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# The sympathetic neural control of the circulation at rest and during exercise: effects of age, biological sex, and sex hormones

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# Abstract

The overall objective of this dissertation was to determine the impact of age, sex, and sex hormones on the discharge behaviours of muscle sympathetic action potentials (APs) as well as the corresponding changes in peripheral vasoconstriction and blood pressure at rest and during exercise. The microneurographic technique was employed to record multi-unit muscle sympathetic nerve activity (MSNA), with a continuous wavelet transform applied *post-hoc* to evaluate APs within the recorded neurogram. *Study One* examined the impact of aging on the central and peripheral arcs of the sympathetic baroreflex under resting conditions. This study revealed that middle-aged-to-older adults demonstrated heightened sympathetic baroreflex control of MSNA and AP discharge (i.e., greater central arc), but attenuated sympathetic transduction into blood pressure (i.e., attenuated peripheral arc). Therefore, it appears that the interactive nature between the central and peripheral baroreflex arcs are preserved with human aging, and that greater baroreflex control of sympathetic discharge may be compensatory for attenuated sympathetic transduction amongst older adults. *Study Two* investigated the interactive effects of age and sex on sympathetic vascular transduction (i.e., the transmission of MSNA into peripheral vasoconstriction) as well as the central and peripheral arcs of the baroreflex at rest. We observed that age, but not sex, affected sympathetic vascular transduction, with older adults (regardless of sex) demonstrating attenuated vasoconstrictor responses following bursts of sympathetic discharge. Additionally, we found that males, but not females (regardless of age) demonstrated a relationship between the central and peripheral baroreflex arcs, indicating that females do not rely on this compensatory neuro-cardiovascular relationship to regulate arterial blood pressure at rest. *Study Three* assessed the interactive effects of age and sex on the neuro-cardiovascular responses to fatiguing exercise. During rhythmic handgrip exercise, we found that older males demonstrated the largest increases in blood pressure and peripheral resistance compared to young adults and older females, despite having the smallest increases in efferent

sympathetic nerve traffic. Notably, amongst males, testosterone was inversely related with the change in blood pressure, but positively related with AP recruitment, indicating that the lower testosterone levels may be driving these larger pressor and attenuated sympathetic responses amongst older males. Conversely, the loss of estradiol following menopause did not affect the neuro-cardiovascular responses to fatiguing rhythmic handgrip exercise amongst women. Finally, *Study Four* aimed to determine the role of biological sex and oral contraception on sympathetic AP discharge as well as the vasoconstrictor responses to changes in AP discharge during fatiguing static handgrip exercise amongst young adults. Here, we found that males had larger increases in AP discharge than females (particularly naturally menstruating females) during static handgrip exercise; however, no differences were observed between females using oral contraceptive pills and naturally menstruating females. Conversely, both males and females using oral contraception demonstrated greater leg vasoconstriction during exercise compared to naturally menstruating females. Therefore, the transduction of sympathetic nerve traffic into vasoconstriction is attenuated in naturally menstruating females compared to males and females using oral contraception. Overall, this series of studies provides new knowledge regarding the impact of age, sex, as well as endogenous and exogenous sex hormones on the regulation of muscle sympathetic AP discharge and the peripheral vasomotor responses to these efferent vasoconstrictor signals.

# Keywords

Aging; biological sex; sex hormones; oral contraception; microneurography; action potential; muscle sympathetic nerve activity; baroreflex; exercise pressor reflex; metaboreflex; sympathetic transduction; vasoconstriction

# Summary for Lay Audience

The sympathetic nervous system, which sends precise, goal-directed messages along nerves to communicate with various organs to ensure that blood pressure is maintained within an optimal range and that blood flow is distributed to vital organs like the brain and the heart. These messages are made up of electrical signals called action potentials. During periods of stress (like exercise), the sympathetic nervous system adjusts these messages toward the heart and the blood vessels by increasing the size, number, and timing of action potentials to permit controlled increases in blood pressure and blood flow, ensuring that sufficient oxygen is delivered throughout the body. Although the sympathetic nervous system plays an important role in human survival and performance, there is growing evidence that its control of the blood vessels in the body change across the lifespan and differ between males and females. This dissertation provides new insight into how factors like human aging, biological sex (i.e., males and females), and sex hormones (i.e., testosterone, estrogen, progesterone) affect the action potential messages used by the sympathetic nervous system to communicate with the blood vessels, and how these factors affect the responsiveness of blood vessels to the carefully crafted messages of the sympathetic nervous system.

# Co-Authorship Statement

Andrew W. D'Souza was the first author and J. Kevin Shoemaker was the senior author on all manuscripts. Qi Fu was a co-senior author on Studies 2-4 (Chapters 3-5). Stephen A. Klassen, Mark B. Badrov, and Sophie Lalande were co-authors on Study 1 (Chapter 2). Ryosuke Takeda and Geoff B. Coombs were co-authors on Studies 2 and 3 (Chapters 3 and 4). Kazumasa Manabe, Sarah L. Hissen, Takuro Washio, and Belinda Sanchez were co-authors on Studies 2-4 (Chapters 3-5). Meghan C. Annis was a co-author on Study 4 (Chapter 5).

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# Epigraph

“Without action, it’s just potential” – Dr. Charles L. Rice

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# List of Abbreviations

ANOVA, Analysis of variance

AP, Action potential

APP, amino peptidase

ATP, Adenosine triphosphate

AU, Arbitrary unit

AVP, Arginine vasopressin

BF, Burst frequency

BMI, body mass index

BP, blood pressure

BSA, Body surface area

COMT, catechol-O-methyltransferase

CVLM, caudal ventrolateral medulla

DBP, diastolic blood pressure

DPPIV, Dipeptidyl peptidase

E2, Estradiol

ECG, Electrocardiogram

EPR, Exercise pressor reflex

ER, Estrogen receptor

FSH, Follicular stimulating hormone

GABA, Gamma aminobutyric acid

GHRH, Growth hormone releasing hormone

GMP, Guanosine monophosphate

H2PO4, dihydrogen phosphate

HPG, Hypothalamic-pituitary gonadal

HPO, Hypothalamic-pituitary ovarian

HR, Heart rate

IP3, Inositol triphosphate

LBF, Leg blood flow

LVC, Leg vascular conductance

MAP, Mean arterial pressure

MRI, Magnetic resonance imaging

MSNA, Muscle sympathetic nerve activity

MVC, Maximal voluntary contraction

NA, nucleus ambiguus

NE, Norepinephrine

NIBP, Non-invasive blood pressure

NO, Nitric oxide

NPY, Neuropeptide Y

NTS, Nucleus tractus solitarius

OCP, Oral contraceptive pill

OF, Older females

OM, Older males

$P2x_1$, purinergic vasoconstrictor receptor

P4, Progesterone

PCOS, Polycystic ovarian syndrome

PECO, Post-exercise circulatory occlusion

PSNS, Parasympathetic nervous system

$\dot{Q}c$, cardiac output

REB, Research ethics board

RNA, Ribonucleic acid

RPE, Rating of perceived exertion

RVLM, Rostral ventrolateral medulla

SBP, Systolic blood pressure

SD, Standard deviation

SFA, Superficial femoral artery

SHBG, Sex hormone binding globulin

SHG, Static handgrip exercise

SNR, Signal-to-noise ratio

SNS, Sympathetic nervous system

TPR, Total peripheral resistance

Y1R, Neuropeptide Y Y1 receptor

Y2, Neuropeptide Y Y2 receptor

YF, Young females

YM, Young males

$\alpha_1$AR, alpha-1 adrenergic receptor

$\alpha_2$AR, alpha-2 adrenergic receptor

# Chapter 1

## 1 Introduction

The concept of homeostasis represents the body's ability to maintain uniform conditions in the face of external stressors (1). Critical to homeostatic function, is the sympathetic branch of the autonomic nervous system. This branch involves the integration of afferent feedback from peripheral receptors located on various organs (e.g., muscle, heart, skin) and feedforward signals generated in the brain to create carefully coordinated circulatory responses to acute physiological stressors that serve to maintain the *milieu interieur,* or internal environment (2, 3). This process is collectively known as the sympathetic neural control of the circulation, and each segment of the sympathetic neurocirculatory pathway (i.e., cortical/subcortical regions, sympathetic ganglia, post-ganglionic c-fibers, neurovascular junction, and target end-organ) represents a unique site of control in which sympathetic outflow can be titrated to maintain homeostasis during a physiological perturbation.

In the late 1960s, Swedish neuroscientists, Karl-Erik Hagbarth and Ake Vallbo, discovered a method to directly access the efferent sympathetic vasoconstrictive messages from postganglionic c-fibers innervating the skeletal muscle vasculature (muscle sympathetic nerve activity; MSNA) called microneurography (4). Since then, microneurography has vastly advanced our understanding sympathetic neural control of the circulation in both health and disease (5), providing insight into the timing, frequency, and size of sympathetic "bursts" that represent efferent action potentials (AP) firing very close together in time (6). However, due to an inherently low signal-to-noise ratio, MSNA analyses have been limited to the integrated neural recording, which conceals information about the AP discharge patterns that represent the neural communication patterns employed by the sympathetic nervous system (7). Recent analytical advances have permitted assessments of AP emission patterns (8), providing novel insight into

the regulation of AP discharge. For example, spontaneous bursts of MSNA vary in size, and this variability in amplitude is attributed to the heterogenous discharge probabilities of synchronized low-threshold APs (9, 10). Furthermore, increases in integrated MSNA during physiological stressors are supported by an ordered pattern of axonal recruitment, with greater firing probability of low-threshold APs being the primary recruitment strategy employed during mild stress (11), whereas during severe stress, the recruitment of previous-silent, larger, and faster conducting APs provide additional support to increase in integrated MSNA burst occurrence and size (11, 12). However, much of the work completed to date assessing sympathetic AP discharge patterns and recruitment has been conducted in young adults – particularly males (9, 11–15) – despite that inter-individual factors such as age and biological sex, affect the regulation of integrated MSNA and blood pressure at rest (16, 17), and during physiological stress (18, 19).

MSNA and blood pressure increase with age (17, 20, 21), and the relationship between resting MSNA and blood pressure is altered with advancing age, such that older adults exhibit a positive relationship between MSNA and blood pressure (i.e., higher MSNA = higher blood pressure), whereas this relationship does not exist in young adults (17). The arterial baroreflex is thought to contribute to the age-related increases in MSNA as the baroreflex exhibits strong control of sympathetic outflow at rest (22). Although the impact of age on the central arc of the baroreflex (i.e., generation of efferent sympathetic nerve traffic in response to changes in blood pressure) is equivocal (23–27), there is clear evidence that the peripheral arc of the baroreflex (i.e., end-organ responses to MSNA changes) is attenuated with aging (28–30). Given that the baroreflex exerts heterogenous control over varying-sized APs that comprise integrated bursts of MSNA in young adults (9), and that older adults exhibit aberrant AP discharge patterns (31), it remains unclear whether these aberrant axonal discharge patterns are mediated by age-related changes in the arterial baroreflex control of sympathetic APs. This background formed the rationale for ***Study 1*** of this dissertation, where we assessed the impact of age on the central and peripheral arcs of

the baroreflex as well as the baroreflex control of AP discharge at rest in young and older normotensive adults.

Following menopause, females exhibit an accelerated rise in cardiovascular disease risk compared to males (32, 33). While the underlying mechanisms are likely multifactorial, sympathetic nervous system dysregulation is purported to be an important contributor, as chronically elevated resting sympathetic outflow is associated with the incidence of cardiovascular disease and an increased risk of mortality (34, 35). Accordingly, most (17, 21), but not all (20) studies have found that the age-related increase in sympathetic nerve activity appears to be steeper in females than males, particularly around the time of menopause. Notably, older females exhibit attenuated central (i.e., less baroreflex-mediated restraint of sympathetic outflow) (36) and peripheral (i.e., blunted sympathetic transduction into blood pressure) baroreflex arcs (29) compared to similarly aged males, suggesting that the greater MSNA may be required to ensure sufficient peripheral vasoconstriction to regulate blood pressure. Furthermore, previous work found that in young adults, the central and peripheral arcs of the baroreflex were inversely related in males, but not females. Given that postmenopausal females and young males exhibit a positive relationship between MSNA and total peripheral resistance (TPR) (16), it is possible that the inverse relationships between the central and peripheral arcs of the baroreflex may exist in females following menopause and the loss of estradiol. This background formed the rationale for ***Study 2*** of this dissertation in which the baroreflex control of MSNA and sympathetic transduction into blood pressure and limb vascular conductance were evaluated at rest in young and older males and females.

Contrary to the resting state, less is known about how age and sex impact the sympathetic neural control of the circulation during physiological stressors such as exercise. Older (postmenopausal) females exhibit greater blood pressure increases during small muscle mass exercise compared to older males, as well as young males and females, primarily driven by larger

increases in systemic vascular resistance (37). Although this larger elevation in systemic vascular resistance was attributed to greater sympathetic nervous system activity, direct measures of MSNA were not completed, and the interactive effects of age and sex on the sympathetic neural control of the circulation during exercise remains unknown. Thus, the objective of ***Study 3*** was to assess the impact of age and sex on sympathetic neural discharge patterns during rhythmic handgrip exercise to fatigue as well as during post-exercise circulatory occlusion (PECO). This model was selected as it provides a unique, and minimally invasive approach in which the central and peripheral determinants of sympathetic vasomotor outflow can be partitioned and studied separately.

Finally, it is well established that endogenous female sex hormones impact the sympathetic nervous system, with estradiol attenuating the central generation of sympathetic activity (38) and the degree of sympathetically mediated vasoconstriction on the peripheral vasculature (39). However, less attention has been directed toward assessing the sympathetic neural control of the circulation in females whose natural production and fluctuations of sex hormones are attenuated by synthetic, exogenous hormones. The paucity of research in this group of individuals is problematic as at least 20% of females of childbearing age use hormonal contraception, and oral hormonal contraception use is associated with a greater risk of developing hypertension (40). To date, a handful of studies have assessed the impact of oral contraception on sympathetic nervous system activity and blood pressure regulation (41–44); however, their impact on AP discharge patterns and sympathetic vasoconstriction during exercise remain unclear. Thus, the purpose of ***Study 4*** was to assess the impact of biological sex, and oral contraception pill use on the sympathetic AP discharge patterns and the subsequent peripheral vasoconstrictor responses during exercise.

Therefore, the **overall objective** of the present dissertation was to investigate the effects of age, biological sex, and sex hormones on the sympathetic neural discharge patterns that serve

to regulate vasomotor tone and blood pressure at rest and during exercise. The **working hypothesis** is that age, sex, and sex hormones impact the discharge behaviour of muscle sympathetic action potentials as well as the corresponding changes in peripheral vascular tone and blood pressure at rest and during exercise. This dissertation includes four distinct studies, each of which addresses an independent aspect of the overall hypothesis:

**Study one:** ***Aging is associated with enhanced central but impaired peripheral arms of the sympathetic baroreflex arc***

Hypothesis: The spontaneous baroreflex control of muscle sympathetic action potentials and the transduction of MSNA into blood pressure would be attenuated in older compared with young adults.

**Study two:** ***Age- and sex-related changes in sympathetic vascular transduction and neuro-hemodynamic balance in humans***

Hypotheses: a) older adults, especially older females, would demonstrate smaller changes in blood pressure and leg vascular conductance following bursts of MSNA, b) an inverse relationship between sympathetic baroreflex sensitivity and sympathetic transduction into leg vascular conductance would exist in all groups except young females, and c) resting sympathetic outflow would be inversely related with sympathetic transduction into mean arterial pressure and leg vascular conductance, but positively related with sympathetic baroreflex sensitivity in all groups except young females.

**Study three:** ***The interactive effects of age and sex on the neuro-cardiovascular responses during fatiguing rhythmic handgrip exercise***

Hypotheses: a) older females will have the greatest increases in blood pressure, integrated MSNA and AP recruitment during exercise and PECO compared to all other groups (i.e., interactive effect

of age and sex), b) young adults will demonstrate greater AP recruitment than older adults during exercise and PECO (i.e., independent effect of age), and c) young males will have larger increases in blood pressure, sympathetic discharge during exercise and PECO compared to young females (i.e., independent effect of sex)

**Study four:** ***The effects of sex, menstrual cycle and oral contraception on muscle sympathetic action potential discharge patterns and vascular transduction during exercise***

Hypothesis: that males and females using oral contraception will have greater muscle sympathetic action potential recruitment and greater sympathetically-mediated vasoconstriction during exercise and post-exercise circulatory occlusion than naturally-menstruating females.

### 1.1 The sympathetic nervous system

The sympathetic nervous system's neural communication pathway starts with signal initiation in the brain and terminates when the signal reaches its end organ target. Specifically, sympathetic activity originates in premotor neurons in the brainstem nuclei (e.g., rostral ventrolateral medulla, ventromedial medulla, caudal raphe nuclei), the midbrain and the hypothalamus (45, 46) that project onto pre-ganglionic neurons in the interomediolateral (IML) column located in the T1-L2 vertebrae of the spinal cord (45). Here, preganglionic neurons synapse onto postganglionic fibers in the paraspinal ganglia (e.g., paravertebral ganglia) (2) via the release acetylcholine which binds to nicotinic cholinergic receptors on the postganglionic neuronal cell body. The binding of acetylcholine stimulates an action potential (AP) in a post-ganglionic neuron that descends along the axon until it reaches its effector organ. The arrival of an AP at the nerve terminal depolarizes the cellular membrane which opens voltage-gated calcium channels, resulting in calcium influx and the exocytosis of vesicles containing neurotransmitters (2). Norepinephrine (NE) is the primary neurotransmitter released from postganglionic c-fibers (47, 48) and binds to post-synaptic

α-1 and α-2 adrenergic receptors on vascular smooth muscle cells. A-1 adrenoreceptor activation induces vasoconstriction through a Gq-protein that stimulates phospholipase C production, leading to inositol triphosphate (IP3) production and sarcoplasmic IP3 receptor binding, which in turn increases intracellular calcium concentrations and protein kinase C activation (49). The intracellular calcium influx activates calmodulin-dependent myosin light chain kinase to increase myosin ATPase activity, which induces vascular smooth muscle cell contraction via actin-myosin binding. Conversely, postsynaptic α-2 adrenergic receptors induce vasoconstriction via inhibitory G-proteins (Gi-protein) that prevent adenylate cyclase activity and the subsequent production of cyclic adenosine monophosphate (cAMP), which provides less inhibition of myosin light chain kinase, resulting in more phosphorylation of myosin light chain, and greater intracellular calcium levels (50). Although norepinephrine is the primary neurotransmitter released from the nerve terminal, it is accompanied by the release of co-transmitters like neuropeptide Y (NPY) and adenosine triphosphate (ATP) which bind to NPY Y1and $P2X_1$ receptors, respectively (50). Like α-2 adrenergic receptors, NPY Y1R causes vasoconstriction via a Gi-protein pathway that inhibits adenylate cyclase, and decreases cAMP production, ultimately increasing intracellular calcium levels (50, 51). Conversely, when ATP binds to $P2X_1$ receptors, it undergoes a conformational change that permits intracellular calcium influx, which depolarizes smooth muscle cells and results in vasoconstriction (51). Importantly, norepinephrine, NPY and ATP also bind to pre-synaptic α-2 adrenergic receptors, NPY Y2 receptors, and P2Y receptors, respectively, that inhibit further pre-synaptic release of these vasoconstrictive neurotransmitters via a negative feedback loop (50–52). This pre-synaptic control of neurotransmitter release plays a pivotal role in tightly governing the degree of sympathetically mediated vasoconstriction. A graphical overview of the neuroeffector junctional control of neurotransmitter release can be found in Figure 1.1.

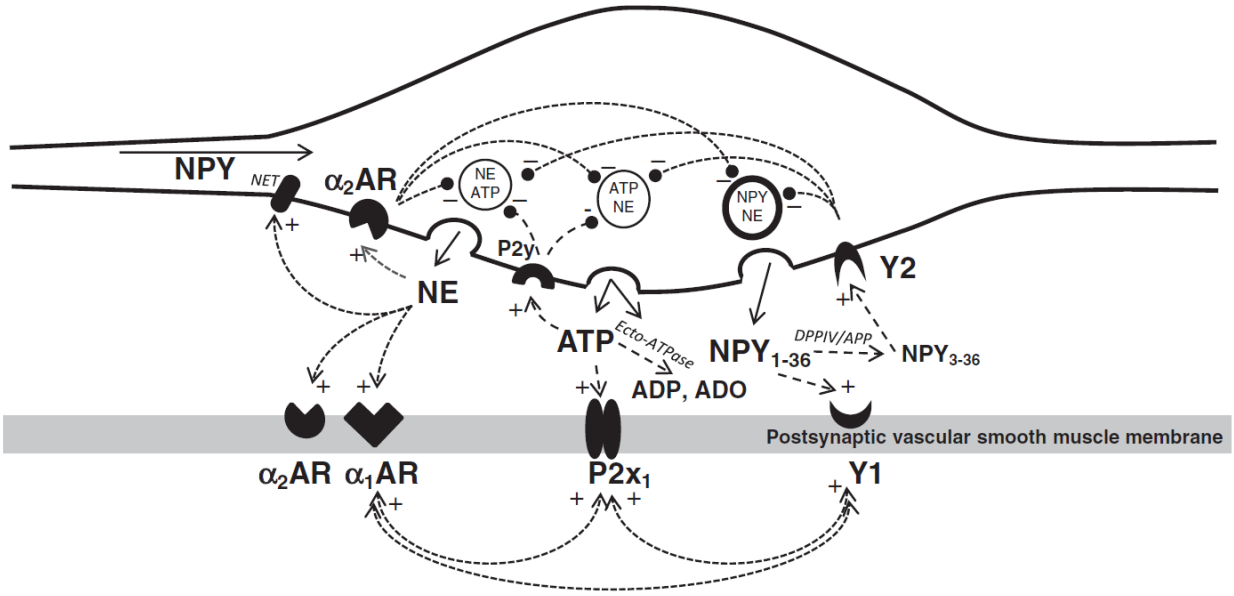


**Figure 1.1** An overview of the mechanisms underlying neurotransmitter release into the neurovascular cleft, the enzymatic control of neurotransmitter concentration in the neurovascular cleft, and the binding of neurotransmitters to their respective post-junctional receptors on the vascular smooth muscle cell. DPPIV; dipeptidyl peptidase IV, APP; amino peptidase, $\alpha_1$AR; alpha 1 adrenergic receptor; $\alpha_2$AR; alpha 2 adrenergic receptor; Y1; neuropeptide Y1 receptor, Y2; Y2 receptor, ATP; adenosine triphosphate, P2x$_1$; purinergic vasoconstrictor receptor, NPY; neuropeptide Y, NE; norepinephrine. +, indicates excitatory actions. -, indicates inhibitory actions. Reused with permissions from Shoemaker and colleagues (52).

In the context of cardiovascular control, the sympathetic nervous system is primarily responsible for regulating arterial blood pressure and vascular tone (53). Although anticipatory "feed-forward" adjustments contribute to neurocirculatory regulation, afferent (feedback) circuitry represents the primary component of the short-term neural regulation of blood pressure and vascular tone (54, 55). In response to changes in the *milieu interieur,* information from peripheral nerve endings regarding blood pressure, metabolic or chemical changes in blood and muscle, lung stretch, or other physiological perturbations (56) are directed centrally, toward the brain, where central sympathetic nuclei integrate this information and generate an appropriate efferent sympathetic response to "correct" the physiological perturbation, via the aforementioned sympathetic neural communication pathway. For example, during a seemingly menial task like

standing upright, the force of gravity results in a re-distribution of blood flow toward the lower limbs, that if not compensated for, would result in a loss of consciousness due to reduced cardiac output (via lower venous return) and cerebrovascular perfusion (57). However, in most healthy individuals, the fall in stroke volume and cardiac output causes a reduction in blood pressure, which leaves the pressure-sensitive nerve endings located in the aortic arch and carotid arteries – the baroreceptors – unperturbed. The lack of baroreceptor activation results in a withdrawal of baroreflex-mediated sympathetic inhibition and a subsequently rapid increase in sympathetic outflow toward the heart and peripheral vasculature (58). As a result, blood pressure is maintained/elevated via increases in heart rate and peripheral vasoconstriction (to limit limb blood flow and increase venous return) (58, 59). From this example alone, it can be appreciated that the sympathetic nervous system plays a pivotal role in ensuring homeostasis via efferent sympathetic messages directed toward peripheral target organs that are finely tuned to the level of stress incurred.

### 1.2 Sympathetic neural control of the circulation at rest

An important aspect of the sympathetic neural control of the circulation is that each segment of the neural communication pathway (as outlined above in section 1.1) can be uniquely modified, be it through neural reflex pathways, structural neuro-anatomical changes, neurochemical signalling, or humoral influences, highlighting the tightly coordinated and integrative nature of the sympathetic neural control of the circulation. Thus, a thorough understanding of each segment is critical to gain insight into the integrative sympathetic neurocirculatory control, and the reader is referred to exhaustive reviews on each of these segments of the neural communication pathway (52, 60, 61). The use of microneurography to assess MSNA discharge patterns – which forms the scope of this dissertation – is commonly used to study the sympathetic neural reflexes (i.e., one aspect along the sympathetic neuro-cardiovascular cascade) governing changes in vasomotor tone and blood pressure at rest and

during acute physiological perturbations. In a resting state, the arterial baroreflex is the primary neural reflex involved in blood pressure regulation, and the following section provides a non-exhaustive review of the arterial baroreflex.

### 1.2.1 The arterial baroreflex: central and peripheral arcs

The arterial baroreflex regulates blood pressure on a beat-by-beat basis via a negative feedback control system that can be broken down into two components: 1) neural/central and 2) peripheral arcs (28, 62) (Figure 1.2). The central arc of the baroreflex represents the feedback responses to changes in blood pressure and the subsequent generation of efferent sympathetic discharge. Arterial baroreceptors are mechanosensitive receptors that detect changes in transmural pressure (or stretch) within the luminal walls of the carotid sinuses and the aortic arch (22, 63). Thus, changes in transmural pressure alter the firing rate of afferent mechanosensitive baroreceptors (64). Carotid baroreceptor afferent discharge is conveyed via the sensory ganglia of cranial nerve IX (the glossopharyngeal nerve), whereas aortic baroreceptor discharge travels via cranial nerve X (the vagus nerve) toward the brainstem, where these afferent neurons synapse in the nucleus tractus solitarius (NTS) of the medulla oblongata (22). Here, NTS neurons provide excitatory synaptic input (via the release of glutamate) onto the nucleus ambiguus and the dorsal motor nucleus of the vagus, which increases efferent parasympathetic outflow toward the heart (22, 55). Conversely, the NTS neuronal projections act via a different pathway in their regulation of efferent sympathetic outflow. Specifically, excitatory synaptic input projects onto the caudal ventrolateral medulla (CVLM), which stimulates the release of γ-amino butyric acid (GABA) from the CVLM interneurons, resulting in an inhibition of preganglionic neurons in the rostral ventrolateral medulla (RVLM) – the primary medullary site involved in the regulation of efferent sympathetic discharge (55). Thus, when blood pressure transiently rises, increased afferent baroreceptor discharge stimulates the NTS, which excites the CVLM, thereby inhibiting RVLM activity and the subsequent generation of efferent sympathetic vasoconstrictor discharge. This

cascade of events serves to limit the rise in blood pressure and permits blood pressure to return to its setpoint. Conversely, when blood pressure transiently falls, there is an insufficient change in transmural pressure to stimulate the arterial baroreceptors, resulting in less NTS excitation, less CVLM activation, and therefore less inhibition of the RVLM. As such, increases in efferent sympathetic vasoconstrictor discharge directed toward the peripheral vasculature are pivotal to drive blood pressure upward to its setpoint to prevent syncope (i.e., fainting).

Paramount to the successful fine tuning of beat-by-beat blood pressure via the arterial baroreflex is the conversion (transduction) of sympathetic outflow into a vasoconstrictor response. Indeed, this phenomenon, termed sympathetic vascular transduction, represents the peripheral arc of the arterial baroreflex (Figure 1.2; Panel C). Briefly, successful sympathetic transduction requires: 1) Efferent neural signals successfully initiating neurotransmitter release into the synaptic cleft, 2) abundant neurotransmitter binding to their target post-synaptic receptors that exceeds the rate of neuronal reuptake (i.e., removal from the synaptic cleft), 3) net positive post-synaptic receptor binding that results in wide-spread signal transduction within the smooth muscle to elicit vasoconstriction, and 5) a sufficient degree of vasoconstriction that appreciably increases blood pressure (65, 66). As seen in Figure 1.2, this increase in blood pressure would feedback into the central baroreflex arc to inhibit further sympathetic neural discharge. Altogether, it is clear that the arterial baroreflex (both central and peripheral arcs) plays a pivotal role in the beat-by-beat regulation of blood pressure.

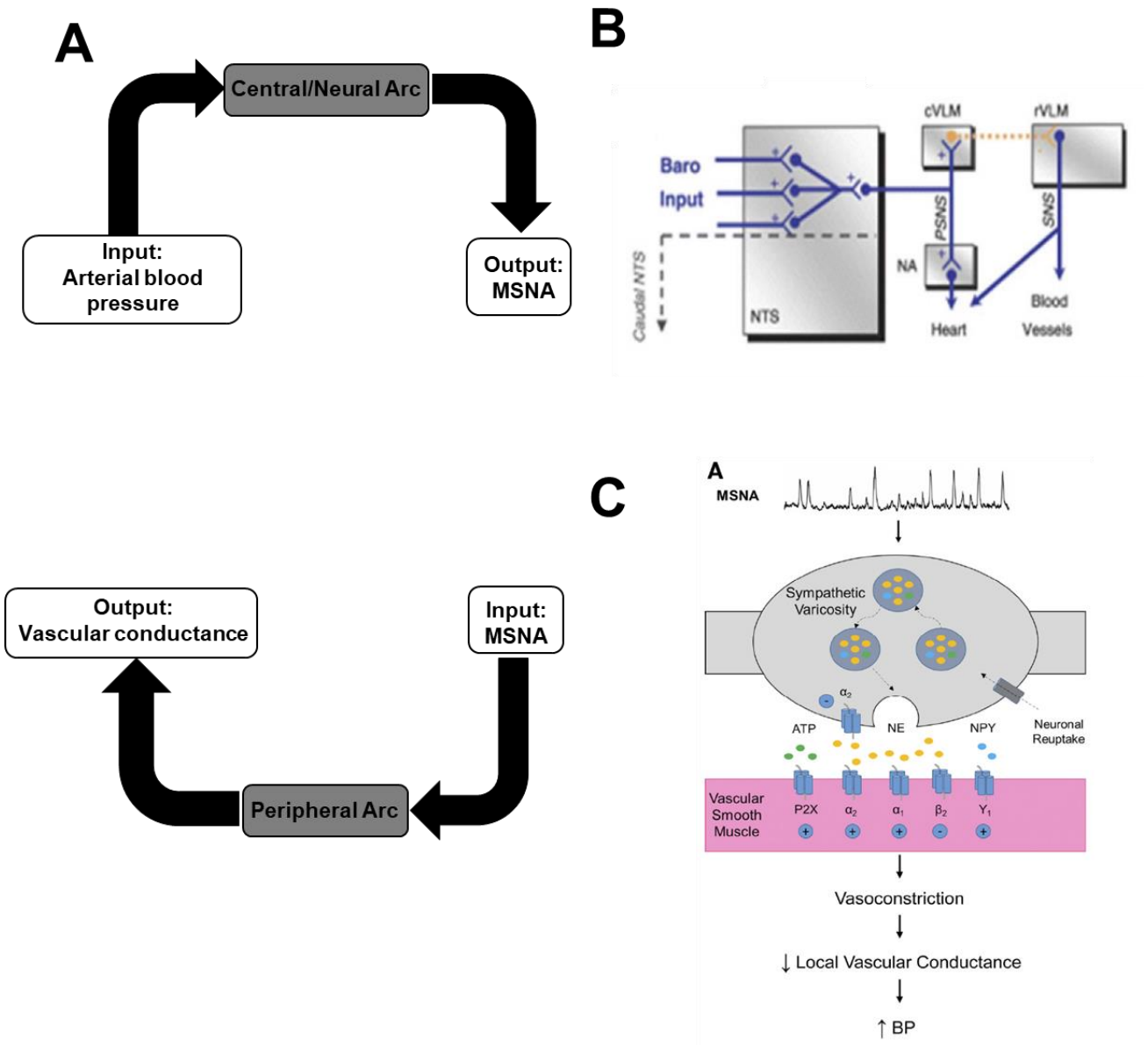


**Figure 1.2.** (A) A schematic of the central and peripheral baroreflex loop. (B) The baroreflex regulation of efferent sympathetic neural discharge at the level of the brainstem. (C) Sympathetic transduction of MSNA into a change in vascular conductance. NTS; nucleus tractus solitarius, NA; nucleus ambiguus, cVLM; caudal ventrolateral medulls, rVLM; rostral ventrolateral medulla, PSNS; parasympathetic nervous system, SNS; sympathetic nervous system $\alpha_1$; alpha 1 adrenergic receptor; $\alpha_2$; alpha 2 adrenergic receptor; $\beta_2$, Beta-2 adrenergic receptor, $Y_1$; neuropeptide Y1 receptor, ATP; adenosine triphosphate, P2x, purinergic vasoconstrictor receptor, NPY; neuropeptide Y, NE; norepinephrine. Panel A is adapted from Ogoh et al. (62), and panel C is reused from Young et al. (67).

### 1.3 Sympathetic neural control of the circulation during exercise

The sympathetic nervous system's response to exercise is mediated by the integration of feedback from peripheral afferents located throughout the body (e.g., muscle mechano/metaboreceptors, the arterial baroreflex), and feed-forward signals from supramedullary brain areas (i.e., central command) (68) in the NTS of the brainstem (Figure 1.3). The integration of these neural signals evokes the appropriate efferent sympathetic and parasympathetic outflows required to maintain blood pressure while re-distributing blood flow to areas of high metabolic demand during exercise (i.e., active skeletal muscle) (55). The following discourse provides a non-exhaustive overview of the neural reflexes that govern the cardiovascular responses to exercise in humans.

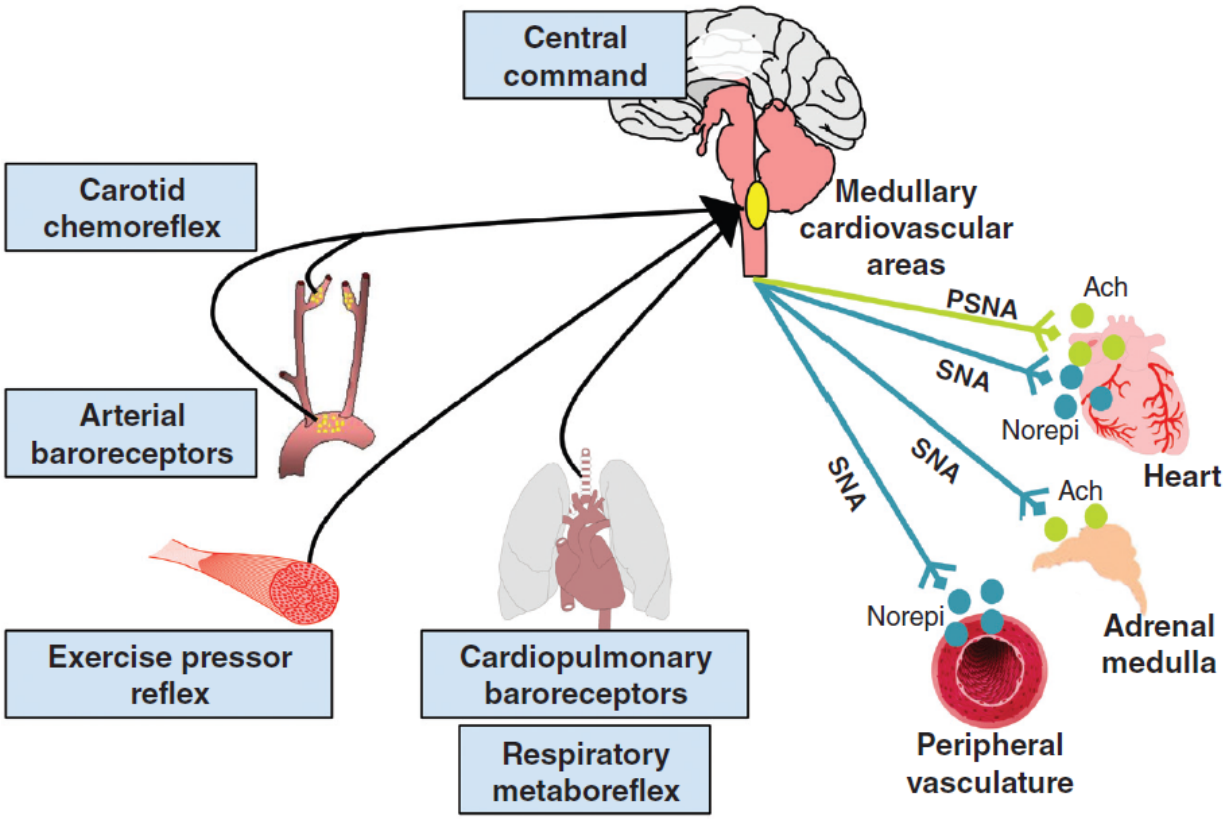


**Figure 1.3.** Graphical summary of the mechanisms involved in mediating the sympathetic neurocirculatory adjustments to exercise. Reused with permissions from Fisher et al. (68).

### 1.3.1. Central command

Central command refers to a feed-forward mechanism whereby motor cortex (i.e., supramedullary) activation and anticipation impact the central efferent and peripheral afferent mechanisms involved in cardiovascular control (69). For example, many groups have found that using antagonistic tendon vibration on an exercising muscle (which produces disynaptic motorneuron inhibition and increases central command), heightens the cardiovascular responses to exercise, whereas agonistic tendon vibration (which assists in muscle tension development and reduces central command), dampens the cardiovascular responses to exercise (70, 71). Furthermore, an elegant series of studies demonstrated that imagining (72, 73) or passively viewing exercise (74, 75), increases blood pressure, respiratory rate, heart rate, and MSNA, indicating that perceived exertion plays a critical role in central command-mediated cardiovascular control. Indeed, strong evidence of descending neural signals involved in the anticipatory aspect of central command comes from stimulation of locomotor brain areas in animals and humans which invoke cardiovascular responses, despite no active muscular contractions (76, 77). Brain imaging studies have also advanced our understanding of the cerebral regions involved in the anticipatory, effort perception, and motor aspects of central command-mediated cardiovascular responses (69). The anticipatory aspect of central command is associated with activation of the periaqueductal grey and anterior cingulate cortex (76–78), effort perception appears to involve the insular cortex (79–81) the anterior cingulate cortex (80, 82–84), and deactivation of subthalamic nuclei (77), whereas the motor aspect of central command involves the thalamus (72, 82, 85), hypothalamus (86), and mesencephalic locomotor region (87, 88). Additionally, central command has been reported to reduce activity in the medial prefrontal cortex and hippocampus (83, 89), likely to dampen parasympathetic outflow and increase sympathetic drive.

Contrary to central command's influence on the cardiovascular responses to exercise, less is known about its role in increasing sympathetic outflow in humans. MSNA increases during

low-intensity electrically-evoked contractions – which bypass central command – but, during voluntary contractions, that engage central command, MSNA is reduced or remains unchanged (90, 91). Conversely, central command appears to contribute to the regulation of efferent sympathetic nerve traffic during high intensity voluntary contractions as the increases and synchronization of sympathetic outflow with muscle contractions (i.e., effortful periods) remained following neuromuscular blockade with curare, which drastically reduced external force production (92). Thus, it is generally thought that central command contributes to the regulation of MSNA only during high intensity static exercise. However, more recent work has demonstrated that central command increases the strength of sympathetic nerve traffic (i.e., MSNA burst size) during zero load single-leg cycling exercise (93), indicating that the impact of central command on MSNA may be dependent on exercise modality.

### 1.3.2. Exercise Pressor Reflex

Evidence of the exercise pressor reflex's impact on blood pressure regulation dates back to 1937 when Alam and Smirk observed a blood pressure increase during rhythmic exercise that was sustained when blood flow was occluded following exercise and exacerbated during ischemic exercise (94). Since then, there has been ample amounts of work examining the mechanisms underlying the exercise pressor reflex and its role in blood pressure regulation and sympathetic outflow (see (95) for a detailed review). In response to mechanical stretch and chemical changes in skeletal muscle during exercise, specialized skeletal muscle nerve endings that are sensitive to mechanical deformation (primarily type III sensory neurons) and metabolic changes (primarily type IV sensory neurons) provide afferent feedback to the cardiovascular control centers (96–101). However, these sensory neurons are polymodal, with some Type III neurons being sensitive to changes in the metabolic milieu of the muscle, and some Type IV neurons being sensitive to mechanical stimuli (99–102). Together, these nerve endings comprise the afferent arm of the exercise pressor reflex, and when stimulated, elicit large increases in blood pressure and

sympathetic nerve activity during exercise (14, 91, 103), albeit after a ~60s delay (104, 105). It is well established that the muscle mechanoreflex contributes to the elevation of sympathetic discharge during exercise as studies using electrical stimulation or passive stretching of a muscle to elicit mechanical deformation in muscle consistently exhibit increases in sympathetic outflow (91, 106). Conversely, isolating the metaboreflex during exercise is difficult, as central command (i.e., central mechanism) and somatic input from skeletal muscle project (i.e., peripheral mechanism) onto the NTS and the RVLM to permit elevations in blood pressure and sympathetic outflow (107). Thus, to non-invasively isolate the muscle metaboreflex's contribution to the generation of sympathetic nerve discharge, many studies have employed a post-exercise circulatory occlusion (PECO) paradigm like Alam and Smirk (94) to trap metabolites produced during exercise. In doing so, many groups have found that blood pressure and MSNA remain elevated following exercise (14, 19, 91, 103, 108). Altogether, these data clearly indicate that group III/IV skeletal muscle afferents contribute greatly to increases in MSNA during exercise.

### 1.3.3. Arterial baroreflex resetting

Given that the baroreflex is inherently a sympathoinhibitory neural reflex, it was initially surprising that heart rate and blood pressure increased at the onset of exercise (109). However, over the past century, experimental evidence in humans has made it clear that the baroreflex is reset towards a higher operating point to permit increases in heart rate and blood pressure in an intensity-dependent manner (55, 107, 110, 111). Pivotal to the resetting of the arterial baroreflex are the central (central command) and peripheral inputs (exercise pressor reflex) that converge onto the NTS to inhibit baroreceptor afferent input (55, 107). Specifically, central command activation via antagonistic tendon vibration (71) and neuromuscular blockade (112, 113), or attenuation via agonistic tendon vibration (71) resets the baroreflex to either a higher or lower operating point, respectively. Similarly, exercise pressor reflex stimulation using medical anti-shock trousers resets the baroreflex to a higher operating point (114), whereas sensory nerve

blockade attenuates group III/IV afferent feedback and resets the baroreflex to a lower operating point (115, 116). Notably, combined augmentation of central command and the exercise pressor reflex results in an even greater resetting of the arterial baroreflex than stimulation of one of these reflexes alone (117). Altogether, it can be appreciated that the sympathetic neural reflexes work in a highly integrative manner to ensure appropriate neuro-cardiovascular adjustments to exercise.

### 1.4 Measuring Muscle Sympathetic Nerve Activity in Humans

Microneurography represents the only direct measure of sympathetic APs in humans. The microneurographic technique involves the insertion of a tungsten microelectrode into a peripheral nerve, typically in the arm (e.g., medial, radial, ulnar) or the leg (e.g., tibial and peroneal), with delicate manipulation of the microelectrode tip until it enters into a fascicle of neurons (Figure 1.4) (118). As such, it can be used to measure several different neuronal firing properties such as sympathetic nerve traffic directed toward muscle, skin, or even the discharge properties of motor axons (118–120). For the measurement of postganglionic c-fiber discharge directed toward the skeletal muscle (i.e., MSNA), the microelectrode tip would be manipulated until it enters a muscle fascicle nearby a discharging bundle of postganglionic axons (121). A key characteristic of MSNA is the bursty nature of sympathetic discharge that is driven by the strong baroreflex control of postganglionic APs, causing them to depolarize very close together in time in a pulse-synchronous manner (122, 123). Indeed, the cardiac entrained nature of MSNA is a distinguishable characteristic from pools of other postganglionic c-fiber axons, such as skin sympathetic nerve activity, which does not exhibit strong arterial baroreflex control, and therefore is not gated to the cardiac cycle (124).

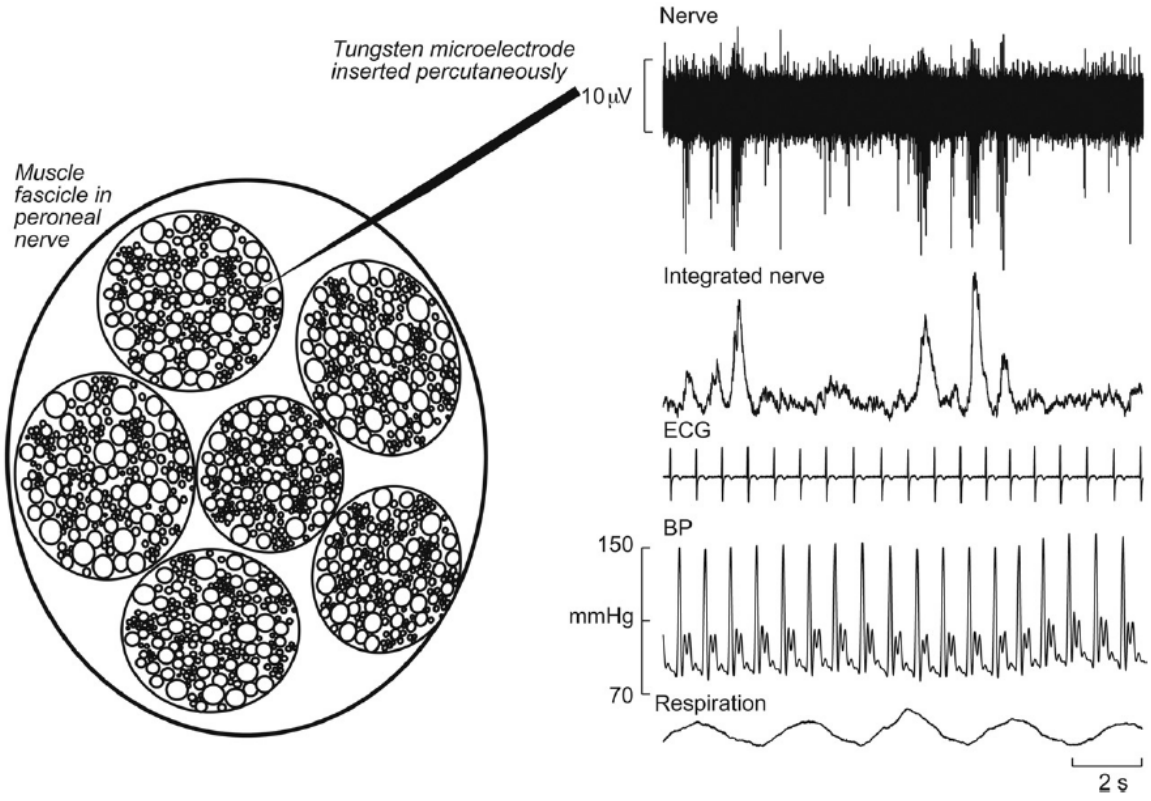


**Figure 1.4.** *Left:* The placement of a tungsten microelectrode into a muscle fascicle of the peroneal nerve to record muscle sympathetic nerve activity. *Right*: The amplified and bandpass filtered raw neurogram is presented on the top right, with the integrated and rectified neurogram below. Electrocardiography (ECG), blood pressure (BP) and respiration are also shown below the neurogram. Reused with permission from Macefield, 2013 (125).

### 1.4.1 Integrated neurogram

MSNA is often measured at the multi-unit level, providing insight into the activity of groups of sympathetic axons. Due to the low signal-to-noise ratio of raw sympathetic neuronal recordings, the sympathetic neurogram is amplified (50,000 to 100,000 times), filtered (700-2,000 Hz band pass), rectified and integrated to provide a smoothed neurogram that displays triangular-shaped bursts of muscle sympathetic action potentials (Figure 1.4) (123). This level of the neural recording is known as the integrated MSNA neurogram and it is the most utilized nerve signal to quantify MSNA. Integrated MSNA burst analysis provides insight into the occurrence and strength

(i.e., size) of sympathetic activity (126). MSNA burst occurrence is commonly expressed as burst frequency (bursts per minute) or burst incidence (bursts per 100 heart beats), which are indicative of the probability of a burst occurring, whereas MSNA burst strength is expressed as the amplitude (i.e., height) of a burst, and reflects the number and size of sympathetic axons firing within the burst of MSNA (7). While MSNA burst occurrence has been shown to be reproducible within an individual between multiple recording sessions (127–129), interpretation of MSNA burst strength can be more complex. Specifically, MSNA burst size is impacted by the proximity of the microelectrode tip to a bundle of postganglionic c-fibres, and the microelectrode position can shift during an experimental session. Although expressing burst strength relative to the strongest burst (i.e., largest burst) within a neural recording has been successful in identifying inter-individual differences (130), many studies have focused on MSNA burst occurrence alone due technical difficulties with maintaining a given electrode position (131). Although MSNA burst occurrence provides important insight into the arterial baroreflex control of sympathetic outflow, (126), it is limited in its interpretation of the broader features of the sympathetic nervous system (7). Integrated MSNA bursts also express latencies that are quantified as the time delay from the initiating cardiac cycle (R-spike in the electrocardiogram) to the peak height of the burst, usually ranging between 1.2-1.5 seconds, with the most influence arising from the slow conduction velocity of unmyelinated axons which is ~1.1 m/s (132, 133). MSNA burst latency reflects the time required for the brain stem generated signal to arrive at the postganglionic recording site, which incorporates all aspects of the sympathetic neural communication pathway outlined section 1.1. A notable feature of MSNA burst latency is that it is inversely related with burst size, such that larger bursts express faster conduction velocities than smaller bursts (134). Thus, it was hypothesized that modifiable central synaptic delays and/or subpopulations of larger, faster conducting sympathetic axons may exist in the architecture of the sympathetic nervous system. However, given that multi-unit integrated MSNA analysis represents the summation of many single neurons firing together, an inherent limitation of integrated multi-unit MSNA analysis is the

assumption that all neurons possess similar activity levels, firing patterns, and response patterns to stressors.

