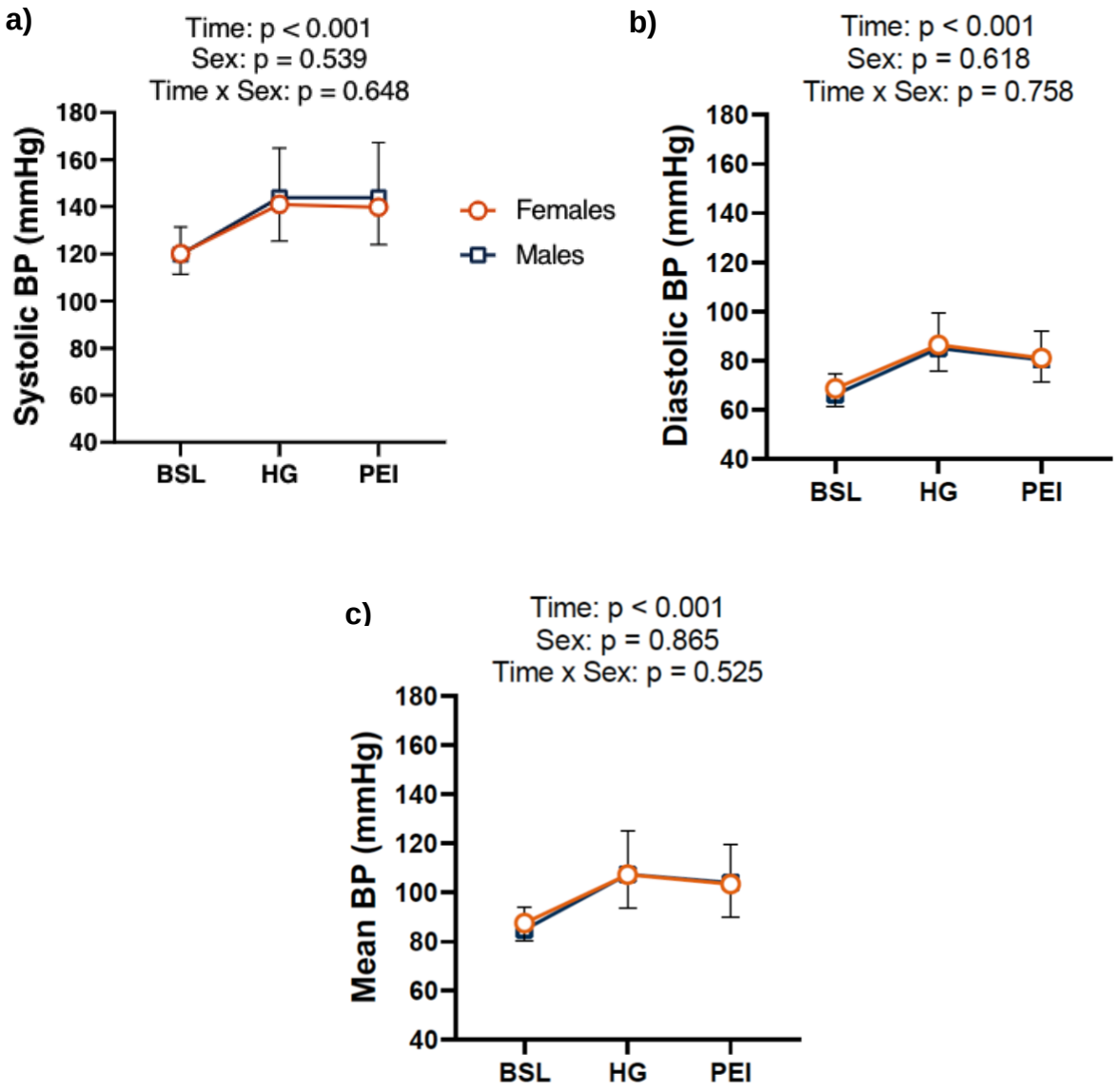


Figure 6. Systolic (graph a), diastolic (graph b), and mean blood pressure (BP) (graph c) responses to isometric 40% handgrip (HG) exercise and post-exercise ischemia (PEI) in strength-matched cohort. Data collected from 40 participants and represented as mean ± SD.