### 1.4.2. Action potential analysis

Advancements in our understanding of neural coding strategies in human efferent sympathetic nerve traffic were made by Macefield and colleagues in the mid-1990s, with the introduction of single-unit (i.e., axon) MSNA recordings (135). Using this methodology, Macefield and colleagues evaluated the discharge properties of single vasoconstrictor axons, finding that, at rest, an axon fires approximately once per integrated MSNA burst in healthy individuals (135), and possibly more often in pathological states characterized by sympathetic hyperactivity (136, 137). Notably however the probability of single-unit firing, and multiple within-burst firing of single axons increases during acute physiological stressors such as an apnea (i.e., breath hold), which contributes to increases in multi-unit MSNA burst size (138). However, only ~40% of bursts exhibited multiple single-unit discharge during apneic stress, indicating that increases in firing probability and multiple firing of already active axons do not fully explain the increases in multi-unit burst size during sympathetic stresses (138). Thus, it was hypothesized that another recruitment strategy must be the primary mechanism involved in increasing sympathetic burst amplitude during acute physiological stressors – the recruitment of larger axons. However, since single-unit MSNA analysis uses a strict AP waveform shape-matching method that sorts APs based on their morphology, instead of peak-to-peak amplitude, this approach is unable to detect the recruitment of larger neurons.

To assess the recruitment of larger, faster conducting sympathetic c-fibers during physiological stress, APs must be extracted from the multi-unit neurogram. Although multiple methodologies have been developed to interrogate multi-unit APs (139–141), majority of the multi-unit sympathetic AP research in the past decade has employed a wavelet-based methodology

that detects APs from a mother wavelet constructed from real human muscle sympathetic APs (8). Thus, the following discourse in this dissertation will focus on this technique and related outcomes. Briefly, the method by Salmanpour et al. (8) identifies and extracts the exact location of individual APs using a modified continuous transform of the raw, filtered neurogram. Groups of APs are then clustered based on their overall shape using a K-means 32-point window length (i.e., 3.2 ms at a 10 kHz sampling rate that provides a sampling frame long enough to represent a human sympathetic AP. Thereafter, extracted APs can be ordered into bins on a peak-to-peak amplitude basis either manually or using Scott's rule (Figure 1.5) (142). This approach permits the assessment of the recruitment of larger, previously silent axons (i.e., high threshold) or increased firing of previously silent axons (i.e., low threshold), provided that the electrode remains stable throughout the baseline and sympathetic stress recording period. Additionally, the timing of an AP within a given integrated burst relative to the R-wave preceding the systolic pulse that inhibited the burst can be identified.

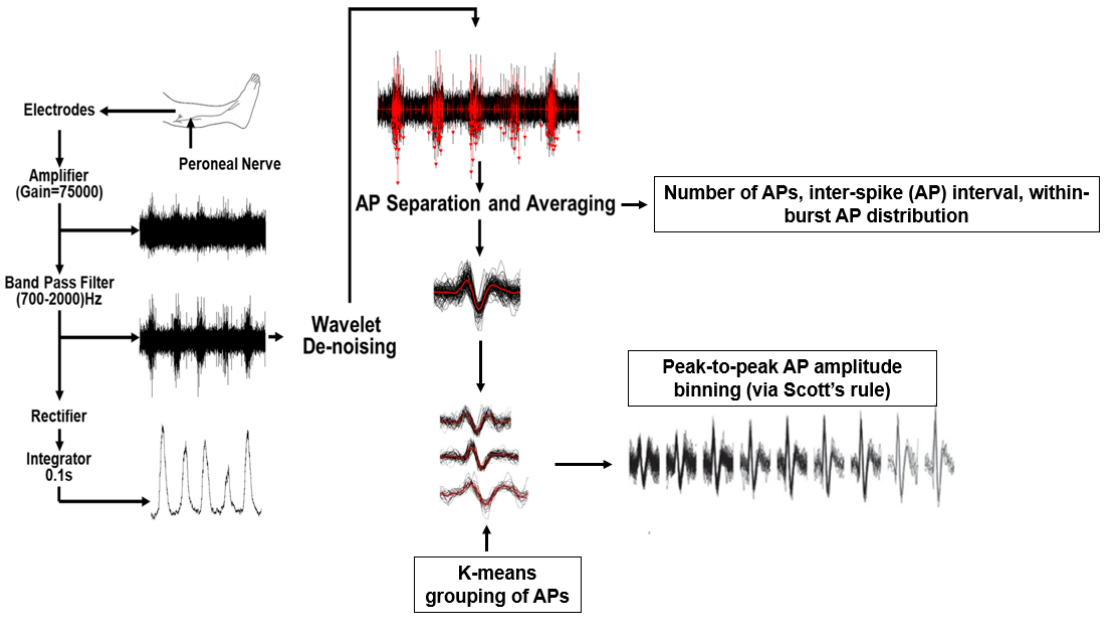


**Figure 1.5** A graphical overview of the procedures involved in extracting muscle sympathetic action potentials (APs) from the amplified, band-pass filtered neurogram using the modified continuous wavelet transform. Adapted from Shoemaker et al. 2018 (7).

Over the last decade, this wavelet approach has exposed the underlying nature of the AP discharge patterns that comprise integrated multi-unit MSNA bursts at rest and during physiological stress. For example, a characteristic property of APs is the heterogenous discharge patterns of varying-sized axons, with medium-sized APs exhibiting the highest firing probabilities, whereas small and larger-sized APs fire less frequently, ultimately resembling an inverted “U” shaped distribution (9, 10). Additionally, this analytical approach has clearly demonstrated an ordered pattern of recruitment within the sympathetic nervous system that serves to increase integrated MSNA burst occurrence and strength during acute physiological stressors. The first recruitment strategy is rate coding, which represents the increased firing probability of APs that were previously active at rest and is reflected in increases in MSNA burst occurrence and greater within-burst AP firing frequency (11, 12). As previously noted, since this wavelet approach uses a multi-unit recording, it does not track APs arising from single units. Thus, rate coding in this context refers to an increase in the firing probability of similarly sized APs (i.e., clusters) that were already active at rest. The second neural communication strategy is population coding, which involves the recruitment of larger, faster conducting AP clusters that were not active at rest (11, 12). Of note, rate- and population coding of muscle sympathetic APs have been observed under baroreflex (10, 11, 143, 144), chemoreflex (12, 13, 145), and metaboreflex (14, 146) stressors, indicating that these strategies are not necessarily reflex-specific; rather, they appear to be specific to the degree of sympathetic stress. Specifically, rate coding is employed during mild sympathetic stresses (11), whereas both rate and population coding strategies are utilized during severe sympathetic stressors, likely to enhance the degree of neurogenic vasoconstriction (11, 12).

This wavelet approach also provides important insight into the relationship between AP cluster size and latency. Like MSNA burst latency, AP latency represents the time required for the neural signal generated in the brainstem to arrive at the axonal recording site and is quantified

as the time between the R-wave associated with AP occurrence and the peak negative deflection of the AP (11). The assessment of AP latency proved critical to the interpretation of axonal size within the sympathetic neurogram, as the presence of larger APs may just reflect movements in the microelectrode position. However, Cable theory indicates that axonal size is proportional to its conduction velocity and AP amplitude (147). Thus, larger APs should express faster conduction velocities (i.e., shorter latencies). Accordingly, an inverse relationship between AP cluster size and latency has been observed, whereby larger APs express shorter reflex latencies than smaller APs (11, 15, 146, 148), providing strong evidence against the notion that larger APs reflect depolarization of axons closer to the microelectrode tip. Notably, Macefield and colleagues demonstrated a high degree of variability in the latency of single-unit axonal recordings (135), suggesting that synaptic delay variations exist in the sympathetic nervous system. In support of this, our laboratory has consistently demonstrated that AP latency is a modifiable feature of multi-unit MSNA discharge. For example, during a Valsalva's maneuver or an apnea, all AP clusters were recruited with a shorter reflex latency compared to a resting state (12, 31, 149), whereas during a passive baroreflex stress (i.e., lower body negative pressure) there was either no change in AP latency, or an upward shift in the AP cluster size-latency relationship, such that all APs were expressed with longer reflex latencies compared to rest (11, 15). While these data suggested that a central perceptual effort aspect was required to alter reflex latency, it was not until more recently that this was assessed. Using an isometric handgrip exercise (i.e., central command and exercise pressor reflex mediated increases in MSNA) and PECO (i.e., metaboreflex-mediated increases in MSNA) model, Badrov and colleagues demonstrated a downward shift in the AP cluster size-latency relationship during exercise, but no change from baseline during PECO (14), clearly indicating that central command/perceptual effort is required to acutely increase the conduction velocity of active sympathetic c-fibers during physiological perturbations.

Altogether, these sympathetic AP discharge patterns represent the language by which the sympathetic nervous system communicates with the peripheral vasculature to ensure that appropriate homeostatic adjustments are made in a timely manner. Although these analyses have provided unique insights into the sympathetic nervous system's communication strategies, there is limited data exploring how these recruitment patterns may be affected by inter-individual factors like age, biological sex, and sex hormones, all of which are known to impact efferent sympathetic nerve traffic and the regulation of arterial blood pressure (150, 151).

## 1.5 Sex hormones and sympathetic neuro-cardiovascular control

There is growing evidence that sex hormones affect both the central generation of sympathetic outflow, as well as the peripheral vasoconstrictor responses to sympathetic stimuli (39, 150, 152). Thus, the following section will provide a non-exhaustive review of the sex steroid hormones and their central and peripheral impact on the sympathetic neural control of the circulation.

### 1.5.1. Central sympathetic neural effects of sex hormones

#### *1.5.1.1. Estradiol*

Several neurons in the central nervous system express estrogen receptors, with estrogen receptor α (Erα) being the predominant estrogen receptor subtype in the brain (153). Estradiol directly impacts the central generation and efferent transmission of neural communications, as intravenous administration of estradiol in a rat model increases efferent parasympathetic outflow and decreases sympathetic outflow (154). However, estradiol's impact on central nuclei is region-specific in the brain. For example, estradiol injected into the amygdala reduced sympathetic outflow, whereas estradiol injected into the lateral hypothalamic area increased efferent sympathetic discharge (155).

The stimulation of central estrogen receptors also alters baroreflex sensitivity (i.e., the baroreflex's ability to effectively buffer changes in arterial blood pressure). Seven days of estrogen supplementation increased cardiovagal baroreflex sensitivity in ovariectomized rats (i.e., rats with surgically removed ovaries), an effect that was blocked when an estrogen receptor antagonist was administered to the nucleus ambiguus (156). Additionally, estrogen affects central adrenergic receptors, which serve to regulate the neuronal release of neurotransmitters. However, its effects are also region specific, with estradiol decreasing pre-synaptic inhibition of norepinephrine release (157) and increasing α-adrenergic receptor mRNA levels (158) in the hypothalamus, but downregulating α-adrenergic receptors in the cortex (158). Whilst these data indicate that the impact of estradiol on adrenergic receptor function is brain region-dependent, the net effect of these estradiol-mediated changes on efferent sympathetic neural communication remains unclear. Nonetheless, evidence from rat models indicates that estradiol attenuates the central generation of efferent sympathetic nerve traffic by enhancing nitric oxide (NO) bioavailability and downregulating the central sympatho-excitatory effects of the renin-angiotensin system (38, 159). Furthermore, in humans, females (pre-menopausal) typically exhibit lower MSNA than males (17, 21), suggesting that estradiol exerts a net central sympathoinhibitory effect.

*1.5.1.2. Progesterone*

Contrary to estradiol, much less is known about the central effects of progesterone on the sympathetic neural control of the circulation. Like estrogen, progesterone receptors have been found in areas of the brain, like the brainstem (160). Although there is clear evidence that progesterone metabolites (e.g., 3a-hydroxy-dihydroprogesterone) impacts central autonomic nuclei involved in baroreflex function (161), the independent effect of progesterone on the central neural control of the circulation remains less clear. Only one study to date has assessed the independent impact of progesterone on sympathetic outflow in humans, finding that the administration of progesterone (following GnRH antagonist administration to attenuate

endogenous sex hormone production), increased MSNA in 66% of individuals (162). While these data indicate that sympathetic postganglionic c-fiber firing rate may be augmented by progesterone, given the small sample size in this study (n=3), further investigations are warranted to confirm these observations. Thus, the complex physiological mechanisms underpinning the independent impact of progesterone on the central neural control of the circulation remains unclear.

*1.5.1.3. Testosterone*

In animal models, androgen receptors have been identified in many brain areas involved in the sympathetic neural control of the circulation such as the nucleus ambiguus, the area postrema, and the nucleus tractus solitarius of the brainstem (163). While the role of testosterone on the central mechanisms governing sympathetic outflow are unclear, animal models have demonstrated that testosterone suppression (via castration) attenuates baroreflex-mediated bradycardia, whereas testosterone supplementation enhances the baroreflex control of the heart (164). Furthermore, testosterone enhances tyrosine hydroxylase activity – an enzyme that is critically involved in norepinephrine synthesis – in the sympathetic ganglia (165). Thus, the positive relationship between testosterone and MSNA discharge in women with polycystic ovarian syndrome (166) and in healthy young males (167), suggests a role for testosterone in the central generation of efferent sympathetic vasomotor discharge.

**1.5.2. The impact of sex hormones on peripheral sympathetic vasoconstriction**

As previously mentioned, vascular smooth muscle cells are innervated by efferent sympathetic postganglionic c-fibers, which form the primary mechanism governing the regulation of vascular tone. Thus, changes in efferent sympathetic nerve traffic and the widespread transduction of that vasoconstrictor signal have significant effects on the regulation of blood pressure and distribution of blood flow. At the postganglionic neuroeffector junction, there are

several sites where sex steroid hormones could impact the transduction of the efferent sympathetic neural messages, including the: 1) Neurotransmitter (norepinephrine, ATP, NPY) release, re-uptake and degradation, and 2) post-junctional, vascular smooth muscle cell receptor activation/sensitivity (168). The following section will provide a non-exhaustive overview of the impact of sex hormones on the postganglionic neuroeffector junction and subsequent regulation of vascular tone.

*1.5.2.1 Estradiol*

Estradiol impacts many steps in the sympathetic vascular transduction cascade at the peripheral neuroeffector junction. For example, estradiol attenuates norepinephrine reuptake in the synaptic cleft (169, 170), and binds to catechol-O-methyltransferase (COMT), which reduces norepinephrine degradation (171). Collectively, the inhibition of neuronal norepinephrine reuptake and reduced norepinephrine degradation should prolong the impact of the sympathetic vasoconstrictor signal and elicit greater vasoconstriction. However, norepinephrine binds to post-junctional α- and β-adrenergic receptors which elicit vasoconstriction and vasodilation, respectively, and estradiol enhances post-synaptic β-adrenergic receptor sensitivity (172). In support of this, Kneale and colleagues demonstrated that, in humans, females had smaller forearm vasoconstrictor responses to norepinephrine infusions compared to males; however, following non-specific β-blocker (propranolol) administration, forearm vasoconstriction was significantly increased in females (39). In addition to estradiol's impact on norepinephrine, it exerts a role on NPY release and post-synaptic NPY Y1R expression. Specifically, using a rat model, Jackson and colleagues found that estradiol attenuated NPY Y1R activation and reduces NPY concentration in females (173), which likely explains why females exhibit less endogenous Y1R control of muscle blood flow compared to male rats (174). Thus, despite potentially prolonging the time of norepinephrine in the synaptic cleft, estradiol attenuates sympathetic vasoconstriction via augmented β-adrenergic vasodilation and attenuated NPY-Y1R interactions.

In humans, assessments of sympathetic neuro-hemodynamic balance indicate that young (premenopausal) females, do not exhibit a relationship between resting MSNA and total peripheral resistance (175) or limb blood flow (176), whereas these relationships exist in young males. Furthermore, following propranolol (β-adrenergic receptor blocker) administration, young females demonstrated a similar relationship between resting MSNA and TPR compared to young males and postmenopausal females (152). While these data provide strong support of the mechanistic evidence derived from animal models indicating that estradiol attenuates the transduction of sympathetically mediated vasoconstriction, evidence regarding the role of estradiol on sympathetic transduction remains equivocal (177).

*1.5.2.2. Progesterone*

Our current understanding of the impact of progesterone on the sympathetic regulation of vascular tone is limited. In humans, much of the work assessing the role of progesterone on vascular function is limited to the cutaneous circulation, with progesterone countering the vasodilatory effects of estradiol (178), and even enhancing adrenergic-mediated vasoconstriction in the cutaneous vasculature of females (179). Furthermore, in ovine uterine arteries, progesterone increased the vasoconstrictor response to nerve stimulation, whereas estradiol reduced vasoconstriction (180). Additionally, progesterone may increase vascular smooth muscle cell contractility via COMT inhibition, which would reduce norepinephrine degradation in the synaptic cleft (181). Importantly however, the possibility that progesterone-mediated sympathoexcitatory effects are driven by increased neurotransmitter release, or progesterone-enhanced post-synaptic adrenergic receptor sensitivity, cannot be excluded.

*1.5.2.3. Testosterone*

Circulating levels of testosterone are thought to play a critical role in the regulation of arterial blood pressure and vascular function (182–184). Indeed, patients with chronically low

(male hypogonadism), or high (individuals abusing anabolic steroids) testosterone levels exhibit vascular and autonomic dysfunction and are at an elevated risk of developing cardiovascular disease (185–189). In males, testosterone is thought to elicit both vasodilatory and vasoconstrictive effects. The reader is directed to (184, 190) for detailed reviews regarding the underlying mechanisms of testosterone-induced vasodilation. However, our understanding of the role of testosterone on sympathetically-mediated vasoconstriction is limited. Indirect evidence in humans implicates testosterone in the enhancement of neurogenic vasoconstriction as males exhibit greater alpha-adrenergic- (39) and NPY-mediated vasoconstriction (174, 191) compared to females. Indeed, these data are supported by direct evidence from animal models wherein the pressor responses to norepinephrine and tyramine (a drug that enhances the endogenous release of norepinephrine) infusions were greater following acute testosterone injections compared to control conditions (192, 193). Additionally, basal plasma norepinephrine levels are attenuated in humans with hypogonadism (194, 195), and castration reduces NPY mRNA levels in rats (196), and sympathetic nerve discharge firing rates in cats at rest and during stress (197). Furthermore, as previously mentioned, testosterone is positively related with MSNA discharge in humans (166, 167), which is reflective of sympathetic vasoconstriction (198, 199). Thus, although in its infancy, there is some evidence indicating that testosterone impacts sympathetic neurocirculatory control.

### 1.5.3. Age-related changes in sex hormones

In males and females, the concentrations of circulating endogenous sex hormones declines as a function of age. However, the rate of hormonal decline exhibits a sex-specific effect (Figure 1.6). Menopause marks the beginning of age-associated female fertility decline driven by less production of ovarian follicles, and typically occurs between 40-55 years of age (200). The stark decline in estradiol production results in an increase in FSH levels as there is not enough circulating estradiol to “turn off” the central generation of FSH via the hypothalamic-pituitary-gonadal (HPG) axis. Conversely, in males, total testosterone levels gradually decline by ~1-2%

annually, starting from ~30 years of age, with an even greater decline in bioavailable testosterone (~3-4% per year), as SHBG concentrations increase with age (201). The age-related decline in testosterone is attributed to a reduction in the number of Leydig cells and attenuated pulsatile release of GnRH – which impacts the HPG axis' regulation of testosterone (202). Notably, the age-related reductions in estradiol in females and testosterone in males are associated with increased risk of developing hypertension (203, 204), suggesting that sex hormones play an important role in blood pressure regulation.

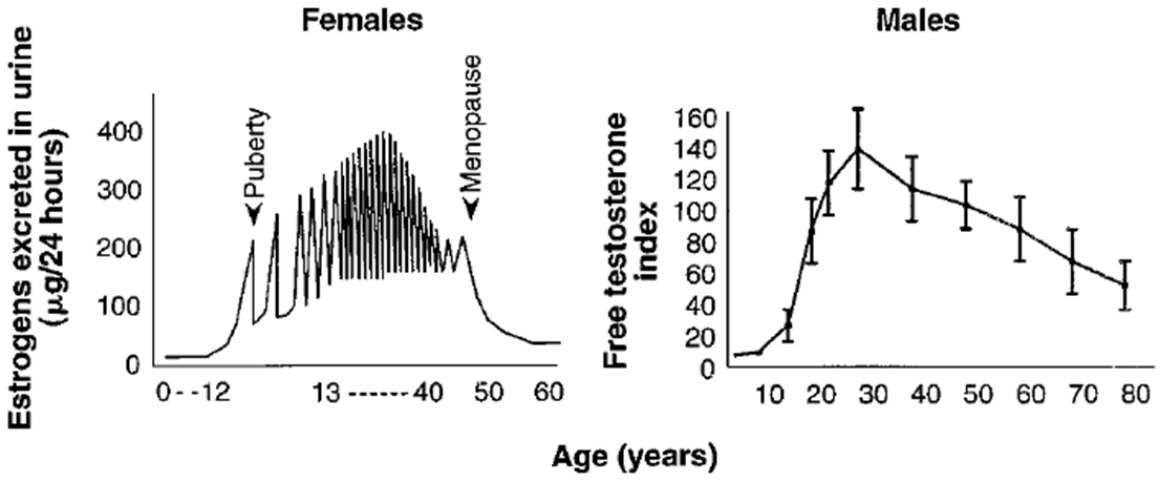


**Figure 1.6** Estradiol in females (left) and free testosterone index in males (right) changes across the lifespan. Note the rapid decline in estradiol concentrations following menopause in females, but the rather steady decline in testosterone in males. Adapted from Lamberts et al. (205).

### 1.5.4. Estradiol and progesterone across the menstrual cycle

The menstrual cycle comprises a monthly series of changes, regulated by sex hormones, that prepares the female body for the possibility of pregnancy. A menstrual cycle is normally ~28 days (range: 26-35 days) and consists of two major phases: a) follicular and b) luteal (Figure 1.7) (206). The follicular phase, which serves to develop an oocyte for ovulation, begins with menstruation, which is triggered by a reduction in circulating estradiol and progesterone. The

early period of the follicular phase is associated with the lowest estradiol and progesterone. These low estradiol levels stimulate an increase in FSH released from the anterior pituitary (via the hypothalamic-pituitary-ovarian [HPO] axis), which promotes grater estradiol production in the ovaries, resulting in greater estradiol concentrations in the late follicular phase. Furthermore, the ovaries also produce testosterone that is rapidly converted to estradiol (via aromatase) which further increases estradiol concentrations. Thus, in the late follicular phase, estradiol concentrations are high, but progesterone remains low. The follicular phase ends with ovulation, during which estradiol levels decrease, and the oocyte is released from an ovary. Following ovulation, the oocyte turns into the corpus luteum, which is a temporary endocrine organ that synthesizes and secretes estradiol and progesterone to prepare the uterine wall for potential implantation and fertilization (207). However, since estradiol and progesterone leak into the systemic circulation, the luteal phase of the menstrual cycle is characterized by gradual increases in circulating levels of these hormones that peak mid-way through the luteal phase (Figure 1.6). If the oocyte is not fertilized, the corpus luteum breaks down, resulting in reductions in estradiol and progesterone, that ultimately trigger the beginning of a new menstrual cycle.

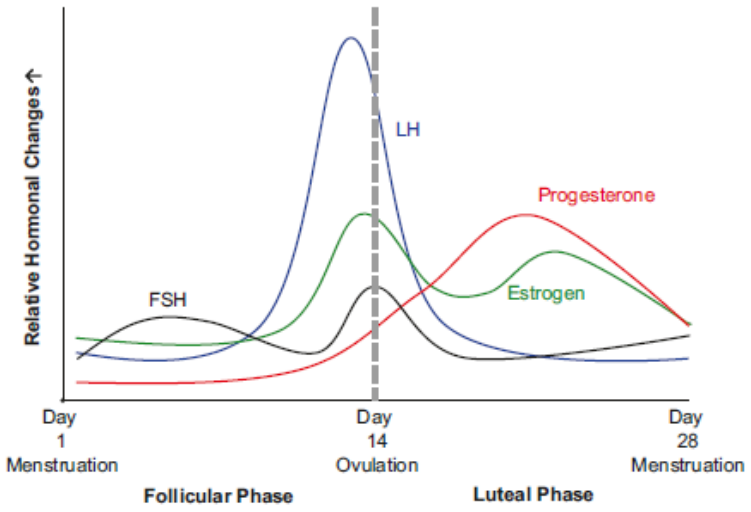


**Figure 1.7.** Hormonal fluctuations across a typical menstrual cycle in healthy eumenorrheic females. Reused with permissions from Springer nature. Davis and Hackney (208).

### 1.5.5. Oral hormonal contraception

Many types of hormonal contraceptives exist (e.g., intrauterine devices, subcutaneous implants, oral pills); however, the discourse in this dissertation will focus on oral hormonal contraception pills (OCP). From 1994-2019, the number of OCP users has increased from 97 to 151 million with more than 20% of reproductive-aged females using this form of contraception (209). To prevent pregnancy, competitive binding to progesterone receptors is critical. However, since progesterone is expensive and difficult to isolate for hormonal supplementation, the development of synthetic progesterone – termed progestins – are the primary form of progesterone in hormonal contraception. Although progestin-only contraceptive pills exist, these are more commonly prescribed for special circumstances such as females who have contraindications to estradiol or females who are breast-feeding (210) and are associated with irregular menstrual bleeding (40). Thus, a combination of synthetic estrogens, like ethinyl estradiol, and progestins is more commonly utilized as the estrogen component was found to help minimize the negative side effects like bleeding in the progestin-only pills (40).

An OCP cycle is typically one month in duration, and they are either monophasic (3 weeks single hormone concentration active hormone pill, 1 week placebo), or triphasic (3 weeks of active pill with incremental increases in hormone concentrations, 1 week placebo). Additionally, there are currently four generations of OCPs, each of which differ in the concentrations of ethinyl estradiol and the type of synthetic progestin. First generation OCPs contained progestins like norethindrone and high doses of (~50µg) ethinyl estradiol but had adverse effects on blood cholesterol levels due to progestin interactions with androgen receptors and placed females at an elevated risk for blood clots due to the high estrogen doses (211–213). Thus, more recent OCP generations typically contain lower doses (~35µg) of ethinyl estradiol and different types of progestins. Second generation progestins, such as levonorgestrel are more potent and exhibit androgenic effects, despite being used in lower doses, whereas third generation progestins (e.g.,

deosorgestrel) are further chemically altered to reduce unwanted androgenic effects (211, 213). Finally, fourth generation progestins (e.g., drosprionone) exhibit anti-androgenic effects, much like endogenous progesterone itself (213). However, despite great advancements in the production of synthetic sex hormones, they do not exhibit the same physiological effects as endogenous hormones, meaning that females using OCPs may exhibit altered physiology compared to naturally menstruating females. Indeed, the incidence of hypertension is greater in females using OCPs (particularly earlier generation OCPs) compared to naturally menstruating females (40), albeit advancements in OCPs (progestin type and hormone concentrations) have mildly reduced the risk of developing hypertension amongst OCP users (40).

## 1.6 Impact of age and biological sex on muscle sympathetic nerve activity and sympathetic transduction

### 1.6.1. Resting State

A hallmark of aging is a gradual increase in blood pressure across the lifespan (214), and heighted tonic sympathetic vasomotor outflow is purported to be a primary contributor (215). Notably, biological sex impacts the age-related increase in MSNA, with most (17, 21), but not all (20), studies demonstrating greater age-related increases in MSNA in females compared to males. The discrepancy in these resting MSNA data may be attributed to the considerable amount of inter-individual variability in resting MSNA amongst healthy, normotensive individuals (17, 21, 216). Despite this wide array of variability, distinct sex- and age-specific patterns emerge between MSNA, blood pressure, and its ohmic determinants (i.e., TPR and cardiac output). Specifically, young males exhibit an inverse relationship between MSNA and cardiac output, and a positive relationship between MSNA and TPR, both of which are absent in older males (217, 218). Conversely, older, but not young, females exhibit a positive relationship between MSNA and TPR, as well as MSNA and blood pressure (152). In addition to these sympathetic-hemodynamic

relationships, both older males and females exhibit greater autonomic support of blood pressure compared to their same-sex younger counterparts (215, 219), indicating that MSNA plays an increasingly important role in the regulation of arterial blood pressure amongst older adults.

While much work has been completed regarding the impact of age on sympathetic neuro-hemodynamic balance, only a handful of studies have assessed the beat-by-beat sympathetic regulation of blood pressure termed sympathetic vascular transduction (29, 30, 220). Sympathetic vascular transduction governs blood pressure on a beat-by-beat basis via changes in vascular tone that are primarily mediated by α-adrenergic vasoconstriction (221). While the concept of sympathetic transduction and the impact of biological sex is well established in young adults (67, 222, 223), only two studies have explored the impact of age and sex on vascular transduction, finding that aging attenuates sympathetic transduction in males, but that females either exhibit augmented or reduced transduction (29, 220). The discrepancy between these studies can be attributed to analytical differences used to evaluate sympathetic vascular transduction, and the reader is directed to the following reviews for a comprehensive methodological overview sympathetic transduction analysis (67, 177). Teleologically, attenuated sympathetic transduction amongst normotensive older adults is unsurprising as older adults exhibit attenuated α-adrenergic vasoconstrictor responsiveness compared to young adults (224). Thus, older adults exhibit attenuated vasoconstriction for a given amount of sympathetic nerve traffic. However, assessments of the impact of age and sex on sympathetic transduction have been completed using blood pressure as the output signal, which is affected by both cardiac output and peripheral vascular resistance. Thus, direct assessments of the impact of age and sex on the beat-by-beat regulation of vascular tone have not been investigated and remains an important area for research.

### 1.6.2. Exercise

As previously mentioned, the exercise pressor reflex is comprised of two reflex components: the mechano- and the metaboreflex. Whilst the impact of biological sex on the sympathetic neural responses to muscle mechanoreflex activation in young adults are unknown (95), clear sex differences have been reported regarding muscle metaboreflex activation, with young males demonstrating greater increases in MSNA during exercise and post-exercise circulatory occlusion compared to young females (19, 225). Several mechanisms have been purported to explain this sex difference including, differences in metabolite concentrations during exercise between males and females (225, 226), absolute contraction force/muscle strength (227), and the vasodilatory actions of estradiol (228). Conversely, the impact of age on MSNA during exercise is equivocal, with studies finding heightened (229), preserved (230, 231), or attenuated (232, 233), sympathetic neural reactivity amongst older adults. The reasons for these heterogenous findings may, in part, be attributed to sex-disparities in neuro-cardiovascular changes associated with aging. Indeed, in primarily male samples, either preserved (230) or attenuated (232, 233) sympathetic and pressor responses to exercise and PECO are reported in older adults. Conversely, post-menopausal females demonstrate exaggerated increases in MSNA and blood pressure during isometric exercise and PECO compared to young, pre-menopausal females, likely due to the loss of estradiol following menopause (229). Only one study to date has assessed the interactive effects of age and sex on the cardiovascular responses to small muscle mass exercise, finding that older females demonstrated larger increases in blood pressure and TPR compared to similarly aged males and young adults (37), further supporting the notion that there may be a sex-specific impact of age on the sympathetic neurocirculatory responses to exercise. Importantly however, direct measures of sympathetic nerve activity were lacking in this study. Thus, it remains unclear how age and sex impact the sympathetic neural vasoconstrictor discharge patterns during exercise.

## 1.7 Impact of menstrual cycle and oral contraception on muscle sympathetic nerve activity and sympathetic transduction in young females

### 1.7.1. Resting State

Over the past two decades, there has been growing interest in the impact of the menstrual cycle (i.e., endogenous hormones) and OCP (i.e., exogenous hormones) on MSNA discharge. The impact of menstrual cycle phase on MSNA is equivocal with some studies finding greater MSNA in the mid-luteal phase compared to the early follicular phase (234–237), and others finding no difference in resting MSNA discharge (238–240). Similarly, within females using OCP, some studies have found greater resting MSNA in the active pill compared to placebo pill phase (41), whereas others have observed no phasic differences in resting MSNA (42, 235, 241). Given that endogenous female sex hormones are thought to exert sympathoinhibitory effects (38, 159), and that exogenous hormones likely do not exert the same physiological effects as endogenous estradiol and progesterone (40, 242), comparisons between naturally menstruating females and those using OCPs could provide important insight into the sympathetic neural regulation of blood pressure. Indeed, in a large retrospective analysis, Harvey et al. found that despite naturally menstruating females (early follicular) and females using OCPs (placebo pill) having similar resting MSNA, blood pressure was higher in OCP users (44). Furthermore, no relationship was observed between MSNA and blood pressure in females using OCP, whereas eumenstruous females exhibited a positive association between these two variables (44). These data suggest that non-neural factors (e.g., renin-angiotensin system) may contribute more to the regulation of resting blood pressure in premenopausal females using oral hormonal contraception. Indeed, OCPs are known to upregulate the renin-angiotensin-aldosterone system (40), which ultimately increases resting arterial pressure.

### 1.7.2. Exercise

Contrary to resting MSNA, natural fluctuations in endogenous sex hormones from the early follicular to mid-luteal phases of the menstrual cycle do not appear to impact the sympathetic neural responses to exercise (19). However, the impact of OCP use on the exercise pressor reflex is equivocal with most (43, 243, 244), but not all (245), studies demonstrating larger increases in blood pressure in females using OCPs compared to naturally menstruating females. To date, only one study has compared the MSNA responses to handgrip exercise in OCP users and eumenstruous females, finding greater increases in MSNA burst occurrence in females using OCP, and augmented sympathetic transduction into TPR at peak exercise compared to naturally menstruating females (43). Importantly however, since Takeda and colleagues (43) only assessed integrated MSNA burst occurrence, the impact of OCP use on MSNA burst amplitude and subsequent sympathetic AP discharge patterns during exercise remains unclear. Additionally, sympathetic transduction was assessed with TPR as the output variable for MSNA. However, given that MSNA reflects sympathetic vasoconstrictor discharge directed toward the skeletal muscle vasculature, assessments of sympathetic vascular transduction would provide more direct insight into the degree of vasoconstriction elicited by MSNA. Furthermore, the size of MSNA bursts is positively related to the degree of neurogenic vasoconstriction (199). Given that MSNA burst amplitude reflects the number and size of individual APs (7), combined assessments of limb blood flow and AP discharge would provide the first insight into how sympathetic AP communication patterns translate into vasoconstriction, and how these vasoconstrictive messages are impacted by sex hormones.

### 1.8 Rationale

With this background in mind, the overall objective of this dissertation is to investigate the effects of age, biological sex, and sex hormones on the muscle sympathetic AP patterns that serve to regulate vasomotor tone and blood pressure at rest and during exercise. The **working hypothesis** is that age, sex, and sex hormones represent important inter-individual factors that

will impact the discharge behaviour of muscle sympathetic APs as well as the corresponding changes in peripheral vasoconstriction and blood pressure at rest and during exercise. The data from this dissertation will highlight the importance of including females and accounting for male and female sex hormones in assessments of sympathetic neurocirculatory control. Additionally, these data will advance our mechanistic insight into the role of the sympathetic nervous system on blood pressure regulation in males and females.

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# Chapter 2

# Aging is associated with enhanced central, but impaired peripheral arms of the sympathetic baroreflex arc

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## 2.1. Introduction

The sympathetic nervous system is fundamental to both the short- and long-term neural regulation of arterial blood pressure. In general, older adults demonstrate chronic elevations in sympathetic vasoconstrictor outflow (i.e., muscle sympathetic nerve activity; MSNA) (1–4), which impairs end organ function and may contribute to the development of hypertension (5). Further, the relationship between MSNA and blood pressure is altered with advancing age such that older adults with higher MSNA demonstrate higher blood pressures, whereas in young adults there is no relationship between these two variables (6). While the increase in sympathetic outflow associated with aging has become a concern, the mechanisms mediating these age-related changes remain unclear.

One potential mechanism underlying age-related increases in MSNA is impaired arterial baroreflex function that fails to inhibit efferent sympathetic outflow when blood pressure is elevated. In this regard, the age-related increase in MSNA is thought to be attributed to a rightward and upward shift in the operating point of the baroreflex threshold relationship in older adults towards a higher diastolic blood pressure and greater MSNA burst occurrence (7–10) with either an unaltered (7, 8, 10, 11) or reduced (4, 12) integrated MSNA baroreflex threshold gain (i.e., the responsiveness of the baroreflex to acute changes in blood pressure). Alternatively, there may be age-related changes in the central (i.e., reflex response of MSNA to changes in arterial blood pressure) and peripheral (i.e., end-organ responses to MSNA) arcs of the baroreflex, such that increases in efferent sympathetic discharge with aging may be compensatory for a reduction in sympathetic transduction. Indeed, the increase in blood pressure in response to a burst of MSNA (i.e., sympathetic transduction) is smaller in older compared to young adults (13), and previous

work that deconstructed the integrated sympathetic baroreflex into its neural and mechanical components (i.e., carotid artery distention) demonstrated that older adults had a greater neural arm of the baroreflex, but a blunted mechanical arm, resulting in a similar integrated MSNA gain compared with young adults (14). Furthermore, Fisher et al. found that older adults exhibited greater responses to carotid hypertension (i.e., neck suction), and blunted responses to carotid hypotension (i.e., neck pressure) compared to young adults, resulting in a similar overall carotid baroreflex gain between young and older adults (15). These data indicate that evaluating the integrated sympathetic baroreflex gain alone may mask age-related changes in baroreflex function, and highlights that our understanding of the mechanisms governing age-related changes in baroreflex regulation of efferent sympathetic discharge and sympathetic transduction remains incomplete.

The arterial baroreflex represents a primary feedback mechanism mediating the dynamic relationship between blood pressure and the sympathetic nervous system, whereby afferent feedback from baroreceptors in the carotid sinuses and aortic arch synchronizes efferent action potential (AP) discharge that comprise the pulse-synchronous bursts of MSNA (16–18). Previous studies have evaluated the baroreflex control of integrated bursts of MSNA only, even though the integration of the sympathetic neurogram conceals information derived from varying-sized APs arising from efferent postganglionic c-fibers, which are the raw sympathetic nerve traffic directed toward the skeletal muscle vasculature (19). Using a continuous wavelet analysis approach to identify the underlying APs firing within the integrated MSNA neurogram, we found that the arterial baroreflex exhibits heterogeneous control over different size-based AP subpopulations, which represent the firing of varying sized sympathetic postganglionic neurons (20). Specifically, medium-sized AP clusters that were present in most integrated MSNA bursts at baseline are tightly governed by the baroreflex, whereas larger AP clusters that have a lower firing probability at baseline, and are recruited during sympathoexcitatory stress, display weaker control of the arterial baroreflex (20, 21). Additionally, we recently found that in young adults, orthostatic stress

reset the baroreflex threshold operating point of both integrated MSNA and individual AP clusters towards greater firing probabilities, whereas the baroreflex threshold gain of medium-sized APs was augmented, without any alteration in the integrated MSNA baroreflex threshold gain (i.e., burst occurrence) (21). While advancing age appears to alter AP discharge patterns (22), it remains unclear whether these aberrant axonal recruitment strategies are mediated by age-related changes in the arterial baroreflex control of postganglionic sympathetic APs.

Therefore, the objective of the current investigation was to evaluate the effect of aging on the central and peripheral components of the baroreflex arc. To do so, we assessed the spontaneous baroreflex control of integrated MSNA and individual AP subpopulations (i.e., central component), as well as the sympathetic transduction of integrated MSNA to blood pressure (i.e., peripheral component) in young and older adults during supine resting conditions. We tested the following hypotheses: 1) The spontaneous baroreflex control of integrated MSNA burst occurrence and AP subpopulations would be blunted in older compared to young adults, and 2) sympathetic transduction will be blunted in older adults relative to young adults.

## 2.2. METHODS

### *2.2.1. Participants*

Thirteen older (7 females, 58 ± 9 years, 166 ± 6 cm, 71 ± 13 kg, BMI: 25.2 ± 3.9) and fourteen young adults (6 females, 24 ± 3 years, 171 ± 7 cm, 69 ± 14 kg, BMI: 23.6 ± 3.9) participated in the current study. All participants were non-smokers, not taking any prescription medications, and free of overt cardiovascular disease and other disorders (e.g., metabolic syndrome, hepatic, and renal disease). Data from the older adults, and four young adults were retrospectively analyzed from studies that addressed unique hypotheses (21, 23). Two of the seven older females were menopausal (self reported ongoing menopausal symptoms), whereas the remaining five were postmenopausal. Of the young females, one was on oral contraceptives, while the others were

either using other forms of hormonal contraception (e.g., intrauterine device) or no hormonal contraception at all. We did not control for menstrual cycle phase as majority of these data were retrospectively analyzed (21, 23) from studies that examined hypotheses unrelated to the impact of sex steroids on efferent sympathetic outflow. Furthermore, most (24–26), but not all (27) previous studies observed no impact of menstrual phase on arterial baroreflex function. Additional studies that focus on these sex-based analyses are required. All participants provided written, informed consent and the experimental protocol was approved by the Human Research Ethics Board (REB) at The University of Western Ontario (REB nos. 17810, 108026), and conforms to the *Declaration of Helsinki*.

*2.2.2. Experimental Protocol and measures*

Participants were studied following at least a 4-hour fast and having refrained from vigorous exercise, caffeine, and alcohol consumption for a minimum of 12 hours. Participants were instructed to empty their bladder to avoid any effects of bladder distention on sympathetic activity and blood pressure (28). Thereafter, measurements of height and weight were recorded, and participants assumed the supine position for the duration of the study. At least 5 minutes after a satisfactory nerve recording site was found, data were collected during spontaneous breathing for a minimum of 5 minutes.

Heart rate (HR) was recorded using a standard Lead II electrocardiogram (BioAmp FE132, ADInstruments; Bella Vista, New South Wales, Australia). Continuous beat-by-beat blood pressure was recorded via finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands) calibrated to the mean of three manual brachial artery blood pressure measures. Stroke volume and cardiac output were estimated from the arterial blood pressure waveform using the Finometer Modelflow algorithm (29). Total peripheral resistance was calculated as the quotient of mean arterial pressure and cardiac output, and pulse pressure was calculated as the difference between systolic and diastolic blood pressure.

Efferent multi-unit muscle sympathetic nerve activity was obtained from the peroneal nerve using a 200-µm diameter, 35-mm long tungsten microelectrode, tapering to an uninsulated 1- to 5 µm tip. The electrode was inserted percutaneously into the nerve, just posterior to the fibular head, and a reference electrode was positioned subcutaneously ~1-3 cm from the recording site. A suitable MSNA site was obtained by manual manipulation of the microelectrode until a pulse-synchronous burst pattern was observed. An MSNA recording site was confirmed by the absence of skin paresthesia, and an increase in sympathetic discharge during a breath-hold, but not in response to a startling loud noise (16, 30). The MSNA neurogram was recorded with a nerve traffic analyzer (662C-3; Bioengineering Dept., University of Iowa, Iowa City, IA). The neural signal was amplified with a gain of 75,000, and band-pass filtered (bandwidth: 700-2000 Hz) before being rectified and integrated (leaky integrator; 0.1-s time constant). The raw, filtered, and integrated MSNA signals were sampled at 10,000 Hz, while all other signals were sampled at 1,000 Hz. All data were collected using LabChart 8 and PowerLab (ADInstruments, Colorado Springs, CO) and were then saved offline for analysis.

*2.2.3. Data analysis*

Integrated MSNA analysis

Integrated bursts of MSNA were identified in accordance with previously published standardized guidelines (31). Specifically, integrated MSNA bursts were included if they demonstrated pulse-synchrony, had a signal-to-noise ratio (SNR) of at least 3:1 relative to the previous period of neural silence, expressed characteristic rising and falling slopes, and individual sympathetic APs were visible in the corresponding raw filtered neurogram. Integrated MSNA was quantified as burst frequency (bursts/min) and burst incidence (bursts/100 heart beats). Burst amplitude was measured in volts and normalized to the largest burst at baseline which was given a value of 100 (AU). Total MSNA was calculated as the product of burst frequency and normalized burst amplitude (AU/min).

AP analysis

Postganglionic sympathetic APs were detected and extracted from the raw filtered neurogram using a wavelet-based methodology, as described previously (32). Briefly, a continuous wavelet transform constructed from real postganglionic sympathetic APs was applied to the filtered neurogram to extract individual APs within an identified burst of MSNA. APs were then ordered based on a peak-to-peak amplitude and histogram analysis was performed to sort APs into amplitude-based clusters using Scott's rule (33). AP behaviour was quantified by AP frequency (spikes/min) and AP incidence (spikes/100 heart beats), the number of active APs per burst (spikes/burst), the number of active AP clusters per integrated burst (clusters/burst) and the number of total AP clusters detected. The average SNR was not different between older and young adults (older: 4.1±0.4, Young: 4.0 ±0.4; Unpaired *t*-test: *P*=0.56), suggesting a correct detection rate of ≥95%, as well as a <3% false positive rate (32).

Baroreflex control of integrated MSNA analysis

Spontaneous baroreflex threshold and sensitivity analyses of integrated MSNA were completed using the methods previously described of Sundölf and Wallin (34) and Kienbaum et al. (35). First, integrated MSNA baroreflex threshold analysis was completed by grouping diastolic blood pressure into 2 mmHg bins and calculating the burst occurrence (%) for each diastolic blood pressure bin (i.e., the percentage of heartbeats per diastolic blood pressure bin associated with an integrated MSNA burst). The relationship between spontaneous changes in MSNA burst occurrence and diastolic blood pressure was assessed using linear regression analyses, with the slope of this relationship representing the integrated baroreflex threshold gain. Diastolic blood pressure bins were weighted based on occurrence. Second, the integrated MSNA baroreflex sensitivity was evaluated by plotting the relationship between normalized integrated MSNA burst amplitude and the corresponding diastolic blood pressure. Pearson's correlation coefficients I were recorded for all participants. Lastly, the operating points of the integrated MSNA baroreflex

threshold and sensitivity diagrams were determined by plotting the point of intersection between mean burst occurrence or amplitude, respectively, and mean diastolic blood pressure. During collection of the current data, Holwerda et al. published an analysis suggesting that the use of 3-mmHg blood pressure bins and a 10-minute recording period were favourable when evaluating spontaneous baroreflex control of MSNA under resting conditions (36). However, no significant differences were found in the percentage of linear regressions with a slope stronger than -0.7 or the percentage of significant ($P<0.05$) linear regressions between 5- and 10-minute recording periods. Additionally, intra-class correlations were strong (0.75-0.9) when comparing separate 5-minute recording durations of spontaneous MSNA baroreflex gains using 2-mmHg blood pressure bins, indicating a strong degree of consistency between 5-minute recording periods (36). Finally, using 3-mmHg blood pressure bins in our 5-minute recording periods resulted in some baroreflex slopes being constructed using only 2 data points. Therefore, while more data may be favourable using 3-mmHg blood pressure bins and a 10-minute recording period, the current analysis using a 2-mmHg bin size and a minimum of 5-minutes of baseline data are expected to provide a robust baroreflex analysis.

Baroreflex control of AP subpopulations

Baroreflex threshold diagrams were developed for each AP cluster describing the probability of an AP cluster firing within a specific bin of diastolic blood pressure (20, 21). Similar to the analysis for the baroreflex control of integrated MSNA, diastolic blood pressures were grouped into 2 mmHg bins and AP probability (%) for each bin was calculated. The baroreflex threshold gain of individual AP clusters were measured as the slope of the linear relationship between AP probability and the mean diastolic blood pressure for each bin (Figure 2.1). In line with previous work (20, 21), diastolic blood pressure bins were not weighted based on occurrence. To allow for between-participant comparisons of AP baroreflex cluster activity, data for each participant were normalized to the largest AP cluster recruited and forced to occupy 10 bins (i.e.,

clusters). In the current study, AP clusters 1-2, 3-7, and 8-10 were categorized as small, medium, and large AP clusters, respectively. AP baroreflex sensitivity was determined by the relationship between the number of active AP clusters per burst and the corresponding diastolic blood pressure. The operating points of the AP cluster baroreflex threshold and sensitivity diagrams were determined by plotting the point of intersection between mean cluster probability or the mean number of active AP clusters per burst, respectively, and the mean diastolic blood pressure.

Sympathetic transduction

The analysis of sympathetic transduction of MSNA into mean arterial pressure was completed using an open-source, Microsoft Excel-based program, as previously described (37). Briefly, spike triggered averaging was performed wherein MSNA bursts acted as a trigger and were followed for the subsequent 12 cardiac cycles to capture the pressor response following a burst of MSNA. All MSNA bursts were used in this analysis, regardless of their proximity to other MSNA bursts. Additionally, the mean arterial pressure responses following periods of sympathetic quiescence (i.e., non-bursts) were evaluated using the average beat-by-beat changes in mean arterial pressure following each cardiac cycle that was not associated with a sympathetic burst. These data provided insight into non-neural hemodynamic regulation during non-burst periods. For each participant, the change in mean arterial pressure was defined as the instantaneous mean arterial pressure at each successive cardiac cycle subtracted from the mean arterial pressure at time point 0 (i.e., the MSNA burst or the first cardiac cycle in a non-burst period).

*2.2.4. Statistical Analysis*

All data are presented as mean (SD). Normality was assessed using Shapiro-Wilk's test. Outlier testing was completed using the ROUT method in Graphpad Prism. Linear regression analyses were used to determine the baroreflex threshold and sensitivity relationships at the integrated MSNA and AP level. Pearson's correlation analyses provided the correlation coefficient

for the regression analyses. Unpaired samples *t*-tests were used to evaluate the effect of age on resting hemodynamic measures, integrated MSNA and AP indices, as well as integrated MSNA baroreflex threshold and sensitivity, and AP cluster baroreflex sensitivity. Two-way analyses of variance (ANOVA) evaluated the effect of group (older vs. Young; fixed variable) and cluster (AP clusters 1-10; repeated measure) on the slope the AP baroreflex threshold relationship and AP probability. Similarly, two-way ANOVAs were conducted to evaluate the effect of age on sympathetic transduction. Bonferroni corrected *post-hoc* comparisons were performed when a significant interaction (i.e., group-by-cluster) was observed. Effect sizes at the ANOVA level are represented by partial $\eta^2$ (small effect: $\eta^2 = 0.01$, medium effect: $\eta^2 = 0.06$, large effect: $\eta^2 = 0.14$), whereas effect sizes for pairwise comparisons are represented by Hedges $g_s$ (small effect: $g_s = 0.2$, medium effect: $g_s = 0.5$, large effect: $g_s = 0.8$). Statistical significance was accepted at $P \leq 0.05$. Statistical analyses were performed using Prism version 9.0 for Windows (GraphPad software, San Diego, CA).

## 2.3. RESULTS

### *2.3.1. Baseline hemodynamics, integrated MSNA and AP metrics*

Baseline hemodynamics, integrated MSNA and AP metrics for older and young adults are presented in Table 2.1. Compared to young adults, older adults had higher mean arterial ($P < 0.001$, $g_s = 1.40$), systolic ($P = 0.011$, $g_s = 1.024$), and diastolic blood pressures ($P < 0.001$, $g_s = 1.208$); however, pulse pressure was similar between the two groups ($P = 0.511$, $g_s = 0.249$). Heart rate ($P = 0.645$, $g_s = 0.174$), stroke volume ($P = 0.960$, $g_s = 0.019$), cardiac output ($P = 0.755$, $g_s = 0.117$), and total peripheral resistance ($P=0.206$, $g_s = 0.485$) were similar between the two groups. Older adults demonstrated greater sympathetic neural outflow when expressed as integrated MSNA burst frequency ($P < 0.001$, $g_s = 1.821$), burst incidence ($P < 0.001$, $g_s = 2.079$),

and total MSNA ($P$ = 0.002, $g_s$ = 1.271), as well as AP frequency ($P$ = 0.002, $g_s$ = 1.22), AP incidence ($P$ = 0.003, $g_s$ = 1.244), and the total number of active AP clusters ($P$ = 0.002, $g_s$ = 1.28) compared to young adults. However, normalized integrated MSNA burst amplitude ($P$ = 0.890, $g_s$ = 0.052), within-burst AP firing frequency ($P$ = 0.669, $g_s$ = 0.162), and the number of active AP clusters/burst ($P$ = 0.110, $g_s$ = 0.614) were not different between the two groups.

*2.3.2. Integrated MSNA baroreflex threshold and sensitivity*

In both older and young adults, pearson's correlation coefficients revealed strong relationships (r > 0.7) between burst occurrence and diastolic blood pressure (older: r = -0.87 ± 0.13, Young: r = -0.85 ± 0.10; Unpaired *t*-tests between groups: $P$ = 0.577, $g_s$ = 0.173), and weak relationships between burst amplitude and diastolic blood pressure (older: r = -0.25 ± 0.15, Young: r = -0.19 ± 0.29; Unpaired *t*-tests between groups: $P$ = 0.515, $g_s$ = 0.257). Figure 2.2 depicts the group mean linear regression outcomes and the individual slopes for the spontaneous integrated baroreflex threshold (Panels A and C) and sensitivity relationships (Panels B and D). Compared to young adults, older individuals demonstrated a rightward and upward shift in the operating point of the arterial baroreflex towards a greater diastolic blood pressure (older: 77 ± 9 mmHg, Young: 66 ± 6 mmHg, $P$ < 0.001, $g_s$ = 1.326) and integrated MSNA burst occurrence (older: 55 ± 15%, Young: 28 ± 10%, $P$ < 0.001, $g_s$ = 2.410). Additionally, integrated baroreflex threshold gain was greater in older compared to young adults (older: -5.7 ± 2.6 %/mmHg, young: -2.7 ± 1.4 %/mmHg, $P$<0.001, $g_s$ = 1.453), whereas integrated baroreflex sensitivity was not different between the two groups (older: -1.8 ± 1.5 AU/mmHg, young: -1.1 ± 1.9 AU/mmHg, $P$ = 0.295, $g_s$ = 0.407).

*2.3.3. Baroreflex control of AP subpopulations*

Baroreflex threshold slopes of the 10 normalized AP clusters from older and young adults are presented in Figure 2.3A. Age modified the strength of the baroreflex control of AP clusters (Figure 2.3A; group-by-cluster interaction: $P$ < 0.001, $\eta^2$ = 0.154). Specifically, *post-hoc* analyses revealed

that older adults demonstrated greater baroreflex threshold slopes – and therefore stronger baroreflex control – of most medium-sized AP clusters compared to young adults (clusters 3-6: all $P$ = 0.003 – 0.026, all $g_s$ = 1.377-1.686). However, aging did not modify the AP baroreflex threshold slopes for small- (clusters 1 and 2: $P$ = 0.999 and 0.425, $g_s$ = 0.325 and 0.825, respectively) or large-sized (clusters 8-10: all $P$ = 0.377 – 0.903, all $g_s$ = 0.745 – 0.894) AP clusters (Table 2.2). Similarly, the operating point of the baroreflex (i.e., the firing probability of individual AP clusters), was modified by age (Figure 2.3B; group-by-cluster interaction: $P$ < 0.001, $\eta^2$ = 0.134), with older adults demonstrating greater AP firing probability of some medium-sized AP clusters (clusters 3 and 4: $P$ = 0.005 and 0.029, $g_s$ = 1.735 and1.434, respectively), but no difference in the firing probability of small- (clusters 1 and 2: $P$ = 0.999 and 0.525, $g_s$ = 0.085 and 0.814, respectively), other medium (clusters 5-7: all $P$ = 0.109 – 0.778, all $g_s$ = 0.724 – 1.108) or large-sized (clusters 8-10: all $P$ = 0.977 – 0.999, all $g_s$ = 0.637 – 0.696) AP clusters. All AP clusters expressed medium-to-strong Pearson correlation coefficients, and age did not modify the strength of the Pearson correlation coefficients (Group-by-cluster interaction: $P$ = 0.854). Bonferroni corrected *t*-test *P*-values for between group (i.e., young versus older) comparisons of the Pearson correlation coefficients of each AP cluster are presented in Table 2.2.

In both older and young adults, weak Pearson correlation coefficients were observed for the baroreflex sensitivity relationships for the number of AP clusters per burst, and statistical comparisons revealed no significant group differences in the strength of these relationships (older: r = -0.11 ± 0.08, Young: r = -0.04 ± 0.15, $P$ = 0.140, $g_s$ = 0.558; Figure 2.4A). Compared to young adults, older individuals demonstrated a rightward shift in the operating point of the arterial baroreflex towards a greater diastolic blood pressure (older: 77 ± 9 mmHg, Young: 66 ± 6 mmHg, $P$ < 0.001, $g_s$ = 1.326); however, no vertical shift in the operating point (i.e., the number of active AP clusters/burst) was observed (older: 6 ± 1 clusters/burst, Young: 5 ± 2 clusters/burst, $P$ = 0.110, $g_s$ = 0.614). Conversely, the gain of the spontaneous AP baroreflex sensitivity relationship

was greater in older compared to young adults (older: -0.12 ± 0.11 clusters/burst/mmHg, young: -0.03 ± 0.11 clusters/burst/mmHg, $P = 0.044$, $g_s = 0.793$; Figure 2.4B).

*2.3.4. Sympathetic transduction*

The beat-by-beat changes in mean arterial pressure following bursts of MSNA and periods wherein no MSNA bursts occurred (i.e., non-bursts) are presented in Figure 2.5. A significant group-by-cardiac cycle interaction was observed for the sympathetic neurovascular transduction responses to bursts ($P < 0.001$, $\eta^2 = 0.206$; Figure 2.5A), and *post-hoc* analyses revealed that young adults demonstrated larger increases in mean arterial pressure during cardiac cycles 4-9 following a burst of MSNA compared to older adults (all $P = 0.0001$ -0.012, all $g_s \geq 1.162 – 1.453$). Similarly, a group-by-cardiac cycle interaction was observed for the sympathetic transduction responses following non-burst periods ($P < 0.001$, $\eta^2 = 0.104$; Figure 2.5B), with young adults demonstrating a smaller fall in mean arterial pressure compared to older adults during cardiac cycles 8-12 (all $P = 0.002 – 0.013$, all $g_s = 0.735 – 2.19$).

***Table 2.1.*** *Hemodynamic, integrated muscle sympathetic nerve activity (MSNA) and action potential (AP) indices in older (n = 13) and young (n = 14) adults during 5 minutes of supine baseline.*

| | Older | Young | *P*-value | Effect size |
|---|---|---|---|---|
| **Hemodynamics** | | | | |
| Heart rate (beats/min) | 58 ± 8 | 59 ± 6 | 0.645 | 0.174 |
| Mean arterial pressure (mmHg) | 91 ± 10 | 79 ± 7 | **<0.001** | 1.402 |
| Systolic blood pressure (mmHg) | 120 ± 15 | 107 ± 10 | **0.011** | 1.024 |
| Diastolic blood pressure (mmHg) | 77 ± 9 | 66 ± 6 | **<0.001** | 1.208 |
| Pulse pressure (mmHg) | 44 ± 12 | 41 ± 9 | 0.511 | 0.249 |
| Stroke volume (mL) | 91 ± 25 | 92 ± 25 | 0.960 | 0.019 |
| Cardiac output (L/min) | 5.2 ± 1.3 | 5.4 ± 1.4 | 0.755 | 0.117 |
| Total peripheral resistance (mmHg/L/min) | 19 ± 7 | 16 ± 5 | 0.206 | 0.485 |
| **Integrated MSNA indices** | | | | |
| Burst frequency (bursts/min) | 32 ± 11 | 17 ± 5 | **<0.001** | 1.821 |
| Burst incidence (bursts/100 beats) | 55 ± 15 | 28 ± 10 | **<0.001** | 2.079 |
| Burst Amplitude (AU) | 46 ± 11 | 47 ± 8 | 0.890 | 0.052 |
| Total MSNA (AU·min) | 1511 ± 715 | 797 ± 317 | **0.002** | 1.271 |
| **AP indices** | | | | |
| AP frequency (bursts/min) | 332 ± 206 | 140 ± 75 | **0.003** | 1.222 |
| AP incidence (bursts/100 beats) | 563 ± 336 | 240 ± 134 | **0.002** | 1.244 |
| AP spikes/burst | 10 ± 3 | 9 ± 6 | 0.669 | 0.162 |
| AP clusters/burst | 6 ± 1 | 5 ± 2 | 0.110 | 0.614 |
| AP total clusters | 22 ± 5 | 16 ± 4 | **0.002** | 1.28 |

Values are mean (SD). Data were analyzed using unpaired samples *t*-tests. Effect size calculations were completed using Hedges $g_s$.

***Table 2.2.*** *Baroreflex threshold regression properties for individual action potential clusters in Older (n = 13) and Young (n = 14) adults during 5 minutes of supine baseline.*

| | **Slope, %/mmHg** | | | **Probability, %** | | | **Correlation coefficient I** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Cluster** | **Older** | **Young** | **Bonferroni-corrected *P* values** | **Older** | **Young** | **Bonferroni-corrected *P* values** | **Older** | **Young** | **Bonferroni-corrected *P* values** |
| 1 | -1.6 (1.8) | -1.0 (1.7) | > 0.999 | 6.5 (5.4) | 7.2 (10.8) | > 0.999 | -0.76 (0.17) | -0.71 (0.15) | > 0.999 |
| 2 | -6.2 (4.8) | -2.8 (3.0) | 0.425 | 47.4 (35.9) | 23.9 (20.1) | 0.525 | -0.77 (0.16) | -0.71 (0.21) | > 0.999 |
| 3 | -10.3 (5.4) | -4.5 (2.2) | 0.026 | 71.6 (29.8) | 33.0.9 (11.4) | 0.005 | -0.86 (0.11) | -0.81 (0.09) | > 0.999 |
| 4 | -8.2 (3.3) | -3.6 (1.9) | 0.003 | 52.2 (25.7) | 25.4 (7.6) | 0.029 | -0.84 (0.09) | -0.81 (0.12) | > 0.999 |
| 5 | -4.8 (2.1) | -2.2 (0.9) | 0.009 | 28.3 (14.2) | 15.8 (7.5) | 0.109 | -0.83 (0.11) | -0.77 (0.13) | > 0.999 |
| 6 | -3.7 (2.4) | -1.2 (0.8) | 0.025 | 15.8 (7.9) | 8.4 (6.4) | 0.140 | -0.84 (0.10) | -0.81 (0.13) | > 0.999 |
| 7 | -1.6 (1.1) | -0.74 (0.3) | 0.146 | 7.7 (3.7) | 5.2 (3.4) | 0.778 | -0.76 (0.18) | -0.72 (0.20) | > 0.999 |
| 8 | -1.1 (1.0) | -0.4 (0.3) | 0.377 | 4.1(2.5) | 2.6 (2.0) | 0.977 | -0.71 (0.25) | -0.67 (0.20) | > 0.999 |
| 9 | -0.7 (0.7) | -0.3 (0.3) | 0.902 | 2.2 (1.7) | 1.3 (0.7) | > 0.999 | -0.73 (0.28) | -0.68 (0.24) | > 0.999 |
| 10 | -0.5 (0.5) | -0.2 (0.1) | 0.819 | 1.2 (0.7) | 0.9 (0.4) | > 0.999 | -0.66 (0.27) | -0.75 (0.13) | > 0.999 |

Values are mean (SD). Repeated measures analysis of variance (ANOVA) revealed a significant group-by-cluster interaction for action potential cluster slope and probability (both $P < 0.001$). Bonferroni-corrected *P*-values represent *post-hoc* analyses when a significant ANOVA interaction was observed.

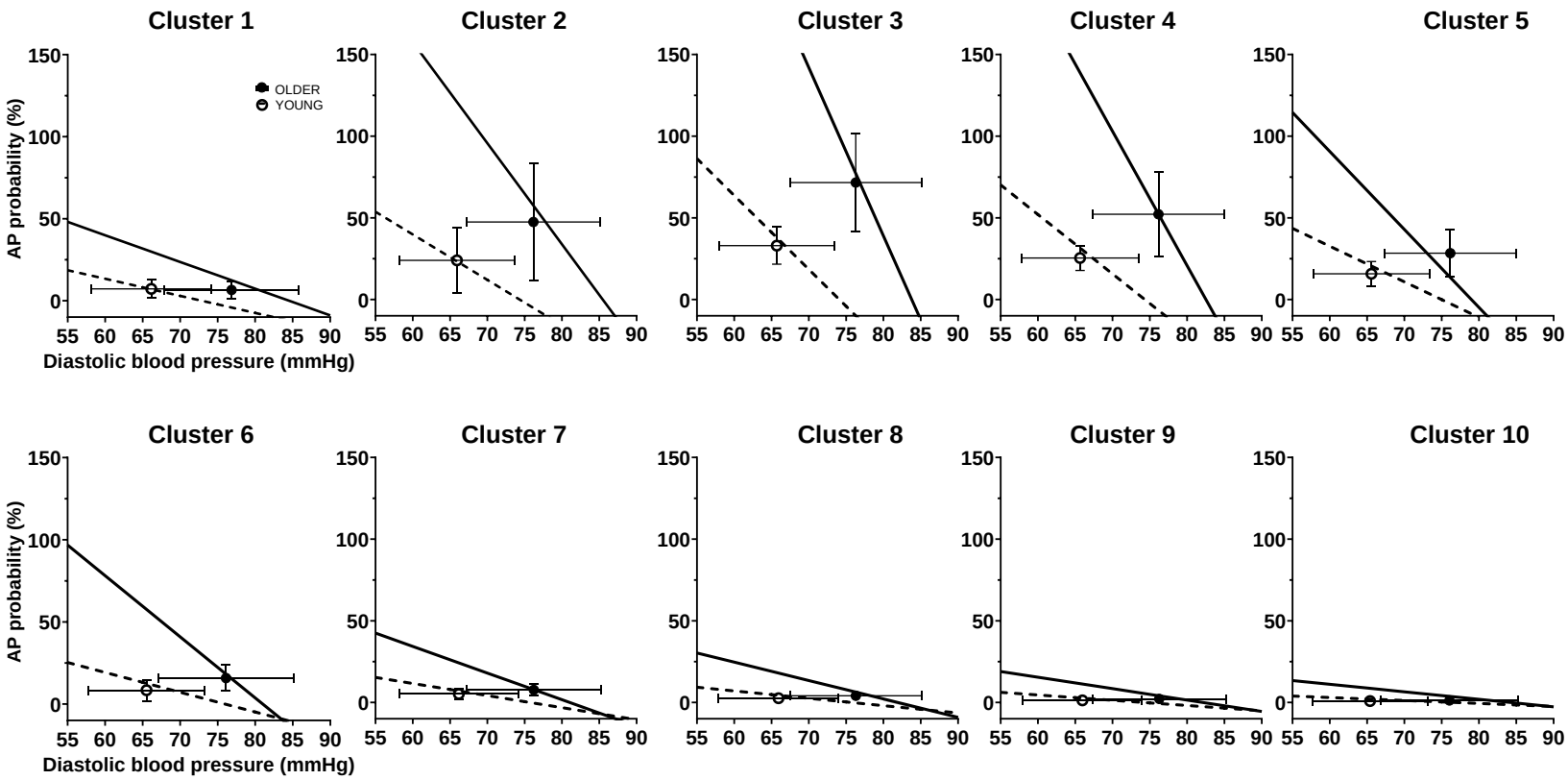


**Figure 2.1: Baroreflex threshold relationships for normalized action potential (AP) clusters.** Mean baroreflex threshold relationships between AP discharge probability and diastolic blood pressure for 10 normalized clusters. Open circles and dashed lines represent the operating points and slopes for each cluster in young adults, respectively. Closed circles and solid lines represent the operating points and slopes for each cluster in older adults, respectively.

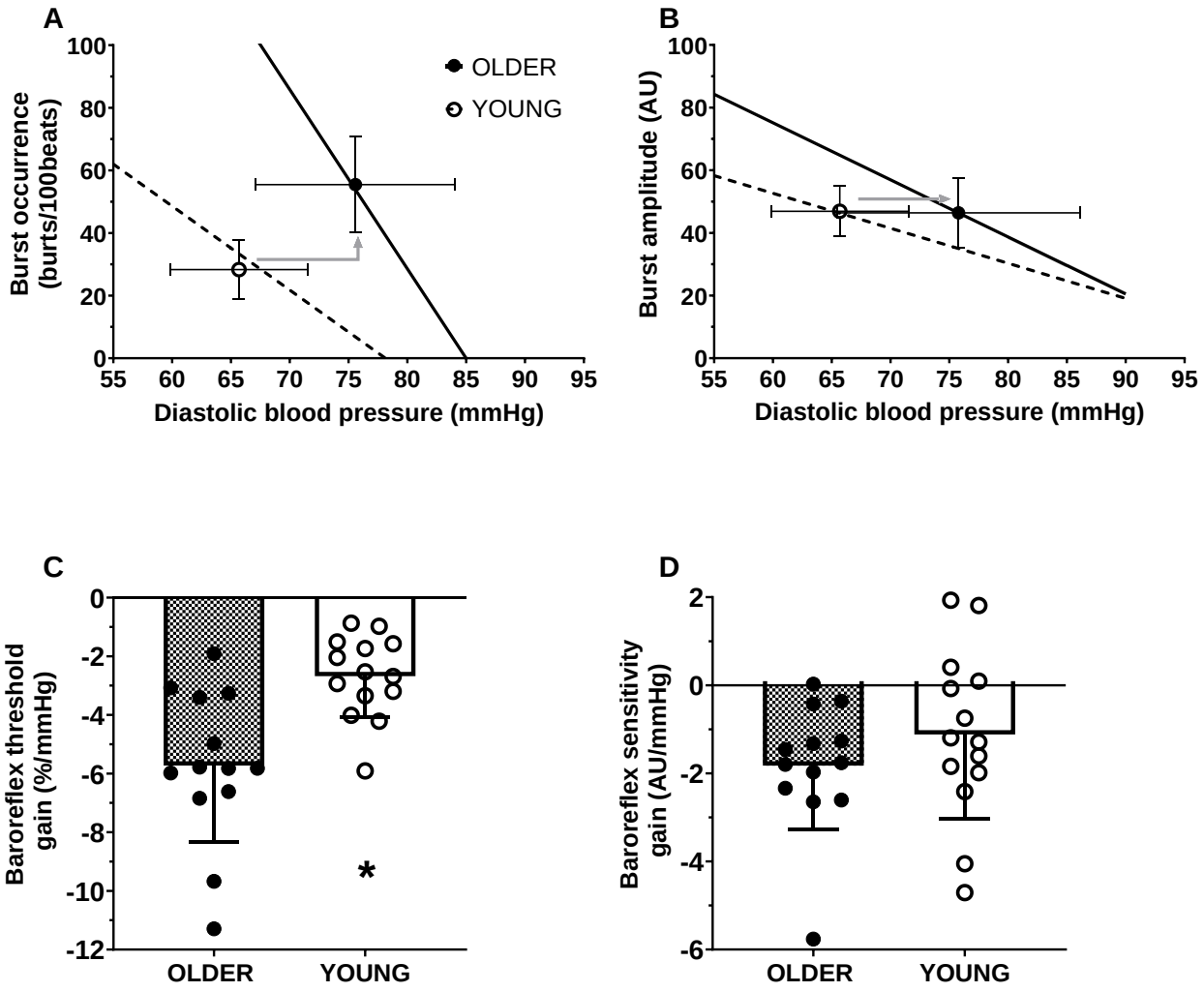


**Figure 2.2: Integrated Muscle sympathetic nerve activity (MSNA) baroreflex threshold and sensitivity relationships in older and young adults.** *A:* Group mean baroreflex threshold relationships between baseline diastolic blood pressure and MSNA burst occurrence are presented with the operating points superimposed on the regression lines. The solid grey arrow indicates that the operating point of the baroreflex was reset rightward and upward to greater diastolic blood pressures and MSNA burst probabilities in older adults. *B:* Group mean baroreflex sensitivity relationships between diastolic blood pressure and normalized integrated MSNA burst amplitude. The solid grey arrow indicates that the baroreflex sensitivity operating point was reset rightward to greater diastolic blood pressures in older adults. *C*: Group mean and individual data points representing the integrated MSNA baroreflex threshold slopes. *D:* Group mean and individual data points representing the integrated MSNA baroreflex sensitivity slopes. Data presented are from thirteen older adults (filled circles) and fourteen young adults (empty circles). Unpaired samples *t*-tests were used to evaluate group differences in baroreflex threshold and sensitivity slopes and operating points. * $P < 0.05$, significantly different from older adults. Values presented are mean (SD).

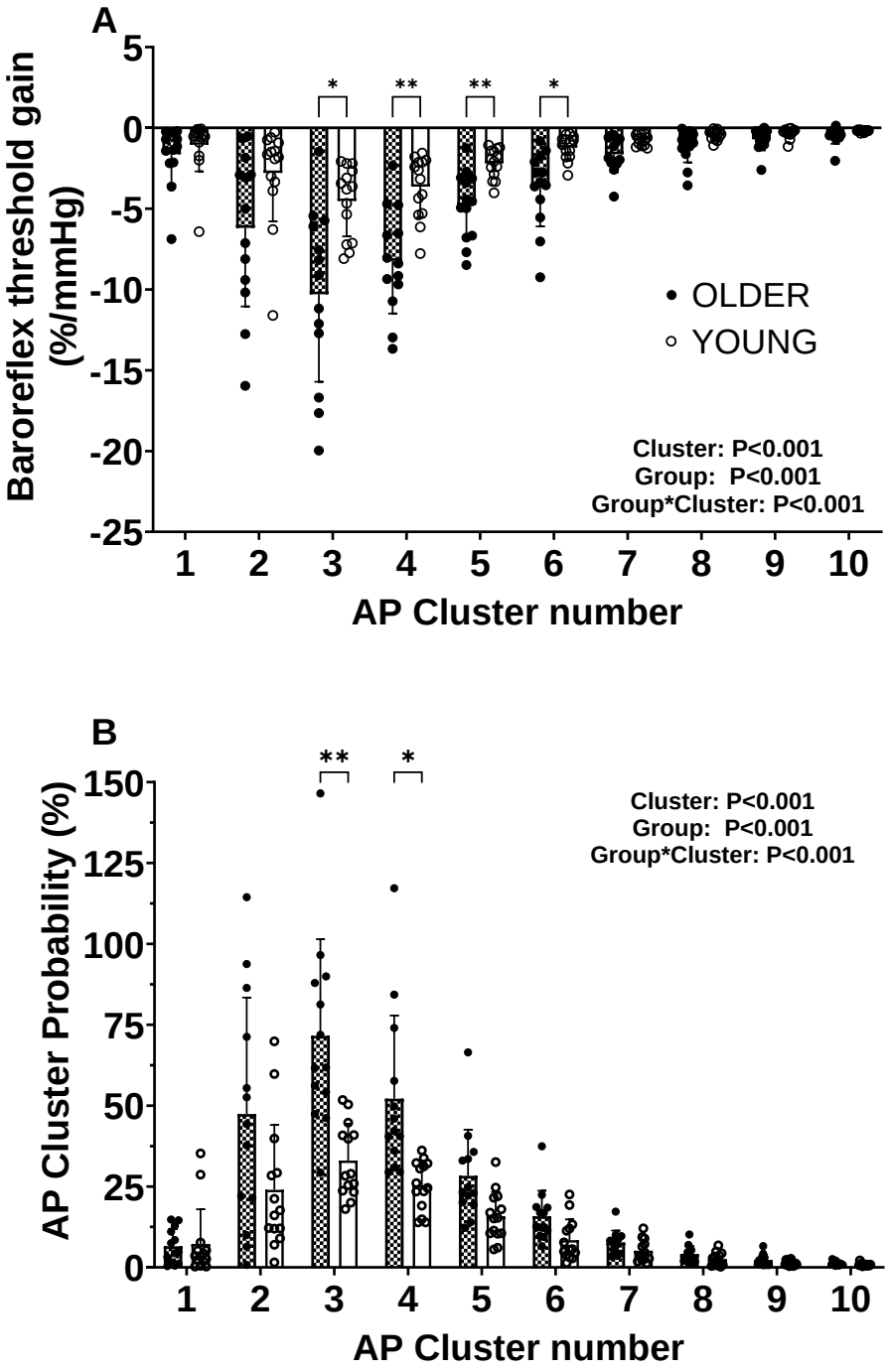


**Figure 2.3: Baroreflex threshold relationships for action potential (AP) discharge in older and young adults.** Baroreflex threshold slopes (A) and probabilities (B) for normalized action potential (AP) clusters in older (*n* = 13; hatched bars and filled circles) and young adults (*n* = 14; empty bars and circles). Two-way repeated measures analyses of variance (ANOVA) were used to determine the impact of aging on the baroreflex control of varying sized AP clusters. Bonferroni-corrected *post-hoc* analyses were performed when a significant ANOVA interaction was observed. Symbols denote significant differences between groups as assessed by *post-hoc* analyses. * $P < 0.05$, ** $P < 0.01$. Values presented are mean (SD).

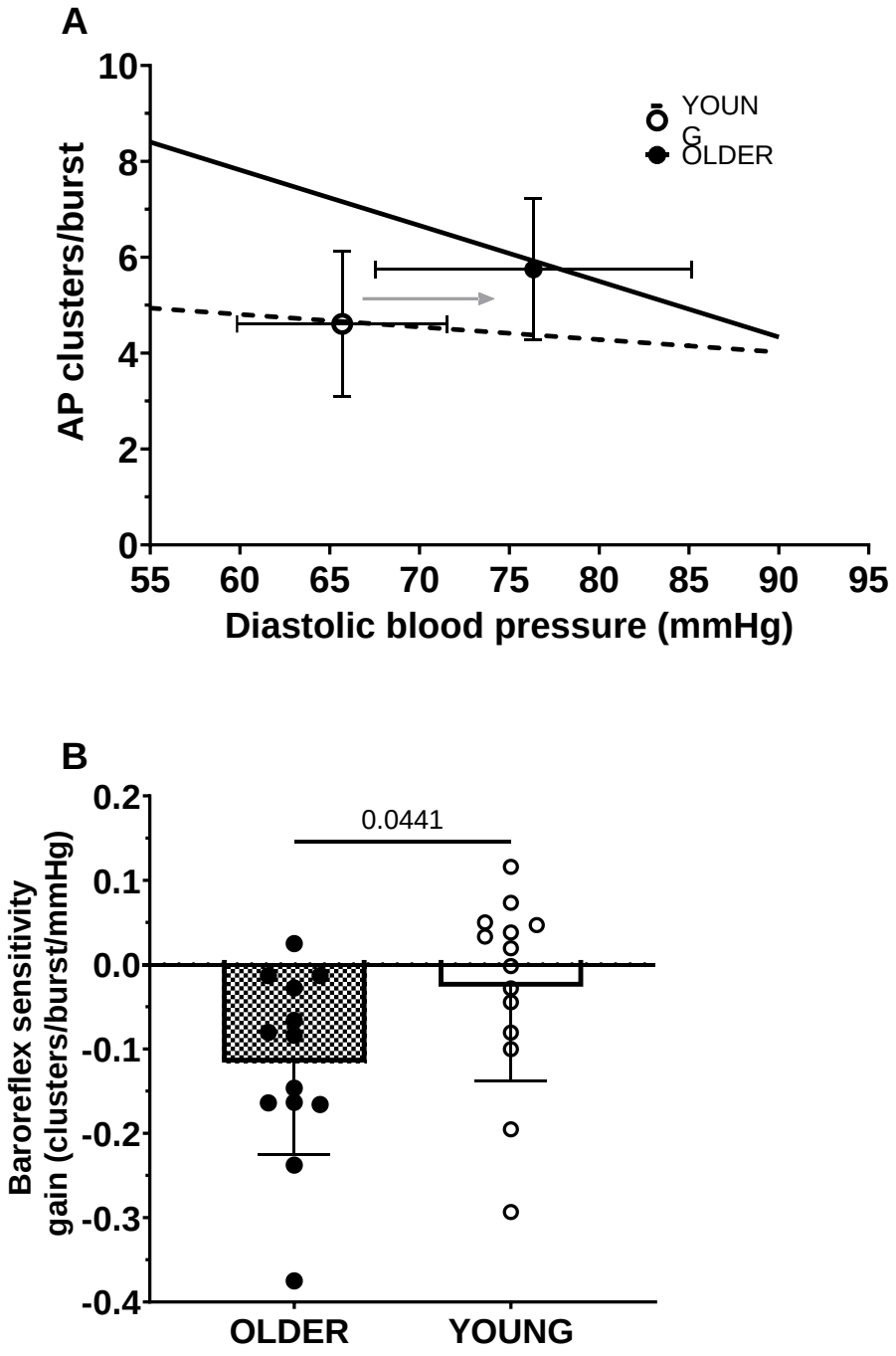


**Figure 2.4: Baroreflex sensitivity relationships for action potential (AP) discharge in older and young adults.** A: Group mean baroreflex sensitivity relationships between the number of AP clusters per burst and diastolic blood pressure. The solid grey arrow indicates that the operating point of the baroreflex was reset rightward to greater diastolic blood pressures in older adults. B: Group mean and individual data points representing the baroreflex sensitivity slopes of AP clusters. Data presented are from thirteen older adults (filled circles) and fourteen young adults (empty circles). Unpaired samples *t*-tests were used to evaluate group differences in baroreflex sensitivity slopes and operating points. Values presented are mean (SD).

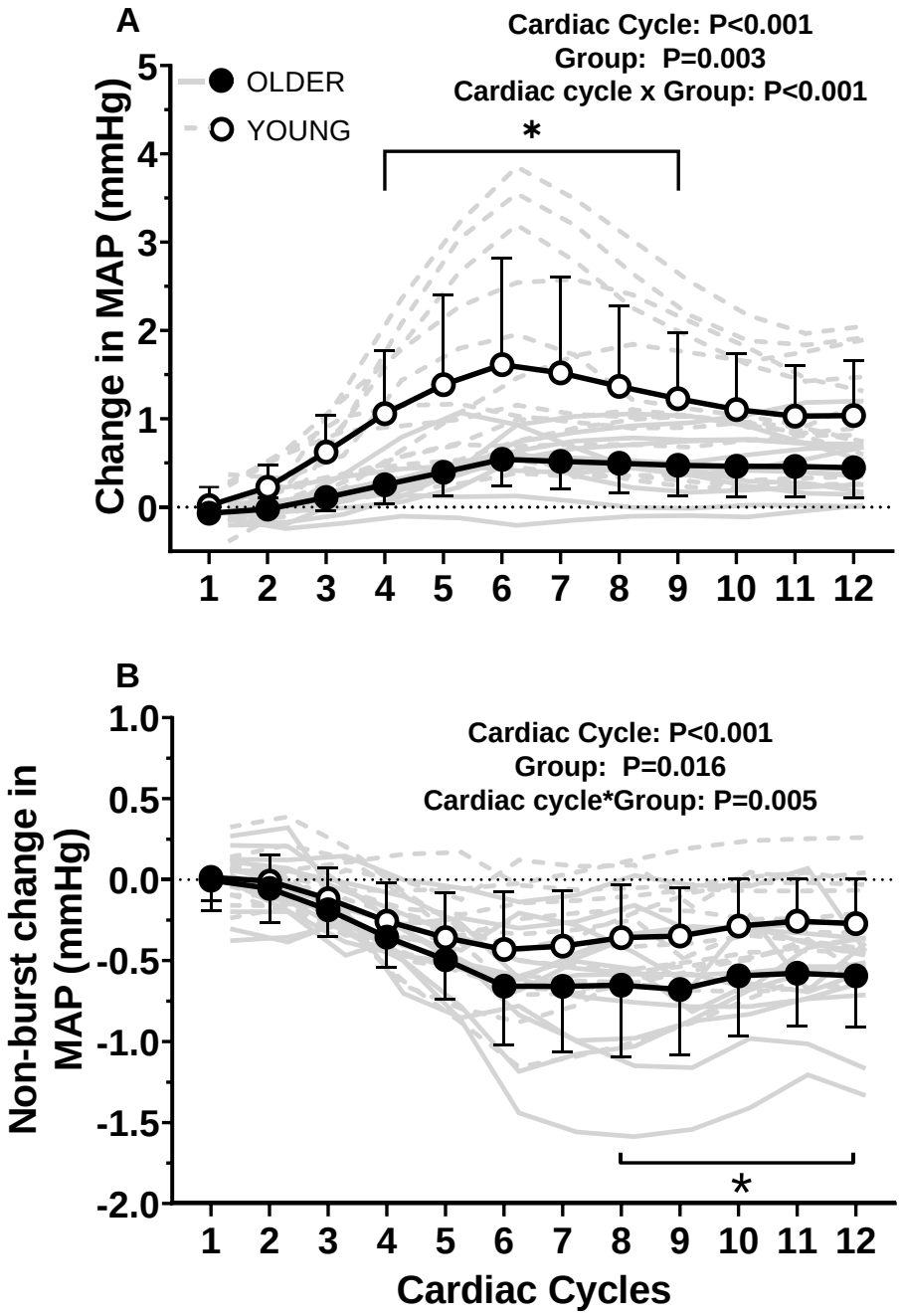


**Figure 2.5: Signal-averaged sympathetic transduction of mean arterial pressure in older and young adults.** A: beat-by-beat changes in mean arterial pressure over 12 cardiac cycles following a burst of muscle sympathetic nerve activity (MSNA). B: beat-by-beat changes in mean arterial pressure over 12 cardiac cycles following a period of sympathetic quiescence (i.e., non-burst). Filled circles denote older adults ($n$ = 13), and open circles denote young adults ($n$ = 14). A repeated measures analysis of variance (ANOVA) was used to evaluate the effect of age on sympathetic transduction (group-by-cluster interaction). Bonferroni-corrected *post-hoc* analyses were performed when a significant ANOVA interaction was observed. * $P$ < 0.05, significant difference between older and young adults. Values presented are mean (SD).

## 2.4. Discussion

The current study provides novel evidence regarding the influence of aging on the spontaneous baroreflex control of sympathetic neural discharge and blood pressure regulation. The major findings are: 1) The integrated MSNA baroreflex threshold gain, and the gain of medium-sized AP clusters were greater in older compared to young adults, 2) compared to young adults, older adults demonstrated a rightward shift of the sympathetic baroreflex threshold operating point to higher diastolic blood pressures, and an upward shift of the integrated MSNA burst occurrence and some medium-sized AP subpopulations towards greater firing probabilities, 3) the integrated MSNA baroreflex sensitivity was not modified by aging; however, older adults exhibited a greater spontaneous AP baroreflex sensitivity compared to young adults, and 4) sympathetic transduction of blood pressure was greater in young compared to older adults. We interpret these findings to indicate that human aging is associated with stronger baroreflex threshold control of integrated MSNA medium-sized AP subpopulations to compensate for a reduction in sympathetic transduction. Phrased differently, to ensure adequate regulation of blood pressure with aging, the central arc of the baroreflex appears to be enhanced in older adults to compensate for age-related impairments in the peripheral arc.

### *2.4.1. The effect of aging on the baroreflex threshold control of integrated MSNA and AP subpopulations.*

Evidence regarding the influence of aging on the baroreflex control of MSNA is equivocal, with most (7, 8, 10, 11) but not all (4, 12) studies demonstrating a preserved ability of the baroreflex to alter MSNA in older compared to young adults. In contrast, we found that older adults demonstrated greater baroreflex threshold gains compared to young adults. These findings were unexpected as arterial stiffness has been associated with reduced sympathetic baroreflex gain among older adults (38). However, compared to the study by Okada et al., the older adults in the current study were relatively younger (~10 years), and therefore the degree of arterial stiffness

amongst the older adults in the current study may have been less than that observed in previous work. Additionally, the discrepancy between our findings and previous work may be attributed to the method of assessing sympathetic baroreflex control of MSNA. In the current study, we assessed the baroreflex control of sympathetic outflow using a closed loop approach (i.e., spontaneous method), whereas others have used open-loop approaches such as vasoactive drug infusions (i.e., phenylephrine and sodium nitroprusside) or a variable pressure neck chamber to force large changes in blood pressure (8, 14, 39). The difference in the range of blood pressures between the open- and closed-loop approaches may be a contributing factor to the disparate conclusions between our study and previous work. An important consideration regarding the use of vasoactive agents in the assessment of arterial baroreflex function is that sodium nitroprusside exerts its depressor effect via nitric oxide. Given the influence of nitric oxide on the central mechanisms involved in the generation of sympathetic outflow (40, 41), some investigators suggest that infusions of this drug may alter the fundamental characteristics of the baroreflex via central neural influences and altered vascular responsiveness to sympathetic discharge (42). Furthermore, not every cardiac cycle is associated with a burst of MSNA, and it is not uncommon for phenylephrine to cause large, rapid inhibition of MSNA bursts, making it difficult to plot baroreflex relationships (43). Although previous work has found strong agreement between the spontaneous baroreflex analysis method and vasoactive drug infusions (44), this work was limited to young adults, and it is unclear whether this agreement exists in an older population. Therefore, to further our understanding of the impact of human aging on the baroreflex regulation of blood pressure within the context of spontaneous cardiovascular conditions, the present study examined the central and peripheral components of the baroreflex in young and older adults under closed-loop conditions.

In the current study we also used a wavelet approach to extract sympathetic APs firing within integrated bursts to explore the baroreflex threshold gain of individual AP clusters. In line

with our previous work (20, 21), we found that the baroreflex demonstrates the strongest control over medium-sized AP clusters, and weaker control over small- and large-sized AP subpopulations. Interestingly, we also found that the baroreflex control over medium-sized AP clusters — which theoretically represent the discharge of a subpopulation of medium diameter sympathetic postganglionic neurons in the microelectrode recording field — was greater in older compared to young adults during supine baseline, whereas the baroreflex threshold gain of smaller and larger AP clusters remained unaltered by human aging. These data support the idea that the baroreflex exerts its strongest control over medium-sized AP clusters and corroborate our previous findings wherein medium-sized AP clusters exhibited modifiable baroreflex threshold gains during lower-body negative pressure, whereas the change threshold gain of smaller and larger AP clusters was minimal (20, 21). Taken together, these data indicate that unlike medium-sized AP clusters, smaller and larger AP clusters appear to be resistant to acute and chronic (e.g., age-related) changes in baroreflex function. We interpret these data to indicate that the ability of the baroreflex to increase or decrease sympathetic vasomotor outflow in response to natural fluctuations in resting blood pressure is augmented in healthy, older adults.

The greater baroreflex threshold gain of integrated MSNA and medium-sized AP clusters in older adults may be compensatory for a reduction in sympathetic transduction. Previous studies have defined the reflex response of MSNA to changes in arterial blood pressure as the central arc of the baroreflex, while the end-organ response to MSNA (i.e., change in mean arterial pressure, total vascular conductance, or limb vascular conductance) has been defined as the peripheral arc of the baroreflex (45). Therefore, it is possible that when there is a reduction in one arm of the sympathetic baroreflex arc (e.g., the peripheral arc in the current group of older adults), the sympathetic nervous system may compensate by augmenting the strength of the other arm of the baroreflex arc (e.g., the neural arc). Indeed, Hissen and colleagues recently demonstrated that in young adults, the integrated sympathetic baroreflex threshold gain is negatively related to

sympathetic vascular transduction (46). However, it is important to note that this relationship was observed in young males, but not females. Our sample size was inadequate to perform a proper sex-based analysis and future work is required to evaluate the interactive effects of sex and aging on the relationship between sympathetic baroreflex threshold gain and sympathetic transduction. Nonetheless, our observations that older adults demonstrated greater sympathetic baroreflex threshold gains and blunted sympathetic transduction compared to young adults, support the idea that the peripheral and central arc of the baroreflex act in concert to ensure effective buffering of blood pressure changes, and that the compensatory relationship between the central and peripheral baroreflex arcs persists in healthy older adults. In line with previous work (13), older adults also demonstrated greater reductions in MAP following cardiac cycles wherein a sympathetic burst did not occur (i.e., non-burst periods) compared to young adults. These data likely reflect the greater sympathetic support of arterial BP with aging (47, 48). Given that older adults have attenuated α-adrenergic receptor sensitivity and/or density relative to young adults (49), it is possible that the higher resting efferent sympathetic outflow in the older adults provides greater sympathetic vasoconstrictor support of arterial BP on a beat-by-beat basis compared to young adults.

In line with prior evaluations (8–11, 39), we found that older adults in the present study, demonstrated an upward and rightward resetting of the integrated MSNA baroreflex threshold operating point towards higher diastolic blood pressures and greater MSNA burst probabilities compared to young adults. Additionally, we demonstrate for the first time that older adults exhibit an increased firing probability of some medium-sized AP subpopulations compared to young adults, further indicating that the baroreflex exerts its strongest control over medium-sized AP clusters. Interestingly, however, not all medium-sized AP subpopulations that exhibited an augmented baroreflex threshold gain in older adults had a greater firing probability. It is possible that spontaneous fluctuations in arterial blood pressure are insufficient to elicit an increase in the

firing probability of all medium-sized AP clusters. Indeed, previous single-unit microneurographic recordings which indicated that a neuron typically only fires once per burst (i.e., 100% AP cluster firing probability) at rest, but can fire multiple times (i.e., greater than 100% AP cluster firing probability) during stress (50, 51) support the idea that larger fluctuations in blood pressure are required to increase the firing probability of all medium-sized AP subpopulations. Additionally, using a multi-unit approach, Limberg and colleagues demonstrated in young adults that pharmacological unloading (using sodium nitroprusside) and loading (via phenylephrine) of the baroreflex, which induces large blood pressure changes, increased, and decreased the firing probability of medium-sized APs, respectively (52). Therefore, it is possible that larger fluctuations in blood pressure are required to elicit significant changes in the firing probabilities of all medium-sized AP clusters that exhibited a greater baroreflex threshold slope with increasing age. However, given that older adults demonstrate impaired sympathetic neural recruitment strategies during sympathoexcitatory stress (22), it is possible that there are age-related impairments in the ability to augment AP cluster firing probability during baroreflex stress as well. This, however, remains to be evaluated.

*2.4.2. The effect of aging on the baroreflex sensitivity control of MSNA and APs.*

In accordance with previous studies (35, 53), we found that the arterial baroreflex strongly governs MSNA burst occurrence but exhibits weak control over MSNA burst size. Furthermore, in line with recent reports from our laboratory, the baroreflex sensitivity relationships for individual AP clusters were weaker than the AP cluster baroreflex threshold relationships (20, 21). Interestingly, however, we found that aging modified the baroreflex sensitivity slope of individual AP clusters but not that of integrated MSNA burst size. The discrepancy between the integrated MSNA and AP cluster baroreflex sensitivity may be due to the integration of the raw, filtered neurogram which masks the nature of the underlying sympathetic APs that comprise integrated bursts of MSNA (19). Alternatively, the process of normalizing burst amplitude to the strongest

burst in both groups is expected to remove sensitivity to changes in the distribution or impact of this variable. Whereas analysis of burst amplitude distribution is thought to better reflect altered sympathetic firing patterns compared with mean normalized burst amplitude (54), assessment of baroreflex function relies on individual burst strength and DBP values. Of potential interest, the greater baroreflex sensitivity of APs indicates that older adults may recruit a wider range of various-sized AP clusters in response to spontaneous fluctuations in DBP compared to young adults. Our observation that older adults had more active AP clusters at rest (I.e., larger range of varying-sized sympathetic APs), without exhibiting a difference in integrated MSNA burst amplitude support this idea. Albeit speculative, these data indicate that there may be an underestimation of the age-related differences in the baroreflex control of integrated MSNA burst size.

### 2.5. Considerations and Limitations

While our study provides novel insight into the influence of aging on the baroreflex control of AP subpopulations, there are several limitations to mention. First, we used the spontaneous baroreflex analysis method which has certain limitations when assessing baroreflex function. For example, baroreflex responsiveness can only be evaluated over a small range of natural fluctuations in BP. Additionally, other factors besides BP, such as respiration, may affect changes in MSNA, and these non-baroreflex factors have less relative influence during the modified Oxford technique, where changes in MSNA are more strongly related to changes in BP. However, given the potential central effects of sodium nitroprusside on efferent sympathetic outflow (42), and the inhibitory effects of phenylephrine on MSNA bursts (52), we believe that the spontaneous baroreflex method provides important information about the sympathetic arm of the baroreflex around the point of its strongest control (i.e., maximal gain). Furthermore, previous work has found a good agreement between the spontaneous baroreflex analysis and the modified Oxford technique, indicating that the spontaneous baroreflex analysis method can be used as an

indicator of sympathetic baroreflex function (44). Second, the current study did not study the hysteresis in the baroreflex control of sympathetic outflow in older and young adults (14, 55). Third, the current results are limited to the age and health of the older group; therefore, the current data may not apply to elderly individuals. Although we found a negative linear relationship between integrated MSNA baroreflex threshold gain and age ($r = -0.50$, $P = 0.017$), which indicates that the strength of the integrated baroreflex threshold gain increases linearly with age (i.e., more negative baroreflex slope with increasing age), future studies are required to evaluate how the baroreflex control of sympathetic outflow changes across the lifespan. Fourth, as previously mentioned, consideration should me made regarding the potential impact of sex on the outcomes of the current study, as biological sex may independently influence the effect of age on both sympathetic baroreflex control of MSNA (38, 56), and sympathetic transduction (13, 57). Whilst it is tempting to speculate the impact sex would have on the current results, future studies are required to directly assess this topic. Fifth, it is important to note that larger APs fire with low probability under baseline conditions making it challenging to discern the extent of the baroreflex control over these AP subpopulations. While this observation is common across various cohorts in our laboratory (20, 21), future work using longer data acquisition periods are required to achieve a greater number of larger APs for deeper analysis such as the reproducibility of probabilities and baroreflex regulation of these AP subpopulations. Lastly, the current study was conducted using a cross-sectional design and therefore cannot determine a cause-and-effect relation between aging and sympathetic baroreflex control. Longitudinal studies would provide important information regarding the impact of human aging on the regulation of efferent sympathetic neural discharge in humans.

### 2.6. Conclusion

The present study demonstrates, for the first time, that the spontaneous baroreflex control varying-sized AP subpopulations is altered by aging. Specifically, older adults demonstrated

greater baroreflex slopes of the medium-sized AP clusters that typically exhibit the strongest baroreflex control, compared to young adults. In line with this, we found that the integrated MSNA baroreflex threshold gain was augmented with aging. The augmented sympathetic baroreflex threshold gain in older adults, may be a compensatory response in the face of an age-related reduction in sympathetic transduction. In contrast to the resetting of the integrated MSNA baroreflex threshold operating point, there was no upward resetting of individual AP clusters towards a greater firing probability. Additionally, while we found no age-related differences in the integrated baroreflex sensitivity relationships, older adults demonstrated an enhanced AP baroreflex sensitivity. In our view, the greater baroreflex threshold gain of integrated MSNA and medium-sized APs in the face of impaired sympathetic transduction provides novel insight into the regulation of sympathetically mediated axons that induce the vascular adjustments that support blood pressure homeostasis in young and older humans.

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# Chapter 3

## Age- and sex-specific changes in sympathetic vascular transduction and neuro-hemodynamic balance in humans

*(Under review at The American Journal of Physiology – Heart and Circulatory Physiology)*

### 3.1. Introduction

The sympathetic nervous system plays a central role in the regulation of arterial blood pressure. However, inter-individual differences exist in the sympathetic neural regulation of blood pressure (1). In young males, reciprocal relationships between the sympathetic neural control of total peripheral resistance (TPR) and cardiac output result in little direct effect between muscle sympathetic nerve activity (MSNA) and arterial pressure (2), likely because young males with higher MSNA have attenuated α-adrenergic receptor responsiveness (3). Conversely, young females do not exhibit any relationship between MSNA, TPR, cardiac output, or blood pressure, perhaps because of estradiol-mediated β-adrenergic vasodilation that offsets α-adrenergic vasoconstriction (4, 5). As humans age, the relationship between MSNA and blood pressure is altered, with older males and females demonstrating a positive relationship between these two variables (5–7). Thus, older adults with higher resting MSNA also have higher blood pressure. While these studies indicate that age and sex impact the relationship between MSNA and blood pressure, less is known about how these inter-individual factors interactively impact the ability of spontaneous bursts of efferent MSNA to evoke beat-by-beat changes in blood pressure and vascular tone (i.e., sympathetic transduction).

Prior assessments of the independent effect of age on sympathetic transduction have found smaller pressor responses to bursts of MSNA amongst older compared to young adults (8, 9). Conversely, the effect of biological sex on sympathetic transduction is equivocal (10), with no sex-related differences observed in young adults (11–14), whereas amongst older adults, females demonstrate either exaggerated (15) or attenuated (11) transduction of MSNA into blood pressure

compared to males. The reason for this discrepancy amongst older adults is likely attributed to the methodology used to assess sympathetic transduction (i.e., spike-triggered averaging vs. weighted linear regression analyses) (16). Nonetheless, these studies have demonstrated sex-specific alterations in sympathetic transduction that occur with aging. Although advancing our understanding of the impact of age and sex on sympathetic transduction into blood pressure, MSNA reflects vasoconstrictive neurotransmitter release onto the skeletal muscle vasculature (17, 18), and there may be discordance between sympathetic transduction into blood pressure versus leg vascular conductance (LVC) (14). Thus, more direct assessments of sympathetic transduction related to MSNA may be achieved through measures of neurogenic vasoconstriction of the peripheral vasculature (e,g., reductions in LVC) (19), and it remains unclear how age and sex interactively impact the vasoconstrictor responses to spontaneous bursts of MSNA.