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267. Watso JC, Babcock MC, Robinson AT, Migdal KU, Wenner MM, Stocker SD, et al. Water deprivation does not augment sympathetic or pressor responses to sciatic afferent nerve stimulation in rats or to static exercise in humans. J Appl Physiol (1985). 2019;127(1):235-45.
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sympathetic outflow and blood pressure responses to exercise. J Neurophysiol. 2018;119(4):1257-65.
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CURRICULUM VITAE

McKenna Tharpe, B.S.
Auburn University, School of Kinesiology
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EDUCATION

M.S. Auburn University – Auburn, AL
Kinesiology, Exercise Physiology
August 2020 – current
Thesis: The Influence of Adjusting for Muscular Strength and Body Size on Sex Differences in Sympathetic Responses to Isometric Handgrip Exercise and Metaboreflex Isolation in Healthy Young Adults

B.S. Auburn University – Auburn, AL
Kinesiology, Exercise Science
Summa cum laude
August 2016 – May 2020

PROFESSIONAL EXPERIENCE

Graduate Student Class Instructor
August 2020 – current
Auburn University - Auburn, AL
School of Kinesiology

Graduate Research/Teaching Assistant
August 2020 – current
Auburn University - Auburn, AL
School of Kinesiology
Neurovascular Physiology Laboratory

AWARDS & HONORS

Graduate Teaching Assistantship
August 2020- current

Dean's List
Spring 2018, Fall 2017, Spring 2017

MANUSCRIPTS

1. Culver MN, Linder BA, **Tharpe MA**, Hutchison ZJ, Robinson AT. Self-reported and actigraphy-derived sleep quality and cardiometabolic health in healthy adults. *Frontiers in Cardiovascular Endocrinology.* In preparation.
2. Linder BA, Barnett AM, **Tharpe MA**. Move it or lose it: Limb immobilisation results in impaired postprandial skeletal muscle glucose uptake. *The Journal of Physiology.* 25 May 2021. DOI: 10.1113/JP281750.

CONFERENCE PRESENTATIONS

1. McIntosh MC, Barnett AM, Linder BA, Culver MN, Jeong S, **Tharpe MA**, Hutchison ZJ, Fuller-Rowell TE, Watso JC, Robinson AT. Rachial differences in urinary hydration markers, but not creatine clearance or urinary NGAL excretion, in healthy young adults. Control of Renal Function in Health and Disease. June 26-30, 2022. Charlottesville, VA.
2. Culver MN, Linder BA, **Tharpe MA,** Barnett AM, Hutchison ZJ, Robinson AT. Actigraphy-derived sleep quality is not related to blood pressure reactivity in young apparently healthy adults. American College of Sports Medicine. San Diego, CA. May 31-June 4, 2022.
3. Jeong S, Linder BA, **Tharpe MA,** Hutchison ZJ, Culver MN, Nichols OI, Fuller-Rowell TE, Robinson AT. Racial differences in night-to-day blood pressure ratio and blood pressure dipping in healthy young adults. American College of Sports Medicine. May 31-June 4, 2022. San Diego, CA.
4. **Tharpe MA,** Watso JC, Babcock MC, Brian M, Linder BA, Pollin KU, Hutchison ZJ, Barnett AM, Farquhar W, Robinson AT. Adjusting for exercise intensity attenuates sex differences in blood pressure during exercise in healthy adults. American College of Sports Medicine. San Diego, CA. May 31-June 4, 2022.
5. Jones SD, Sanchez SO, **Tharpe MA**, Culver MN, Linder BA, Hutchison ZJ, Robinson AT. The Influence of habitual dietary sodium and potassium on blood pressure in young adults. American Heart Association Annual HBCU Scholars Research Symposium. Nashville, TN. April 6-7, 2022.
6. Culver MN, Linder BA, Lyons DE, **Tharpe MA,** Hutchison ZJ, Jeong S, Garrett CL, Robinson AT. The influence of self-reported and actigraphy-derived sleep quality on cardiometabolic health in diverse cohort of healthy adults. Experimental Biology. Philadelphia, PA. April 2-5, 2022.
7. Jeong S, Linder BA, **Tharpe MA,** Hutchison ZJ, Culver MN, Nichols OI, Fuller-Rowell TE, Robinson AT. Pilot study on the influence of race and sex on beat-to-beat blood pressure variability in healthy young adults. Experimental Biology. Philadelphia, PA. April 2-5, 2022.

8. Linder BA, Barnett AM, Hutchison ZJ, **Tharpe MA**, Kavazis AN, Gutierrez OM, Robinson AT. The influence of acute high dose MitoQ on urinary kidney injury markers. Experimental Biology. Philadelphia, PA. April 2-5, 2022.

9. Jeong, S, Linder BA, Hutchison ZJ, Barnett AM, **Tharpe MA**, Robinson AT. Racial differences on ambulatory blood pressure variability in healthy young adults. Auburn Research Student Symposium. Auburn, AL. March 28, 2022.

10. Culver MN, Linder BA, **Tharpe MA,** Barnett AM, Hutchison ZJ, Robinson AT. Actigraphy-derived sleep quality is not related to blood pressure reactivity in young apparently healthy adults. Southeast American College of Sports Medicine. Greenville, SC. February 17-19, 2022.

11. Hutchison ZJ, **Tharpe MA,** Barnett AM, Linder BA, Culver MN, Brown MD, Kavazis AN, Robinson AT. Influence of acute supplementation with mitochondrial antioxidant MitoQ on vascular function in healthy adults. Southeast American College of Sports Medicine. Greenville, SC. February 17-19, 2022.