Furthermore, although MSNA drives changes in blood pressure, it is also impacted by spontaneous blood pressure fluctuations through baroreflex feedback (20, 21). In closed loop conditions, the arterial baroreflex regulation of blood pressure can be decomposed into two components: 1) the peripheral arc (i.e., sympathetic transduction) and 2) the central arc (i.e., arterial baroreflex control of MSNA) (22). Although the coordinated interplay between the central and peripheral arcs of the baroreflex are critical to the regulation of sympathetic outflow, vascular tone, and blood pressure, many studies have either assessed the central (23–25) or peripheral arcs (11, 12, 26) in isolation. Recently however, an inverse relationship between the central and peripheral arcs of the sympathetic baroreflex at rest was observed in young males, but not females (13). Furthermore, while we found attenuated sympathetic transduction into blood pressure (i.e., peripheral arc), but greater spontaneous baroreflex control of MSNA (i.e., central arc), in older compared to young adults (9), others found no relationship between sympathetic transduction and sympathetic baroreflex sensitivity in a mixed-sex group of older adults (27). The lack of relationship between the central and peripheral baroreflex arcs previously observed

amongst older adults may be attributed to the lack of sex-based analyses. Indeed, older females demonstrate attenuated baroreflex control of MSNA (28) and sympathetic transduction into blood pressure (11) compared to males, suggesting that sex differences in the central and peripheral arcs of the baroreflex exist amongst older adults. However, no study to date has evaluated how age and sex interactively impact the compensatory neuro-cardiovascular relationships between the central and peripheral baroreflex arcs that serve to regulate blood pressure and sympathetic vasoconstrictor tone on a beat-by-beat basis.

Accordingly, the purpose of the current study was to evaluate the impact of age and sex on sympathetic vascular transduction and sympathetic transduction into blood pressure, as well as the relationship between the central and peripheral arcs of the baroreflex. We hypothesized that a) older adults, especially older females, would demonstrate smaller changes in blood pressure and LVC following bursts of MSNA, b) an inverse relationship between sympathetic baroreflex sensitivity and sympathetic transduction into LVC would exist in all groups except young females, and c) resting sympathetic outflow would be inversely related with sympathetic transduction into mean arterial pressure (MAP)/LVC, but positively related with sympathetic baroreflex sensitivity in all groups except young females.

## 3.2. METHODS

### *3.2.1. Ethical approval*

Written and informed consent was obtained from all participants prior to testing. Approval of experimental protocols was granted by the institutional review boards of the University of Texas Southwestern Medical Center, Texas Health Presbyterian Hospital Dallas (File no. STU-2022-0433), and The Western University Health Sciences Research Ethics Board (File no. 119380).

The study was conducted in accordance with the *Declaration of Helsinki*, except for registration in a database.

### *3.2.2. Participants*

52 individuals (Young males (YM): *n*=14, Young females (YF): *n*=13, Older males (OM): *n*=12, Older females (OF): *n*=13) participated in the current study in a fasted state (≥8 hours), and having refrained from caffeine, alcohol, and vigorous exercise for a minimum of 12 hours. Data were collected at the Neurovascular Research Laboratory at Western University (n=16; 10 YM and 6 YF) and the Women's Heart Health Laboratory at The Institute for Exercise and Environmental Medicine (Texas Health Presbyterian Hospital) (n=36; 4 YM, 7 YF, 12 OM, 13 OF). Of the 14 young males: 8 self-identified as Caucasian, 1 as African-American, 3 as South Asian, 1 as Middle-eastern, and 1 as East Asian. Of the 13 young females: 1 self-identified as Middle-eastern, 1 as East Asian, 2 as African-American, 2 as Latin-American, 1 as South Asian, and 6 as Caucasian. All 12 older males self-identified as Caucasian, and 11 of the 12 older females as Caucasian. 1 older female self-identified as African-American. All participants were non-smokers, and free of overt cardiovascular disease, metabolic syndrome, hepatic and renal disease, neurological disease, pulmonary disease, and not pregnant (within the last 12 months). To minimize the impact of exogenous sex hormones on neural and cardiovascular outcomes, no participants were using hormonal replacement therapy or contraception (e.g., intrauterine device, oral contraception, implant), and young females were tested in the mid-luteal phase of the menstrual cycle (as determined by self-report [n=13], blood draw [n=12] and ovulation kits [n=9]). Microneurographic recordings were unsuccessful in one older male and one older female. Thus, data are reported for 11 older males and 12 older females.

### *3.2.3. Experimental measures and data analysis*

<u>Cardiovascular and hematological measures</u>

Heart rate (HR) was measured using a standard lead II electrocardiogram (BioAmplifier, ADInstruments, Dunedin, New Zealand). Beat-by-beat blood pressure was measured continuously via finger photoplethysmography (Finapres Medical Systems, Amsterdam, The Netherlands, and Human NIBP Nano system, ADInstruments). Finger-derived blood pressure measures were calibrated to the mean of three brachial artery blood pressure measures that were completed just prior to data collection. Blood samples were collected from the antecubital vein for the assessment of estradiol, progesterone, total testosterone, sex hormone binding globulin, and albumin via chemiluminescence (ARUP laboratories, Salt Lake City, UT, USA, and LifeLabs, London, Ontario, Canada). Bioavailable testosterone was calculated from total testosterone, sex hormone binding globulin, and albumin using a published equation (29). Hematological data were previously reported in our recent work (30).

Muscle Sympathetic Nerve Activity

Efferent postganglionic multiunit MSNA was recorded using standard microneurographic techniques, as previously described (31). Briefly, a 15-30-mm long tungsten microelectrode, with a 200-µm diameter, tapering to an uninsulated 1- to 5-µm tip was inserted percutaneously into the common peroneal nerve at the popliteal fossa (Frederick Haer Corporation, Bowdoin, ME, USA) and a reference electrode was positioned subcutaneously ~1-3 cm from the recording site. The presence of sympathetic nerve traffic innervating skeletal muscle vasculature was confirmed based on pulse synchronous bursts at rest and that increased in frequency and size during an apnea (i.e., breath hold), but not during skin stroking or auditory perturbations. The MSNA neurogram was recorded with a nerve traffic analyzer (662C-3; Bioengineering Dept., University of Iowa, Iowa City, IA). Neural signals were amplified (gain: 70,000-160,000-fold), and band-pass filtered (bandwidth: 700-2000 Hz) before being rectified and integrated (leaky integrator; 0.1-s time constant). The raw, filtered, and integrated MSNA signals were sampled at 10,000 Hz, while

all other signals were sampled at 1,000 Hz. All data were collected using LabChart 8 and PowerLab (ADInstruments, Colorado Springs, CO) and were then saved offline for analysis.

The integrated MSNA neurogram was analyzed on a beat-to-beat basis to determine the presence or absence of MSNA bursts. Bursts were identified in accordance with previously published standardized guidelines (32). Specifically, integrated MSNA bursts were included if they demonstrated pulse-synchrony, had a signal-to-noise ratio (SNR) of at least 3:1 relative to the previous period of neural silence, expressed characteristic rising and falling slopes, and individual sympathetic APs were visible in the corresponding raw filtered neurogram. Integrated MSNA was quantified as burst frequency (bursts/min) and burst incidence (bursts/100 heart beats). Burst amplitude was measured in volts and normalized to the largest burst at baseline which was given a value of 100 (AU). Total MSNA was calculated as the product of burst frequency and normalized burst amplitude (AU/min).

Leg Blood Flow

Superficial femoral artery (SFA) blood flow was obtained using a duplex Doppler ultrasound (uSmart 3300, Terason, Burlington, MA;, Teratech). The SFA was imaged ~3cm distal to the bifurcation of the common femoral artery. Continuous measures of SFA diameter and velocity were obtained simultaneously in B-mode and pulse wave, respectively, and operating at a linear frequency of 4-15 MHz (15L4 Smart Mark). In accordance with recent ultrasound technical guidelines (33), the sample volume encompassed the entire vessel lumen without extending the vessel walls, the insonation angle was set to 60°, and the ultrasound probe was steered such that the ultrasound beam was parallel to the vessel walls. Since high quality Doppler ultrasound measures were unable to be obtained in 1 YM and 1 OF, blood flow data were included for a total of 48 participants (13 YM, 13 YF, 11 OM and 11 OF).

A video image of the ultrasound screen was recorded and saved as an Audio Video Interleave (AVI) file using Camtasia (TechSmith). SFA diameter and blood velocity were analyzed using a custom-designed edge detection, and wall-tracking software (BloodFlow Analysis, version 4.0). and the video recording of the ultrasound was used to extract continuous diameter and velocity of the SFA at a frequency of 30 Hz. This approach is independent of investigator bias and has been previously validated with an interobserver coefficient of variation of 0.36% (34). Leg blood flow (LBF) was calculated as: (peak envelope blood velocity/2) X ($\pi$(0.5 X diameter)$^2$ X 60). LVC was determined by dividing LBF/ MAP, and was used to indicate the degree of neurogenic vasoconstriction as changes in conductance are proportional to changes in blood flow and may better represent the importance of regional vasomotor changes in the context of blood pressure regulation (35, 36). Additionally, as noted in a recent methodological review, percent changes in vascular conductance should be used to represent sympathetic vascular transduction (37).

Sympathetic Transduction into Leg Vascular Conductance and Blood Pressure

The analysis of sympathetic transduction of MSNA into MAP and LVC was completed using an open-source, Microsoft Excel-based program, as previously described (38). Briefly, a spike-triggered averaging methodology was performed wherein MSNA bursts acted as a trigger and were followed for the subsequent 12 cardiac cycles to capture the pressor and leg vascular responses following a burst of MSNA. This time frame was selected as previous work has found that the peak increase in blood pressure and fall in LVC occur within ~5-7 cardiac cycles following bursts of MSNA (39). For each participant, the change in MAP and LVC were defined as the MAP or LVC at each successive cardiac cycle subtracted from the MAP or LVC at time point 0 (i.e., the MSNA burst or the first cardiac cycle in a non-burst period). Absolute changes in MAP and percent changes in LVC were averaged for each participant, and a group mean was determined. The peak or nadir changes in MAP and LVC were used to provide an estimate of the magnitude of the transduction response, whereas the beat-to-beat changes over 12 cardiac cycles were used to

provide a more detailed evaluation of the time-course changes in sympathetic vascular transduction. Additionally, the responses following periods of sympathetic silence (i.e., non-burst periods) were evaluated using the average beat-by-beat changes in MAP and LVC following all cardiac cycles that were not associated with an MSNA burst. These data provided insight into the reliance of sympathetic vasoconstrictor tone in the maintenance of blood pressure.

MSNA burst amplitude reflects the number and size of synchronously firing postganglionic c-fibers (40, 41), and larger bursts are associated with greater changes in MAP and LVC (19, 26, 42). Thus, we examined the influence of MSNA burst amplitude on the ensuing MAP and LVC responses. Bursts were categorized into quartiles based on their normalized burst amplitudes, wherein quartile 1 represented the smallest 25% of all bursts, and quartile 4 represented the largest 25% of bursts. The slope of the linear regression between MSNA burst amplitude quartile and sympathetic transduction (i.e., the peak MAP and nadir LVC changes plotted as a function of mean quartile burst amplitude) was also used as an additional metric of sympathetic transduction. Furthermore, norepinephrine spillover is positively related with muscle sympathetic axonal firing frequency (18, 43). Thus, greater sympathetic transduction following bursts firing in combination (i.e., multiples) are likely attributed to greater vasoconstrictive neurotransmitter(s) release into the synaptic cleft. To examine the impact of MSNA burst firing pattern on sympathetic transduction, bursts were classified as singlets (i.e., surrounded by cardiac cycles without MSNA bursts), doublets (i.e., 2 MSNA bursts firing directly adjacent), or triplets + (three or more bursts firing in consecutive cardiac cycles), and serial and peak or nadir changes in MAP and LVC, respectively, across 12 cardiac cycles were determined. For bursts occurring within each burst cluster pattern, the amplitudes of all bursts were summed and the peak or nadir changes in MAP and LVC, respectively, were plotted as a function of mean cluster amplitude using linear regression analyses.

Sympathetic baroreflex sensitivity

Spontaneous baroreflex threshold and sensitivity analyses of integrated MSNA were completed using the methods previously described (44). Spontaneous baroreflex control of MSNA was calculated over a 3-mmHg diastolic blood pressure bin and statistically weighted for the number of cardiac cycles in each bin to reduce the impact of non-baroreflex variability (e.g., respiration) and minor variation of bin width and position (24). Pearson's correlation coefficients (r) were recorded for all participants. In accordance with published guidelines (44), one young female and one older female were excluded from analyses due to inadequate sympathetic baroreflex sensitivity relationships ($r<0.70$).

Experimental Protocol

After instrumentation for all measurements, participants rested for ~10 minutes. Cardiovascular and sympathetic neural measures were then recorded for a 10-minute baseline period in a quiet, dimly lit, climate-controlled (~22°C) room. Care was taken to ensure participants remained awake and quiet throughout the recording period.

*3.2.4. Statistical analysis*

All data are presented as mean (SD). Based on previous work (11) power calculations were performed to determine the group differences in peak sympathetic transduction between young males, young females, older males, and older females. With an effect size of 1.25, a β of 0.8, and a two-sided α of 0.05 indicated that a sample of 48 participants were required to see age- and sex-related differences in sympathetic transduction. Normality was assessed using the Shapiro-Wilk test, and when appropriate, non-parametric testing was performed. Two-way analyses of variance (ANOVA) were used to evaluate the effects of age and sex on participant characteristics, resting hemodynamics, sex hormones and peak or nadir sympathetic transduction into MAP and LVC, respectively. The time course effects of age, sex, and cardiac cycle on sympathetic transduction into MAP and LVC were assessed using a three-factor linear mixed

model analysis. Bonferroni-corrected *post-hoc* comparisons were performed to evaluate specific differences between means in the event of a significant interaction effect. *A priori* pairwise comparisons were restricted to within age and sex. Thus, young males were not compared with older females and young females were not compared with older males. Linear regression analyses were used to determine sympathetic transduction slopes. Age-related differences in the sympathetic transduction slopes were assessed using unpaired *t*-tests. Statistical significance was accepted at $P \leq 0.05$. Statistical analyses were performed using SPSS statistics (Version 28.0; IBM, Armonk, NY).

## 3.3. RESULTS

### *3.3.1. Participant characteristics, sex hormones, resting hemodynamics and sympathetic activity*

Participant characteristics, resting hemodynamics and sex hormone data are presented in Table 3.1. Estradiol and progesterone concentrations were greater in young females relative to older females (both $P<0.001$) and young males (both $P<0.001$). Bioavailable testosterone was greater in young compared to older males ($P<0.001$), but not different between young and older females ($P=0.836$). Sex-based comparisons within each age group revealed that both older and young males had greater levels of bioavailable testosterone compared to similarly aged females (both $P<0.001$). Heart rate was higher in young compared to older adults ($P=0.017$) and was higher in females compared to males ($P=0.005$). Older adults had higher systolic ($P<0.001$) and diastolic blood pressure ($P<0.001$), as well as higher MAP ($P<0.001$) than young adults, regardless of sex. Significant age-by-sex interactions were observed for resting MSNA burst frequency ($P=0.034$) and burst incidence ($P=0.004$). *Post-hoc* analyses revealed that older females and older males had greater MSNA burst occurrence compared to young females (burst frequency: $P=0.049$, burst incidence: $P=0.051$) and young males (burst frequency: $P<0.001$, burst

incidence: *P*<0.001), respectively. Furthermore, older males had greater MSNA burst frequency (*P*=0.002) and incidence (*P*<0.001) compared to older females. Thus, total MSNA was greater in older adults (*P*<0.001) compared to young adults, regardless of sex. A main effect of sex was observed for sympathetic baroreflex sensitivity, indicating that males had greater spontaneous baroreflex control of MSNA compared to females (*P*=0.037). Lastly, significant age-by-sex interactions were observed for LBF (*P*=0.040) and LVC (*P*=0.027), with *post-hoc* analyses revealing that young males had greater LBF and LVC than young females and older males (range: *P*≤0.001-0.004). No differences in LBF or LVC were observed between young and older females (range: *P*=0.212-0.345) or between older males and females (range: *P*=0.594-0.769).

*3.3.2. Sympathetic transduction into MAP and LVC*

Figure 3.1A shows the beat-by-beat increases in MAP following spontaneous bursts of MSNA. In all groups, there was clear increase in MAP following MSNA bursts (main effect of cardiac cycle: *P*<0.001). However, an age-by-cardiac cycle interaction effect was observed for the beat-by-beat changes in sympathetic transduction into MAP (Figure 3.1A; *P*<0.001), with young adults demonstrating larger increases in MAP following spontaneous bursts of MSNA compared to older adults from cardiac cycles 2-12 (range: *P*≤0.001-0.023). Thus, young adults demonstrated larger peak changes in MAP following MSNA bursts than older adults, regardless of sex (Figure 3.1B; main effect of age: *P*<0.001). LVC decreased in all groups following spontaneous bursts of MSNA (Figure 3.1C; main effect of cardiac cycle *P*<0.001). An age-by-sex-by-cardiac cycle interaction was observed for the reduction in LVC, with significant differences between older and young females from cardiac cycles 4-8, and at cardiac cycle 10 (range: *P*≤0.001-0.022). Additionally, significant differences between older and young males at cardiac cycle 2 (*P*=0.008), as well as between cardiac cycles 6-12 (range: *P*≤0.001-0.05). No sex differences were observed between older males and females (range: *P*=0.330-0.948) whereas young males had a smaller reduction in LVC compared to young females at cardiac cycles 3

(*P*=0.038) and 4 (*P*=0.036), only. Consequently, the nadir change in LVC was attenuated in older compared to young adults (Figure 3.1D; main effect of age: *P*<0.001); however, no age-by-sex interaction effect was observed (*P*=0.743).

Contrary to MSNA bursts, cardiac cycles that did not contain a burst of MSNA resulted in a gradual reduction in MAP in all groups (Figure 3.2A; main effect of cardiac cycle: *P*<0.001). An age-by-sex-by-cardiac cycle interaction was observed for the reduction in MAP (*P*=0.012), with *post-hoc* analyses revealing that older females had larger reductions in MAP compared to young females between cardiac cycles 7-12 (range: *P*=0.008-0.029) and older males had larger reductions in MAP compared to young males between cardiac cycles 6-12 (range: *P*≤0.001-0.004). Furthermore, older males demonstrated larger reductions in MAP compared to older females between cardiac cycles 8-12 (range: *P*=0.002-0.019). When assessing the nadir change in MAP following cardiac cycles that lacked MSNA (Figure 3.2B), main effects of age was observed indicating that older adults had greater reductions in MAP following periods of sympathetic quiescence compared to young adults (*P*<0.001). Additionally, a significant main effect of sex (*P*=0.048) indicated that males, regardless of sex, had greater nadir MAP responses following non-burst periods.

Following cardiac cycles without MSNA bursts, LVC increased in all groups (main effect of cardiac cycle: *P*<0.001). Significant sex-by-cardiac cycle (*P*<0.001) and age-by-cardiac cycle (*P*<0.001) interaction effects were observed. *Post-hoc* analyses revealed that males had larger increases in LVC than females during cardiac cycles 9-11 (range: *P*=0.004-0.05), whereas older adults demonstrated larger increases in LVC compared to young adults between cardiac cycles 4-12 (range: *P*≤0.001-0.022). Thus, older adults (*P*<0.001) and males (*P*=0.021) demonstrated greater increases in LVC compared to young adults and females, respectively.

*3.3.3. Effect of MSNA burst amplitude on sympathetic transduction into MAP and LVC*

The mean amplitude of bursts in quartile 1 was slightly greater in older compared to young adults (*P*=0.032; Table 3.2), but no effects of age or sex were observed for bursts in quartiles 2-4 (range: *P*=0.198-0.957). When MSNA bursts were binned into quartiles based on burst amplitude, significant main effects of age were observed within each quartile (all *P*<0.001) such that young adults had larger increases in MAP following MSNA bursts of all sizes. No effect of sex (range: *P*=0.533-0.854) or interaction effects (range: *P*=0.060-0.721) were found for MAP. Similarly, young adults demonstrated greater reductions in LVC following bursts of MSNA across all quartiles compared to older adults, regardless of sex (Figure 3.3A-D; all *P*<0.001).

Strong linear relationships were observed for the increases in MAP and decreases in LVC as a function of MSNA burst amplitude. The slope of sympathetic transduction into MAP as a function of MSNA burst amplitude tended to be greater in young males compared to older males (YM: 0.033 mmHg/AU vs. OM: 0.024 mmHg/AU; *P*=0.092) and in young females compared to older females (YF: 0.039 mmHg/AU vs. OF: 0.020 mmHg/AU; *P*=0.053). Similarly, young males and females demonstrated greater reductions in LVC as a function of MSNA quartile burst amplitude compared to older males (Figure 3.3E; *P*=0.032), and older females (Figure 3.3F; *P*=0.004), respectively.

*3.3.4. Effect of MSNA burst pattern on sympathetic transduction into MAP and LVC*

The number of MSNA bursts firing as singlets, doublets, and triplets+ and total amplitude of MSNA in each firing pattern cluster are presented in Table 2. Young adults demonstrated greater increases in MAP and larger reductions in LVC (Figure 4A-C) following singlet, doublet and triplet+ bursts of MSNA (range: *P*<0.001-0.006). No main effect of sex or age-by-sex interaction effects were observed for the changes in MAP or LVC following singlet, doublet, or triplet + MSNA bursts (range: *P*=0.288-0.988). The changes in MAP and LVC increased in a graded manner, proportional to MSNA burst firing pattern. Notably, however, older adults (males and females) demonstrated smaller changes in MAP (YM: 0.017 vs. OM: 0.007 mmHg/AU;

*P*=0.01, YF: 0.020 vs. OF: 0.010 mmHg/AU; *P*=0.004) and LVC (Figure 3.4D and E; range: *P*=0.004-0.007) compared to young adults of the same sex.

*3.3.5. Relationship between the central and peripheral arcs of the arterial baroreflex, as well as resting MSNA and sympathetic transduction*

Sympathetic baroreflex gain was inversely related to sympathetic vascular transduction (i.e., sympathetic transduction into LVC) in young (Figure 3.5A, r=-0.59; *P*=0.032) and older (Figure 3.5B, r=-0.71; *P*=0.014) males. However, this relationship was not observed in young (Figure 3.5C, r=0.16; *P*=0.621) or older (Figure 3.5D, r=0.26; *P*=0.445) females. Notably, no relationships were observed in any group when the peripheral baroreflex arc was assessed as sympathetic transduction into blood pressure, (Figures 3.5D-H, range: *P*=0.373-0.732). Resting MSNA burst incidence was not related to sympathetic transduction into MAP or LVC in older males (MAP: Figure 3.6A, r=0.17; *P*=0.60; LVC: Figure 6C, r=0.23; *P*=0.50), or young females (MAP: Figure 3.6B, r=0.16; *P*=0.603; LVC: Figure 6D, r=0.28; *P*=0.36). Conversely, the peak change in MAP and LVC were inversely related with MSNA burst incidence in young males (MAP: Figure 3.6A, r=0.55; *P*=0.04; LVC: Figure 6C, r=0.60; *P*=0.03) and older females (MAP: Figure 3.6B, r=0.58; *P*=0.046; LVC: Figure 6D, r=0.62; *P*=0.04), such that young males and older females with higher MSNA burst incidence had lower sympathetic transduction into MAP and LVC.

***Table 3.1.*** *Participant characteristics, resting blood pressure, and sex hormones.*

| | Young | | Older | | ANOVA *P*-values | | |
|---|---|---|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** | **Age** | **Sex** | **Age x Sex** |
| **Demographics** | | | | | | | |
| Age (yrs) * | 25 (3) | 24 (4) | 71 (5) | 70 (4) | **<0.001** | 0.629 | 0.697 |
| Height (cm) † | 178 (8) | 164 (6) | 172 (8) | 166 (7) | 0.338 | **<0.001** | 0.053 |
| Weight (kg) † | 78 (11) | 67 (12) | 78 (7) | 71 (15) | 0.437 | **0.012** | 0.621 |
| BMI (kg/m$^2$) | 24.4 (3.0) | 25.0 (4.1) | 26.6 (2.1) | 25.8 (4.3) | 0.149 | 0.931 | 0.489 |
| **Hematology** | | | | | | | |
| Estradiol (pmol/L) | 91 (25) | 687 (386)$^{\alpha}$ | 86 (21) | 15 (11)‡ | **<0.001** | **<0.001** | **<0.001** |
| Progesterone (nmol/L) | 0.9 (0.6) | 38 (18)$^{\alpha}$ | <0.3$^{\beta}$ | <0.3‡$^{\beta}$ | **<0.001** | **<0.001** | **<0.001** |
| Bioavailable testosterone (nmol/L) | 11 (2) $^{\alpha}$ ‡ | 0.3 (0.2) | 7 (2)$^{\alpha}$ | 0.2 (0.2) | **<0.001** | **<0.001** | **<0.001** |
| **Hemodynamics** | | | | | | | |
| Heart rate (beats/min) *† | 63 (9) | 65 (5) | 55 (3) | 64 (7) | **0.017** | **0.005** | 0.074 |
| Brachial SBP (mmHg) * | 113 (10) | 114 (12) | 133 (11) | 131 (9) | **<0.001** | 0.879 | 0.541 |
| Brachial DBP (mmHg) * | 69 (6) | 71 (8) | 79 (2) | 75 (4) | **<0.001** | 0.735 | 0.053 |
| Brachial MAP (mmHg) * | 83 (7) | 86 (7) | 97 (4) | 94 (5) | **<0.001** | 0.766 | 0.120 |
| **Integrated MSNA** | | | | | | | |
| MSNA burst frequency (bursts/min) | 16 (6) | 15 (6) | 34 (12) $^{\alpha}$ ‡ | 22 (11) ‡ | **<0.001** | **0.012** | **0.034** |
| MSNA burst incidence (bursts/100 heart beats) | 25 (10) | 23 (9) | 61 (21) $^{\alpha}$ ‡ | 34 (16) ‡ | **<0.001** | **<0.001** | **0.004** |
| Total MSNA (AU/min) * | 706 (304) | 701 (269) | 1535 (560) | 1087 (563) | **<0.001** | 0.073 | 0.079 |
| Sympathetic baroreflex sensitivity (bursts/100 heart beats/mmHg) † | -3.6 (2.2) | -3.0 (1.2) | -3.7 (1.9) | -2.1 (1.5) | 0.403 | **0.037** | 0.327 |
| **Superficial femoral artery measurements** | | | | | | | |
| LBF (ml/min) | 90 (15) $^{\alpha}$ ‡ | 60 (20) | 57 (15) | 53 (28) | **0.001** | **0.006** | **0.040** |
| LVC (ml/min/mmHg) | 1.1 (0.2) $^{\alpha}$ ‡ | 0.8 (0.3) | 0.6 (0.1) | 0.6 (0.3) | **<0.001** | 0.070 | **0.027** |

Data are mean (standard deviation). BSA, body surface area. BMI, body mass index. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial pressure. LBF, leg blood flow. LVC, Leg vascular conductance. Statistical comparisons were carried out using linear mixed model analyses. Significance was set to $P \leq 0.05$. * Indicates significant difference between young and

older adults, independent of sex (main effect of age). † Indicates significant difference between males and females, independent of age (main effect of sex). ‡ Indicates significant difference between young males and older males or between young and older females (within sex *post-hoc* comparison for the interaction term). ᵅ indicates significant difference between young males and females or older males and females (within age *post-hoc* comparison for the interaction term). ᵝprogesterone values for all older males and females were below the lower limit of our assay. For statistical comparisons, a value of 0.3 nmol/L was assigned to each older adult. Due to participant comfort (n=2) and technical difficulties (n=1), blood was drawn in 12 young males and 12 young females.

***Table 3.2.*** *Muscle sympathetic nerve activity (MSNA) burst distribution throughout the 10-minute supine recording period.*

| | YM | Range | YF | Range | OM | Range | OF | Range | ANOVA *P*-values Age | Sex | Age x Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Burst count** | | | | | | | | | | | |
| **MSNA burst pattern** | | | | | | | | | | | |
| Singlet * | 80 (18) | [58-114] | 85 (18) | [51-107] | 62 (18) | [29-86] | 71 (25) | [34-114] | **0.006** | 0.194 | 0.706 |
| Doublet * | 49 (23) | [6-91] | 41 (18) | [14-68] | 61 (18) | [28-90] | 62 (28) | [18-108] | **0.012** | 0.597 | 0.437 |
| Triplet+ †* | 39 (44) | [0-139] | 22 (32) | [3-115] | 158 (101) | [18-293] | 92 (83) | [3-304] | **<0.001** | **0.052** | 0.234 |
| **MSNA burst size quartile (AU)** | | | | | | | | | | | |
| Quartile 1 | 24 (34) | [1-86] | 15 (20) | [1-63] | 22 (47) | [1-159] | 14 (15) | [1-43] | 0.955 | 0.346 | 0.937 |
| Quartile 2* | 76 (32) | [23-122] | 76 (33) | [40-145] | 145 (48) | [77-250] | 115 (74) | [15-242] | **<0.001** | 0.242 | 0.345 |
| Quartile 3* | 56 (39) | [9-153] | 47 (30) | [4-101] | 98 (78) | [0-246] | 76 (55) | [6-173] | **0.023** | 0.372 | 0.589 |
| Quartile 4 | 12 (8) | [5-32] | 10 (7) | [1-21] | 14 (10) | [1-28] | 16 (15) | [1-49] | 0.198 | 0.957 | 0.560 |
| **Mean Amplitude** | | | | | | | | | | | |
| **MSNA burst pattern** | | | | | | | | | | | |
| Singlet | 47 (8) | [30-59] | 48 (9) | [31-61] | 46 (10) | [24-58] | 49 (9) | [32-63] | 0.881 | 0.353 | 0.638 |
| Doublet | 97 (19) | [59-128] | 97 (17) | [60-116] | 93 (17) | [49-114] | 99 (19) | [66-128] | 0.859 | 0.636 | 0.590 |
| Triplet+ * | 156 (34) | [98-216] | 155 (37) | [105-215] | 193 (52) | [110-280] | 175 (39) | [126-229] | **0.023** | 0.430 | 0.482 |
| **MSNA burst size quartile (AU)** | | | | | | | | | | | |
| Quartile 1* | 21 (2) | [18-25] | 20 (3) | [14-23] | 22 (2) | [19-24] | 22 (1) | [21-24] | **0.032** | 0.650 | 0.543 |
| Quartile 2 | 38 (3) | [34-43] | 39 (3) | [34-44] | 39 (4) | [30-43] | 40 (4) | [35-46] | 0.693 | 0.324 | 0.949 |
| Quartile 3 | 59 (1) | [57-61] | 59 (3) | [53-63] | 59 (2) | [56-61] | 59 (2) | [54-62] | 0.758 | 0.880 | 0.588 |
| Quartile 4 | 85 (3) | [81-100] | 88 (6) | [81-100] | 87 (7) | [82-100] | 87 (7) | [80-100] | 0.947 | 0.490 | 0.523 |

Data are mean (standard deviation). YM, young males. YF, young females. OM, older males. OF, older females. Statistical comparisons were carried out using two-way analyses of variance (ANOVA). Data are mean (standard deviation). * Indicates significant difference between young and older adults, independent of sex (main effect of age). † Indicates significant difference between males and females, independent of age (main effect of sex). ‡ indicates significant difference between young males and older males or between young and older females (within sex *post-hoc* comparison for the interaction term).

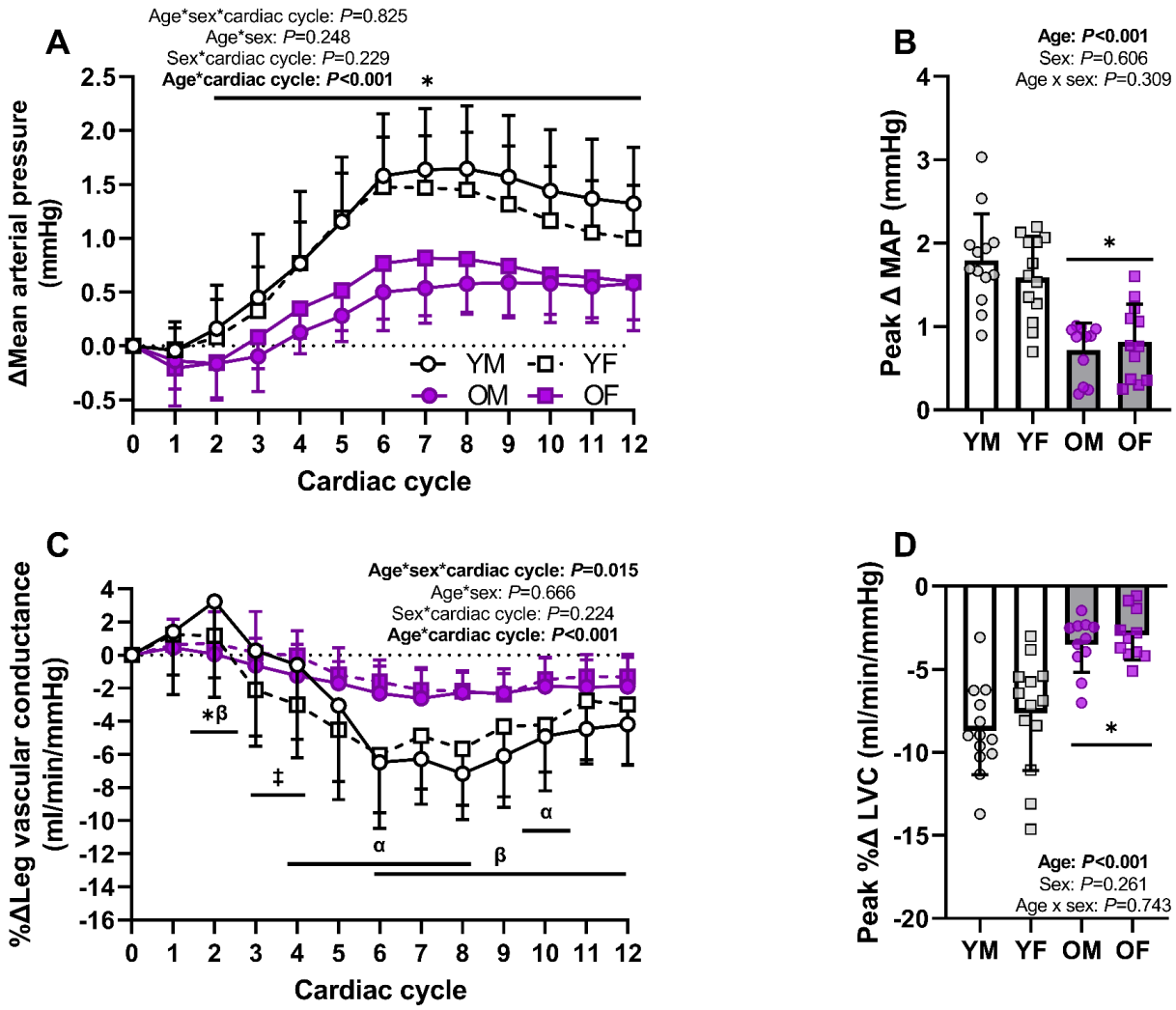


**Figure 3.1. Mean arterial pressure (MAP) and leg vascular conductance (LVC) responses following spontaneous bursts of muscle sympathetic nerve activity (MSNA).** Time course as well as peak changes in MAP (A and B), and nadir LVC (C and D) following spontaneous MSNA bursts in young males (YM, *n*=14; open circles), young females (YF, *n*=13; open squares), older males (OM, *n*=11; purple circles) and older females (OF, *n*=12; purple squares). Time course data (panels A and C) were analyzed using three-factor (Age, Sex, Stage) linear mixed model analyses, whereas peak data (panels B and D) were analyzed using two-way analyses of variance (ANOVA). *Post-hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. *; significant difference between young and older adults, ‡; significant difference between young males and females, α; significant difference between young and older females, β; significant difference between young and older males. Data are presented as mean (standard deviation). Note that data for sympathetic transduction into LVC are presented in 13 YM and 11 OF due to difficulties obtaining high quality ultrasound images for the 10-minute recording period.

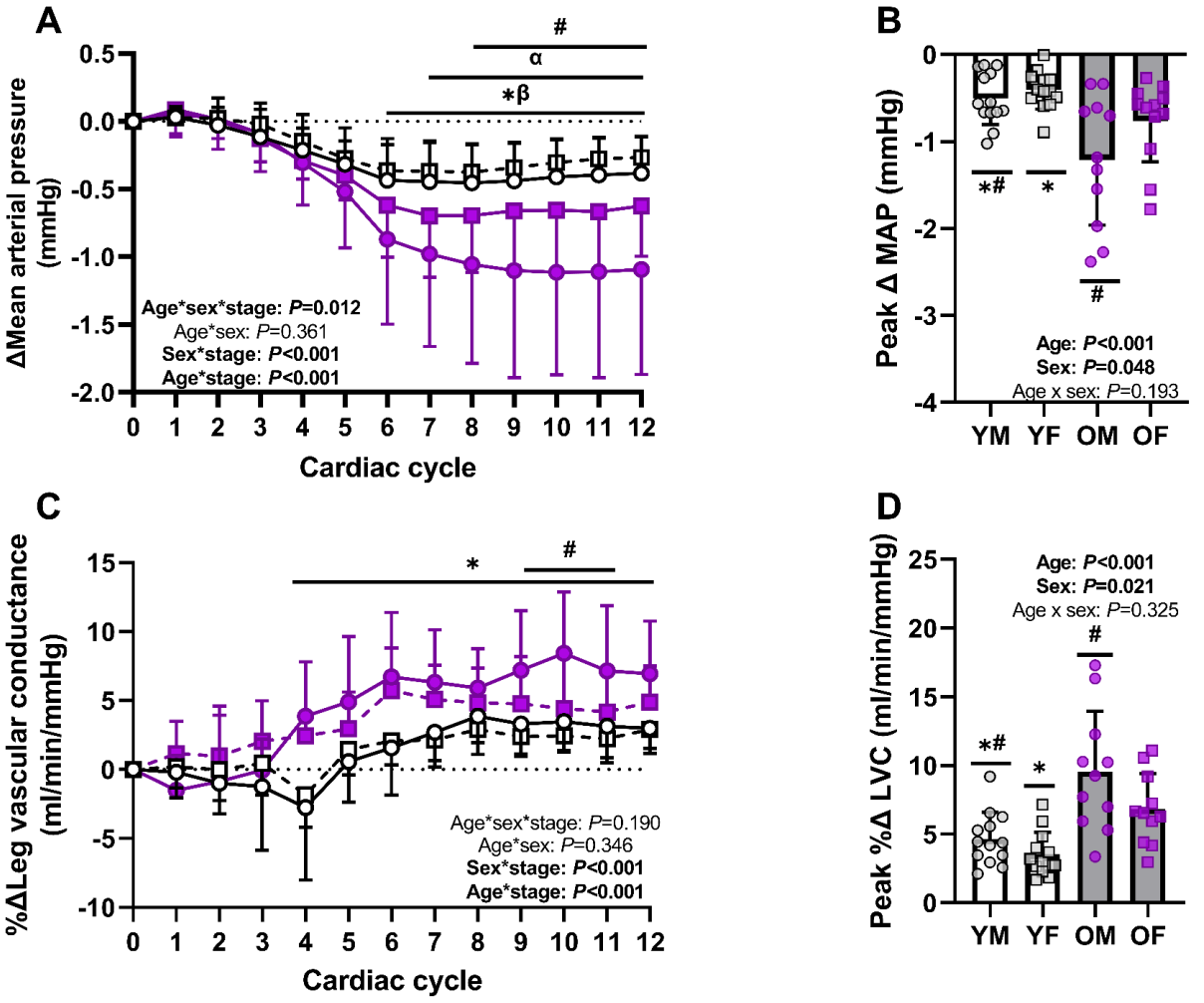


**Figure 3.2. Mean arterial pressure (MAP) and leg vascular conductance (LVC) responses following cardiac cycles without bursts of muscle sympathetic nerve activity (MSNA).** Time course, as well as nadir changes in MAP (A and B), and peak LVC (C and D) following spontaneous MSNA bursts in young males (YM, *n*=14; open circles), young females (YF, *n*=13; open squares), older males (OM, *n*=11; purple circles) and older females (OF, *n*=12; purple squares). Time course data (panels A and C) were analyzed using three-factor (Age, Sex, Stage) linear mixed model analyses, whereas peak data (panels B and D) were analyzed using two-way analyses of variance (ANOVA). *Post-hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. *; significant difference between young and older adults. #; significant difference between males and females. A; significant difference between young and older females, β; significant difference between young and older males. Data are presented as mean (standard deviation). Note that data for sympathetic transduction into LVC are presented in 13 YM and 11 OF due to difficulties obtaining high quality ultrasound images for the 10-minute recording period.

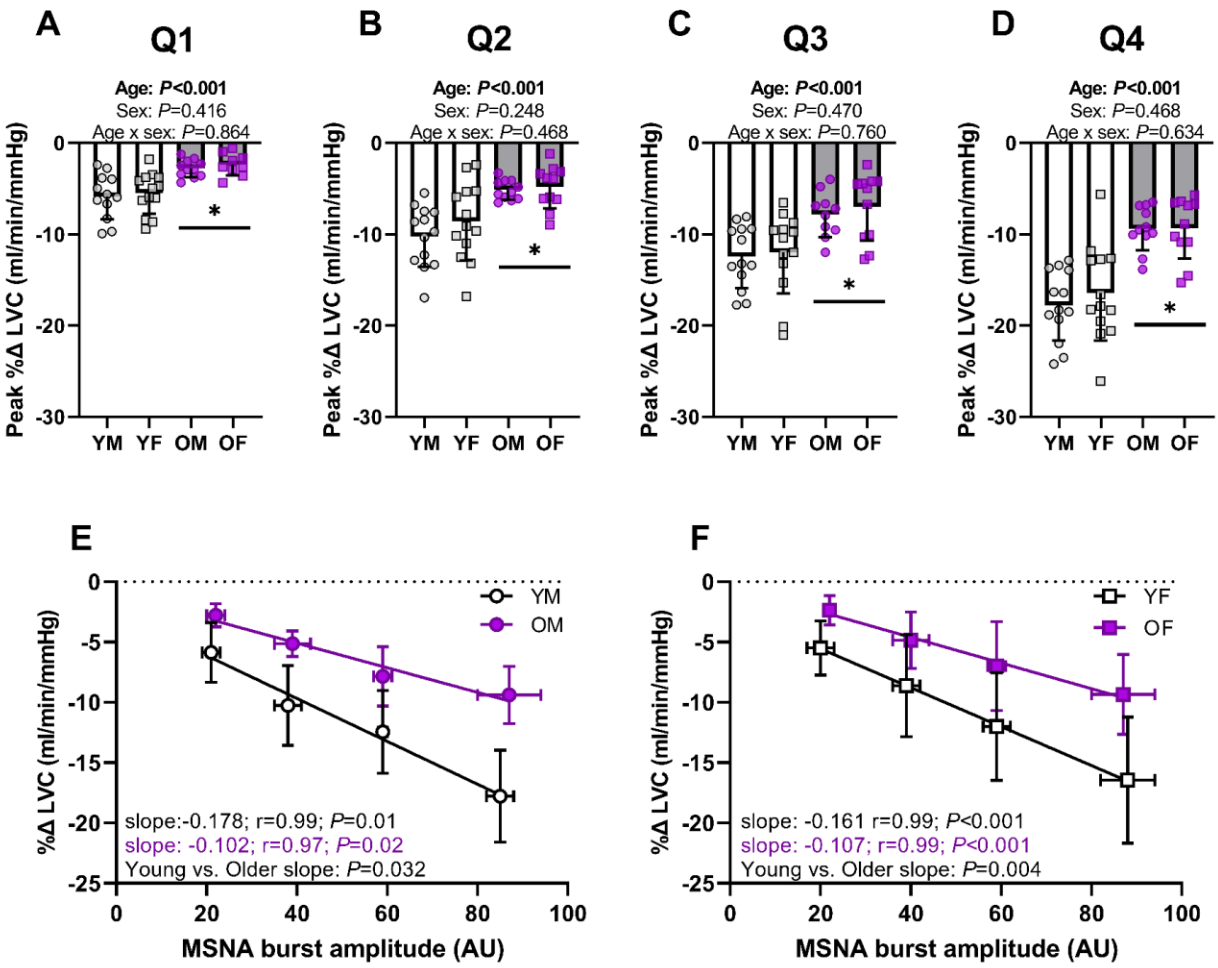


**Figure 3.3. The effect of muscle sympathetic nerve activity (MSNA) burst amplitude on sympathetic transduction into leg vascular conductance (LVC).** Nadir changes in LVC following MSNA bursts binned into quartiles (i.e., 25% bins) based on burst size (A-D), and the slope of sympathetic transduction into LVC when plotted against MSNA burst amplitude quartiles in males (E) and females (F). Data were collected in 13 young males (YM; open circles), 13 young females (YF; open squares), 11 older males (OM; purple circles) and 11 older females (OF; purple squares). Data in panels A-D were analyzed using two-way analyses of variance (ANOVA), with *post-hoc* comparisons carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. The sympathetic transduction slopes (E and F) were analyzed using linear regression analyses. Age comparisons of the sympathetic transduction slopes between the young and older males or young and older females were carried out using unpaired *t*-tests. *; significant difference between young and older adults. Data are presented as mean (standard deviation). Note that data for sympathetic transduction into LVC are presented in 13 YM and 11 OF due to difficulties obtaining high quality ultrasound images for the 10-minute recording period.

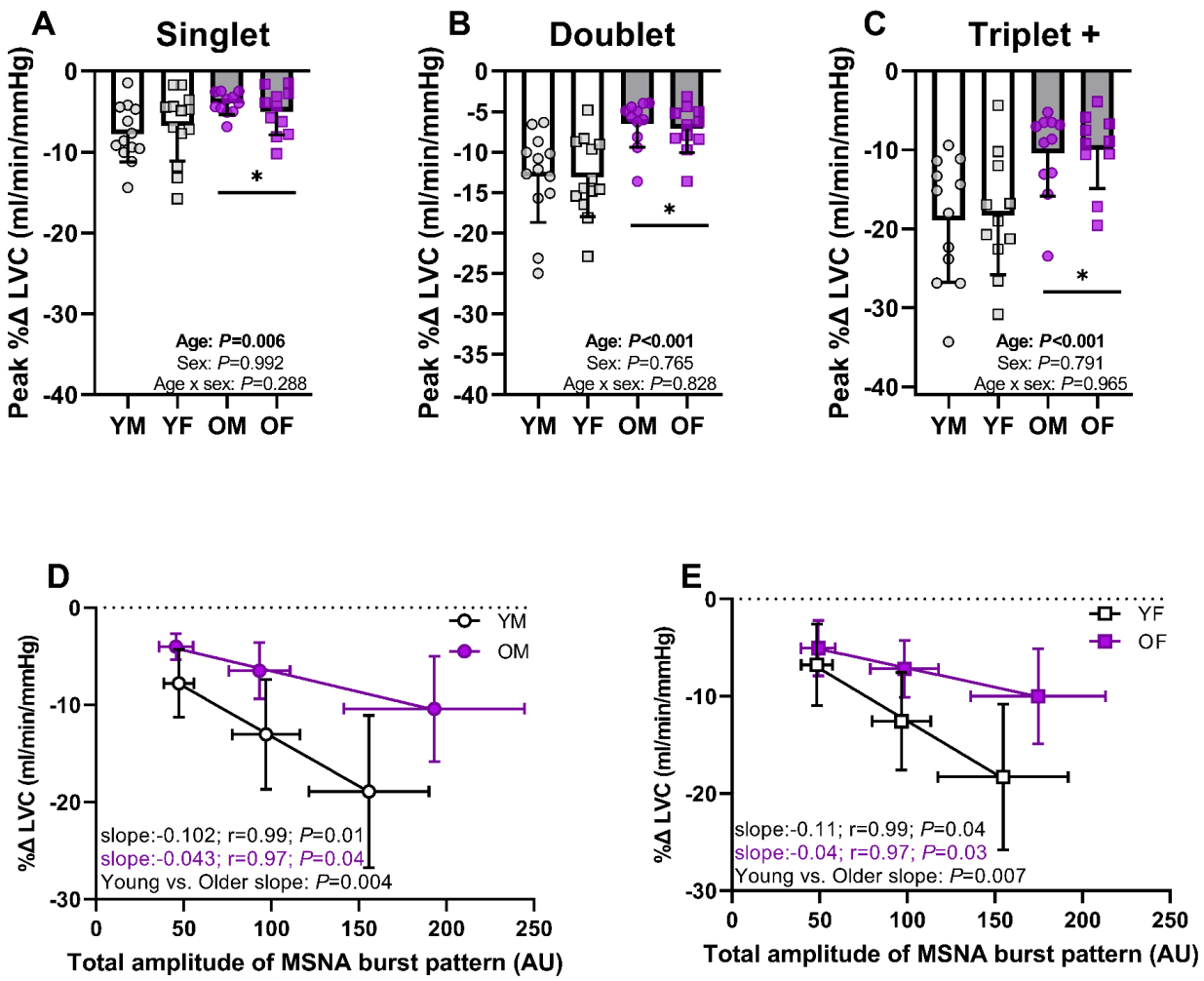


**Figure 3.4. The effect of muscle sympathetic nerve activity (MSNA) burst firing pattern on sympathetic transduction into leg vascular conductance (LVC).** Nadir changes in LVC (A-C) following MSNA bursts firing in isolation (singlets), in pairs (doublets), or in a sequence of three or more (triplets+), and the slope of sympathetic transduction into LVC when plotted against the total amplitude of MSNA burst firing pattern in males (D) and females (E). Data in panels A-C were analyzed using two-way analyses of variance (ANOVA), with *post-hoc* comparisons carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. The sympathetic transduction slopes (D and E) were analyzed using linear regression analyses. Age comparisons of the sympathetic transduction slopes between the young (YM) and older males (OM) or young (YF) and older females (OF) were carried out using unpaired *t*-tests. *; significant difference between young and older adults. Data are presented as mean (standard deviation). Note that data for sympathetic transduction into LVC are presented in 13 YM and 11 OF due to difficulties obtaining high quality ultrasound images for the 10-minute recording period.

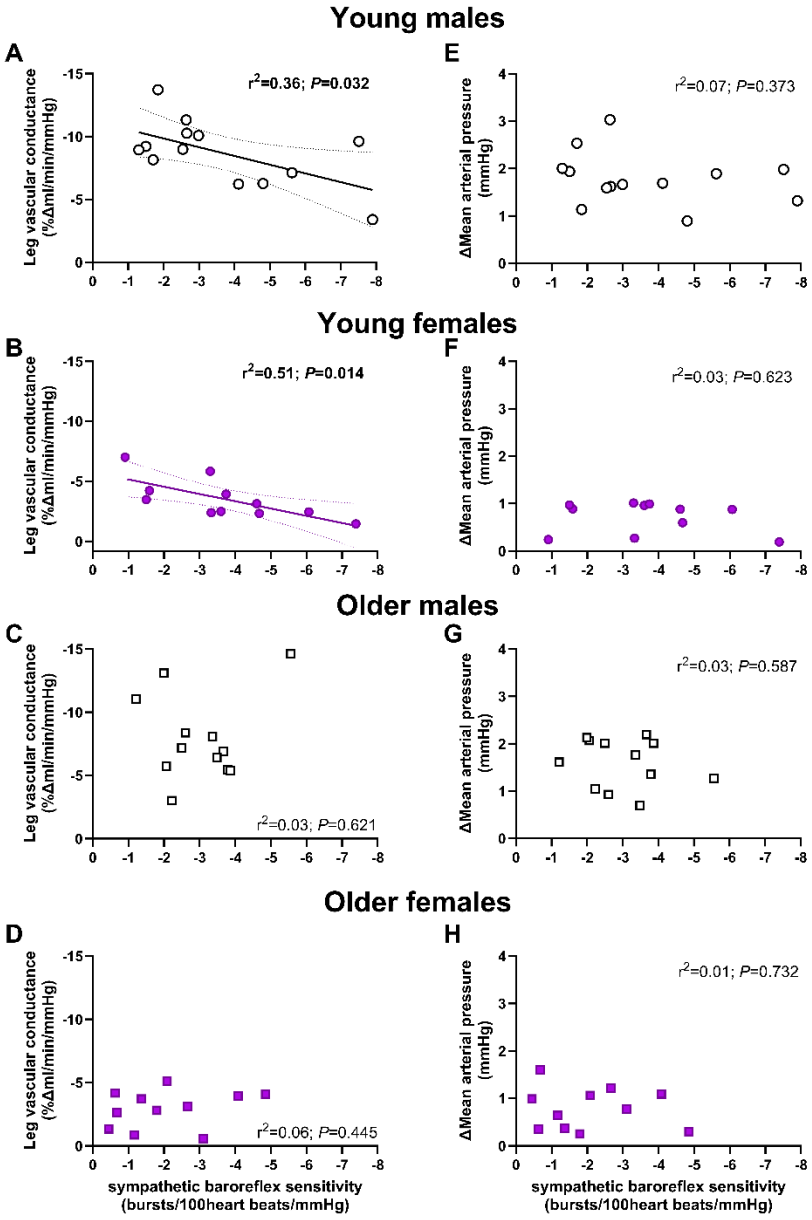


**Figure 3.5**. **Sex-related differences in the relationship between the central and peripheral arcs of the arterial baroreflex.** Linear regression analyses of the relationship between sympathetic transduction into leg vascular conductance and sympathetic baroreflex sensitivity in young males (YM, *n*=13; open circles; A), young females (YF, *n*=13; open squares and bars; B), older males (OM, *n*=11; purple circles; C) and older females (OF, *n*=11; purple squares; D). Linear regression analyses of the relationship between sympathetic transduction into blood pressure and sympathetic baroreflex sensitivity in young males (YM, *n*=13; open circles; E), young females (YF, *n*=13; open squares and bars; F), older males (OM, *n*=11; purple circles; G) and older females (OF, *n*=11; purple squares; H). Note that data for sympathetic transduction into LVC and MAP are presented in 13 YM due to difficulties obtaining high quality ultrasound images for the 10-minute recording period. The central and peripheral baroreflex arcs are only presented in 11 OF as one OF had a sympathetic baroreflex sensitivity relationship of <0.70. Furthermore, one young female was excluded due to a poor sympathetic baroreflex sensitivity relationship (r < 0.5).

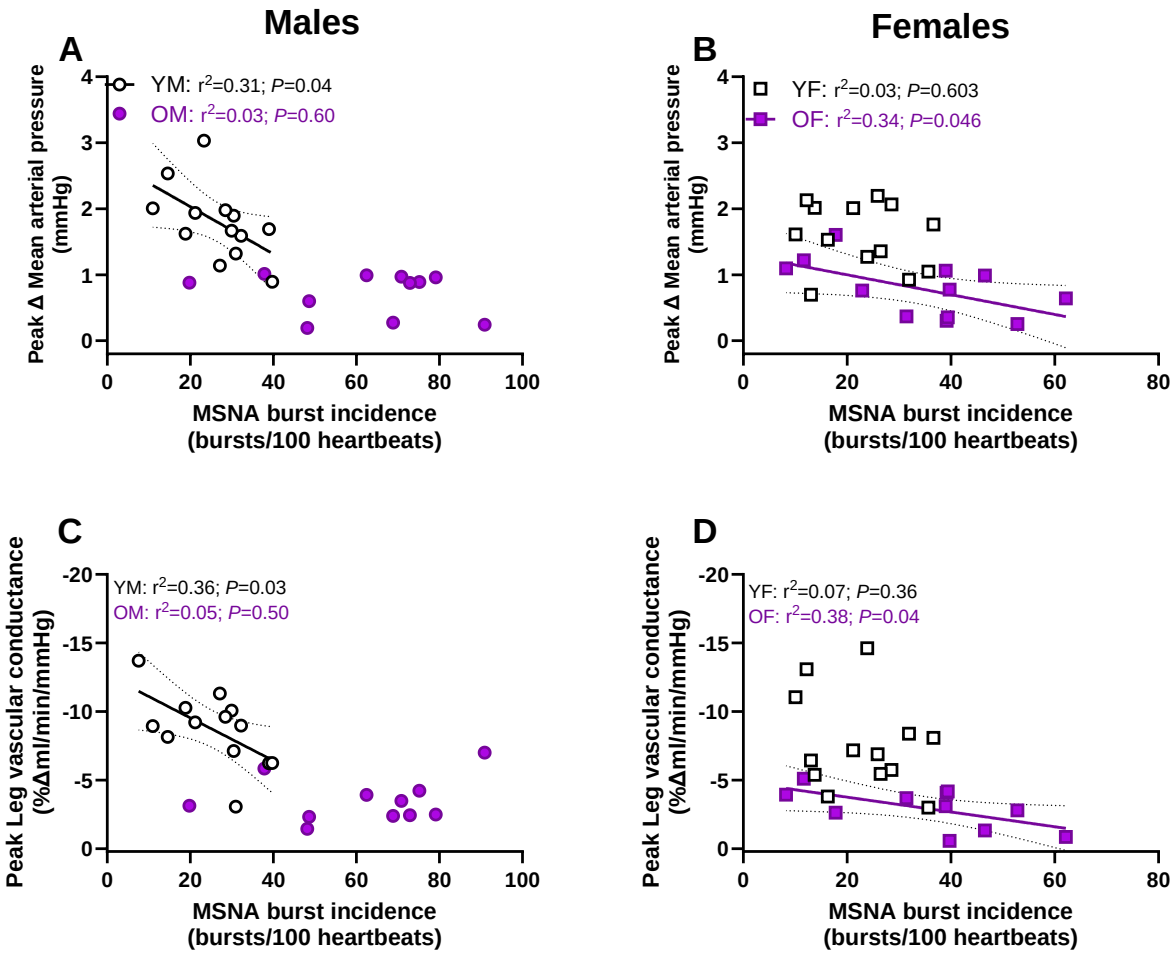


**Figure 3.6. Linear regression analyses of the relationship between resting MSNA burst incidence and mean arterial pressure (MAP; A and B), and leg vascular conductance (LVC; C and D).** Pearson's product-moment correlation analyses were carried out to determine the strength of the relationship between resting MSNA and the peak changes of sympathetic transduction into MAP and LVC. Data were collected in 13 young males (YM; open circles), 12 young females (YF; open squares), 11 older males (OM; purple circles) and older females (OF; purple squares).

### 3.4. Discussion

This study provides novel insight into the impact of age and sex on sympathetic transduction into MAP and LVC and how these factors are related to sympathetic neuro-hemodynamic balance in humans. The major new findings are that: 1) age, but not sex, impacts

sympathetic transduction, such that young adults have larger changes in MAP and LVC following spontaneous bursts of MSNA compared to older adults, 2) young adults have larger increases in sympathetic transduction into MAP and LVC as a function of MSNA burst size and MSNA burst firing pattern, 3) males but not females demonstrate an inverse relationship between sympathetic baroreflex sensitivity (central arc) and sympathetic vascular transduction (peripheral arc), 4) young males and older females exhibit an inverse relationship between resting sympathetic outflow and sympathetic transduction into MAP as well as LVC. We interpret these data to indicate that age affects the peripheral arm of the baroreflex (i.e., sympathetic transduction into MAP and LVC), whereas both sex and age affect the tonic relationship between spontaneous sympathetic discharge and baroreflex regulation of MAP and LVC.

*3.4.1. Age, but not sex, impacts sympathetic transduction into MAP and LVC*

Previous work found that young adults demonstrated larger increases in MAP following spontaneous bursts of MSNA compared to older adults (9, 11, 45). The results of the current study support these findings and extend them to the level of the peripheral vasculature, demonstrating that young adults have larger reductions in LVC following MSNA bursts compared to older adults. We further assessed the pressor and vasoconstrictor responses to spontaneous bursts of MSNA of varying amplitudes, and bursts firing in isolation (singlets) or in multiples (doublets, triplets +) because sympathetic transduction increases as a function of MSNA burst size and firing pattern (19, 26). In doing so, we found that older adults (regardless of sex) exhibited smaller pressor and vasoconstrictor responses regardless of MSNA burst size (Figure 3) or firing pattern (Figure 4), compared to young adults.

The mechanisms underpinning the attenuated sympathetic transduction in older adults observed here cannot be determined from the current data; however, given that norepinephrine release per integrated MSNA burst likely is unaffected by increasing age (46, 47), differences in the degree of pressor and vasoconstrictor responses to MSNA bursts of varying sizes and firing

patterns may reflect an age-related reduction in postjunctional α-adrenergic receptor responsiveness (48). In support of this, we found that the magnitude of increase in sympathetic transduction (i.e., the slope of the relationship) as a function of MSNA burst amplitude (Figure 3) and burst firing pattern (Figure 4) were smaller in older compared to young adults. Furthermore, despite older adults having more bursts firing as triplets+ compared to young adults – and consequently more vasoconstrictive neurotransmitter release (18, 43) – older adults still demonstrated attenuated vasoconstrictor and pressor responses compared to young adults. Given that sympathetic vascular transduction appears to be primarily mediated by α-adrenergic vasoconstriction (49), our observation that age-related reductions in sympathetic transduction are not specific to MSNA burst pattern or size support prior evidence that postjunctional vasoconstrictor responsiveness is reduced with increasing age (48, 50). While these data indicate that altered postjunctional responsiveness is likely the primary cause of attenuated sympathetic transduction among older adults, future work with pharmacological blockade of α-adrenergic receptors, as well as consideration of other sympathetic neurotransmitters and potential competitive vasodilatory signals are warranted to directly confirm this hypothesis. Notably, despite having attenuated sympathetic vascular transduction, resting arterial blood pressure was still higher in older adults. Resting MSNA burst frequency is positively associated with blood pressure (51), arterial stiffness (52, 53), and vascular smooth muscle cell hypertrophy in older adults (54, 55). Additionally, chronically elevated sympathetic nervous system activation, vascular smooth muscle cell hypertrophy, and arterial stiffness are all associated with attenuated vascular reactivity (56–58). Thus, greater sympathetic drive amongst older adults may support higher tonic vasoconstrictor tone, and therefore higher resting blood pressure. However, these sustained elevations in MSNA likely attenuate vasoreactivity to a given degree of sympathetic vasoconstrictor drive, resulting in a discordance between steady-state blood pressure and beat-by-beat sympathetic transduction.

Contrary to the effect of age, we did not observe any sex-related differences in resting sympathetic transduction into blood pressure or LVC following MSNA bursts. The current data align with prior evaluations in young males and females using spike-triggered sympathetic transduction analyses during spontaneous breathing (12–14). However, sex differences in sympathetic transduction into blood pressure (using signal averaging analyses) have been previously observed among older adults, with older females demonstrating smaller increases in MAP compared to older males following spontaneous bursts of MSNA (11). The reasons for these differing results are unclear. One possibility is that older females in the current study had lower MSNA burst incidence compared to older females in the study by Vianna et al. (Current: 34 beats/100 heart beats vs. Vianna: 45 bursts/100 heart beats). Given that MSNA burst occurrence at rest is thought to be inversely associated with sympathetic transduction (59), the lower MSNA burst incidence in older females in the current study may have attenuated the magnitude of reduction in sympathetic transduction in older females. This remains an important avenue for future research with large sample sizes that could account for the high degree of variability in resting MSNA among older females (60). Additionally, given that previous work using weighted regression analyses found that older females exhibit greater transduction of MSNA into blood pressure (15), no consensus can be stated regarding sympathetic transduction among older females.

In addition to changes in the vascular and pressor responses to spontaneous MSNA bursts, we found that older adults (regardless of sex) and males (regardless of age) have greater falls in MAP and larger increases in LVC in cardiac cycles without MSNA bursts (Figure 2B and D). The observations pertaining to age-related differences in sympathetic support of blood pressure are in accordance with previous work (11) and support prior reports of larger reductions in blood pressure following ganglionic blockade in older compared to young adults (61, 62). Furthermore, previous assessments in young males and females have found greater reductions

in blood pressure following cardiac cycles without MSNA bursts (12, 14), and following ganglionic blockade (63) in young males compared to females. In line with these observations, we also found larger reductions in blood pressure and larger increases in LVC following cardiac cycles without MSNA bursts in males compared to females (regardless of age). These data support the conclusion, and previous work, which indicates that males exhibit greater tonic sympathetic support of blood pressure, largely via sex-related differences in neurogenic vasoconstrictor tone.

*3.4.2. Sex-related differences in the relationship between the central and peripheral components of the sympathetic baroreflex arc*

The arterial baroreflex plays a critical role in blood pressure regulation via its involvement in the generation of MSNA bursts and beat-by-beat changes in vascular tone (64). The sympathetic arm of the baroreflex can be dissected into two components: the central and peripheral arcs. The central arc represents the ability of the baroreflex to modulate sympathetic outflow (i.e., sympathetic baroreflex sensitivity), whereas the peripheral arc represents changes in blood pressure or vascular tone in response to bursts of MSNA (i.e., sympathetic transduction) (22). Recently, Hissen et al. demonstrated an inverse relationship between the central and peripheral arcs of the baroreflex in young males, but not females, indicating that young males with higher sympathetic baroreflex sensitivity have lower sympathetic vascular transduction (13). Additionally, we reported that middle-aged-to-older adults (45-75 years) exhibited heightened sympathetic baroreflex sensitivity and attenuated sympathetic transduction into MAP compared to young adults (9), whereas O'Brien and colleagues demonstrated no relationship between sympathetic baroreflex sensitivity and sympathetic transduction amongst older adults (27). Importantly however, these studies were performed in mixed sex samples of middle-aged-to-older adults (45-75 years), and detailed analyses regarding the interactive effects of age and sex of the relationships between the central and peripheral arcs of the baroreflex could not have been completed. Furthermore, unlike Hissen and colleagues (13), assessments of the peripheral

baroreflex arc among older adults were completed using sympathetic transduction into blood pressure. Notably, in the current study, we replicated the observations of Hissen and colleagues (13), finding that young males, but not females demonstrated a relationship between the central and peripheral baroreflex arcs when assessed using sympathetic vascular transduction. However, when we evaluated the peripheral arc using sympathetic transduction into blood pressure, no relationships were observed in young males. We advanced these observations to older adults, finding that older males, but not females, exhibit an inverse relationship between central and peripheral baroreflex arcs when assessed using sympathetic vascular transduction, not sympathetic transduction into blood pressure. These data indicate the importance of making more direct associations between levels of MSNA and the most direct end organ target, that is, sympathetic vascular transduction. Collectively, our data indicate that, in males (regardless of age), a reduction in one arm of the sympathetic baroreflex is compensated for by an increase in the other arm. Conversely, females do not appear to rely on this compensatory neurovascular relationship to regulate vasomotor tone at rest.

The lack of relationship between sympathetic baroreflex sensitivity and sympathetic vascular transduction in young females may be attributed to estradiol-mediated increases in central nitric oxide synthesis and peripheral vascular $\beta_2$-adrenergic receptor sensitivity, which would impact the generation of MSNA bursts (65) and offset α-adrenergic vasoconstriction (66), respectively. However, the novel finding that older females also exhibit no relationship between the central and peripheral baroreflex arcs argues against a primary role of estradiol. This observation may be related to the fact that MSNA burst size appears to contribute more to sympathetic BRS than burst occurrence among older females (67), suggesting a shift toward a greater reliance on MSNA burst size rather than occurrence to regulate resting blood pressure amongst older females. Future work is warranted to confirm this observation and elucidate the

mechanisms underpinning the sex differences observed in the compensatory interactions between the central and peripheral arcs of the baroreflex.

*3.4.3. Age- and sex-specific impact on the relation between resting MSNA, sympathetic vascular transduction*

In accordance with previous work in young adults (13, 14), we found that resting MSNA burst occurrence (i.e., burst incidence) was inversely associated with sympathetic transduction into MAP and LVC, and positively associated with sympathetic baroreflex sensitivity in young males, but not young females. Furthermore, these data support the concept that resting MSNA is inversely associated with vascular adrenergic responsiveness in young males (3). Thus, young males with higher MSNA have attenuated vasoconstriction following bursts of MSNA, but for a given change in blood pressure, they experience a greater central baroreflex arc mediated change in MSNA (Figure 5). Conversely, the lack of relationship in young females may be attributed to the central and peripheral effects of estradiol, which would attenuate the baroreflex control of MSNA and the vasoconstrictive effects of sympathetic nerve traffic, respectively (5, 65, 66).

Contrary to young adults, previous work found relationships between resting MSNA, and MAP/TPR in older females (5) but not older males (7). Furthermore, in a mixed-sex sample of older adults, resting MSNA was inversely related with sympathetic transduction into MAP (27). The current study supports these data and provides new information by demonstrating that older females exhibit an inverse relationship between resting sympathetic outflow and sympathetic transduction into MAP/LVC, whereas these relationships were not present in older males. Taken together, we interpret the data to indicate that older females with higher MSNA have smaller reflex increases in blood pressure and peripheral vasoconstriction following bursts of MSNA because they are operating at a higher tonic MAP and TPR. Conversely, older females with low resting MSNA and steady-state blood pressure and TPR require larger beat-by-beat changes in MAP and LVC following MSNA bursts to prevent dangerous falls in blood pressure. This relationship

between basal sympathetic outflow and sympathetic transduction into MAP/LVC among older females is likely driven by the loss of estradiol during menopause, which would result in less β-adrenergic offsetting of α-adrenergic vasoconstriction (5). However, the lack of relationship between MSNA and sympathetic transduction into MAP and LVC in older males in our study, and between resting MSNA and TPR in previous work (7), indicate that MSNA may not be a primary determinant of MAP and vascular tone in this group of individuals. Indeed, previous work found that heightened MSNA was not a primary contributor to TPR in older males (61), indicating that, non-neural factors, such as greater local circulating vasoconstrictor substances (e.g., endothelin-1) may contribute more to peripheral resistance in older males than MSNA.

### 3.5. Experimental considerations

First, spontaneous analyses assess baroreflex function across a small range of natural fluctuations in blood pressure whereas methods such as the modified oxford technique (68, 69) or the variable neck pressure chamber (70–72) evaluate arterial baroreflex function across a range of blood pressures. Additionally, in a resting state, other factors like respiration have more of an impact on changes in MSNA, and these non-baroreflex factors have less relative influence during the modified Oxford technique. However, spontaneous analyses assess the functional operating range of baroreflex control. Also, previous work demonstrated good agreement between spontaneous baroreflex analysis and the modified oxford technique (69). Second, spike-triggered averaging may underestimate sympathetic transduction into MAP and LVC in individuals with high MSNA, as resting MSNA is inversely related with sympathetic transduction in healthy young adults (59). However, a definitive study to determine the degree to which signal averaged sympathetic transduction is compromised because of high resting MSNA has not been completed to date, and there is ongoing debate surrounding the interpretation of normalized sympathetic transduction data (73). To address this, we completed analyses on a subset of young and older adults with similar resting MSNA levels (Supplemental Table 1; 10.6084/m9.figshare.23638851)

which revealed that sympathetic vascular transduction remained attenuated amongst older adults (Supplemental Figure 1; https://doi.org/10.6084/m9.figshare.23638848.v1). Nonetheless, this question would benefit from studies with an *a-priori* design to determine the impact of resting MSNA on age-related changes in sympathetic transduction. Additionally, more data are required to determine whether the attenuated transduction in older adults is partly attributed to a greater proportion of bursts firing above the blood pressure operating point (which results in a paradoxical depressor and vasodilatory response) (74). In this sense, accounting for the arterial pressure at which a burst of MSNA occurs may offer further insight into age- and sex-related differences in sympathetic transduction. Third, albeit adequately powered to detect group differences in sympathetic vascular transduction, some degree of caution should be exerted when assessing correlational analyses with relatively small sample sizes. Notably, despite significant linear relationships being observed between the central and peripheral baroreflex arcs in young and older males, a large proportion of the variance in this relationship remains unexplained (49-64%). Fourth, although blood flow to skin is minimal during rest in thermoneutral conditions, doppler ultrasound measures of superficial femoral artery blood flow cannot isolate the contribution of muscle and skin vascular conductance, potentially confounding interpretation of the MSNA-LVC transduction outcomes.

### 3.6. Conclusions

The current study demonstrates that age, but not sex, affects the pressor and vascular responses to spontaneous bursts of MSNA, with older adults (regardless of sex) demonstrating attenuated sympathetic transduction into MAP and LVC compared to young adults. Furthermore, as expected, we found that older adults exhibited a smaller magnitude of increase in sympathetic transduction into MAP and LVC as a function of MSNA burst size and burst firing pattern compared to young adults. The age-related reduction in the pressor and vasoconstrictor

responses to MSNA bursts may serve a functional purpose to minimize transient surges in blood pressure. Additionally, we demonstrated for the first time that males but not females (regardless of age) exhibited an inverse relationship between the central (i.e., sympathetic baroreflex sensitivity) and peripheral (i.e., sympathetic vascular transduction) arcs of the baroreflex. Future research is warranted to determine the mechanisms underpinning the sex-related differences in these relationships. Lastly, we found age- and sex-specific relationships between resting sympathetic outflow and sympathetic transduction. Specifically, young males and older females demonstrated inverse relationships between sympathetic transduction into MAP/LVC and resting MSNA burst incidence, whereas these relationships were not present in young females and older males. Collectively, the current study provides confirmatory and novel insight into the impact of age and sex on the beat-by-beat sympathetic regulation of arterial blood pressure and vasoconstrictor tone, and sympathetic neuro-hemodynamic balance in humans. Additionally, these data highlight many areas of future research in women's cardiovascular control that remain to be interrogated.

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# Chapter 4

## 4 The interactive effects of age and sex on the neuro-cardiovascular responses during fatiguing rhythmic handgrip exercise

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### 4.1. Introduction

The sympathetic nervous system plays a pivotal role in the homeostatic responses to exercise via increases in efferent sympathetic nerve discharge that serve to regulate BP whilst redistributing blood flow to active muscle to meet metabolic demand (1, 2). These neurocirculatory adjustments are mediated by central feed-forward signals originating from higher brain centers (i.e., central command) (3, 4), as well as peripheral feedback from group III and group IV skeletal muscle afferents (i.e., the exercise pressor reflex) (5–7). Increases in BP and sympathetic nerve traffic during moderate-intensity exercise are primarily driven by the exercise pressor reflex, whereas during high-intensity exercise, central command also contributes to increases in sympathetic outflow directed toward inactive skeletal muscle vasculature (8). To partition the central versus peripheral determinants of sympathetic discharge during fatiguing exercise, many studies have used a post-exercise circulatory occlusion (PECO) as it represents a period where muscle sympathetic nerve activity (MSNA; a direct measure of sympathetic neural discharge directed toward skeletal muscle vasculature) remains elevated due to stimulation of chemically sensitive muscle afferents, independent of central processes related to effort or muscle mechanoreceptor stimulation (5, 7).

Among young adults, males demonstrate greater increases in MSNA and BP compared to females during both exercise and PECO (9, 10), possibly due to a greater proportion of type I muscle fibers (and oxidative metabolism) in females (11), central sympatho-inhibitory effects of estradiol (12, 13), or estradiol-mediated offsetting of sympathetic α-adrenergic vasoconstriction (14). Conversely, the independent impact of age on the sympathetic neuro-cardiovascular

responses to exercise and isolated muscle metaboreflex activation remains equivocal. Specifically, in primarily male samples, either no impact of age (15) or attenuated (16–18) sympathetic and pressor responses to exercise and PECO are reported in older adults. Conversely, post-menopausal females demonstrate exaggerated increases in MSNA and BP during isometric exercise and PECO compared to young, pre-menopausal females, likely due to the loss of estradiol following menopause (19). Taken together, these data suggest that there may be a sex-specific impact of age on the sympathetic neurocirculatory responses to exercise and PECO.

Thus far, only one study has assessed the impact of age and sex on the cardiovascular responses to rhythmic small muscle mass exercise, finding a greater rise in BP in older females compared to young females and similarly aged males that was driven primarily by increases in total peripheral resistance (TPR) (20). Given that BP and TPR are positively associated with MSNA in older females (21), and that females exhibit greater age-related increases in MSNA than males (22–24), it was thought that the exaggerated exercise pressor responses in older females was attributed to heightened sympathetic neural reactivity. However, direct assessments of sympathetic outflow were not conducted. Thus, the interactive effects of age and sex on the sympathetic neural responses to exercise remain unknown. Furthermore, it is unclear whether these exaggerated pressor responses in older females were primarily driven by central or peripherally mediated increases in sympathetic neural discharge.

To date, assessments of both age- and sex-related changes in sympathetic outflow during exercise have been completed using traditional, integrated MSNA analysis. Yet, bursts of MSNA represent periods of synchronously firing efferent action potentials (AP) that, in response to acute physiological stress, exhibit unique recruitment patterns that cannot be detected in the integrated MSNA neurogram (25). We have previously found reduced AP discharge and recruitment, but not integrated MSNA responses, during apneic stress in older compared to young adults (26),

indicating that the central features governing sympathetic neural emissions directed toward the skeletal muscle vasculature are attenuated with age. However, whether this is the case during exercise, and whether sex impacts age-related changes in axonal discharge and recruitment patterns remains unknown.

Therefore, the objective of the current study was to determine the impact of age and sex on the sympathetic and cardiovascular responses during incremental rhythmic handgrip exercise to fatigue and PECO. Incremental rhythmic handgrip exercise was used because MSNA increases during fatiguing, high intensity – but not moderate-intensity – rhythmic exercise, primarily due to central command activation (8, 27). Thus, unlike isometric exercise, where the progressive increases in MSNA are largely attributed to muscle mechano- and metaboreflex, with less contribution of central command (7, 28, 29), incremental rhythmic handgrip exercise provides a unique opportunity to assess the role of central command on sympathetic neural discharge patterns during exercise. Our hypotheses are that 1) older females will have the greatest increases in BP, integrated MSNA and AP recruitment during exercise and PECO compared to all other groups (i.e., interactive effect of age and sex), 2) young adults will demonstrate greater AP recruitment than older adults during exercise and PECO (i.e., independent effect of age), and 3) young males will have larger increases in BP, sympathetic discharge during exercise and PECO compared to young females (i.e., independent effect of sex).

## 4.2. METHODS

### *4.2.1. Ethical approval*

Written and informed consent was obtained from all participants prior to testing. Approval of experimental protocols was granted by the institutional review boards of the University of Texas

Southwestern Medical Center, Texas Health Presbyterian Hospital Dallas (File no. STU-2022-0433), and The Western University Health Sciences Research Ethics Board (File no. 119380). The study was conducted in accordance with the *Declaration of Helsinki*.

*4.2.2. Participants*

Fifty individuals (Young males [YM]: *n*=12, Young females [YF]: *n*=13, Older males [OM]: *n*=12, Older females [OF]: *n*=13) participated in the current study. Studies were conducted in a research laboratory at Western University (n=13; 7 YM and 6 YF) and a clinical research laboratory at The Institute for Exercise and Environmental Medicine (n=37; 5 YM, 7 YF, 12 OM, 13 OF). All participants were non-smokers, free of overt cardiovascular disease, metabolic syndrome, hepatic and renal disease, neurological disease, and pulmonary disease, and were not pregnant. To minimize the impact of exogenous sex hormones on neural and cardiovascular outcomes, no participants were using hormonal replacement therapy or hormonal contraception (e.g., intrauterine devices, oral hormonal contraception). Young females were tested in the mid-luteal phase of the menstrual cycle (as determined by self-report [n=13], blood draw [n=12] and ovulation kits [n=9]). Microneurographic recordings were unsuccessful in one older male and one older female. Thus, data are reported for 11 older males and 12 older females.

*4.2.3. Experimental Procedures*

Participants reported to the laboratory in a fasted state (≥8 hours), and having refrained from caffeine, alcohol, and vigorous exercise for a minimum of 12 hours. Prior to beginning the study, participants emptied their bladder to avoid any effects of bladder distention on sympathetic activity and BP (30). Thereafter, participants laid in the supine position and blood samples were collected from the antecubital vein (estradiol, progesterone, testosterone, sex hormone binding globulin, and albumin). Participants were then instrumented for the study with an electrocardiogram and a finger BP cuff. Prior to microneurography, participants performed three brief (~3 s) maximal

contractions using a handgrip dynamometer with the dominant hand to determine their maximal voluntary contraction (MVC) force. Data collection began after at least 10 minutes of quiet rest, and at least 10 minutes after an acceptable microneurographic nerve recording was obtained. Baseline data were collected for one minute, and participants began exercise thereafter. The exercise protocol consisted of rhythmic handgrip exercise using a 50% duty cycle (2-s contraction, 2-s relaxation) at a rate of 15 contractions per minute. Participants began contractions at 10% MVC for one minute. Each stage thereafter, the target intensity was increased by 10% MVC every minute until task failure. Task failure was defined as the inability to maintain the target force output for two consecutive contraction:relaxation cycles, or volitional fatigue. Once the participant was unable to meet the target force output, a BP cuff was inflated rapidly around the exercising forearm (distal to the elbow) to suprasystolic pressure (~250 mmHg) four seconds before the cessation of handgrip exercise to induce PECO for two minutes. Ratings of perceived exertion (RPE) were noted at the end of exercise using the 6-20 Borg scale (31). All experimental sessions were conducted in a thermoneutral environment (~24°C).

*4.2.4. Experimental measures*

Estradiol, progesterone, testosterone, sex hormone binding globulin, and albumin were analyzed via chemiluminescence (ARUP laboratories, Salt Lake City, UT, USA, and LifeLabs, London, Ontario, Canada). Bioavailable testosterone was calculated from total testosterone, sex hormone binding globulin and albumin using a previously validated equation (32). Heart rate (HR) was recorded using a standard lead II electrocardiogram (BioAmplifier, ADInstruments, Dunedin, New Zealand). Brachial BP was measured by electrosphygmomanometry (model 4240; SunTech Medical Instruments, Raleigh, NC) with a microphone placed over the brachial artery to detect Korotkoff sounds. Beat-by-beat BP was determined via finger photoplethysmography (Finapres Medical Systems, Amsterdam, The Netherlands, and Human NIBP Nano system, ADInstruments). Multi-unit MSNA was obtained via microneurography of the peroneal nerve, as

previously described (10). Briefly, a 200-µm-diameter tungsten microelectrode, tapering to an uninsulated 1- to 5-µm tip, was inserted percutaneously into the common peroneal nerve at the popliteal fossa, and a reference electrode was positioned subcutaneously ~1-3 cm from the recording site. A suitable MSNA site was obtained by manual manipulation of the microelectrode until a pulse-synchronous burst pattern was observed. An MSNA recording site was confirmed by the absence of skin paresthesia, and an increase in sympathetic discharge during a maximal apnea but not in response to a startling loud noise (33). The MSNA neurogram was recorded with a nerve traffic analyzer (662C-3; Bioengineering Dept., University of Iowa, Iowa City, IA). The neural signal was amplified (gain: 70,000-160,000-fold), and band-pass filtered (bandwidth: 700-2000 Hz) before being rectified and integrated (leaky integrator; 0.1-s time constant). The raw, filtered, and integrated MSNA neurograms were sampled at 10,000 Hz, and stored for offline analysis using Powerlab (Labchart 8; ADInstruments, Colorado Springs, CO).

*4.2.5. Data analysis*

Finger photoplethysmography-derive BP waveforms were calibrated to the average of three brachial BP measures. Calibrated BP waveforms were then extracted on a beat-by-beat basis to determine systolic BP (SBP), diastolic BP (DBP), and mean arterial pressure (MAP), as defined as the maximum, minimum, and mean BP of each waveform. Stroke volume and cardiac output were estimated from the BP waveform using the Modelflow method (Labchart, Adinstruments), which incorporates age and biological sex, and were presented as stroke volume index and cardiac index to account for differences in body size between individuals. TPR was calculated as the quotient of MAP and cardiac output and multiplied by 80.

Integrated bursts of MSNA were identified in accordance with recently published guidelines (34), and quantified as burst frequency (bursts/min) and incidence (bursts/100 heart beats). Burst amplitude was measured in volts and normalized to the largest burst at baseline,

which was given a value of 100. Total activity of MSNA was calculated as the product of burst amplitude and burst frequency.

Postganglionic sympathetic APs were detected and extracted from the raw filtered neurogram using a wavelet-based methodology, as described previously (25). Briefly, APs were binned on peak-to-peak amplitude and histogram analysis was performed to group APs into amplitude-based clusters using Scott's rule (35). As such, the number of total clusters varied between participants. Within participant cluster characteristics were normalized to ensure that bin width, maximum bin center and the total number of AP clusters would be identical across conditions (i.e., baseline to 10%, 20%, 30% MVC, etc.). This normalization process assures that corresponding clusters across conditions contain APs with similar peak-to-peak amplitudes. This process was done separately for exercise and PECO, guaranteeing that an increase in the number of active clusters at peak exercise or during PECO represents recruitment of subpopulations of previously silent, larger-sized AP clusters.

AP discharge was quantified as follows: 1) AP frequency (spikes/min) and AP incidence (spikes/100 heart beats) as well as the number of active APs firing within a MSNA burst (spikes/burst), reflecting total sympathetic discharge; 2) the number of active AP clusters per integrated burst (clusters/burst) and the number of total AP clusters detected, reflecting the recruitment of larger axonal subpopulations; and 3) the conduction latency of individual APs, established as the time delay between the R-wave of the preceding cardiac cycle and the negative deflection of the AP waveform. AP cluster latency was determined for each cluster. Because the number of AP clusters recruited varied between individuals, the number of total clusters was normalized to 10 bins, each containing 10% ranges (i.e., 10-20%, 20-30% MVC, etc.) of the largest detected cluster, which was given a value of 100% (36).

*4.2.6. Statistical Analysis*

All data are presented as mean (SD). Data are expressed as a one-minute average of peak exercise (defined as the final completed one-minute stage of exercise) or an average of each minute of PECO (i.e., PECO1, PECO2). Analysis for outliers was conducted using the ROUT method (GraphPad Prism version 9.3, San Diego, CA). Two-factor linear mixed models with a compound symmetry structure were used to evaluate the effects of age and sex on participant characteristics, resting hemodynamics, sex hormones and peak exercise neuro-cardiovascular outcomes. The time course effects of age, sex, and stage (%MVC or PECO1 and 2) on all cardiovascular and neural outcomes were assessed using a three-factor linear mixed model analyses with a compound symmetry structure. Bonferroni-corrected *post-hoc* comparisons were performed to evaluate specific differences between means when applicable. Pairwise comparisons were restricted to within age and sex. Thus, young males were not compared with older females and young females were not compared with older males. For the AP analysis, one young female was excluded due to poor signal-to-noise ratio (<3.7) in the MSNA neurogram. Additionally, blood samples were obtained in 11 of 12 young males and 12 of 13 young females due to participant discomfort (n=1) and technical difficulties (n=1). Partial regression analyses were conducted to assess the relationship between bioavailable testosterone and MAP, TPR, and AP recruitment while adjusting for age. Statistical significance was accepted at $P \leq 0.05$. Statistical analyses were performed using SPSS statistics (Version 28.0; IBM, Armonk, NY).

## 4.3. RESULTS

### *4.3.1. Participant characteristics, resting hemodynamics, and sex hormones*

Participant characteristics, resting hemodynamics and sex hormone data are presented in Table 4.1. Regardless of age, females were shorter ($P<0.001$), and had a lower body mass ($P=0.009$) and smaller body surface area ($P<0.001$) than males, whereas body mass index was

not impacted by age ($P$=0.247) or sex ($P$=0.730). Older adults had higher SBP, DBP and MAP compared to young adults (all $P$<0.001); however, sex did not impact resting BP (range: $P$=0.408-0.557). MVC was higher in males ($P$<0.001) and young adults ($P$=0.009) compared to females and older adults, respectively. Estradiol and progesterone concentrations were greater in young females relative to older females (both $P$<0.001) and young males (both $P$<0.001). Bioavailable testosterone was greater in young compared to older males ($P$<0.001), but not different between young and older females ($P$=0.785). Sex-based comparisons within each age group revealed that both older and young males had greater levels of bioavailable testosterone compared to similarly aged females (both $P$<0.001).

*4.3.2. Incremental exercise to fatigue*

The peak hemodynamic responses to incremental handgrip exercise to fatigue are presented in Figure 4.1, and absolute hemodynamic data at peak exercise are presented in Table 4.2. The increase in HR at peak exercise was greater in young, relative to older adults (Figure 4.1A; $P$=0.012). Significant age-by-sex-by-stage interactions were observed for the peak increase in MAP from baseline (Figure 4.1B; $P$<0.001), with *post-hoc* analyses revealing a larger rise in MAP in older males than similarly aged females and young males (both $P$<0.001). At peak exercise, young males demonstrated larger increases in cardiac index (Figure 4.1C) and stroke volume index (Figure 4.1D), and larger reductions in TPR (Figure 4.1E) than older males (all $P$<0.001) and young females (range: $P$<0.001-0.014). Furthermore, older females demonstrated smaller increases in cardiac index than young females ($P$=0.007), and smaller changes in TPR than older males ($P$=0.009). Young adults (regardless of sex) had a higher group mean absolute HR ($P$<0.001), cardiac index ($P$<0.001), and stroke volume index ($P$=0.016) at peak exercise. Furthermore, a significant age-by-sex interaction was observed for MAP ($P$<0.001), with *post-hoc* analyses revealing greater MAP in older males compared to young males ($P$<0.001) and older females ($P$<0.001). Similarly, a significant age-by-sex interaction was found for peak exercise

TPR ($P$=0.011), and *post-hoc* analyses indicated that young males had lower TPR than young females ($P$=0.024) and older males ($P$<0.001). RPE at peak exercise was not different in all participants (YM: 18[1], YF: 17[2], OM: 17[2], and OF: 17[3]; $P_{interaction}$=0.669). Furthermore, a significant positive relationship was observed between MVC and the peak change in MAP across all participants (Figure 4.2; $P$=0.005).

Peak integrated MSNA responses to incremental handgrip exercise to fatigue are presented in Figure 4.3. The peak change in MSNA burst frequency (Figure 4.3A; $P$=0.024), incidence (Figure 4.3B; $P$=0.003) and amplitude (Figure 4.3C; $P$=0.048) were smaller in older compared to young adults. Furthermore, females demonstrated greater increases in MSNA burst incidence than males, regardless of age ($P$=0.015). Neither age ($P$=0.116) nor sex ($P$=0.318) impacted the peak change in total MSNA activity (Figure 4.3D). However, absolute MSNA burst frequency ($P$<0.001) and total MSNA activity ($P$=0.005) were higher in older compared to young adults at peak exercise (Table 4.2). Furthermore, *post-hoc* analyses on a significant interaction for MSNA burst incidence ($P$=0.016) revealed that older males ($P$<0.001) and older females ($P$=0.002) had higher absolute MSNA burst incidence compared to young males and young females, respectively. Additionally, older males had higher MSNA burst incidence relative to older females at peak exercise ($P$=0.006).

The peak AP discharge responses to incremental handgrip exercise to fatigue are presented in Figure 4.4. The peak changes in AP frequency (Figure 4.4A) and incidence (Figure 4.4B) from baseline were smaller in older males than older females and young males (range: $P$=0.001-0.052). Additionally, young males demonstrated greater increases in APs/burst (Figure 4.4C) and AP clusters/burst (Figure 4.4D) than older males (both $P$<0.001) and young females (range: $P$=0.001-0.010), whereas the number of total AP clusters recruited was lower in older compared to young adults, regardless of sex (Figure 4.4E; $P$=0.045). When assessing the peak absolute AP discharge and recruitment during exercise (Table 4.2), AP incidence was higher in

older compared to young adults (*P*=0.017). Age and sex did not impact any other indices of absolute AP discharge or recruitment (range: *P*=0.053-0.770).

In all groups, at baseline (YM: $r^2$=0.69, YF: $r^2$=0.89, OM: $r^2$=0.83, and OF: $r^2$=0.90; range: *P*=0.003-0.033) and during peak exercise (YM: $r^2$=0.91, YF: $r^2$=0.81, OM: $r^2$=0.92, and OF: $r^2$=0.82; range: *P*=0.001-0.020), a pattern emerged whereby AP cluster latency decreased as normalized AP cluster size increased (Figure 4.5). The AP cluster size-latency relationship profile was shifted downward at peak exercise in young (range: -15 to -44 ms, mean: -27 [9]) and older males (range: -5 to -39 ms, mean: -26 [13]) (both *P*<0.001) whereby APs of all sizes expressed faster conduction velocities. Conversely, females did not demonstrate a shift in the mean AP cluster size-latency relationship during exercise (YF: range: 11 to -21 ms, mean: -13 [20], *P*=0.116; OF range: 20 to -48 ms, mean: 0.6 [27], *P*=0.955).

*4.3.3. Post-exercise circulatory occlusion*

The HR, BP, integrated MSNA, and AP recruitment responses to PECO are presented in Figure 4.6. No age-by-sex-by-stage interactions were observed for any cardiovascular or sympathetic neural outcome during PECO (range: *P*=0.462-0.883). The changes in HR (Figure 4.6A; range: *P*=0.342-0.845) and MAP (Figure 4.6B; range: *P*=0.056-0.942) during PECO were not impacted by age or sex. The changes in MSNA burst frequency (Figure 4.6C) and burst incidence (Figure 6D) were smaller in older males and females compared to their younger counterparts throughout PECO (both *P*<0.001). Changes in MSNA burst amplitude during PECO were not impacted by age or sex (range: *P*=0.109-0.880), whereas the change in total MSNA activity was smaller in older compared to young adults throughout PECO (*P*=0.001), but unaffected by sex (*P*=0.876). Older adults had higher absolute MSNA burst frequency, incidence and total MSNA activity than young adults throughout PECO (Table 4.3; all *P*≤0.05). AP discharge and recruitment patterns during PECO are presented in Figure 4.7. Neither age, nor sex impacted the changes in any index

of AP discharge or recruitment (range: *P*=0.299-0.923). However, absolute AP incidence was higher in older males compared to young males throughout PECO (Table 4.3; *P*=0.003). No other indices of absolute AP discharge or recruitment were impacted by age or sex (range: *P*=0.059-0.928). In all groups, at baseline (YM: $r^2$=0.79, YF: $r^2$=0.67, OM: $r^2$=0.71, and OF: $r^2$=0.83; range: *P*=0.001-0.039) and during PECO2 (YM: $r^2$=0.87, YF: $r^2$=0.93, OM: $r^2$=0.86, and OF: $r^2$=0.78; range: *P*<0.001-0.001), a pattern emerged whereby AP cluster latency decreased as normalized AP cluster size increased. Contrary to exercise, the AP cluster size-latency relationship profile was not altered during PECO in any group (range: *P*=0.547-0.740).

*4.3.4. Relationships between endogenous sex hormones and hemodynamics, and AP recruitment*

After accounting for age, partial regression analyses revealed that bioavailable testosterone was inversely related with ΔMAP ($r_{adj}$ = -0.463; *P*=0.035) and trended toward a significant negative relationship with ΔTPR ($r_{adj}$ = -0.414; *P*=0.062) in males (Figure 4.8A and B). Additionally, bioavailable testosterone was positively related to ΔAP clusters/burst ($r_{adj}$ = 0.469; *P*=0.032) but not ΔAPs/burst ($r_{adj}$ = 0.045; *P*=0.848) in males (Figure 4.8C and D). Conversely, bioavailable testosterone was not related to ΔMAP ($r_{adj}$ = -0.212; *P*=0.332; Figure 4.8E), ΔTPR ($r_{adj}$ = 0.064; *P*=0.776; Figure 4.8F), ΔAP clusters/burst ($r_{adj}$ = -0.259; *P*=0.245; Figure 4.8G), or ΔAPs/burst ($r_{adj}$ = -0.306; *P*=0.166; Figure 8H) in females. Estradiol was not related to ΔAPs/burst (YF: r=0.05; *P*=0.882; Figure 4.9A, OF: r=-0.36; *P*=0.25, Figure 4.9B) or ΔAP clusters /burst (YF: r=0.13; *P*=0.715; Figure 4.9C, OF: r=-0.22; *P*=0.492, Figure 4.9D) in young or older females.

***Table 4.1.*** *Participant characteristics, resting blood pressure, and sex hormones.*

| | Young | | Older | | ANOVA *P*-values | | |
|---|---|---|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** | **Age** | **Sex** | **Age x Sex** |
| Age (yrs)* | 26 (5) | 25 (4) | 71 (5) | 70 (4) | **<0.001** | 0.346 | 0.890 |
| Height (cm) † | 178 (8) | 164 (6) | 172 (8) | 166 (7) | 0.384 | **<0.001** | 0.067 |
| Weight (kg) † | 79 (11) | 67 (12) | 78 (7) | 71 (15) | 0.587 | **0.009** | 0.489 |
| BSA ($m^2$) † | 2.0 (0.2) | 1.7 (0.2) | 1.9 (0.1) | 1.8 (0.2) | 0.744 | **<0.001** | 0.174 |
| BMI ($kg/m^2$) | 24.9 (2.9) | 25.0 (4) | 26.6 (2.1) | 25.8 (4.3) | 0.247 | 0.730 | 0.676 |
| Heart rate (beats/min)* | 67 (11) | 68 (7) | 57 (4) | 65 (7) | **0.011** | 0.067 | 0.090 |
| Brachial SBP (mmHg)* | 116 (7) | 114 (12) | 133 (11) | 131 (9) | **<0.001** | 0.557 | 0.861 |
| Brachial DBP (mmHg)* | 70 (6) | 71 (8) | 79 (2) | 75 (4) | **<0.001** | 0.448 | 0.110 |
| Brachial MAP (mmHg)* | 85 (6) | 86 (7) | 97 (4) | 94 (5) | **<0.001** | 0.408 | 0.255 |
| Cardiac index ($L/min/m^2$)* | 2.9 (0.7) | 3.1 (0.6) | 2.6 (0.3) | 2.8 (0.3) | **0.032** | 0.116 | 0.728 |
| Stroke volume index ($ml/m^2$) | 44 (9) | 47 (9) | 45 (6) | 44 (7) | 0.790 | 0.530 | 0.346 |
| Total peripheral resistance ($dynes/s/cm^5$)* | 1247 (389) | 1403 (275) | 1592 (243) | 1481 (177) | **0.014** | 0.788 | 0.113 |
| MVC (kg)*† | 42 (5) | 32 (8) | 39 (6) | 25 (5) | **0.009** | **<0.001** | 0.274 |
| Estradiol (pmol/L) | 93 (25)$^{\alpha}$ | 687 (386)‡ | 86 (21) | 15 (11) | **<0.001** | **<0.001** | **<0.001** |
| Progesterone (nmol/L) | 0.8 (0.5)$^{\alpha}$ | 38 (18)‡ | <0.3$^{\beta}$ | <0.3‡$^{\beta}$ | **<0.001** | **<0.001** | **<0.001** |
| Total testosterone (nmol/L) † | 21 (7) | 1 (0.5) | 18 (7) | 0.5 (0.4) | 0.193 | **<0.001** | 0.376 |
| Albumin (g/L)* | 46 (3) | 44 (5) | 41 (3) | 39 (2) | **<0.001** | 0.071 | 0.985 |
| Sex hormone binding globulin (nmol/L) | 29 (13) $^{\alpha}$ | 78 (51) | 49 (16) | 59 (26) | 0.909 | **0.002** | **0.032** |
| Bioavailable testosterone (nmol/L) | 11 (2) $^{\alpha}$ ‡ | 0.3 (0.2) | 7 (2) $^{\alpha}$ | 0.2 (0.2) | **<0.001** | **<0.001** | **<0.001** |

Data are mean (standard deviation). BSA, body surface area. BMI, body mass index. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial pressure. MVC, maximum voluntary contraction force. Statistical comparisons were carried out using linear mixed model analyses. Significance was set to $P < 0.05$. * indicates significant difference between young and older adults, independent of sex (main effect of age). † indicates significant difference between males and females, independent of age (main effect of sex). ‡ indicates significant difference between young males and older males or between young and older females (within sex *post-hoc* comparison for the interaction term). $^{\alpha}$indicates significant difference between young males and females or older males and females (within age *post-hoc* comparison for the interaction term). $^{\beta}$progesterone values for all older males and females were below the lower limit of detection of our assay (0.3 nmol/L). For statistical comparisons, a value of 0.3 nmol/L was assigned to each older adult. Due to participant comfort (n=1) and technical difficulties (n=1), blood was drawn in 11 young males and 12 young females.