12. Jeong S, Linder BA, **Tharpe MA,** Hutchison ZJ, Culver MN, Nichols OI, Fuller-Rowell TE, Robinson AT. Racial differences in night-to-day blood pressure and blood pressure dipping in healthy young adults. Southeast American College of Sports Medicine. Greenville, SC. February 17-19, 2022.

13. **Tharpe MA,** Watso JC, Babcock MC, Brian M, Linder BA, Pollin KU, Hutchison ZJ, Barnett AM, Farquhar W, Robinson AT. Adjusting for exercise intensity attenuates sex differences in blood pressure during exercise in healthy adults. Southeast American College of Sports Medicine. Greenville, SC. February 17-19, 2022.

14. **Tharpe MA,** Watso JC, Babcock MC, Migdal KU, Wenner MM, Farquhar WB, Robinson AT. Physiological determinants of salt-sensitivity in young, female adults in a randomized, crossover, controlled feeding study. American College of Sports Medicine. Remote. June 1-5, 2021.

15. **Tharpe MA,** Hutchinson ZH, Barnett AM, Linder BA, Robinson AT. Effects of MitoQ on central hemodynamics, arterial stiffness, and oxidative stress in healthy, young adults. Experimental Biology. Remote. April 27-30, 2021.

16. Hutchison ZJ, **Tharpe MA,** Farquhar WB, Robinson AT. The influence of race on the relation between serum sodium and the renin-angiotensin-aldosterone system in response to a hypertonic saline infusion. Auburn Health Disparities Initiative Research. Virtual Symposium. July 30, 2020.

TEACHING EXPERIENCE

Auburn University- Auburn, AL

- Class Instructor for PHED1103, *Wellness* – Instructed a course on general health and fitness practices and programs. 08/2021 – current.

- Class Instructor for PHED 1003, *Active Auburn* - Instructed a walking-based group fitness class. 08/2020 – current
- Class Instructor for PHED 1640, *Performance Activities: Yoga* - Instructed a yoga and meditation group class. 08/2020 – current

CERTIFICATES & MEMBERSHIPS

10/2020 – present	Member - American College of Sports Medicine (ACSM)
10/2020 – present	Member - The American Physiological Society (APS)

SCIENTIFIC SERVICE

09/2021	Supervised review for Medicine & Science in Sports & Exercise: MSSE-D-21-00703
02/2020	Supervised review for European Journal of Applied Physiology: EJAP-D-21-00011

RELEVANT MEETINGS & WORKSHOPS ATTENDED

05/2022	American College of Sports Medicine. May 31-June 4, 2022. San Diego, CA.
02/2022	Southeast American College of Sports Medicine. February 17-19, 2022. Greenville, SC.
06/2021	American College of Sports Medicine. June 1-5, 2021. Virtual.
04/2021	Experimental Biology. April 27-30, 2021. Virtual.

COMMUNITY SERVICE

06/2021-06/2022	21st Century Community Learning Centers Camp
02/2020	Tallapoosa County Girls Ranch Volunteer
10/2019	Little HAPIE Tree Daycare Volunteer
10/2018	Food Bank of East Alabama Volunteer
04/2018, 10/2019	Morningside Nursing Home Volunteer
04/2018, 03/2019	Miracle League Volunteer
03-04/2018	Auburn University Campus Kitchens Volunteer
02/2018, 04/2019	Storybook Farms Volunteer
08/2016 – 05/2020	Numerous on-campus service projects via membership in Omega Phi Alpha National Service Sorority

TECHNICAL SKILLS

- Nutritional analysis via University of Minnesota's Nutrition Data System for Research (NDSR)
- Ultrasound assessment of flow-mediated dilation (FMD) of the brachial artery
- Measurement of arterial stiffness via Sphygmocor pulse wave analysis (PWA) and velocity (PWV)

- Preparation and centrifugation of blood samples
- Determination of hemoglobin and hematocrit
- Analysis of urine and serum electrolyte levels via SmartLyte Plus
- Determination of urine and serum osmolality via Advanced Instruments Osmometer
- Analysis of urine specific gravity via refractometry
- Evaluation of serum superoxide dismutase (SOD) levels via Cayman Chemical colorimetric assay kit
- Evaluation of β-Hydroxybutyric acid (BHB) levels via Cayman Chemical colorimetric assay kit
- Preparation of whole blood electron paramagnetic resonance (EPR) samples
- Isolation of peripheral blood mononuclear cells (PBMCs)
- Collection of beat-to-beat blood pressure via photoplethysmography (Finapres NOVA)
- Analysis of cardiac baroreflex sensitivity (cBRS) via Hemolab
- Data processing via Lab Chart
- Statistical analysis via R, Jamovi, and GraphPad
- Determination of body composition via dual-energy X-ray absorptiometry (DEXA)
- Trained in phlebotomy (certified through Auburn University Department of Clinical Sciences, January 2022)