***Table 4.2.*** *Absolute hemodynamics, integrated MSNA and AP discharge/recruitment patterns in young and older males and females at peak exercise (i.e., final completed stage of exercise).*

| | Young | | Older | | ANOVA *P*-values | | |
|---|---|---|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** | **Age** | **Sex** | **Age x Sex** |
| **Hemodynamics** | | | | | | | |
| Heart rate (beats/min) | 88 (14) | 86 (8) | 72 (10)* | 78 (9)* | **<0.001** | 0.664 | 0.182 |
| Mean arterial pressure (mmHg) | 98 (6) | 105 (11) | 123 (16) ‡α | 107 (9) | **<0.001** | 0.155 | **<0.001** |
| Cardiac index (L/min/m$^2$) | 4.3 (1.1) | 3.8 (0.8) | 2.8 (0.3)* | 3.1 (0.4)* | **<0.001** | 0.604 | 0.079 |
| Stroke volume index (mL/m$^2$) | 49 (10) | 45 (10) | 41 (6)* | 40 (8)* | **0.016** | 0.479 | 0.549 |
| Total peripheral resistance (dynes/s/cm$^5$) | 526 (159) α | 795 (213) | 961 (360) ‡ | 927 (360) | **<0.001** | 0.555 | **0.011** |
| **Integrated MSNA** | | | | | | | |
| MSNA burst frequency (bursts/min) | 20 (8) | 21 (8) | 37 (9)* | 30 (10)* | **<0.001** | 0.280 | 0.157 |
| MSNA burst incidence (bursts/100 heart beats) | 23 (8) | 25 (10) | 52 (13)‡ α | 39 (11)‡ | **<0.001** | 0.110 | **0.016** |
| MSNA burst amplitude (AU) | 92 (24) | 93 (32) | 76 (17) | 83 (17) | 0.072 | 0.601 | 0.659 |
| Total MSNA activity (AU/min) | 1819 (906) | 1934 (876) | 2748 (700)* | 2523 (975)* | **0.005** | 0.830 | 0.509 |
| **AP discharge and recruitment** | | | | | | | |
| AP frequency (spikes/min) | 302 (306) | 223 (166) | 475 (341) | 289 (138) | 0.110 | 0.078 | 0.465 |
| AP incidence (spikes/100 heart beats) | 332 (304) | 275 (222) | 646 (394)* | 372 (177)* | **0.017** | 0.052 | 0.195 |
| APs/burst | 14 (10) | 10 (5) | 12 (6) | 10 (4) | 0.642 | 0.107 | 0.673 |
| AP clusters/burst | 6 (2) | 4 (1) | 6 (3) | 5 (1) | 0.569 | 0.053 | 0.770 |
| Total AP clusters | 14 (5) | 12 (2) | 17 (7) | 14 (5) | 0.090 | 0.113 | 0.684 |

Data are mean (standard deviation). MSNA, muscle sympathetic nerve activity. AP, action potential. Statistical comparisons were carried out using linear mixed model analyses. Significance was set to $P < 0.05$. * Indicates significant difference between young and older adults, independent of sex (main effect of age). † Indicates significant difference between males and females, independent of age (main effect of sex). ‡ Indicates significant difference between young males and older males or between young and older females (within sex *post-hoc* comparison for the interaction term). αindicates significant difference between young males and females or older males and females (within age *post-hoc* comparison for the interaction term). *N*=12 for the AP indices in young females as one participant was excluded due to a poor signal-to-noise ratio.

***Table 4.3.*** *Absolute integrated MSNA and AP discharge patterns in young and older males and females during post-exercise circulatory occlusion.*

| | Stage | | | P-values | | | |
|---|---|---|---|---|---|---|---|
| | **BSL** | **PECO1** | **PECO2** | **Age*Stage** | **Sex*Stage** | **Age*Sex** | **Age*Sex*stage** |
| **MSNA burst frequency (bursts/min)** | | | | | | | |
| Young males | 10 (7) | 20 (9) | 21 (12) | **<0.001** | 0.363 | 0.062 | 0.873 |
| Young females | 10 (6) | 20 (8) | 19 (8) | | | | |
| Older males | 33 (12)* | 32 (11)* | 35 (11)* | | | | |
| Older females | 22 (11)* | 24 (11)* | 23 (11)* | | | | |
| **MSNA burst incidence (bursts/100 heart beats)** | | | | | | | |
| Young males | 15 (9) | 29 (12) | 32 (17) | **<0.001** | 0.400 | **0.010** | 0.754 |
| Young females | 15 (10) | 30 (10) | 29 (12) | | | | |
| Older males† | 57 (20) * | 52 (17) * | 59 (18) * | | | | |
| Older females | 34 (15) * | 35 (16) * | 36 (16) * | | | | |
| **MSNA burst amplitude (AU)** | | | | | | | |
| Young males | 64 (15) | 75 (25) | 74 (22) | 0.119 | 0.840 | 0.859 | 0.626 |
| Young females | 67 (12) | 81 (26) | 82 (23) | | | | |
| Older males | 61 (9) | 63 (10) | 68 (15) | | | | |
| Older females | 66 (7) | 70 (10) | 70 (10) | | | | |
| **Total MSNA activity (AU*min)** | | | | | | | |
| Young males | 598 (307) | 1407 (686) | 1522 (999) | **<0.001** | 0.404 | 0.066 | 0.883 |
| Young females | 620 (312) | 1609 (609) | 1558 (753) | | | | |
| Older males | 2005 (793)* | 1940 (663) | 2398 (1062)* | | | | |
| Older females | 1396 (606)* | 1625 (737) | 1640 (759)* | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AP frequency (spikes/min)** | | | | | | | |
| Young males | 138 (216) | 270 (356) | 304 (358) | 0.107 | 0.372 | 0.146 | 0.820 |
| Young females | 87 (72) | 185 (164) | 181 (160) | | | | |
| Older males | 432 (307) | 393 (233) | 505 (419) | | | | |
| Older females | 148 (89) | 197 (105) | 190 (90) | | | | |
| **AP incidence (spikes/100 heart beats)** | | | | | | | |
| Young males | 190 (258) | 388 (454) | 447 (477) | 0.069 | 0.379 | 0.059 | 0.659 |
| Young females | 130 (108) | 265 (227) | 267 (227) | | | | |
| Older males | 755 (538) | 647 (377) | 846 (679) | | | | |
| Older females | 234 (155) | 296 (142) | 291 (131) | | | | |
| **APs/burst** | | | | | | | |
| Young males | 10 (8) | 12 (9) | 12 (9) | 0.980 | 0.928 | 0.525 | 0.462 |
| Young females | 8 (4) | 8 (5) | 9 (7) | | | | |
| Older males | 12 (6) | 12 (4) | 13 (7) | | | | |
| Older females | 7 (3) | 8 (3) | 8 (3) | | | | |
| **AP clusters/burst** | | | | | | | |
| Young males | 4 (2) | 5 (2) | 5 (2) | 0.442 | 0.650 | 0.262 | 0.860 |
| Young females | 4 (1) | 4 (1) | 4 (2) | | | | |
| Older males | 5 (2) | 6 (2) | 6 (2) | | | | |
| Older females | 4 (1) | 4 (1) | 4 (1) | | | | |
| **Total AP clusters** | | | | | | | |
| Young males | 9 (4) | 11 (4) | 12 (4) | 0.094 | 0.622 | 0.225 | 0.719 |
| Young females | 8 (3) | 10 (4) | 11 (5) | | | | |
| Older males | 14 (5) | 14 (3) | 16 (5) | | | | |
| Older females | 10 (3) | 12 (3) | 11 (3) | | | | |

Data are mean (standard deviation). MSNA, muscle sympathetic nerve activity. AP, action potential. Data are presented as a function of exercise intensity until the last common time point (i.e., 50%) and the final exercise stage (i.e., Peak) in all participants. Statistical comparisons were carried out using three-factor (age, sex and stage) linear mixed model analyses. *Post-hoc* comparisons were performed in the event of a significant interaction effect. Significance was set to $P < 0.05$. * indicates significant difference between young and older adults, independent of sex (i.e., *post-hocs* for the age-by-stage interaction term). $N$=12 for the AP indices in young females as one participant was excluded due to a poor signal-to-noise ratio.

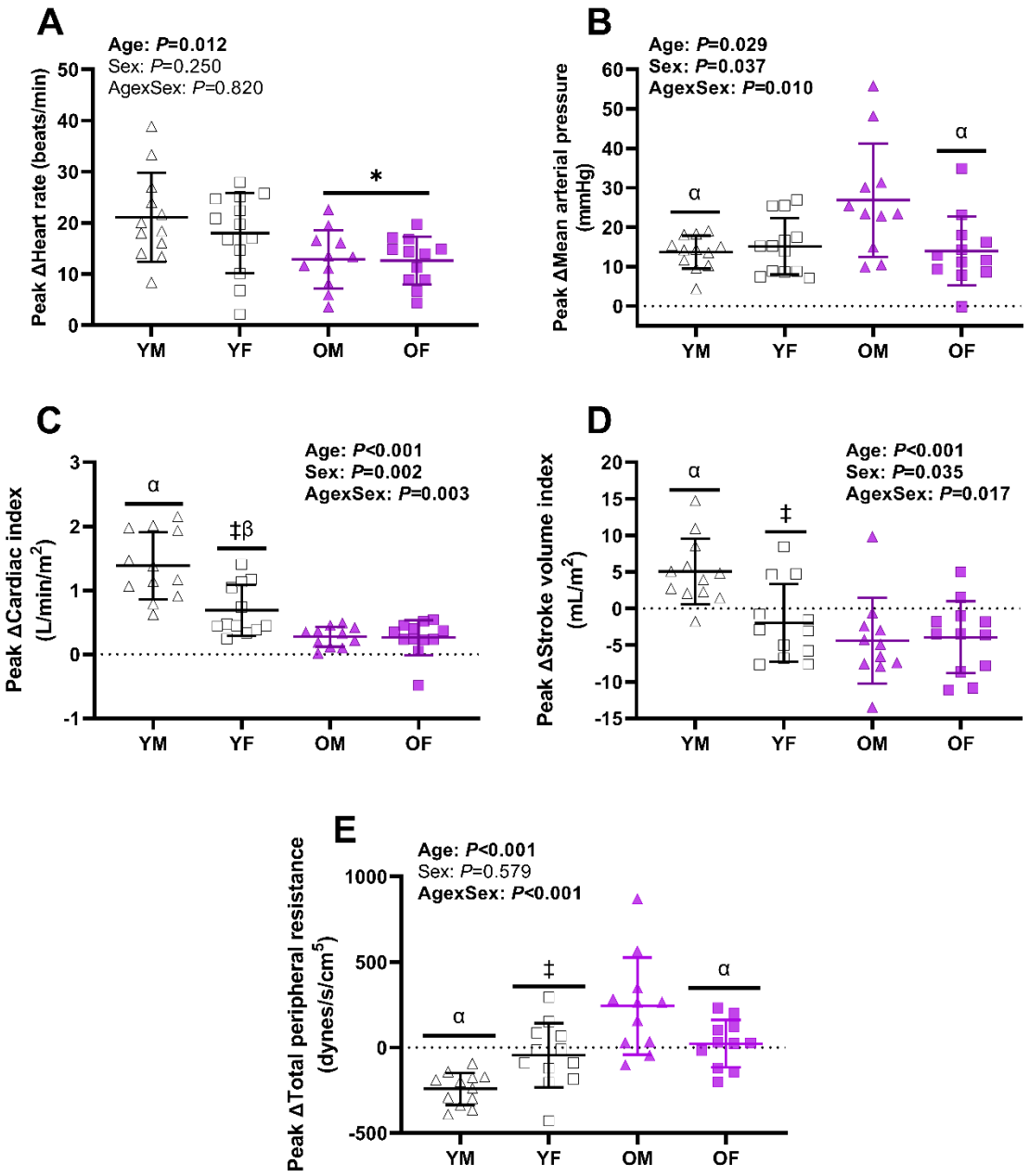


**Figure 4.1. PEAK CHANGES IN HEMODYNAMICS DURING EXERCISE.** Peak changes (Δ) in heart rate (A), mean arterial pressure (B), cardiac index (C), stroke volume index (D), and total peripheral resistance (E) in young males (YM, *n*=12; open triangles and solid black lines), young females (YF, *n*=13; open squares and solid black lines), older males (OM, *n*=11; purple triangles and dashed lines) and older females (OF, *n*=12; purple squares and dashed lines). Data were analyzed using two-factor (age and sex) analyses of variance (ANOVA). *Post-hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. *; significantly different between young and older adults, independent of sex. *#;* significant difference between older males and females. ‡; significantly different versus young males. α; significantly different versus older males. β; significantly different versus older females. Significance was set to $P \leq 0.05$.

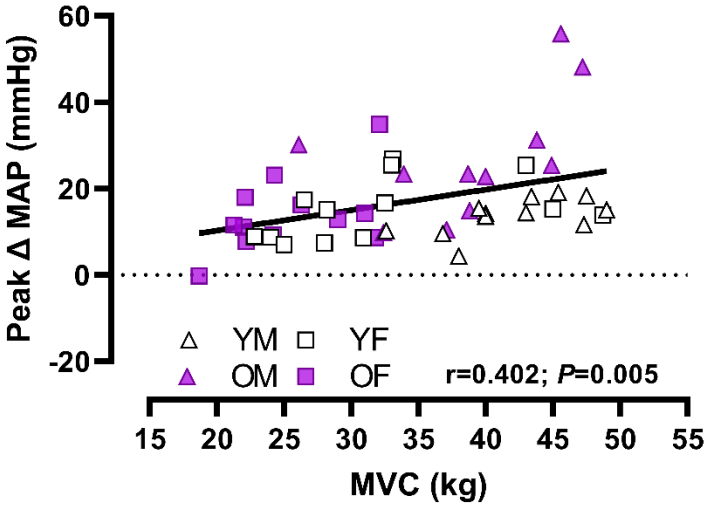


**Figure 4.2. RELATIONSHIP BETWEEN MAXIMAL VOLUNTARY CONRACTION FORCE (MVC) AND PEAK EXERCISE MEAN ARTERIAL PRESSURE (MAP).** Young males (YM; open triangles), young females (YF; open squares), older males (OM; purple triangles), and older females (OF; purple squares). A positive linear relationship was observed between MVC and the peak change in MAP during exercise across all participants.

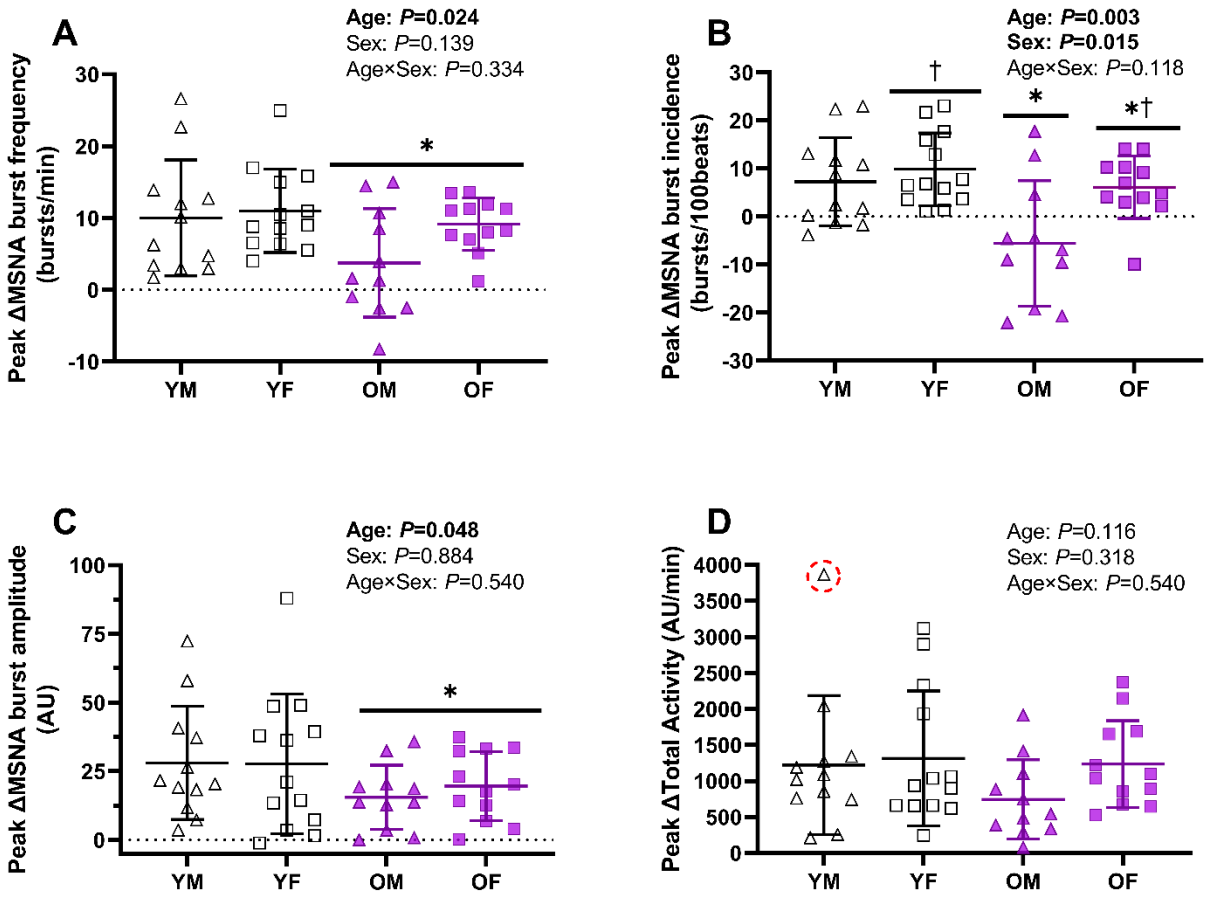


**FIGURE 4.3. PEAK CHANGES IN INTEGRATED MUSCLE SYMPATHETIC NERVE ACTIVITY (MSNA) DURING EXERCISE.** Peak changes (Δ) in MSNA burst frequency (A), burst incidence (B), burst amplitude (C), and total MSNA activity (D) in young males (YM, *n*=12; open triangles and solid black lines), young females (YF, *n*=13; open squares and solid black lines), older males (OM, *n*=11; purple triangles and dashed lines) and older females (OF, *n*=12; purple squares and dashed lines). Data were analyzed using two-factor (age and sex) analyses of variance (ANOVA). *Post-hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. *; significantly different between young and older adults, independent of age. †; significant difference between males and females, independent of age. Significance was set to $P \leq 0.05$. One outlier was identified (dashed red circle) in the peak change in total MSNA activity; however, these data were incorporated in the statistical model as exclusion of their data did not change the statistical outcomes.

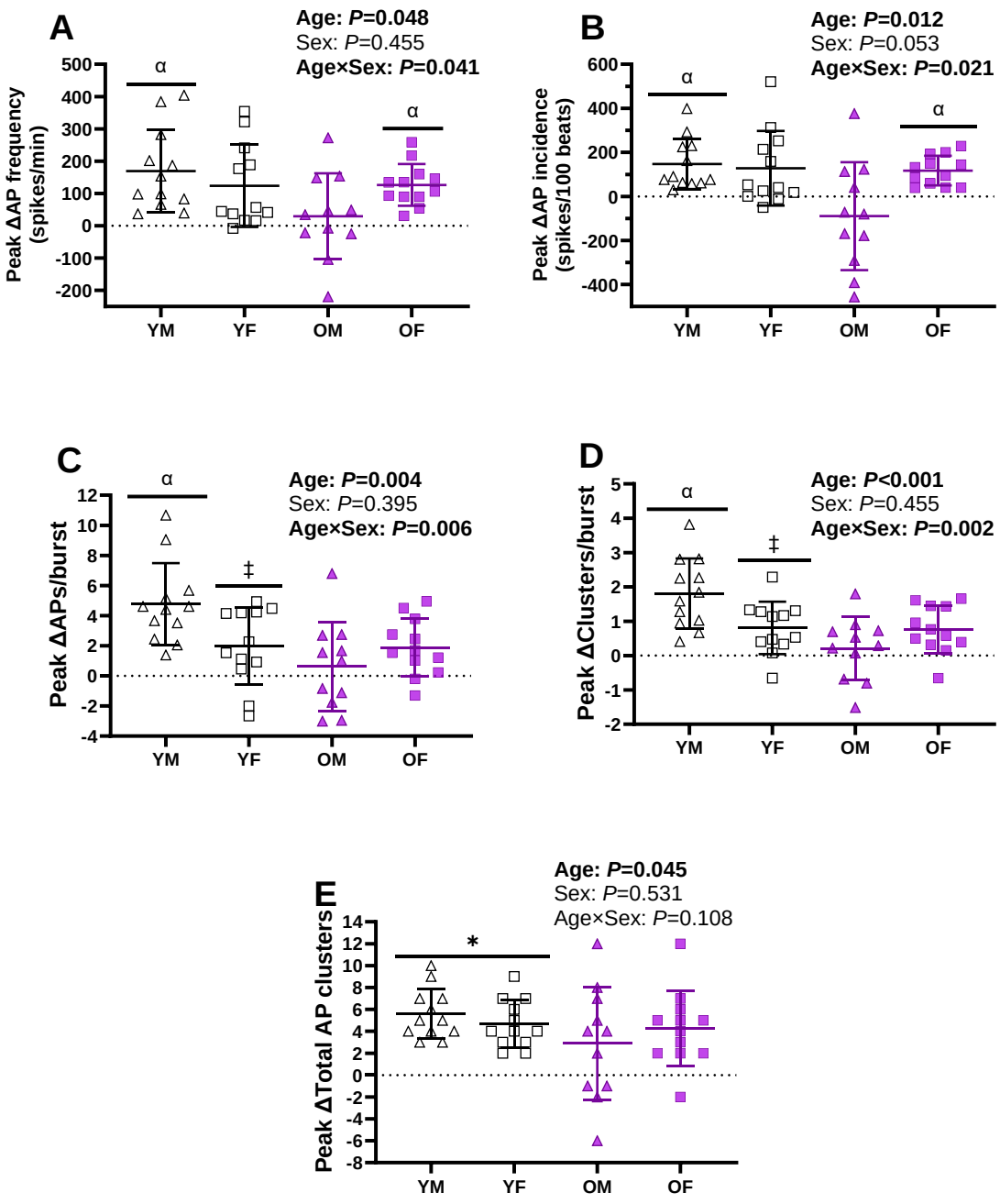


**Figure 4.4. PEAK CHANGES IN ACTION POTENTIAL (AP) RECRUITMENT DURING EXERCISE.** Peak changes (Δ) in AP frequency (A), AP incidence (B), APs/burst (C), AP clusters/burst (D), and total AP clusters (E) in young males (YM, *n*=12; open triangles and solid black lines), young females (YF, *n*=12; open squares and solid black lines), older males (OM, *n*=11; purple triangles and dashed lines) and older females (OF, *n*=12; purple squares and dashed lines). Data were analyzed using two-factor (age and sex) analyses of variance (ANOVA). *Post-hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant interaction effect. *; significantly different between young and older adults, independent of age. ‡; significantly different versus young males. α; significantly different versus older males. Significance was set to $P \leq 0.05$.

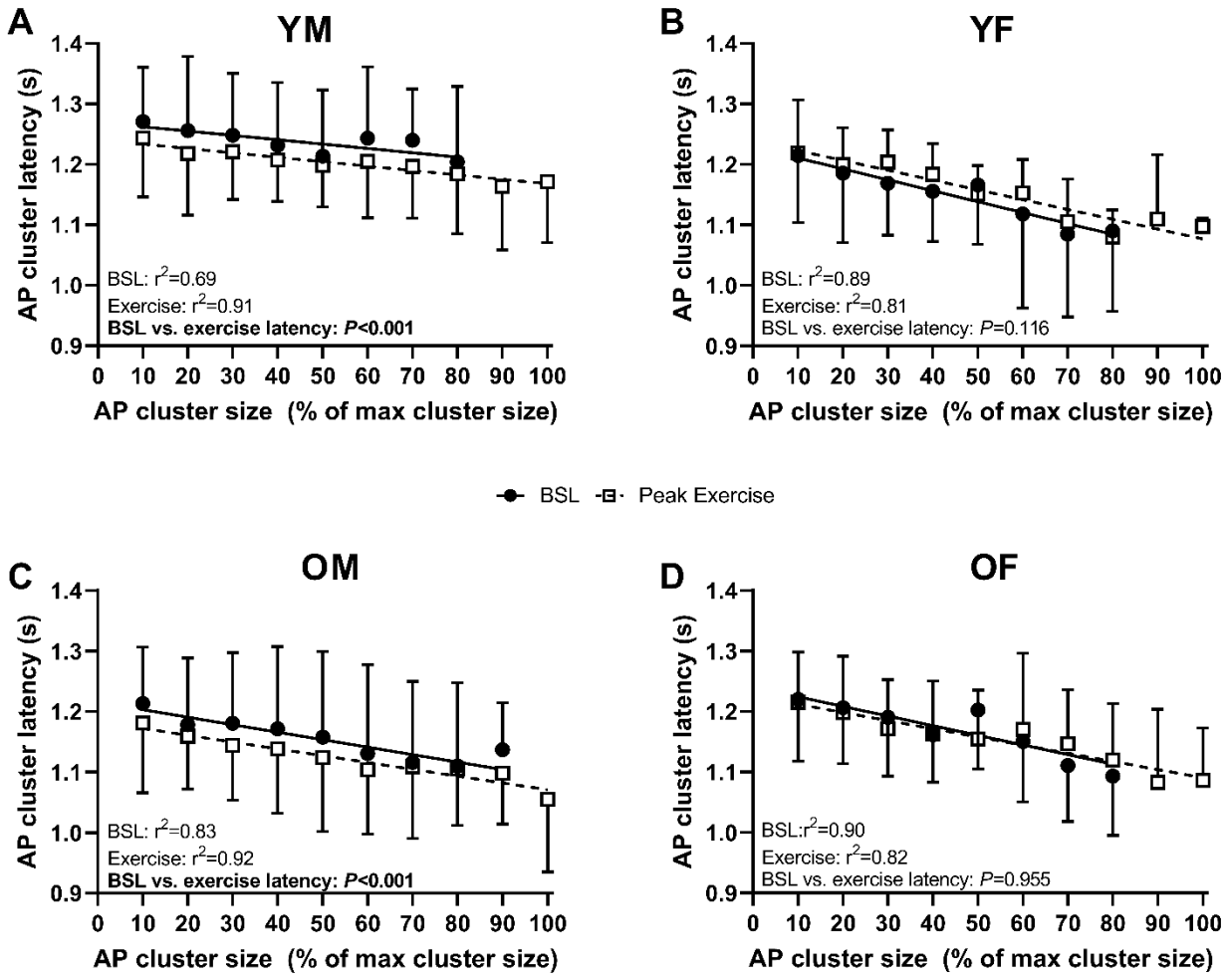


**Figure 4.5. ACTION POTENTIAL (AP) LATENCY DURING EXERCISE.** AP cluster latency as a function of AP cluster size at baseline (BSL; filled circles) and peak exercise (open squares) in young males (YM, *n*=12; panel A), young females (YF, *n*=12; panel B), older males (OM, *n*=11; panel C), older females (OF, *n*=12; panel D). The AP cluster size-latency relationship was fit with an exponential decay function.

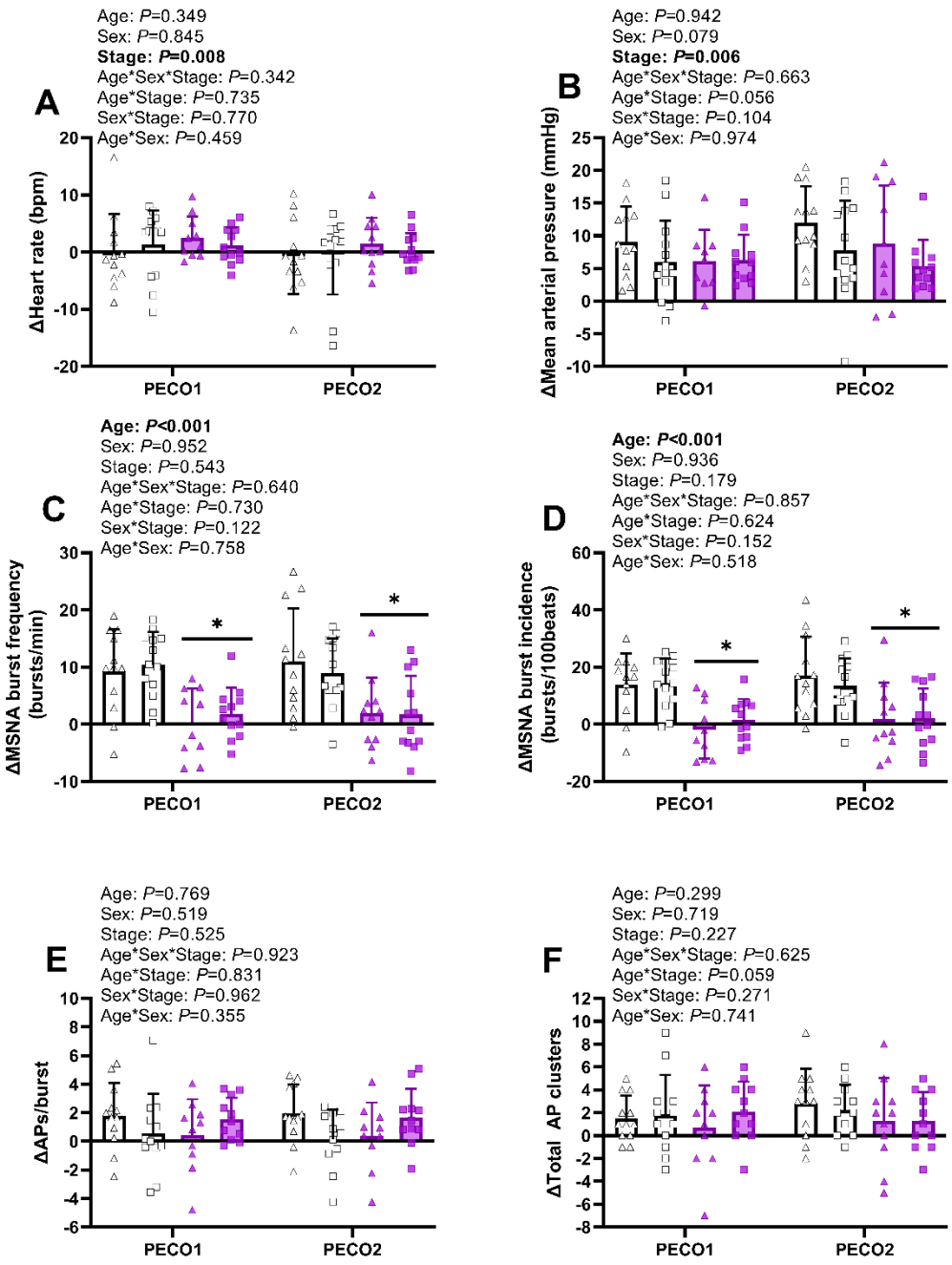


**Figure 4.6**. **CHANGES IN HEMODYNAMICS, INTEGRATED MUSCLE SYMPATHETIC NERVE ACTIVITY (MSNA), AND ACTION POTENTIAL (AP) RECRUITMENT DURING POST-EXERCISE CIRCULATORY OCCLUSION (PECO).** Changes (Δ) in heart rate (A), mean arterial pressure (B), MSNA burst frequency (C), MSNA burst incidence (D), APs/burst (E), and Total AP clusters (F) in young males (YM, *n*=12; open triangles and bars), young females (YF, *n*=12; open squares and bars), older males (OM, *n*=11; purple triangles and bars) and older females (OF, *n*=12; purple squares and bars). Data were analyzed using three-factor (Age, Sex, Stage) linear mixed model analyses with a compound symmetry structure. *Post-hoc* comparisons were carried out using Bonferroni multiple comparisons tests in the event of a significant main effect or interaction. *; main effect of age. Significance was set to $P \leq 0.05$.

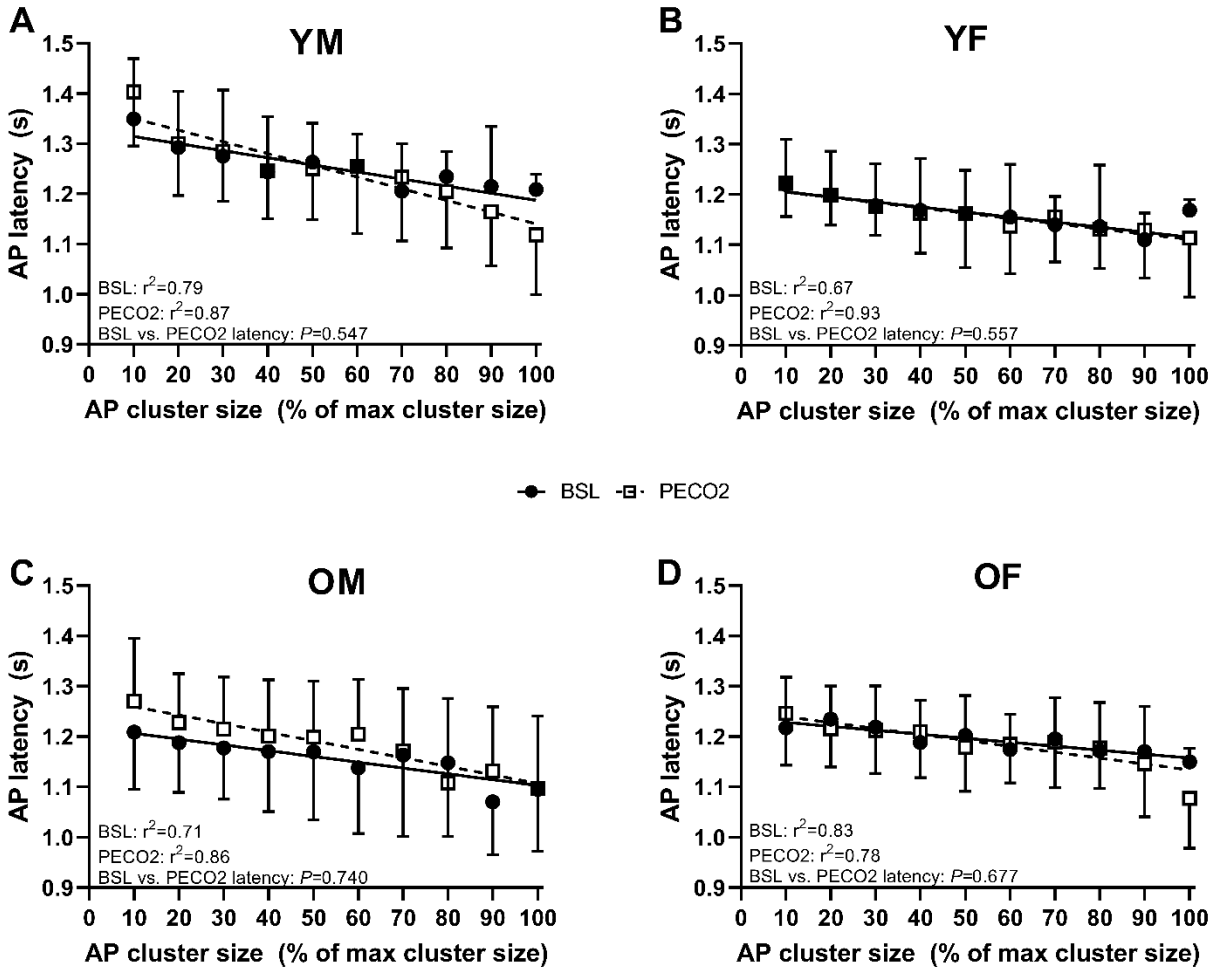


**Figure 4.7. ACTION POTENTIAL (AP) LATENCY DURING POST-EXERCISE CIRCULATORY OCCLUSION (PECO).** AP cluster latency as a function of AP cluster size at baseline (BSL; filled circles) and the second minute of PECO (PECO2; open squares) in young males (YM, *n*=12; panel A), young females (YF, *n*=12; panel B), older males (OM, *n*=11; panel C), older females (OF, *n*=12; panel D). The AP cluster size-latency relationship was fit with an exponential decay function.

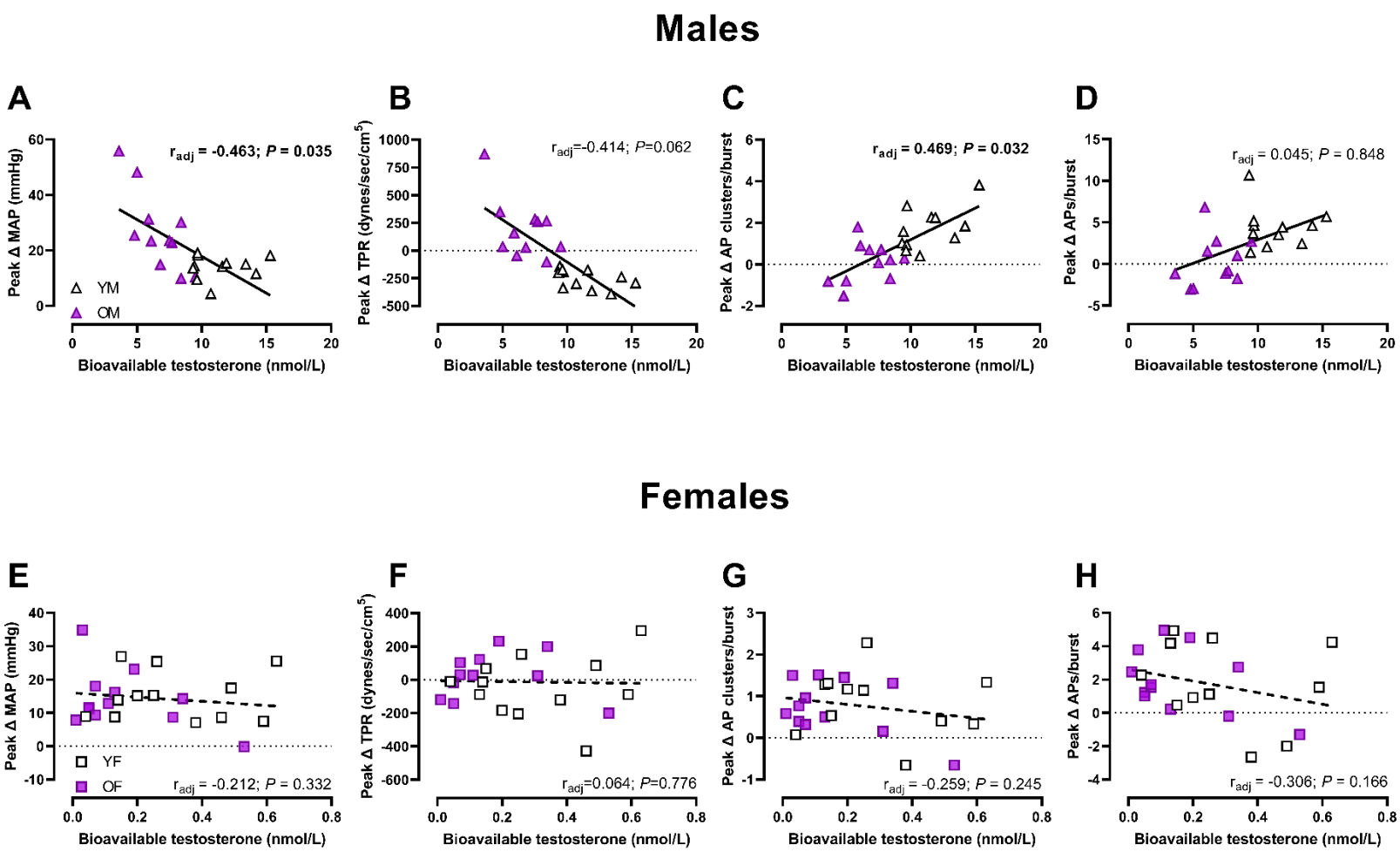


**Figure 4.8**. **RELATIONSHIP BETWEEN BIOAVAILABLE TESOSTERONE, BLOOD PRESSURE, TOTAL PERIPHERAL RESISTANCE, AND ACTION POTENTIAL (AP) DISCHARGE PATTERNS DURING EXERCISE.** Linear relationships between bioavailable testosterone and mean arterial pressure (MAP), total peripheral resistance (TPR), as well as APs/burst and AP clusters/burst in males (A, B, C, and D; *n*=23) and females (C, D, E, and F; *n*=24). Partial regression analyses were used to determine the linear relationships between bioavailable testosterone and sympathetic/hemodynamic variables of interest, while accounting for the impact of age.

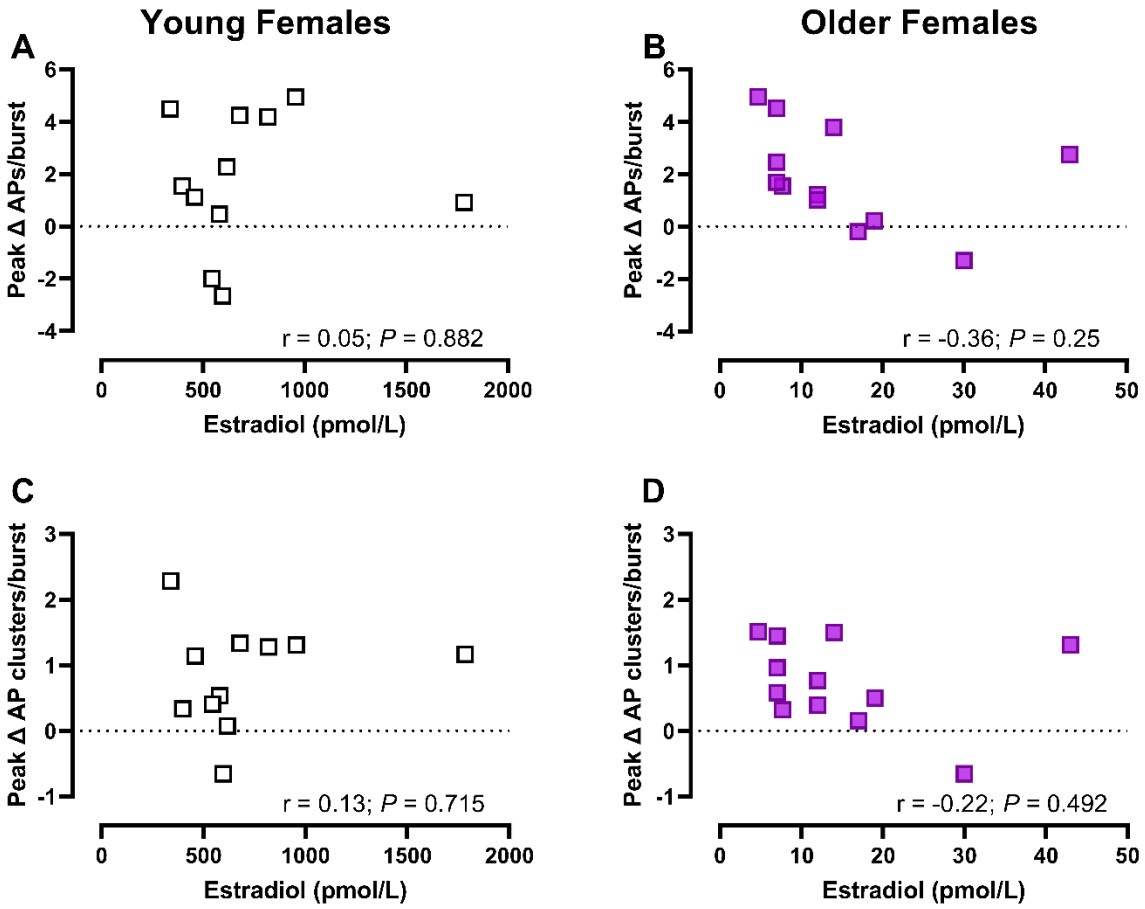


**Figure 4.9**. **RELATIONSHIP BETWEEN ESTRADIOL AND ACTION POTENTIAL (AP) DISCHARGE PATTERNS DURING EXERCISE.** Linear relationships between estradiol and APs/burst, as well as AP clusters/burst in young (A and C; *n*=12) and older females (B and D; *n*=12). Linear regression analyses were used to determine the relationships between estradiol and sympathetic AP discharge patterns.

## 4.4. Discussion

The present study examined the independent and interactive effects of age and biological sex on the sympathetic neural and hemodynamic responses during fatiguing rhythmic handgrip exercise and isolated skeletal muscle metaboreflex activation in humans. Overall, this study indicates that age and sex interactively impact neuro-cardiovascular responses to exercise. The major findings of the current study were: 1) during exercise, older males, not females, demonstrated larger increases in BP despite smaller increases in AP discharge (frequency,

incidence, and APs/burst) and recruitment (AP clusters/burst) compared to young males. Furthermore, compared to young females, young males demonstrated greater increases in APs/burst and AP clusters/burst compared during exercise, whereas sex differences in AP recruitment were not observed in older adults. 2) regardless of sex, older adults demonstrated smaller increases in integrated MSNA burst amplitude and total AP clusters compared to young adults during exercise, and smaller increases in MSNA burst frequency and incidence compared to young adults during exercise and PECO, and 3) regardless of age, males, but not females, demonstrated reflex changes in AP conduction velocity during exercise. Collectively, these data provide novel evidence for sex-specific impacts of aging on the sympathetic and cardiovascular responses to exercise; however, contrary to our hypothesis, older females did not exhibit exaggerated neuro-cardiovascular reactivity to exercise.

*4.4.1. Interactive effects of sex and age on the neuro-cardiovascular responses to incremental handgrip exercise and PECO*

Reports regarding the impact of age on the pressor and sympathetic neural responses to exercise and PECO are inconsistent, likely due to either single-sex studies or a lack of consideration for the interactive effects of age and sex. To date, only one study has examined the effects of age and sex on the cardiovascular responses during exercise, finding larger increases in BP and TPR in older females compared to similarly aged males and young females during rhythmic plantar flexion exercise, but no age-related differences in males (20). Although the authors attributed the exaggerated TPR and BP responses in older females to heightened sympathetic vasoconstrictor drive, MSNA was not measured. In contrast, in the current study, we found no age-related differences in BP, TPR or sympathetic outflow in females during exercise, whereas older males exhibited greater pressor and TPR responses to rhythmic handgrip exercise compared to young males and similarly aged females despite smaller increases in integrated MSNA and AP recruitment.

The reasons for the discrepancy between our work and that of Trinity and colleagues (20) are unclear but warrant discussion. BP is primarily determined by cardiac output and TPR (i.e., Ohm's law). Compared to young adults, cardiac output responses to exercise decline with age due to smaller increases in HR and stroke volume (37). Thus, to raise BP during exercise, there is a shift from a cardiac output-driven increase in BP to greater TPR-mediated pressor responses in older adults. Indeed, like Trinity et al. (20) this was observed in the current study; however, contrary to their work, we found that older males exhibited the largest increases in BP and TPR. This discrepancy may be explained by group differences in MVC. Specifically, in the current study, MVC was not different between young and older males (42 [5] vs. 38 [6] kg; *P*=0.231) but was lower in older compared to young females (25 [5] vs. 32 [8] kg; *P*=0.018), whereas older males in the study by Trinity et al. had a lower plantar flexion MVC than young males, and no difference in MVC was observed in females (20). In the current study, RPE at peak exercise were not different in all participants, indicating that differences in effort are unlikely to contribute to the discrepancy between studies. Rather, less active muscle mass in older compared to young females would have required a smaller proportion of cardiac output, thus minimizing the need for larger TPR increases to redistribute blood to the active muscle. Indeed, when assessing a subset of young (n=7) and older females (n=7) with MVCs that were not different (YF: 26.8 [3] vs. OF: 26.6 [4] kg; *P*=0.907, *d*=0.057), young females demonstrated slightly smaller increases in MAP (YF: 11 [4] vs. OF: 17 [9] mmHg; *P*=0.097, *d*=0.862), and larger reductions in TPR (YF: -60 [88] vs. OF: 70 [141] mmHg; *P*=0.068, *d*=1.11) compared to older females. However, these comparisons did not reach statistical significance, likely because of the small sample size of this sub-analysis. Furthermore, in line with previous work in young males and females (38, 39), a positive relationship was observed between MVC and BP at peak exercise across all groups (Figure 2), indicating that individuals with a higher MVC had a greater pressor response. Thus, the lower MVC in our older females compared to young females may have masked age-related increases in exercise pressor reflex activation in females.

The larger BP and TPR responses observed in older males suggests that more sympathetically mediated vasoconstriction was required to redistribute the limited cardiac output towards active muscle. Thus, it was expected that older males would have greater overall MSNA responses that include larger levels of AP recruitment, because larger bursts of MSNA elicit greater neurogenic vasoconstriction (40) and are comprised of more and larger APs than smaller bursts (41, 42). However, AP discharge was lower in older compared to young males during exercise. This discrepancy between sympathetic neural recruitment and hemodynamics may be explained by numerous factors. First, sympathetic neural discharge between organs is non-uniform (43, 44), and older males may have experienced larger increases in sympathetically mediated vasoconstriction of other vascular beds during exercise, such as the kidney (45). Second, age- and sex-specific changes in muscle metabolic responses to incremental fatiguing exercise may contribute to the smaller sympathetic AP discharge in older males. Older adults demonstrate smaller reductions in muscle cell pH and produce fewer metabolites (e.g., $H_2PO_4$) during fatiguing rhythmic exercise compared to young adults (effect of age), and females demonstrate smaller skeletal muscle metabolic perturbations during exercise compared to males (effect of sex) (46). However, females (young and older) exhibit greater sensitivity to metabolic perturbations than males, with older females possibly demonstrating the greatest sensitivity to changes in the muscle metabolic milieu compared to young males and females as well as older males (46). Therefore, smaller reductions in muscle cell pH without compensatory enhancement of metabolic sensitivity in older males may result in less group III/IV muscle afferent stimulation and subsequently less efferent sympathetic neural discharge. Third, compared to static contractions, rhythmic handgrip exercise induces a considerably smaller reduction in muscle pH, despite similar increases in MSNA, indicating that the skeletal muscle metaboreflex likely contributes less to the MSNA responses during rhythmic exercise (47). Thus, other mechanisms, such as central command or the muscle mechanoreflex, may mediate sympathetic AP discharge and recruitment during rhythmic handgrip exercise. Indeed, central command plays a prominent role in the regulation of

sympathetic neural discharge during high-intensity intermittent handgrip exercise in young adults (8). However, the impact of age and sex on central command during exercise remains unknown. Further, the lack of difference in RPE between groups suggests that central command activation was unaffected by age or sex in the current study. Nonetheless, the current data extend our previous observations of blunted AP recruitment in a mixed sex sample of older adults during an apneic stress (26), as we demonstrate that males may experience larger age-related changes in the central features governing sympathetic neural discharge during fatiguing rhythmic exercise than females. Specifically, during PECO, a period of peripherally-mediated elevations in BP and sympathoexcitation (7), AP discharge and recruitment, as well as BP were not affected by age or sex. These data provide further support that the exaggerated pressor and attenuated axonal discharge responses in older males during exercise are likely driven by central, not peripheral, mechanisms.

Alternatively, the smaller increases in AP discharge, as well as the greater increases in BP and TPR in older males compared to young males during exercise may have been driven by age-related reductions in testosterone. Previous work found a positive relationship between testosterone and sympathetic outflow (48) and a negative relationship between testosterone and resting BP in males (49). We extend these observations by demonstrating a positive relationship between bioavailable testosterone and sympathetic neural recruitment, but not total AP discharge (Figure 8). Furthermore, we found a negative relationship and a trend toward a significant negative relationship between bioavailable testosterone and exercise MAP and TPR, respectively, in males, but not females (Figure 8). It is currently unknown how testosterone impacts efferent sympathetic nerve traffic in males. The significant relationship between testosterone and the number of active AP clusters per burst, but not mean AP content per burst, in males suggests that testosterone may impact the ability to recruit larger axons more than the ability to reflexively increase total axonal discharge during stress. This may also partly explain the

greater increases in AP clusters per integrated burst in young males compared to young females during exercise. Conversely, low testosterone is associated with greater arterial stiffness (50) and reduced endothelial function (51, 52), which may contribute to the larger increases in exercise BP and TPR observed in older males. Future studies with larger sample sizes are warranted to determine the impact of testosterone on exercise BP and its determinants (e.g., preload, afterload, contractility).

In females, estradiol exhibits central sympatho-inhibitory effects (53, 54) and is inversely related to resting sympathetic neural outflow in young females (55). Furthermore, exogenous estradiol therapy dampens the sympathetic neural responses to isometric handgrip exercise in postmenopausal females (19). Thus, the reduction in estradiol following menopause is thought to contribute to exaggerated sympathetic neural discharge in older females during exercise. However, in the current study, estradiol was not related to AP recruitment during exercise in older or young females (Figure 9). It is unclear why no relation between estradiol and sympathetic neural discharge was observed in the current study. However, contrary to isometric exercise (19), estradiol treatment does not impact the MSNA responses to rhythmic handgrip exercise (56) suggesting that exercise mode may impact the relationship between estradiol and MSNA. Nonetheless, the impact of sex hormones on sympathetic neural reactivity to exercise remains an emerging area of research, and our data highlight the important need for further assessments in this domain.

*4.4.2. Independent effect of age on integrated MSNA and AP discharge during incremental handgrip exercise*

Older adults demonstrated smaller increases in MSNA burst occurrence (frequency and incidence), burst strength (amplitude), and the number of AP clusters recruited compared to young adults during exercise. The smaller rise in MSNA burst occurrence among older adults may reflect an age-related increase in the ability of the baroreflex to defend against elevations in BP

during exercise (57). Contrary to MSNA burst occurrence, the arterial baroreflex exerts weak regulation of integrated MSNA burst strength (58) and large AP clusters (59, 60). Thus, the smaller increases in MSNA burst amplitude and less recruitment of larger AP clusters in older adults is likely not explained via a baroreflex-mediated mechanism. However, our data align with previous work which demonstrated smaller increases in integrated MSNA burst amplitude during a cold pressor test (61), and smaller increases in AP recruitment during an apneic stress (26), in older compared to young adults. Taken together, the reduced ability to increase integrated MSNA burst amplitude and recruit larger axons in older adults may be attributed to age-related changes in the central features governing sympathetic neural recruitment.

*4.4.3. Independent impact of sex on sympathetic neural discharge responses to incremental handgrip exercise*

Regardless of age, we found that AP latency was reduced in males during exercise but not females. AP latency reflects the time required for the brainstem-generated neural signal to reach the postganglionic recording site. The ability to alter AP latency represents a fundamental recruitment pattern employed by the sympathetic nervous system (62). The current results are consistent with previous findings that physiological challenges with an intense volitional effort component (e.g., exercise, apnea) cause a downward shift in the AP cluster size-latency relationship (26, 63). Of note, a downward shift in AP latency during exercise was previously observed in a male-only sample (63) whereas we recently demonstrated that females with and without posttraumatic stress disorder did not modify AP conduction velocity during handgrip exercise (64). Although these prior findings suggest that sex differences in AP latency shifts exist, the current data provide the first direct evidence of a sex difference in the ability to modify AP conduction velocity.

Central command is speculated to contribute to AP latency modifications as a downward shift in the AP cluster size-latency relationship is observed during effortful tasks but not during

passive sympathetic stressors (e.g., cold pressor test) (36, 65). Although estradiol reduces the autonomic responses to central command activation (i.e., mesencephalic locomotor region stimulation) in cats (66), it is unlikely that sex hormones primarily mediate the sex differences in central processing times during rhythmic handgrip exercise because the similar resting and exercise AP latencies were also observed in older post-menopausal females who have low estradiol levels. Furthermore, central command-mediated neuro-cardiovascular responses to exercise are dictated more by effort perception than force production (67). Thus, despite smaller MVCs in females compared to males, RPE was not different between all groups, suggesting similar levels of central command activation during exercise. As such, we do not believe that sex differences in central command explain the lack of shift in AP latency in females during exercise. While this feature of modifiable AP latency is consistent across many of our studies, the mechanisms governing this neural communication strategy, and its functional relevance in the context of BP regulation, remain to be determined.

### 4.5. Limitations

We acknowledge certain limitations in the current study. First, strength training status was not strictly controlled for, which may have contributed to the variability in the sympathetic neural responses to handgrip exercise (68, 69). Second, Modelflow-derived cardiac index and stroke volume index may have underestimated the change in hemodynamics during exercise (70). Third, measures of skeletal muscle metabolite production were not made. Additional research is required to quantify concomitant measures of sympathetic AP discharge and local metabolite production and examine the degree of metaboreflex activation in young and older males and females.

### 4.6. Conclusions

The present study demonstrated that sex and age interactively impact muscle sympathetic AP recruitment patterns and systemic hemodynamics during rhythmic exercise, with age-related reductions in the AP discharge responses, and age-related increases in exercise MAP and TPR observed in males, but not females. Furthermore, sex differences in AP recruitment were observed in young (young males > young females) but not older adults. Additionally, regardless of sex, older adults demonstrated smaller increases in integrated MSNA burst amplitude and total AP clusters compared to young adults during exercise, as well as smaller increases in integrated MSNA burst frequency, incidence and total MSNA activity during PECO (i.e., independent effect of age). Thus, older adults (especially older males) exhibit a reduced ability to reflexively alter sympathetic neural recruitment strategies during rhythmic handgrip exercise, indicating that there may be age-related changes in the central features governing sympathetic vasoconstrictor discharge. Lastly, we found that males, but not females (i.e., independent effect of sex), reflexively modify AP conduction velocity during exercise. Altogether the interactive effects of age and sex on the neural and cardiovascular homeostatic adjustments to fatiguing small muscle mass exercise highlight the critical importance of considering biological sex in the interpretation of age-related changes in sympathetic neuro-cardiovascular responses to exercise.

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# Chapter 5

# 5 The effect of biological sex and oral contraception on the sympathetic neurocirculatory adjustments to static handgrip exercise in humans

*(Under revision in The American Journal of Physiology – Regulatory, Integrative and Comparative Physiology)*

## 5.1 Introduction

Increases in sympathetic nervous system activity during exercise are largely governed by central command and the exercise pressor reflex (EPR). The central command hypothesis postulates that descending neural signals from higher brain centers evoke cardiovascular changes during muscle contraction through stimulation of medullary and spinal neuronal circuits (1), whereas the EPR reflects the activation of group III and IV skeletal muscle nerve endings which emit afferent impulses toward the nucleus tractus solitarius of the brainstem to permit elevations in blood pressure and efferent sympathetic vasoconstrictor discharge directed toward the skeletal muscle vasculature (i.e., muscle sympathetic nerve activity; MSNA) (2). These homeostatic adjustments collectively serve to regulate arterial blood pressure while ensuring sufficient oxygen delivery to contracting skeletal muscle during exercise (2–4).

Amongst young adults, biological sex affects EPR activation, with most (5–8), but not all (9–12) studies demonstrating attenuated increases in blood pressure and MSNA during exercise in premenopausal females compared to males. Whilst the mechanisms underpinning this sex-related difference in EPR activation are likely multifactorial (13), the central sympathoinhibitory effect of estradiol (14, 15) is purported to be one of the primary factors. However, recent work found greater pressor and MSNA reactivity amongst females using combined (ethinyl estradiol and progestins) oral contraceptive pills (OCP) compared to naturally menstruating females during exercise (16–18), as well as post-exercise circulatory occlusion (PECO) (17, 18). Given that exogenous estrogens and progestins in OCPs downregulate the hypothalamic-pituitary-ovarian

axis' production of endogenous estradiol and progesterone, the greater pressor and sympathetic responses to exercise amongst females using OCPs indicate that *endogenous* female sex hormones (particularly estradiol) may drive the sex-related differences in EPR activation, whereas suppression of endogenous estradiol may augment the EPR in females.

Pivotal to arterial blood pressure regulation is the transduction of a central efferent sympathetic signal into a peripheral vasoconstrictor response (19, 20). In this regard, prior work demonstrated that premenopausal females exhibit less vasoconstriction (assessed as calf vascular resistance) per unit increase in MSNA during static handgrip exercise (SHG) compared to young males (11), likely due to estradiol-mediated upregulation of β-adrenergic vasodilation (21, 22). Contrary to these data, the transduction of MSNA into total peripheral resistance (TPR) during SHG is greater in females using OCPs compared to naturally menstruating females (18), suggesting that exogenous estradiol does not confer the same degree of β-adrenoreceptor mediated vasodilation as endogenous estradiol during exercise. However, MSNA represents the activity of vasoconstrictor neurons that supply the skeletal muscle vasculature, and it remains unclear how OCPs affect the peripheral vasomotor responses to given increases in efferent sympathetic nerve traffic during exercise.

Bursts of MSNA represent periods of synchronously firing postganglionic action potentials (APs) that serve the rise in overall sympathetic outflow during exercise via increasing the content of AP discharge within an integrated MSNA burst, and recruiting larger, high threshold axons, that are not present at baseline (23, 24). Notably, these discharge patterns represent deterministic messages from the central nervous system that govern circulatory homeostasis during acute physiological perturbations (25). However, it remains unknown how these AP discharge behaviours are impacted by biological sex and endogenous vs. exogenous female sex hormones. Additionally, although the patterning and magnitude of efferent sympathetic outflow dictate

vasoconstrictor signalling (20, 26, 27), the impact of efferent AP emission patterns on vasomotor control in humans is unclear.

With this background in mind, the current investigation aimed to determine the impact of sex and endogenous versus exogenous female sex hormones on the magnitude and patterning of sympathetic AP recruitment and the ensuing limb vasoconstrictor responses during exercise and PECO. PECO was utilized to isolate the contribution of the skeletal muscle metaboreflex to sympathetic outflow from central command and the muscle mechanoreflex (3). We tested the hypotheses that during exercise and PECO: 1) young males and females using OCPs would demonstrate greater sympathetic AP recruitment and sympathetic vascular transduction compared to naturally menstruating females, and 2) AP recruitment patterns would be related to the degree of limb vasoconstriction (i.e., sympathetic vascular transduction) in males and females using OCPs, but not naturally menstruating females.

## 5.2. METHODS

### *5.2.1. Ethical approval*

Written and informed consent was obtained from all participants prior to testing. Approval of experimental protocols was granted by the institutional review boards of the University of Texas Southwestern Medical Center, Texas Health Presbyterian Hospital Dallas (File no. STU-2022-0433), and The Western University Health Sciences Research Ethics Board (File no. 119380). The study was conducted in accordance with the *Declaration of Helsinki*.

### *5.2.2. Participants*

Forty-eight individuals between the ages of 18-35 years participated in the current study: 18 naturally menstruating females, 16 females using OCPs, and 14 males. Participant characteristics are presented in Table 1. Data were collected at the Neurovascular Research

Laboratory at Western University (n=20) and the Women's Heart Health Laboratory at The Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital (n=28). All participants were non-smokers, and free of overt cardiovascular disease, metabolic syndrome, hepatic and renal disease, neurological disease, pulmonary disease, and not pregnant. Participants were not taking any medications that could alter autonomic or vascular function (except OCPs). Naturally menstruating females were tested in the mid-luteal phase of the menstrual cycle (as determined by self-report [n=18], blood draw [n=16] and ovulation kits [n=16]), whereas females using OCPs were tested in the active-pill (high hormone) phase of their pill cycle. All naturally menstruating females had regular menstrual cycles (range: 27-33 days), and females using OCPs had been taking the same OCP for a minimum of 4 months. Some data (n=9) were published previously in studies that addressed independent research questions (18, 28).

*5.2.3. Experimental Procedures*

Participants arrived at the laboratory after an 8 hour fast and having refrained from vigorous exercise as well as caffeine and alcohol consumption for a minimum of 24 hours. All experiments were performed in a quiet, thermoneutral (~23ºC) room. Prior to beginning the study, participants were instructed to empty their bladder to avoid any effects of bladder distention on sympathetic activity and blood pressure (29). Thereafter, participants assumed the supine position and were instrumented for the study. Prior to microneurography, participants performed three brief (~3 seconds) maximal contractions using a handgrip dynamometer with the dominant hand to determine their maximal voluntary contractile force (MVC). The largest of the three contractions was used as the MVC. Data collection began at least 15 minutes following the acquisition of an acceptable microneurographic nerve recording. After a 1-3 min baseline period, participants completed SHG exercise at 40% of MVC until task failure. Task failure was defined as the inability to maintain at least 80% of the target handgrip force for longer than 2 seconds. Approximately 5 seconds prior to exercise termination, a blood pressure cuff was rapidly inflated

around the biceps to suprasystolic pressures (250mmHg) for a 2-minute PECO period. Participants were instructed to avoid breath holding throughout the experiment. Ratings of perceived exertion (RPE) were noted at the end of exercise using the 6-20 Borg scale (30). Participants were instructed to provide an assessment of effort in relation to the forearm muscles performing the task.

*5.2.4. Experimental measures*

Estradiol, progesterone, and testosterone were analyzed via chemiluminescence (ARUP laboratories, Salt Lake City, UT, USA, and LifeLabs, London, Ontario, Canada). Blood work was obtained in 16 of the 18 naturally menstruating females as well as a subset of young males (n=7). Heart rate was measured using a standard lead II electrocardiogram (BioAmplifier, ADInstruments, Dunedin, New Zealand). Beat-by-beat blood pressure was measured continuously via finger photoplethysmography (Finapres Medical Systems, Amsterdam, The Netherlands, and Human NIBP Nano system, ADInstruments). Superficial femoral artery (SFA) blood flow was obtained using a duplex Doppler ultrasound (uSmart 3300, Terason, Burlington, MA; Teratech). The SFA was imaged ~3 cm distal to the bifurcation of the common femoral artery. Continuous measures of SFA diameter and velocity were obtained simultaneously in B-mode and pulse wave, respectively, and operating at a linear frequency of 4-15 MHz (15L4 Smart Mark). In accordance with recent ultrasound technical guidelines (31), the sample volume encompassed the entire vessel lumen without extending the vessel walls, the insonation angle was set to 60°, and the ultrasound probe was steered such that the ultrasound beam was parallel to the vessel walls. Blood flow measures were conducted in a subset of 36 participants (naturally menstruating females: n=12, OCP: n=13, males: n=11).

Multi-unit MSNA was obtained via microneurography of the peroneal nerve (32). Briefly, a 200-µm diameter, tungsten microelectrode, tapering to an uninsulated 1- to 5-µm tip was inserted percutaneously into the common peroneal nerve at the popliteal fossa, and a reference electrode

was positioned subcutaneously ~1-3 cm from the recording site. A suitable MSNA site was obtained by manual manipulation of the microelectrode until a pulse-synchronous burst pattern was observed. An MSNA recording site was confirmed by the absence of skin paresthesia, and an increase in sympathetic discharge during a breath hold (i.e., apnea) but not in response to a startling loud noise (4). The MSNA neurogram was recorded with a nerve traffic analyzer (662C-3; Bioengineering Dept., University of Iowa, Iowa City, IA). The neural signal was amplified (gain: 70,000-160,000-fold), and band-pass filtered (bandwidth: 700-2000 Hz) before being rectified and integrated (leaky integrator; 0.1-s time constant). All data were collected using LabChart 8 and PowerLab (ADInstruments, Colorado Springs, CO) and were then saved offline for analysis.

*5.2.5. Data and statistical analysis*

Baseline hemodynamics, integrated MSNA and AP indices were averaged over the entire first minute of the baseline period. Since handgrip exercise was completed to the point of task failure, the duration of exercise varied between individuals. To account for inter-individual variability in the time to task failure, the total handgrip time was evenly divided into four stages, and presented as SHG 25, 50, 75 and 100%. PECO data were analyzed as a change from baseline to the first (PECO 1) and second (PECO 2) minute of cuff inflation.

*Hemodynamics*. Beat-by-beat heart rate was calculated from the R-R interval of the electrocardiogram. Finger photoplethysmography-derived blood pressure waveforms were calibrated to the average of three brachial blood pressure measures and extracted on a beat-by-beat basis to determine systolic blood pressure, diastolic blood pressure as defined as the maximum and minimum blood pressure of each waveform. Mean arterial pressure was calculated for each blood pressure waveform as 1/3*systolic blood pressure + 2/3*diastolic blood pressure. Cardiac output was estimated from the blood pressure waveform using a three-element Windkessel model (Labchart, ADinstruments), which incorporates age and biological sex, and was presented as cardiac index (calculated as cardiac output/body surface area) to account for

differences in body size between individuals. TPR was calculated as the quotient of mean arterial pressure and cardiac output.

*Integrated MSNA and AP analyses.* MSNA bursts were identified in accordance with recently published guidelines (33), and quantified as burst frequency (bursts/min), burst amplitude (AU; normalized to the largest burst at baseline which was given a value of 100), and total MSNA (AU/min; burst amplitude * burst frequency). Postganglionic APs that fired within integrated MSNA bursts were detected and extracted from the raw, filtered MSNA neurogram using a wavelet-based methodology, as described previously (34). Sympathetic APs were ordered on peak-to-peak amplitude basis and sorted into cluster bins using Scott's rule (35). To enable comparisons within maneuvers and individuals, AP bin characteristics across conditions (e.g., baseline vs. SHG 25%, baseline vs. SHG 50%, baseline vs. PECO1, etc…) were normalized within each individual to ensure identical bin width, maximum bin center and the total number of AP clusters across conditions. This normalization process assures that corresponding clusters across conditions contain APs with similar peak-to-peak amplitudes. This process was done separately for exercise and PECO, guaranteeing that an increase in the number of active clusters at peak exercise or during PECO represents recruitment of subpopulations of previously silent, larger-sized AP clusters. AP discharge was quantified as AP frequency (spikes/min), the number of active APs firing within a MSNA burst (spikes/burst), the number of active AP clusters per integrated burst (clusters/burst) and the number of total AP clusters detected. A high signal-to-noise ratio in the neural recordings (>3.7) is required to ensure accurate AP detection within the filtered neurogram (34). Due to technical difficulties in maintaining a stable, high-quality recording during fatiguing volitional exercise, successful muscle sympathetic AP recordings were collected in 43 of the 48 participants (16 naturally menstruating, 14 OCP, 13 males).

*Leg blood flow and sympathetic vascular transduction*. A video image of the ultrasound screen was recorded and saved as an Audio Video Interleave (AVI) file (Camtasia Studio,

TechSmith, MI, USA). SFA diameter and blood velocity were analyzed using a custom-designed edge detection, and wall-tracking software (BloodFlow Analysis, version 4.0), and the video recording of the ultrasound was used to extract continuous diameter and velocity of the SFA at a frequency of 30 Hz. This approach is independent of investigator bias and has been previously validated with an interobserver coefficient of variation of 0.36% (Woodman *et al.*, 2001). Leg blood flow (LBF) was calculated as: (peak envelope blood velocity/2) X ($\pi$(0.5 X diameter)$^2$ X 60). LVC was determined as LBF divided by mean arterial pressure. LVC was used to indicate the degree of neurogenic vasoconstriction as changes in conductance are proportional to changes in blood flow and may better represent the importance of regional vasomotor changes in the context of blood pressure regulation (36, 37). In accordance with previous work (38), sympathetic vascular transduction was assessed as the quotient of the change in LVC and integrated MSNA or AP recruitment during 100% of SHG.

Data are presented as mean (SD), unless otherwise stated. Normality was assessed using the Shapiro-Wilk's test. Non-parametric data were analyzed using a Mann-Whitney U test and presented as median and the 95% confidence intervals. One-way analyses of variance (ANOVA) were used to evaluate baseline hemodynamics, integrated MSNA and AP indices. Split-plot ANOVAs were used to determine the effect of group (mid-luteal, OCP, males) and stage (SHG 25, 50, 75, 100% or PECO 1 and 2) on the hemodynamic, vascular, and sympathetic responses to handgrip exercise and PECO. *Post-hoc* comparisons were performed using Bonferroni multiple comparisons test when a significant interaction (i.e., group-by-stage) was observed. Linear regression analyses were conducted to determine the relationships between the changes in LVC and integrated MSNA/AP discharge patterns at peak exercise and during PECO. Analysis for outliers was conducted using the ROUT method (GraphPad Prism version 9.3, San Diego, CA). One-way ANOVAs were also used to determine group differences in hemodynamics, integrated MSNA, AP discharge patterns, and sympathetic vascular transduction at peak exercise. Effect

sizes at the ANOVA level are presented as partial $\eta^2$, where small, medium and large effects are determined by $\eta^2$=0.01, $\eta^2$=0.06, and $\eta^2$=0.14, respectively. Additionally, effect sizes for pairwise comparisons were represented by Hedges $g_s$ (small effect: $g_s$=0.2, medium effect, $g_s$=0.5, large effect $g_s$=0.8). Pairwise comparison effect sizes were determined when exact *P*-values (not a range of *P*-values) were presented to aid in power and meta-analysis calculations in future studies. Statistical significance was accepted at $P \leq 0.05$, and analyses were performed using SPSS statistics (Version 28.0; IBM, Armonk, NY).

## 5.3. RESULTS

### *5.3.1. Participant characteristics, baseline hemodynamics and muscle sympathetic nerve traffic*

Participant characteristics are presented in Table 5.1. Males were taller than both groups of females (both *P*<0.001) and were heavier than females using OCP (*P*=0.012), but not naturally menstruating females (*P*=0.168). Nonetheless, body surface area was greater in males compared to both groups of females (both *P*<0.001), and body mass index was not different between groups (*P*=0.213). MVC was greater in males compared to both groups of females (both *P*<0.001), whereas the time to task failure during SHG was not different between groups (*P*=0.845). Females in the mid-luteal phase of the menstrual cycle had higher concentrations of estradiol (Mid-luteal: 607 [531-902] vs. Males: 79 [70-112] pmol/L; *P*<0.001) and progesterone (Mid-luteal: 36 [30-47] vs. Males: 0.3 [0.23-0.80] nmol/L; *P*<0.001) compared to males. Conversely, males had greater plasma concentrations of testosterone compared to naturally menstruating females (Mid-luteal: 1.2 [0.8-1.4] vs. Males: 17 [13-28] nmol/L; *P*<0.001). Baseline hemodynamic and sympathetic outflow data are presented in Table 5.2. Baseline hemodynamics (range: *P*=0.114-0.959) and sympathetic nerve traffic (range: *P*=0.286-0.999) were not significantly different between groups.

### *5.3.2. Hemodynamic responses during exercise and PECO*

A group-by-stage interaction effect was observed for absolute heart rate ($P$=0.006; $\eta^2$=0.110), whereby males and females using OCP demonstrated increases in heart rate above baseline from 25% of SHG until task failure (all $P$<0.001), whereas naturally menstruating females demonstrated increases in heart rate from 50% of SHG until task failure (all $P$<0.001) (Figure 5.1A). Although no group differences were observed in absolute heart rate throughout exercise (range: $P$=0.170-0.999; range), the change in heart rate (i.e., delta) at peak exercise was greater in males compared to naturally menstruating females ($P$=0.019) (Figure 5.1E). No differences were observed between males and females using OCP ($P$=0.663) or between naturally menstruating females and those using OCP ($P$=0.322). Absolute mean arterial pressure increased throughout exercise ($P$<0.001; $\eta^2$=0.845); but no main effect of group ($P$=0.607; $\eta^2$=0.022) or interaction effect ($P$=0.595; $\eta^2$=0.036) was observed (Figure 5.1B). However, the peak change in mean arterial pressure was greater in males compared to naturally menstruating females ($P$=0.041; $\eta^2$=0.128) (Figure 5.1F). No differences were observed between males and females using OCP ($P$=0.434; $g_s$=0.523) or between naturally menstruating females and those using OCP ($P$=0.844; $g_s$=0.041). A group-by-stage interaction was observed for absolute cardiac index ($P$=0.005; $\eta^2$=0.641), which increased in males and females using OCP from 25% of SHG until task failure (range: $P$<0.001-0.011), whereas naturally menstruating females demonstrated increases in cardiac index from 50% of SHG until task failure (range: $P$<0.001-0.005) (Figure 5.1C). Although no group differences were observed in absolute cardiac index throughout exercise (range: $P$=0.189-0.999), the peak change in cardiac index from baseline to peak exercise was greater in males compared to females who were naturally menstruating ($P$=0.025; $g_s$=0.977) (Figure 5.1G). Lastly, absolute TPR fell from baseline throughout exercise in all groups ($P$<0.001; $\eta^2$=0.188); however, TPR was lower in males compared to females using OCP ($P$=0.020; $g_s$=1.028) and tended to be lower compared to naturally menstruating females ($P$=0.062; $g_s$=0.828) (Figure 5.1D). No group differences were observed for the change in TPR (i.e., delta) at peak (100% SHG) exercise ($P$=0.284; $\eta^2$=0.032) (Figure 5.1H). During PECO, there was a

trend toward a main effect of group for the change in mean arterial pressure ($P$=0.063; $\eta^2$=0.118, Table 5.3) but no group differences were observed for the changes in heart rate ($P$=0.295; $\eta^2$=0.054), cardiac index ($P$=0.997; $\eta^2$=0.011) or TPR ($P$=0.727; $\eta^2$=0.014).

*5.3.3. Integrated MSNA responses during exercise and PECO*

Absolute integrated MSNA burst frequency ($P$<0.001; $\eta^2$=0.672, Figure 5.2A), MSNA burst incidence ($P$<0.001; $\eta^2$=0.596), amplitude ($P$<0.001; $\eta^2$=0.466, Figure 5.2B), and total MSNA ($P$<0.001; $\eta^2$=0.674, Figure 5.2C) increased throughout exercise, and this time course increase in MSNA did not differ between groups (range: $P$=0.393-0.928). As such, the peak changes in MSNA burst frequency ($P$=0.786; $\eta^2$=0.011, Figure 5.2D), burst incidence (mid-luteal: $\Delta$23[13] vs. OCP: $\Delta$23 [15] vs. males: $\Delta$16 [8] bursts/100 heart beats, One-way ANOVA $P$=0.181; $\eta^2$=0.073), amplitude ($P$=0.594; $\eta^2$=0.037, Figure 5.2E), and total MSNA ($P$=0.951; $\eta^2$=0.001, Figure 5.2F) were not different between groups. When assessing the impact of biological sex alone (i.e., all females pooled into one group), no differences in the peak changes in any integrated MSNA indices were observed (range: $P$=0.285-0.902; Figure 5.3A-C). During PECO, a significant interaction effect was observed for the change in burst amplitude ($P$=0.012; $\eta^2$=0.183, Table 5.3), with females using OCPs demonstrating a smaller increase in burst size in the second minute of PECO compared to the first minute ($P$=0.009; $g_s$=0.630). However, no interaction effects were observed for MSNA burst frequency ($P$=0.415; $\eta^2$=0.039). Therefore, the change in total MSNA was not different between groups ($P$=0.175; $\eta^2$=0.076).

*5.3.4. AP discharge and recruitment patterns during exercise and PECO*

Absolute AP frequency increased throughout exercise ($P$<0.001; $\eta^2$=0.529, Figure 5.4A), in a similar manner amongst all three groups ($P$=0.583; $\eta^2$=0.040), and the peak change in AP frequency from baseline was not different between groups ($P$=0.453; $\eta^2$=0.035, Figure 5.4E). Similarly, AP incidence increased throughout exercise ($P$<0.001; $\eta^2$=0.483), with no group

differences observed ($P$=0.768; $\eta^2$=0.030). As such, the peak increases in AP incidence were not different between groups (mid-luteal: ∆257 [259] vs. OCP: ∆274 [81] vs. males: ∆317 [194] spikes/100 heart beats, One-way ANOVA $P$=0.808; $\eta^2$<0.001). Significant group-by-stage interaction effects were observed for absolute APs/burst ($P$=0.007; $\eta^2$=0.121, Figure 5.4B), AP clusters/burst ($P$=0.002; $\eta^2$=0.139, Figure 5.4C), and total AP clusters ($P$=0.049; $\eta^2$=0.091, Figure 5.4D). *Post-hoc* comparisons revealed that males demonstrated increases in APs/burst, AP clusters/burst and total AP clusters from baseline at 75% and 100% of SHG (range $P$<0.001-0.007). Similarly, females using OCP demonstrated increases in AP clusters/burst at peak exercise ($P$=0.008; $g_s$=0.623) and increases in total AP clusters at 75 ($P$=0.021; $g_s$=0.660) and 100% of SHG compared to baseline ($P$<0.001; $g_s$=0.899). Conversely, naturally menstruating females did not demonstrate significant increases in AP clusters/burst (range: $P$=0.373-0.999), despite demonstrating increases in total AP clusters at 100% SHG only ($P$=0.042 $g_s$=0.509). Neither females using OCP, nor naturally menstruating females had significant increases in APs/burst throughout exercise (range $P$=0.055-0.999). No group differences were observed in absolute APs/burst (range: $P$=0.194-0.999), AP clusters/burst (range: $P$=0.148-0.999) or total AP clusters (range: $P$=0.544-0.999) during exercise. When females were grouped together to assess the effect of biological sex on the AP discharge and recruitment patterns during exercise, we found that males demonstrated larger changes in APs/burst ($P$=0.029; $g_s$=0.701), AP clusters/burst ($P$=0.003; $g_s$=1.07) and total AP clusters ($P$=0.017; $g_s$=0.861) compared to females from baseline to peak exercise (Figure 5.3D-F). However, when females were partitioned based on OCP use, males demonstrated greater changes in APs/burst ($P$=0.028; $g_s$=0.933, Figure 5.4D), AP clusters/burst ($P$=0.004; $g_s$=1.27, Figure 5.4E), and total AP clusters ($P$=0.008; $g_s$=1.12, Figure 5.4F) at peak exercise compared to naturally menstruating females, but not females using OCPs (range: $P$=0.096-0.455). Furthermore, no differences in AP discharge or recruitment were observed between naturally menstruating females and females using OCPs (range: $P$=0.282-

0.999). No group differences were observed for the AP discharge or recruitment responses to PECO (range: *P*=0.510-0.872; Table 5.3).

*5.3.5. Sympathetic vascular transduction during exercise and PECO*

Figure 5.5 depicts the LBF and LVC responses to exercise and PECO. A significant interaction effect was observed for LBF during exercise (*P*=0.006; $\eta^2$=0.178), with naturally menstruating females demonstrating smaller reductions in LBF at 75 (*P*=0.050; $g_s$=0.979) and 100% of SHG compared to males (*P*=0.043; $g_s$=1.19), as well as females using OCP at 100% SHG (*P*=0.050; $g_s$=1.07) (Figure 5.5A). Similarly, the changes in LVC were greater in males and females using OCPs at 75 and 100% of SHG compared to naturally menstruating females (range: *P*=0.022-0.050) (Figure 5.5B). Conversely, during PECO, a significant effect of group was observed for the changes in LBF (*P*=0.04; $\eta^2$=0.193, Figure 5.5C) and LVC (*P*=0.015; $\eta^2$=0.245, Figure 5.5D), with *post-hoc* analyses revealing larger reductions in LBF (*P*=0.036; $g_s$=.1.27) and LVC (*P*=0.012; $g_s$=.1.04) in young males compared to naturally menstruating females. No differences in LBF or LVC were observed between females using OCPs and naturally menstruating females (both *P*≥0.529) or females using OCPs and males (both *P*≥0.23) during PECO. At 100% of SHG, males and females using OCPs demonstrated significant linear, inverse relationships between the changes in LVC and MSNA burst frequency, burst amplitude, APs per burst and total AP clusters at peak exercise (range: *P*<0.001-0.043; Figure 5.6A-D). However, these relationships were not observed amongst naturally menstruating females (range: *P*=0.402-0.865). Furthermore, sympathetic vascular transduction (as assessed by ΔLVC/ΔSNA) of MSNA burst frequency at peak exercise was greater in males compared to naturally menstruating females (*P*=0.004; $g_s$=1.44), but no differences were observed between females using OCPs and males (*P*=0.169; $g_s$=0.792) or females using OCP and naturally menstruating females (*P*=0.197; $g_s$=0.963) (Figure 5.6E). Conversely, males and females using OCPs demonstrated greater sympathetic vascular transduction of MSNA burst amplitude, APs per burst, and total AP clusters

compared to naturally menstruating females at peak exercise (range: $P$<0.001-0.046; Figure 5.6F-H). Contrary to exercise, sympathetic nerve traffic was not related to LVC during PECO in any group (range: $P$=0.08-0.949; Figure 5.7).

***Table 5.1.*** *Participant characteristics*

| | Mid-luteal n=18 | OCP n=16 | Males n=14 | *P*-value |
|---|---|---|---|---|
| Age, years | 26 (4) | 24 (6) | 25 (5) | 0.507 |
| Height, cm | 163 (6)* | 164 (5)* | 176 (6) | **<0.001** |
| Weight, kg | 67 (12) | 62 (9)* | 75 (12) | **0.014** |
| BSA, $m^2$ | 1.7 (0.1)* | 1.7 (0.1)* | 1.9 (0.2) | **<0.001** |
| BMI, $kg/m^2$ | 25.3 (5) | 23.1 (3) | 24.1 (3) | 0.213 |
| MVC, kg | 30 (7)* | 30 (4)* | 44 (6) | **<0.001** |
| SHG time to task failure, s | 157 (76) | 160 (61) | 147 (48) | 0.845 |
| **Oral contraception generation, *n* (%)** | | | | |
| First | -- | 3 (19) | -- | -- |
| Second | -- | 5 (31) | -- | -- |
| Third | -- | 1 (6) | -- | -- |
| Fourth | -- | 7 (44) | -- | -- |

Data are mean (standard deviation). Mid-luteal represents naturally menstruating females. OCP, oral contraceptive pill. BSA, body surface area. BMI, body mass index. MVC, maximal voluntary contraction. SHG, static handgrip. Statistical comparisons were carried out using one-way analyses of variance, with Bonferroni *post-hoc* comparisons. *, indicates significant difference from males. Significance was set to $P \leq 0.05$.

***Table 5.2.*** *Hemodynamics, integrated MSNA and AP indices during supine baseline.*

| | Mid-luteal n=18 | OCP n=16 | Males n=14 | *P*-value |
|---|---|---|---|---|
| **Hemodynamics** | | | | |
| Heart rate (beats/min) | 69 (9) | 68 (7) | 66 (9) | 0.449 |
| Mean arterial pressure (mmHg) | 85 (9) | 88 (8) | 84 (9) | 0.497 |
| Cardiac index (L/min/m$^2$) | 2.5 (0.6) | 2.5 (0.5) | 2.5 (0.5) | 0.959 |
| Systemic vascular conductance (ml/min/mmHg) | 52 (14) | 47 (11) | 57 (11) | 0.103 |
| Leg blood flow (ml/min) | 54 (19) | 63 (37) | 72 (24) | 0.216 |
| Leg vascular conductance (ml/min/mmHg) | 0.6 (0.4) | 0.7 (0.4) | 0.9 (0.3) | 0.236 |
| **Integrated MSNA and AP indices** | | | | |
| Burst frequency (bursts/min) | 10 (4) | 10 (6) | 13 (9) | 0.286 |
| Total activity (AU/min) | 577 (181) | 553 (354) | 707 (437) | 0.359 |
| AP frequency (spikes/min) | 80 (67) | 65 (79) | 97 (116) | 0.633 |
| APs per burst | 6 (3) | 6 (4) | 6 (3) | 0.999 |
| AP clusters per burst | 4 (1) | 3 (2) | 3 (1) | 0.785 |
| AP total clusters | 10 (3) | 8 (4) | 8 (3) | 0.433 |

Data are mean (standard deviation). Mid-luteal represents naturally menstruating females. OCP, oral contraceptive pill. MSNA, muscle sympathetic nerve activity. AP, action potential. AU, arbitrary unit. Statistical comparisons were carried out using one-way analyses of variance (ANOVA). *Post-hoc* comparisons were carried out using a Bonferroni corrected *t*-tests. Significance was set to $P \leq 0.05$.

***Table 5.3.*** *Hemodynamic, Integrated MSNA, and AP responses during PECO.*

| | Stage | | *P*-values | | |
|---|---|---|---|---|---|
| | **PECO 1** | **PECO 2** | **Group** | **Stage** | **Interaction** |
| **Hemodynamics** | | | | | |
| *∆Heart rate (beats/min)* | | | | | |
| Mid-luteal | 1 (6) | 0 (7) | | | |
| OCP | 3 (7) | 2 (7) | 0.295 | 0.494 | 0.936 |
| Males | 0 (5) | 1 (6) | | | |
| *∆Mean arterial pressure (mmHg)* | | | | | |
| Mid-luteal | 9 (7) | 10 (7) | | | |
| OCP | 11 (6) | 12 (6) | 0.063 | **<0.001** | 0.085 |
| Males | 12 (6) | 15 (6) | | | |
| *∆Cardiac index (L/min/m$^2$)* | | | | | |
| Mid-luteal | 0.4 (0.6) | 0.4 (0.6) | | | |
| OCP | 0.5 (0.3) | 0.4 (0.3) | 0.997 | 0.707 | 0.779 |
| Males | 0.4 (0.3) | 0.4 (0.3) | | | |
| *∆Total peripheral resistance (mmHg/ml/min)* | | | | | |
| Mid-luteal | -1 (3) | -1 (3) | | | |
| OCP | -1 (3) | -1 (4) | 0.727 | 0.084 | 0.786 |
| Males | 0 (3) | 0 (2) | | | |
| **Integrated MSNA** | | | | | |
| *∆Burst frequency (bursts/min)* | | | | | |
| Mid-luteal | 12 (9) | 14 (8) | | | |
| OCP | 10 (7) | 13 (8) | 0.690 | 0.078 | 0.415 |
| Males | 11 (6) | 11 (8) | | | |
| *∆Burst amplitude (AU)* | | | | | |
| Mid-luteal | 12 (14) | 16 (20) | | | |
| OCP | 14 (14) | 7 (10)* | 0.743 | 0.283 | **0.012** |
| Males | 15 (18) | 12 (21) | | | |
| *∆Total activity (AU/min)* | | | | | |
| Mid-luteal | 1059 (867) | 1353 (994) | | | |
| OCP | 869 (482) | 897 (532) | 0.436 | 0.198 | 0.175 |
| Males | 954 (694) | 921 (874) | | | |
| **AP discharge and recruitment** | | | | | |
| *∆AP frequency (spikes/min)* | | | | | |
| Mid-luteal | 114 (132) | 159 (154) | | | |
| OCP | 140 (244) | 144 (248) | 0.996 | 0.115 | 0.510 |
| Males | 127 (111) | 149 (138) | | | |
| *∆APs per burst* | | | | | |
| Mid-luteal | 2 (2) | 3 (4) | | | |
| OCP | 2 (4) | 1 (4) | 0.276 | 0.469 | 0.872 |
| Males | 2 (3) | 2 (4) | | | |
| *∆AP clusters per burst* | | | | | |
| Mid-luteal | 0.3 (0.5) | 0.7 (1) | | | |
| OCP | 0.5 (0.9) | 0.3 (0.9) | 0.576 | 0.133 | 0.797 |

| | | | | | |
|---|---|---|---|---|---|
| Males | 0.6 (0.8) | 0.6 (0.9) | | | |
| ∆*Total AP clusters* | | | | | |
| Mid-luteal | 1 (3) | 1 (3) | | | |
| OCP | 1 (3) | 0 (4) | 0.753 | 0.974 | 0.634 |
| Males | 1 (2) | 1 (4) | | | |

Data are mean (standard deviation). Mid-luteal represents naturally menstruating females. OCP, oral contraceptive pill. MSNA, muscle sympathetic nerve activity. AP, action potential. PECO, postexercise circulatory occlusion. AU, arbitrary unit. Statistical comparisons were carried out using split-plot analyses of variance (ANOVA) with Bonferroni corrected *post-hoc* comparisons in the event of a significant interaction effect. *, indicates significant difference from PECO 1. Significance was set to $P \leq 0.05$.

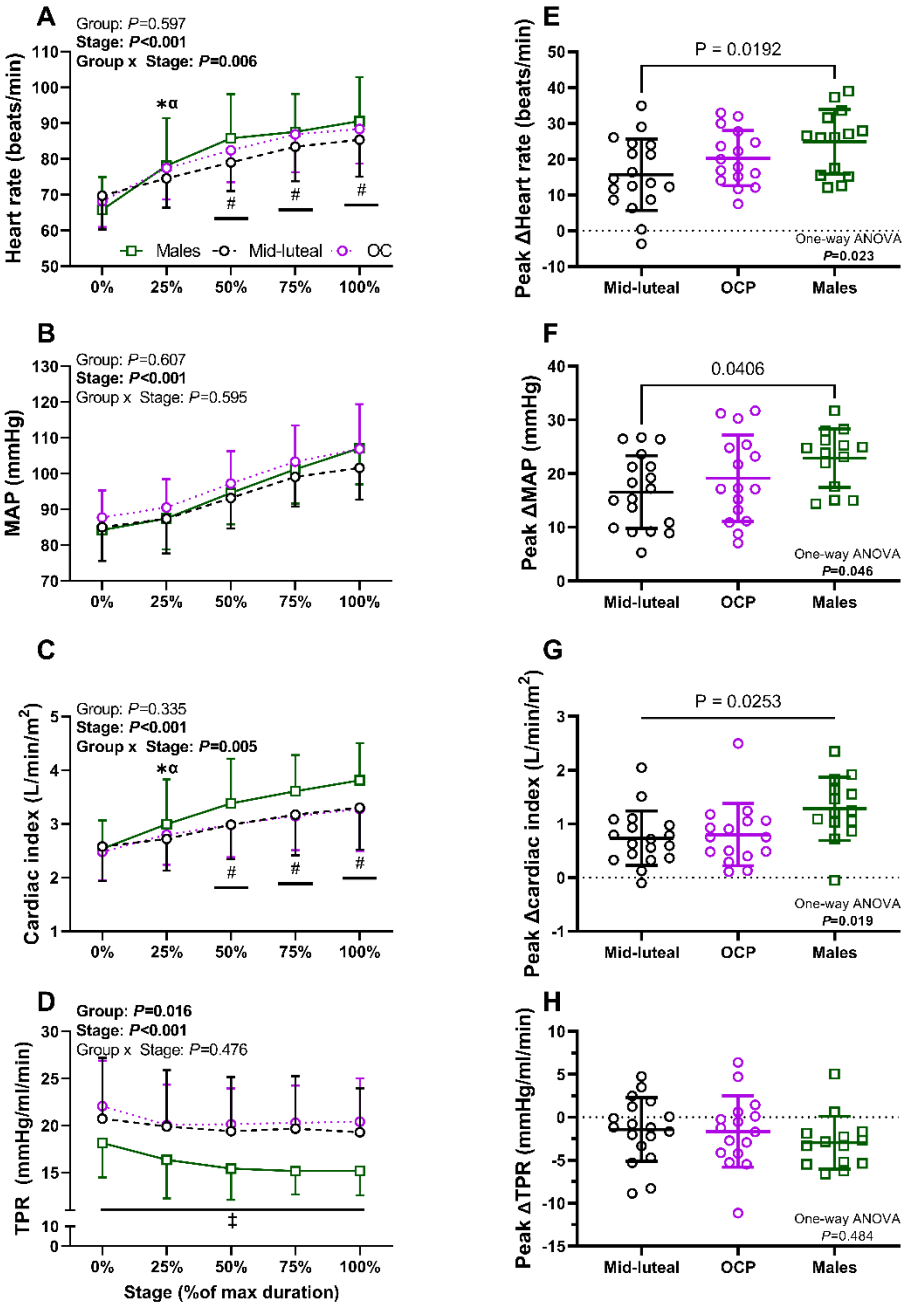


**Figure 5.1.** Time course and peak exercise increases in heart rate (A and E), mean arterial pressure (MAP; B and F), cardiac index (C and G), and total peripheral resistance (TPR; D and H) during static handgrip exercise in naturally menstruating females (mid-luteal; open black circles; n=18), females using oral contraception (OCP; open purple circles; n=16), and males (open green squares; n=14). Time course data were analyzed using split-plot analyses of variance (ANOVA), whereas the peak exercise responses were analyzed using one-way ANOVAs. *Post-hoc* analyses were carried out using Bonferroni corrected *t*-tests. *, significant difference from baseline among males. α, significant difference from baseline among females using OCPs. #, significant difference from baseline in all groups. ‡, significant difference between males and females using OCPs. Data are presented as mean and standard deviation.

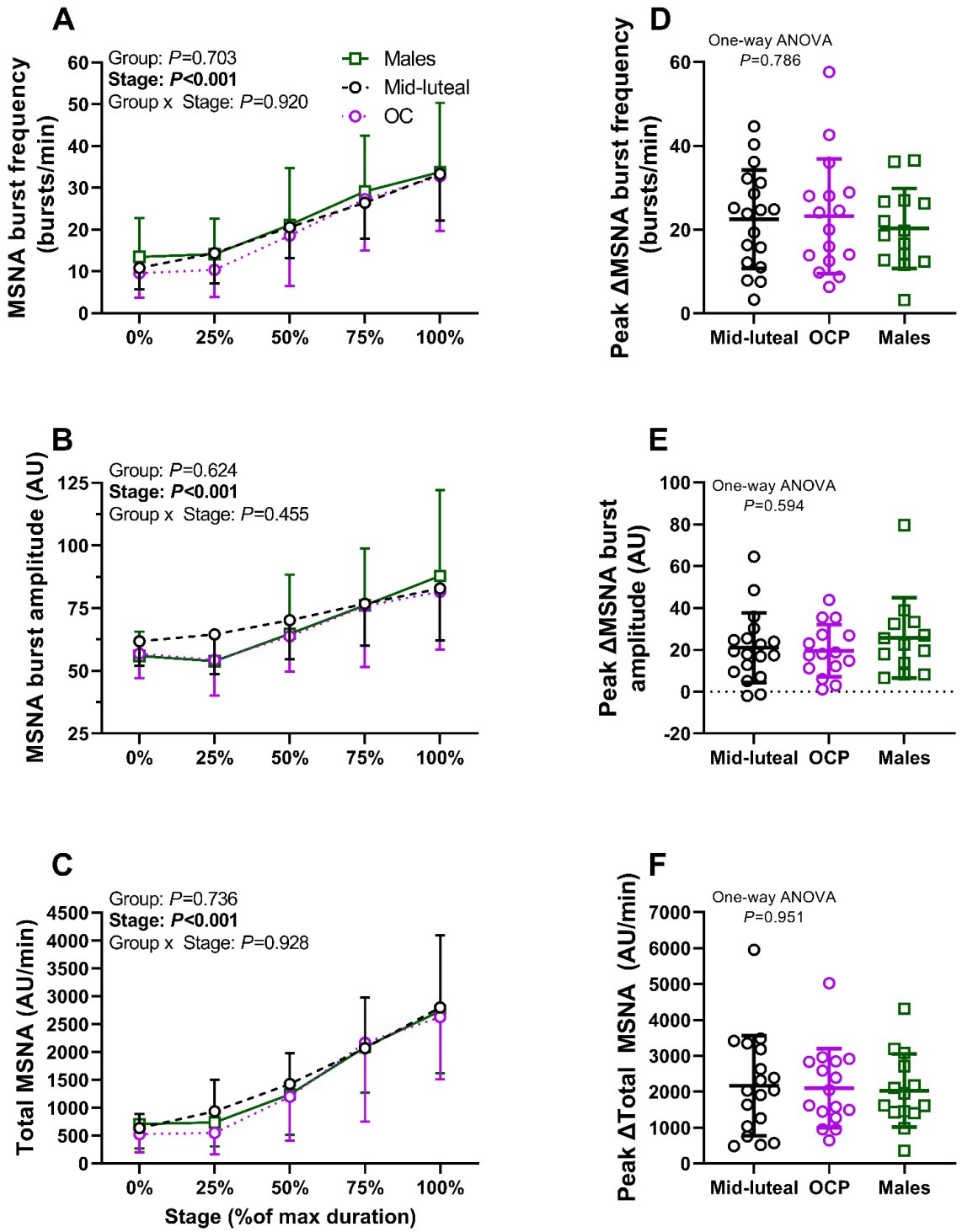


**Figure 5.2.** Time course and peak exercise increases in muscle sympathetic nerve activity (MSNA) burst frequency (A and D), burst amplitude (B and E), and total MSNA (C and F) during static handgrip exercise in naturally menstruating females (mid-luteal; open black circles; n=18), females using oral contraception (OCP; open purple circles; n=16), and males (open green squares; n=14). Time course data were analyzed using split-plot analyses of variance (ANOVA), whereas the peak exercise responses were analyzed using one-way ANOVAs. Data are presented as mean and standard deviation.

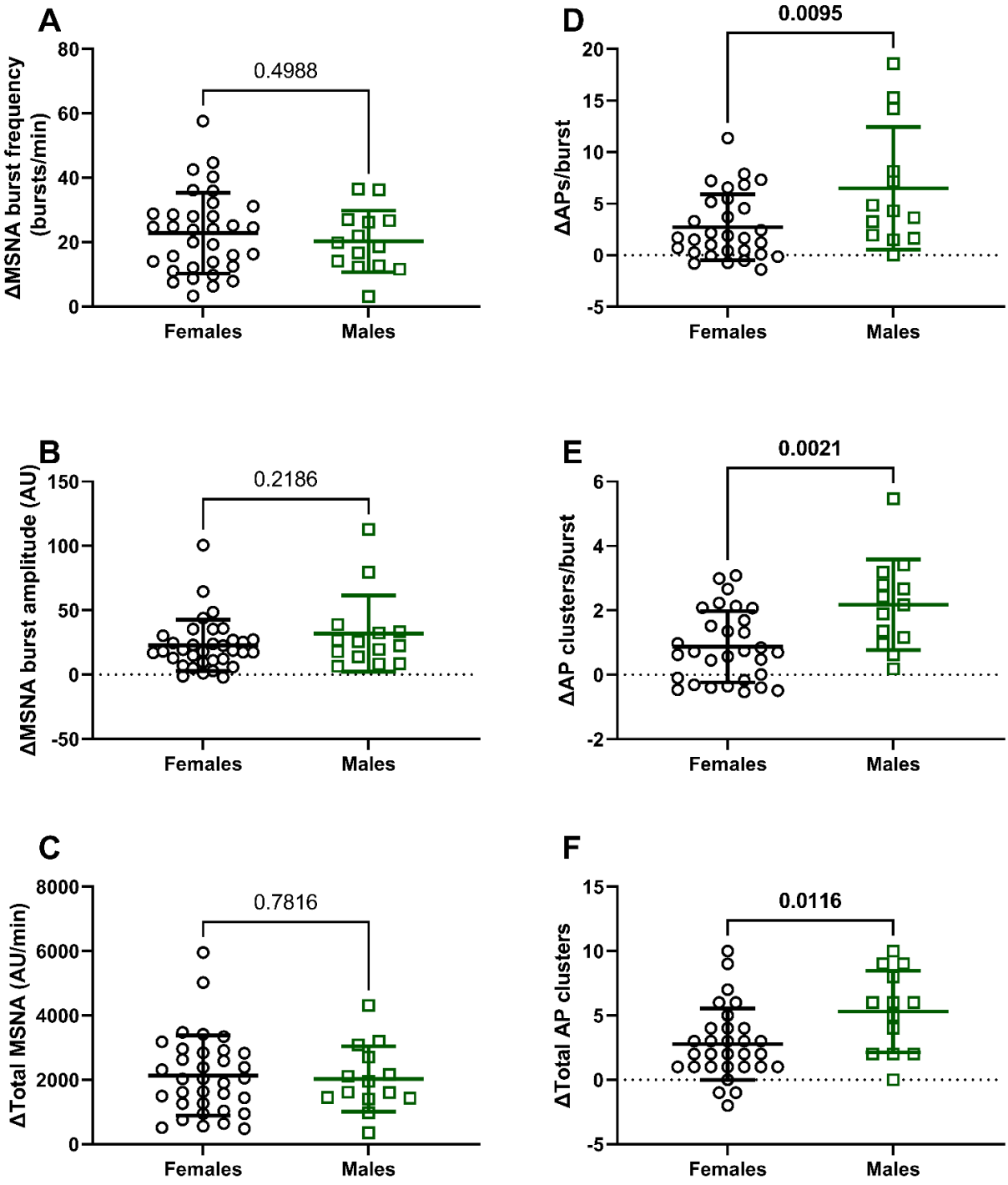


**Figure 5.3.** Peak exercise changes in integrated muscle sympathetic nerve activity (MSNA) burst frequency (A), burst amplitude (B), total MSNA (C), action potentials (APs) per burst (D), AP clusters per burst (E), and total AP clusters (F) in females (oral contraceptive pill and naturally menstruating, combined; open circles; n=34) and males (open green squares; n=14). Data were analyzed using unpaired, two-tailed *t*-tests. Data are presented as mean and standard deviation.

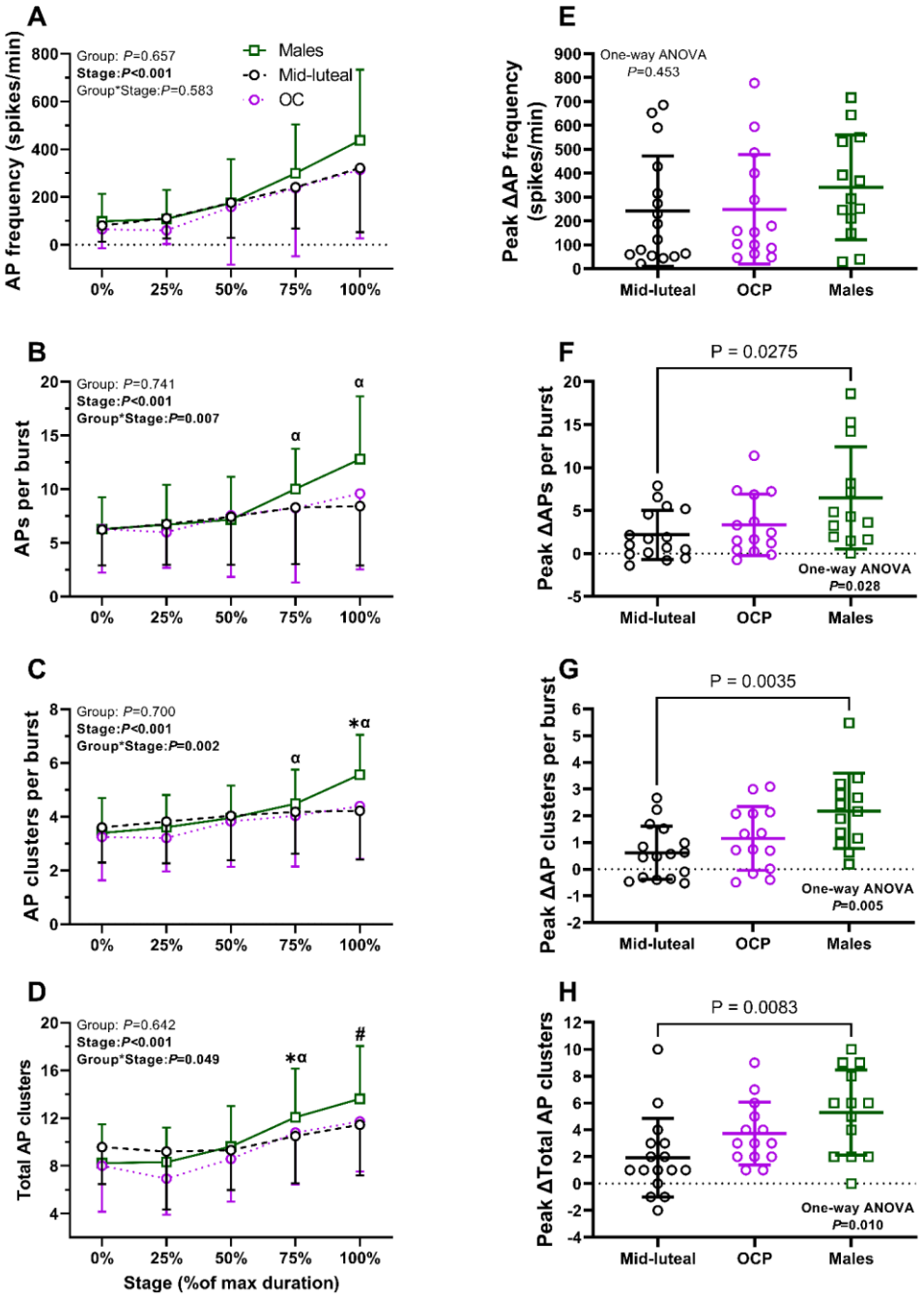


**Figure 5.4.** Time course and peak exercise increases in action potential (AP) frequency (A and E), APs per burst (B and F), AP clusters per burst (C and G), and the total number of active AP clusters (D and H) during static handgrip exercise in naturally menstruating females (mid-luteal; open black circles; n=16), females using oral contraception (OCP; open purple circles; n=14), and males (open green squares; n=13). Time course data were analyzed using split-plot analyses of variance (ANOVA), whereas the peak exercise responses were analyzed using one-way ANOVAs. *Post-hoc* analyses were carried out using Bonferroni corrected *t*-tests. α, significant difference from baseline among males. *, significant difference from baseline among females using OCPs. #, significant difference from baseline in all groups. Data are presented as mean and standard deviation.

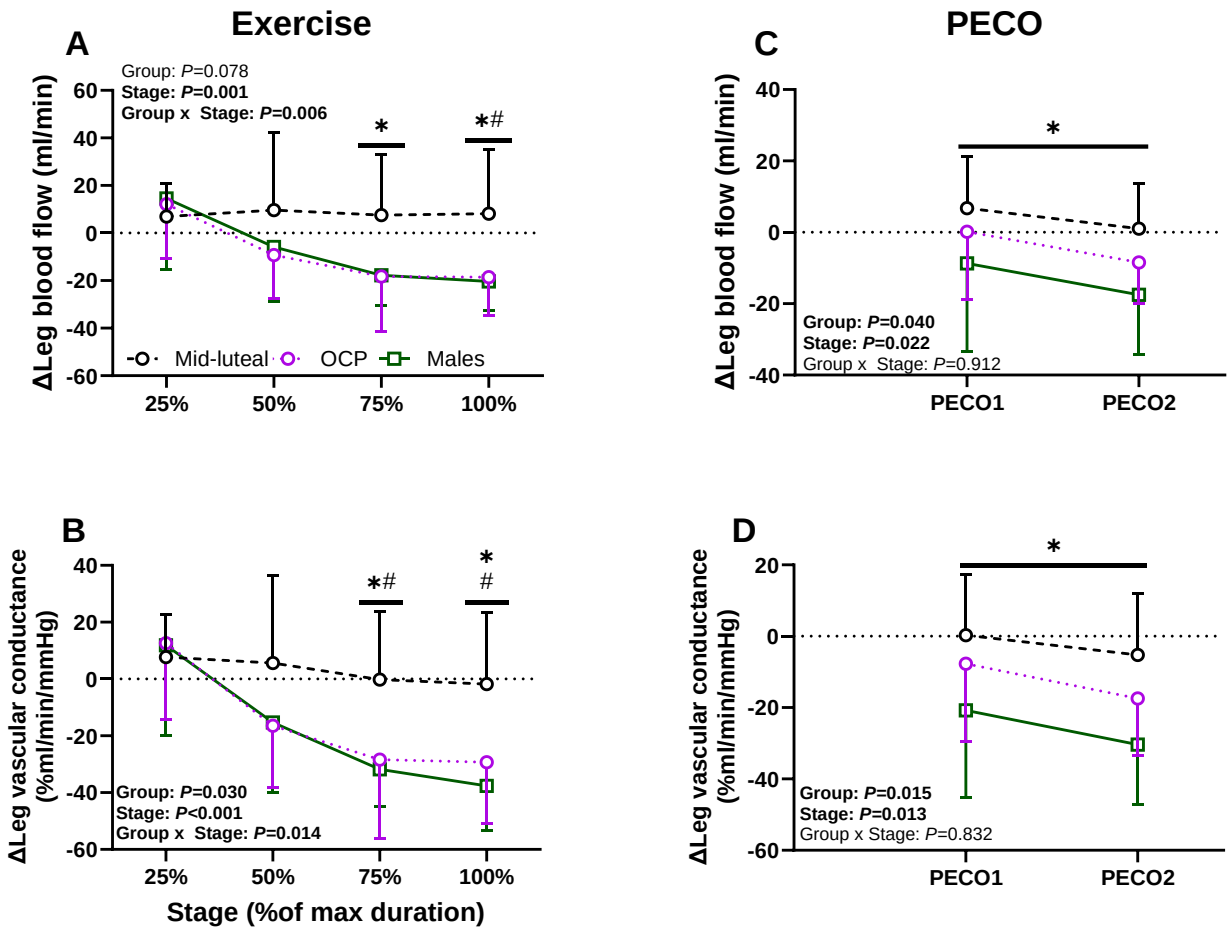


**Figure 5.5.** The changes in leg blood flow and percent changes in leg vascular conductance during static handgrip exercise (A and B) and post-exercise circulatory occlusion (PECO; C and D) in naturally menstruating females (mid-luteal; open black circles; n=11), females using oral contraception (OCP; open purple circles; n=13), and males (open green squares; n=11). Data were analyzed using split-plot analyses of variance, with *post-hoc* Bonferroni corrected *t*-tests carried out in the event of a significant interaction effect. *, significant difference between mid-luteal and males. #, significant difference between mid-luteal and OCP. Data are presented as mean and standard deviation.

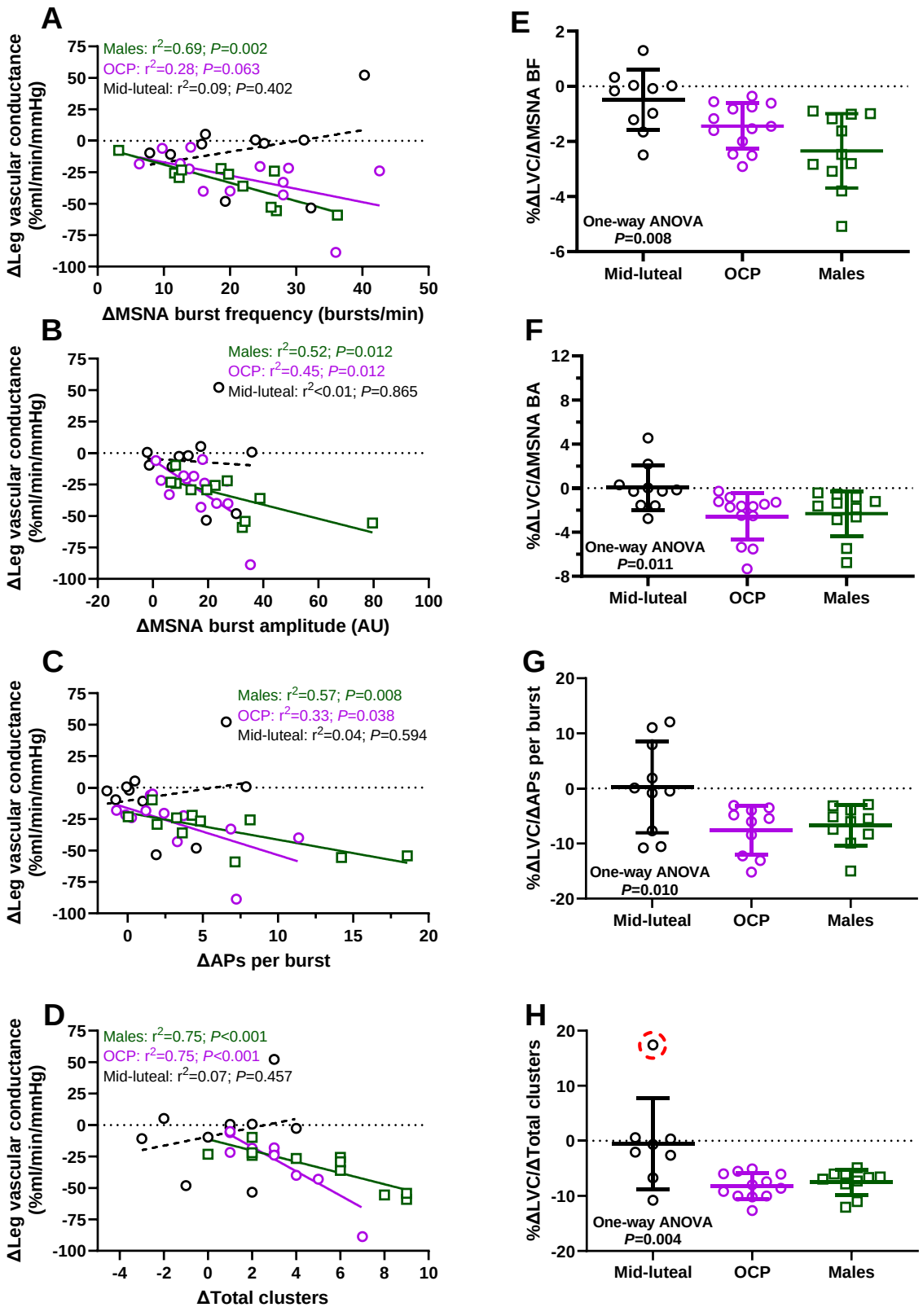
A
Males: r²=0.69; P=0.002
OCP: r²=0.28; P=0.063
Mid-luteal: r²=0.09; P=0.402
ΔLeg vascular conductance (%ml/min/mmHg)
ΔMSNA burst frequency (bursts/min)
B
Males: r²=0.52; P=0.012
OCP: r²=0.45; P=0.012
Mid-luteal: r²<0.01; P=0.865
ΔMSNA burst amplitude (AU)
C
Males: r²=0.57; P=0.008
OCP: r²=0.33; P=0.038
Mid-luteal: r²=0.04; P=0.594
ΔAPs per burst
D
Males: r²=0.75; P<0.001
OCP: r²=0.75; P<0.001
Mid-luteal: r²=0.07; P=0.457
ΔTotal clusters
E
%ΔLVC/ΔMSNA BF
One-way ANOVA P=0.008
F
%ΔLVC/ΔMSNA BA
One-way ANOVA P=0.011
G
%ΔLVC/ΔAPs per burst
One-way ANOVA P=0.010
H
%ΔLVC/ΔTotal clusters
One-way ANOVA P=0.004
Mid-luteal
OCP
Males

**Figure 5.6.** Relationships between the change in leg vascular conductance and the changes in muscle sympathetic nerve activity (MSNA) burst frequency (A), burst amplitude (B), action potentials (AP) per burst (C), and the total number of active AP clusters (D) at peak static handgrip exercise in naturally menstruating females (mid-luteal; open black circles; n=11), females using oral contraception (OCP; open purple circles; n=13), and males (open green squares; n=11) as well as sympathetic vascular transduction of MSNA burst frequency (A; mid-luteal: n=11; OCP: n=13; males: n=11), burst amplitude (B; mid-luteal: n=11; OCP: n=13; males: n=11), action potentials (AP) per burst (C; mid-luteal: n=11; OCP: n=10; males: n=10), and the total number of active AP clusters (D; mid-luteal: n=10; OCP: n=12; males: n=11). Linear regression analyses were used to assess the relationships between the changes in leg vascular conductance and sympathetic outflow, whereas group differences in sympathetic vascular transduction were analyzed using one-way analyses of variance (ANOVA) with Bonferroni corrected *t*-tests employed as *post-hoc* multiple comparisons tests. Outliers were removed using the ROUT method in Graphpad Prism. One outlier was left in the data presentation and analysis for Total AP clusters (Panel D – dashed red circle) as inclusion of this outlier did not alter the statistical analysis. Sympathetic nerve traffic was significantly related to the degree of vasoconstriction (i.e., reduction in leg vascular conductance) in males and females using OCPs, but not in naturally menstruating females, and sympathetic vascular transduction of MSNA burst amplitude and AP recruitment was greater in males and females using OCPs compared to naturally menstruating females.

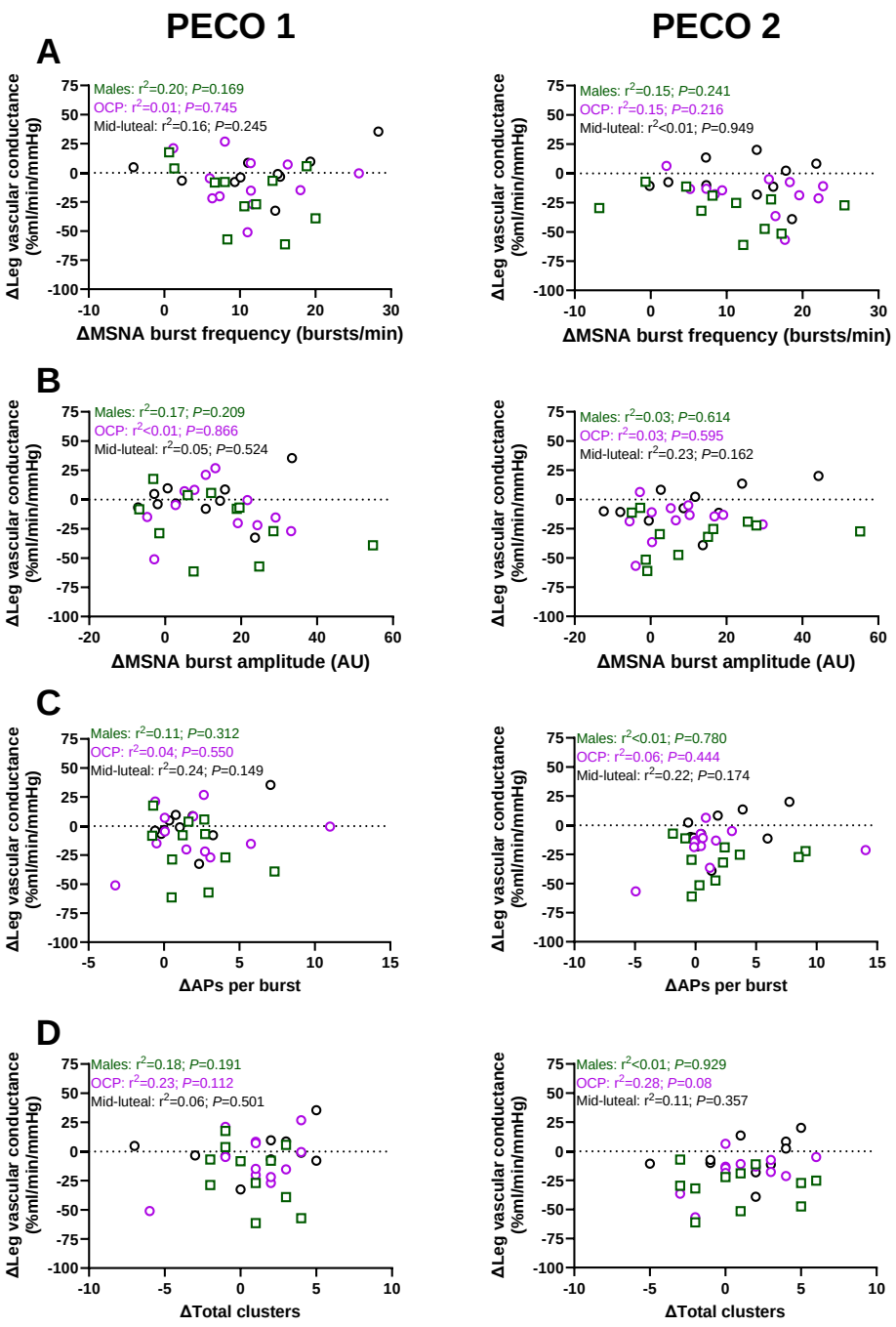


**Figure 5.7.** Relationships between the change in leg vascular conductance and the changes in muscle sympathetic nerve activity (MSNA) burst frequency (A), burst amplitude (B), action potentials (AP) per burst (C), and the total number of active AP clusters (D) during post-exercise circulatory occlusion (PECO) in naturally menstruating females (mid-luteal; open black circles; n=11), females using oral contraception (OCP; open purple circles; n=13), and males (open green squares; n=11). Data were analyzed using linear regression analyses. No relationships were observed between any index of sympathetic nerve traffic and the degree of vasoconstriction (i.e., reduction in leg vascular conductance) during PECO in any group.

## 5.4. Discussion

The present study assessed the impact of biological sex, as well as endogenous and exogenous female sex hormones on the sympathetic neurocirculatory responses to fatiguing small muscle mass exercise. Our data demonstrate that the integrated MSNA responses to fatiguing handgrip exercise and PECO are not affected by biological sex or OCP use. However, when assessing the discharge patterns of sympathetic nerve traffic at the multi-unit AP level, males exhibited larger increases in AP content per MSNA burst, and greater recruitment of latent, high threshold axons at peak exercise compared to females. No group differences were observed in the AP discharge responses to PECO. Notably, when females were partitioned based on OCP use, we found that only naturally menstruating females had smaller increases in AP discharge and recruitment compared to males. Furthermore, males and females using OCP demonstrated greater reductions in LVC compared to naturally menstruating females in the latter half of fatiguing SHG exercise. As a result, sympathetic vascular transduction was greater in males and females using OCPs compared to naturally menstruating females at peak exercise. Conversely, during PECO, the changes in sympathetic nerve traffic were not related to the degree of leg vasoconstriction, suggesting that PECO-related vasoconstriction is not of a neurogenic origin. Altogether, these data indicate that endogenous female sex hormones attenuate sympathetic AP recruitment as well as sympathetic vascular transduction during exercise compared to males, and that the long-term suppression of endogenous female sex hormones due to OCP use may enhance the vasoconstrictor responses to sympathetic neuronal discharge amongst females.

Contrary to previous work (5, 7), we did not find any impact of biological sex on the integrated MSNA responses to fatiguing SHG exercise or PECO. One possibility is that the time to fatigue during SHG in both males and females was considerably shorter in the current study (average: ~2.5 minutes) compared to prior work (average: ~3.5 minutes) (7). Notably, most (11,

12) but not all (5) studies that have employed a two minute SHG exercise at a similar exercise intensity (~30% MVC) reported no sex differences in MSNA. Time to fatigue is inversely related with exercise blood pressure, regardless of biological sex (39). Although males had a larger pressor response than naturally menstruating females, this was driven by larger increases in cardiac output and possibly handgrip strength (12), the latter of which does not explain sex-related differences in MSNA (5). It is unclear whether time to fatigue is related with the MSNA responses to exercise; however, given that sex differences appear to be present after ~3 minutes of SHG exercise (7), it is possible that longer exercise durations are required to observe sex-related differences in sympathetic reactivity. Alternatively, sex-related differences of the increase in MSNA may be observed at lower exercise intensities, during which females appear to be less fatigable than males (40). Indeed, recent work demonstrated that during static knee extension exercise at 10% MVC, males experienced greater increases in deoxy hemoglobin (i.e., greater vascular occlusion); however, at 30% MVC these sex-related differences were not apparent, despite males having a higher MVC (41). Notably, the changes in deoxy hemoglobin were positively related with the exercise-induced increases in blood pressure at 10, but not 30% MVC (41). Furthermore, using $^{31}$P-nuclear magnetic resonance to assess the degree of metabolic perturbation during exercise, Ettinger and colleagues observed no sex-related differences in active muscle pH during 2 minutes of SHG at 30% MVC (5). Given that the cellular pH of active skeletal muscle is tightly coupled with sympathetic outflow (42), these data indicate that at higher exercise intensities (e.g., ~30-40% MVC), males and females may have exceeded their threshold for limiting intramuscular blood flow (i.e., similar muscle ischemia during static contractions), resulting in similar group III/IV afferent neuron stimulation.

Despite integrated MSNA responses not being different between males and females, it is important to note that the integration process of the raw MSNA neurogram conceals the discharge properties and behaviours of heterogenous AP subpopulations (25). Thus, exclusive

assessments of integrated MSNA limit our understanding of the fundamental communication strategies employed by the sympathetic nervous system that serve to maintain circulatory homeostasis. Indeed, at peak exercise, females (pooled OCP and naturally menstruating) demonstrated smaller increases in both AP content per burst and the recruitment of larger AP clusters compared to males. However, when females were partitioned based on OCP use, we found that males only exhibited greater increases in AP discharge and recruitment compared to naturally menstruating females. These sex-related differences in neuronal coding strategies between males and all females may be partly attributed to sex hormones, as we recently demonstrated that bioavailable testosterone is positively associated with the peak increases in AP content per burst during fatiguing incremental rhythmic handgrip exercise in males (9). Thus, the higher levels of bioavailable testosterone in males may augment sympathetic nerve traffic. However, the differences in exercising AP discharge patterns between males and naturally menstruating females may be attributed to the interaction between estradiol and progesterone in females. Amongst naturally menstruating females, Ettinger et al. (5) found larger increases in MSNA during SHG in the preovulatory phase of the menstrual cycle (i.e., elevated estradiol with minimal change in progesterone) compared to the early follicular phase, whereas Jarvis et al. (7) found no difference in MSNA during SHG between the mid-luteal and early-follicular phases of the menstrual cycle. Recently, Coovadia and colleagues demonstrated that, during a hypercapnic-hypoxic apnea, the predominance of progesterone over estradiol in the mid-luteal phase of the menstrual cycle was associated with attenuated sympathetic neural recruitment relative to the early follicular phase (43). Phrased differently, the shift from a high estradiol:progesterone ratio ($E_2/P_4$ ratio) in the early follicular phase to a low $E_2/P_4$ ratio in the mid-luteal phase of the menstrual cycle was associated with attenuated AP recruitment. Given that females were tested in the mid-luteal/active pill phase, our data support the notion that a low $E_2/P_4$ ratio may contribute to attenuated sympathetic neural recruitment in naturally menstruating females.

Contrary to the sex differences in the pressor and sympathetic AP discharge patterns observed between males and naturally menstruating females, no differences were observed between males and females using OCPs. These data align with previous hemodynamic assessments of the EPR in females using OCPs, wherein females using OCPs demonstrated similar increases in blood pressure compared to males (16, 17). The similar pressor and axonal discharge patterns between males and females using OCPs may be attributed to genomic changes associated with long-term suppression of endogenous estradiol, like upregulated renin-angiotensin-aldosterone system activity (44), that may enhance EPR sensitivity (45). However, direct assessments of these mechanistic interactions are warranted to confirm this hypothesis.

To date, only one study has assessed the impact of OCPs on sympathetic reactivity to SHG exercise amongst females, finding that females using OCPs had larger increases in MSNA burst frequency compared to naturally menstruating females (18). The reasons for this discrepancy are unclear; however, it is possible that due to large inter-individual variability in sympathetic reactivity to SHG (46, 47), the larger sample size of naturally menstruating females in the current study (Takeda: n=8 vs. current study: n=18 naturally menstruating females) may have eliminated the group differences previously observed. Notably, we found a weak effect size (Cohen's *d*=0.07) for the change in MSNA burst frequency from baseline to peak exercise between females using OCPs and naturally menstruating females, indicating that there was no meaningful difference between groups. Furthermore, no differences in AP discharge/recruitment were observed between females using OCPs and naturally menstruating females during exercise. The similar AP discharge between the two groups of females in the current study may be driven by the high prevalence of fourth generation OCP use in the current study (~45%) as these OCPs comprise synthetic progestins that exert anti-androgenic effects as well as similar cardiovascular effects as endogenous progesterone (48). However, sub-analysis of females using fourth (anti-androgenic) and second (most androgenic; ~30% of the OCP group) OCPs, indicated that

females using fourth generation OCPs tend to exhibit greater increases in AP content per burst compared to females using second generation OCPs (Fourth gen: 1.9 [1.3] clusters/burst vs. Second gen: 0.4 [1.1] clusters/burst; unpaired two-tailed *t*-test: *P*=0.09, *d*=1.25). Although these data appear to be contradictory to the role of progesterone on sympathetic discharge (43), it is important to note that synthetic progestins are not identical to endogenous progesterone (48). Thus, the interaction between synthetic progestins and ethinyl estradiol may not be akin to the relationship between endogenous estradiol and progesterone. Notably, however, our data align with prior studies that found no differences in sympathetic outflow between females using OCPs and naturally menstruating during various acute sympathoexcitatory stressors (49, 50). The impact of hormonal contraception on physiological outcomes during acute physiological perturbations remains in its infancy, and more work is required to determine the mechanisms underlying their role in regulating efferent sympathetic nerve traffic.

In addition to the central sympathetic discharge responses to exercise, we sought to determine the effects of biological sex and OCP use on the peripheral vasoconstrictor responses to muscle sympathetic AP emission patterns. Males, as well as females using OCPs, had greater reductions in LVC (i.e., more vasoconstriction) during exercise compared to naturally menstruating females in the latter half of exercise. Furthermore, the changes in AP emission patterns at peak exercise were positively associated with the degree of vasoconstriction amongst males and females using OCPs, but not naturally menstruating females. These data indicate that efferent sympathetic impulses may not effectively translate into a vasoconstrictor response amongst naturally menstruating females. The current observations align with previous work using integrated MSNA burst frequency (11), and extend these findings to indicate that the transduction of AP emission patterns into vasoconstriction is also attenuated amongst naturally menstruating females. The lack of relationship between postganglionic AP discharge and limb vasoconstriction, amongst naturally menstruating females may be attributed to estradiol-mediated enhancement of

$\beta_2$-adrenergic vasodilation, which offsets the degree of sympathetic α-adrenergic vasoconstriction (21, 22).

Contrary to naturally menstruating females, the impact of OCP use on vascular transduction remains equivocal. During a cold pressor test, females using OCPs exhibited a paradoxical vasodilation of the forearm vascular bed (51) that was attributed to heightened β-adrenoreceptor mediated vasodilation amongst females using OCPs (52). Conversely, during exercise, the current data, as well as prior work (18) found that females using OCP exhibit greater sympathetic transduction into leg vascular conductance as well as systemic vascular resistance compared to naturally menstruating females, respectively. It is unclear why sympathetic vascular transduction is heightened in females using OCPs during exercise, but attenuated during a cold pressor test; however, differences in the generation of OCPs may explain some of the discrepancy between studies. Specifically, 80% of females in the study by Jacob et al. (51) were using a third generation OCP, whereas nearly ~50% of the females in the current study were using a fourth generation OCP and ~30% were using a second generation OCP. Fourth generation OCPs contain synthetic progestins that are most similar to endogenous progesterone, whereas second generation OCPs contain the most androgenic progestins of all OCPs (48). Although the effects of progesterone on peripheral vascular function are poorly understood, it appears to counteract the vasodilatory effects of estradiol (53, 54). Additionally, androgens heighten vasoconstrictor tone in females (55). Thus, the similar properties of fourth and second generation OCP progestins compared to endogenous progesterone and androgens, respectively, may augment sympathetic vascular transduction to a greater degree than other generations of oral contraception. Nonetheless, future work is warranted to determine the impact of OCP generation on both the central generation of efferent sympathetic nerve traffic as well as the transduction of this sympathetic vasoconstrictor signal.

While the current study supports the concept of sympathetically-mediated vasoconstriction during exercise (11, 56, 57), no relationships were observed between sympathetic nerve traffic and LVC during PECO. Thus, it appears that the vasoconstriction incurred during PECO may not be sympathetically mediated. Indeed, these data align with previous work which found a dissociation between sympathetic outflow and leg vasoconstriction during PECO, and suggested that PECO-related vasoconstriction may be driven by myogenic mechanisms (58, 59). Notably, prior work has found that females demonstrate attenuated myogenically-mediated vasoconstriction compared to males (60), which aligns with the current LVC responses to PECO. Taken together, our data support the hypothesis that vasoconstriction during PECO is likely of myogenic, not neurogenic origin.

**5.5. Experimental considerations**

First, although females using OCPs were all using monophasic OCPs, there was variability in OCP generation. Although the impact of OCP generation on the central generation of efferent sympathetic discharge is unknown, there is growing evidence that OCP generation affects vascular endothelial function (61). Thus, the heterogeneity of OCP generations in the current study may have resulted in greater inter-individual variability in AP discharge and LVC during exercise. Second, the observations from the current study are limited to females using OCPs, not other types of hormonal contraception (i.e., intrauterine devices or hormonal implants). It remains unclear how these other forms of hormonal contraception impact the sympathetic neurocirculatory adjustments to acute physiological perturbations amongst females. Third, strength training status was not controlled for, which can affect the MSNA responses to fatiguing exercise (62). Fourth, finger photoplethysmographic estimates of cardiac output are likely underestimated during SHG exercise (63). Given that TPR was calculated from cardiac output, it is also possible that changes in TPR were underestimated in the current study. Future work with more direct measures of

cardiac output like doppler ultrasound or acetylene rebreathing would circumvent the errors associated with modeled estimates of cardiac output and TPR.

### 5.6. Summary

The current study provides a robust assessment of the impact of biological sex as well as female sex hormones (endogenous and exogenous) on both the central generation of efferent muscle sympathetic AP emission patterns, as well as sympathetic vascular transduction. We found that, despite no group differences in integrated MSNA responses, males demonstrate larger increases in axonal discharge and recruitment compared to females at peak exercise. Furthermore, when females were separated into groups of those using OCPs and naturally menstruating females, we found that males demonstrated larger exercise-induced increases in AP content per MSNA burst as well as greater recruitment of larger, high threshold axons compared to naturally menstruating females only. However, these differences were not apparent during PECO. Additionally, both males and females using OCPs demonstrated greater reductions in LVC (i.e., greater leg vasoconstriction) at peak exercise compared to naturally menstruating females. Thus, males and females using OCPs had greater sympathetic vascular transduction of MSNA, and AP discharge/recruitment compared to naturally menstruating females at peak exercise. Conversely, no relationships were observed between postganglionic c-fiber discharge and leg vasoconstriction during PECO, suggesting that this vasoconstriction may be due to myogenic, not neurogenic mechanisms. Overall, these data indicate that both biological sex and female sex hormones affect the central generation of efferent sympathetic nerve traffic, as well as the peripheral vasomotor responses to these descending sympathetic vasoconstrictor signals during fatiguing exercise, highlighting the importance of considering biological sex as well as female sex hormones in assessments of sympathetic neurocirculatory control during exercise.

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# Chapter 6

## 6 General Discussion

A hallmark characteristic of human aging is an increase in sympathetic nerve traffic directed toward the skeletal muscle vasculature (i.e., MSNA) (1–3). This is thought to be one of the mechanisms contributing to the age-related increase in the risk of developing cardiovascular disease (1, 4–6). However, over the last few decades, there has been growing appreciation that sex disparities exist in these age-related changes. Specifically, females, who at a young age, have lower MSNA (2, 7) and cardiovascular disease risk (9) compared to males, demonstrate larger increases in MSNA (2, 7) and cardiovascular disease risk (8, 9) compared to males throughout the lifespan. The question then remains, what is driving these sex-specific changes in cardiovascular control with aging? One hypothesis is that the reduction in endogenous female sex hormones throughout menopause may explain these differences. Indeed, females demonstrate the most rapid increases in basal MSNA and blood pressure during (and immediately after) menopause compared to any other period of life (2) Furthermore, sex hormones play a pivotal role in the regulation of blood pressure in pre-menopausal females. For example, pre-menopausal females using OCPs – which attenuates the endogenous production of estradiol and progesterone – exhibit increases in resting blood pressure from pre-contraceptive measures (10), and altered vascular function compared to naturally menstruating females (11). However, despite great advances in our understanding of how these inter-individual factors affect the sympathetic neural control of the circulation, little is known about how age, biological sex, and sex hormones affect the central generation of efferent muscle sympathetic AP discharge and the ensuing end-organ vasoconstrictor responses that dictate changes in blood pressure and blood flow at rest and during acute physiological perturbations like exercise. Therefore, the current dissertation is comprised of a series of studies that aimed to advance our understanding of how age, biological sex, and sex hormones affect the behaviour and discharge patterns of sympathetic APs and how these deterministic efferent neural messages affect peripheral vascular tone.

## 6.1 Major findings

A major finding of this dissertation was that the inverse relationship between the arterial baroreflex control of MSNA discharge (i.e., central arc) and sympathetic transduction into blood pressure and leg vascular conductance (i.e., peripheral arc) appeared to be maintained with human aging (Study 1). Amongst young adults, sex-related disparities in this relationship have been observed, with males, but not females, demonstrating an inverse relationship between the central and peripheral baroreflex arcs (12). The absent relationship amongst young females was thought to be driven by estradiol's central sympathoinhibitory (13) and peripheral vasodilatory effects (14, 15). However, we found sex-related disparities in this relationship continue to exist amongst older adults, arguing against a primary role of estradiol in offsetting this relationship (Study 2). Thus, it appears that males, but not females (young and older), exhibit an inverse relationship between the central and peripheral arcs of the arterial baroreflex at rest. Although it remains possible that estradiol attenuates this relationship amongst young females, other factors like attenuated baroreflex control of MSNA discharge, greater central arterial stiffness (6), and/or attenuated endothelial function (16) may impact the relationship between the central and peripheral baroreflex arcs amongst older females. Thus, future work is warranted to determine the mechanisms underpinning the sex-related differences observed in the compensatory interactions between the central and peripheral arcs of the baroreflex.

Beyond the control of MSNA discharge at rest, studies 3 and 4 aimed to address how age, sex, and sex hormones affect the discharge patterns of individual APs that serve elevations in total sympathetic outflow during acute physiological perturbations. Collectively, study 3 provided the first evidence that both age and sex exert independent and interactive effects on the central generation of efferent sympathetic AP emission patterns during fatiguing rhythmic handgrip exercise. As such, several novel findings emerged from this study. First, contrary to prior work (17), older males demonstrated larger increases in blood pressure as well as systemic

vascular resistance compared to young adults and similarly aged females during exercise. However, despite the larger rise in systemic vascular resistance, the increases in sympathetic nerve traffic directed toward the skeletal muscle vasculature was attenuated amongst older males. Notably, the attenuated sympathetic vasomotor reactivity and exaggerated blood pressure responses observed in older males appeared to be associated with lower levels of circulating bioavailable testosterone. Second, we found that older adults (regardless of sex) demonstrated attenuated increases in the ability to reflexively augment integrated MSNA burst amplitude and recruit larger, higher threshold axons. These data align with prior work during apneic stress, which found reduced sympathetic AP recruitment amongst older compared to young adults (18), indicating that there may be a generalized (i.e., non-reflex specific) attenuation of AP recruitment associated with human aging. Third, we found that males, but not females (regardless of age) demonstrated increases in the conduction velocity (i.e., reduced latency) of APs during exercise. These data align with prior work in a female-only cohort, wherein both females with posttraumatic stress disorder and healthy controls did not alter AP conduction velocity during fatiguing SHG exercise (19). Taken together, these data indicate that the ability to acutely modify synaptic delays and/or central processing times is not a neural coding strategy employed by females during exercise. Whether this sex-related difference exists during stimulation of other autonomic reflexes (i.e., baroreflex, chemoreflex) remains to be determined.

Finally, the objective of study 4 was to determine the impact of biological sex as well as endogenous and exogenous female sex hormones on AP emission patterns in young adults. Furthermore, on the basis that MSNA discharge is vasoconstrictive in nature (20), we sought to determine the peripheral vasoconstrictor responses to efferent sympathetic AP discharge during reflex sympathoexcitation (i.e., fatiguing SHG exercise). Several novel findings emerged from the current study. First, although integrated MSNA responses were not different between males and females, sex-related differences in AP emission patterns were observed during SHG exercise,

with males demonstrating larger increases in AP content per burst as well as the recruitment of larger, high threshold APs compared to females. However, when females were separated based on OCP use, the sex-related differences were only observed between males and naturally menstruating females. These data indicate that the long-term suppression of estradiol and progesterone via OCPs may mildly enhance the EPR in females. Additionally, females using OCPs and males demonstrated significantly larger peripheral vasoconstrictor responses during SHG exercise compared to naturally menstruating females. As such, the transduction of efferent muscle sympathetic AP discharge into a peripheral vasoconstrictor response was attenuated in naturally menstruating females compared to both males and females using OCPs. Altogether, these data indicate that the administration of exogenous female sex hormones may augment the vasoconstrictor responses to efferent sympathetic nerve traffic amongst females, rendering their vasomotor behaviour to resemble more closely that of males, rather than naturally menstruating females. Furthermore, this study provides the first evidence linking the patterning and magnitude of efferent muscle sympathetic APs and vasomotor control in humans.

### 6.2 Perspectives and future directions

The current series of studies advances our understanding of the inter-individual factors of age, biological sex, as well as female sex hormones affect the behaviour of sympathetic AP discharge directed toward the skeletal muscle vasculature, as well as the subsequent vasomotor changes in response to these neural patterns. However, these studies generated several opportunities for future work that may offer additional insight into the complex nature of reflex cardiovascular control. For example, the relationships between bioavailable testosterone and the pressor and sympathetic AP responses during rhythmic handgrip exercise in males suggest an important role of testosterone in the homeostatic adjustments during exercise. Taken together with prior studies in sleep deprived young adults (21) and females with polycystic ovarian syndrome (PCOS) (22), it is possible that testosterone exerts sympathoexcitatory actions in

humans. To address the independent role of testosterone, short-term (7-30 day) suppression of circulating sex hormones can be experimentally induced using gonadotropin releasing hormone (GnRH) antagonist treatments, and can be superimposed with the add-back of exogenous testosterone (23). Indeed, this technique has been frequently utilized in females to assess the independent (and interactive) roles of estradiol and progesterone (24–26). This area of research is particularly interesting as males with clinically low testosterone (i.e., male hypogonadism) exhibit a larger risk of developing cardiovascular disease compared to eugonadal males (27) – albeit it is somewhat unclear if this is a causal relationship or due to low testosterone being a biomarker of overall poor health (28). Notably, previous work found that males with heart failure and low testosterone exhibit higher levels of resting MSNA (29), whereas our data suggest that higher levels of testosterone enhance the ability to modify AP discharge and recruitment patterns during acute physiological stressors. Thus, it is possible that lower testosterone may limit the ability to increase sympathetic discharge firing rates and recruitment due to a ceiling effect from elevated baseline levels. This, however, remains to be determined. Additionally, males with low testosterone demonstrate attenuated vascular endothelial function (30), and greater arterial stiffness compared to males with normal (age-specific) levels of testosterone (31). However, whether testosterone affects the vasoconstrictor responses to efferent sympathetic nerve traffic (I.e., sympathetic vascular transduction) in males remains unknown. As briefly mentioned earlier, females with PCOS often have elevated testosterone levels and exhibit both vascular dysfunction (32–34) and heightened MSNA outflow (22). Thus, contrary to males, it seems that a hyperandrogenic environment may be pathophysiological in females. As such, the study of testosterone and its impact on the central generation of sympathetic outflow as well as peripheral vasoconstrictor responsiveness remain an important area of future research that could provide important basic scientific and clinical information for males and females.

Additionally, our data from study 4 support recent work that the interaction between estradiol and progesterone in naturally menstruating females may affect sympathetic neural recruitment (35). However, using a GnRH antagonist and hormone add-back model would provide more precise control of sex hormones and likely deeper insight into the independent and interactive roles of estradiol and progesterone on muscle sympathetic AP emission patterns. Furthermore, athough our study assessed the role of oral hormonal contraception on sympathetic outflow and vasoconstrictor responsiveness, it remains unclear how other forms of hormonal contraception (e.g., intrauterine device, hormonal implant) impact the sympathetic nervous system and peripheral vasculature. Given that these alternate forms of contraception are being prescribed more often (36), understanding their impact on human physiological function as it pertains to blood pressure regulation remains an important area of future work in the domain of women's cardiovascular health.

### 6.3 Conclusion

Collectively, the studies that comprise this dissertation clearly highlight the impact of age, biological sex, as well as sex hormones on the regulation of efferent muscle sympathetic AP discharge and recruitment patterns, as well as the peripheral vasoconstrictor responses to these sympathetic impulses. Specifically, we found that middle-aged-to-older adults exhibit greater baroreflex control of medium-sized APs that fire within most integrated bursts of MSNA, and that this higher sympathetic baroreflex control of MSNA discharge may be compensatory for attenuated vascular transduction of these efferent sympathetic vasoconstrictor signals (Study 1). However, when assessing the effects of age and sex on the interrelationship between the baroreflex control of MSNA and sympathetic vascular transduction, we found that males (young and older), but not females demonstrate inverse relationships between the central and peripheral baroreflex arcs (Study 2). Together, these data indicate that both age and sex affect both the regulation of efferent sympathetic nerve traffic and the homeostatic circulatory adjustments in

response to these coded neural messages under resting conditions. Furthermore, we sought to determine how age and biological sex affect the sympathetic AP recruitment strategies that serve to increase total sympathetic outflow during acute physiological perturbations. In doing so, we found that age-related impairments in sympathetic neural recruitment were most apparent amongst males during fatiguing rhythmic handgrip exercise, and that these attenuated sympathetic discharge responses were associated with circulating levels of bioavailable testosterone. Conversely, estradiol did not dictate sympathetic neural recruitment during rhythmic handgrip exercise in females (Study 3). Finally, we found that, amongst young adults, biological sex affects the central generation of efferent sympathetic nerve traffic during static, small muscle mass exercise, with young males demonstrating larger increases in AP discharge and recruitment compared to young females. However, synthetic hormones in OCPs did not have any effect on AP emission patterns during SHG exercise. Conversely, the vasoconstrictor responses to these efferent sympathetic impulses are greater in males and females using OCPs compared to naturally menstruating females (Study 4). Collectively, these data highlight the important role of endogenous and exogenous sex hormones on both the magnitude and patterning of muscle sympathetic AP discharge employed during acute physiological stress, as well as the peripheral vasomotor responses to these efferent vasoconstrictor signals. These data provide novel and important advancements towards understanding the impact of the SNS on age-related increase in the incidence of hypertension and cardiovascular disease (1,8), as well as the higher levels of blood pressure seen in women using OCPs (10). The current series of studies have shone a light on how much is unknown in the field of reflex cardiovascular control, particularly among women. Indeed, this remains an area of research that waits to be spearheaded with great enthusiasm and rigor – proving an exciting time to be an integrative physiologist.

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# Appendix A: Ethical approval

Western Research

**Date:** 2 February 2022

**To:** Dr. Kevin Shoemaker

**Project ID:** 119380

**Study Title:** The effects of Sex and Sex hormones on Autonomic and Vascular function during Exercise

**Reference Number/ID:** N/A

**Application Type:** HSREB Amendment Form

**Review Type:** Delegated

**Full Board Reporting Date:** 22/February/2022

**Date Approval Issued:** 02/Feb/2022

**REB Approval Expiry Date:** 06/Aug/2022

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Dear Dr. Kevin Shoemaker ,

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

**Documents Approved:**

| Document Name | Document Type | Document Date |
|---|---|---|
| 220123_SSAVE_LOI_Amend | Consent Form | 23/Jan/2022 |
| 220131_SSAVE_protocol_Changes | Protocol | 31/Jan/2022 |

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Karen Gopaul , Ethics Officer on behalf of Dr. Philip Jones, HSREB Chair

***Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).***

Western Research

**Date:** 7 October 2022

**To:** Dr. Kevin Shoemaker

**Project ID:** 119380

**Review Reference:** 2022-119380-71798

**Study Title:** The effects of Sex and Sex hormones on Autonomic and Vascular function during Exercise

**Application Type:** HSREB Amendment Form

**Review Type:** Delegated

**Full Board Reporting Date:** 25/Oct/2022

**Date Approval Issued:** 07/Oct/2022 11:13

**REB Approval Expiry Date:** 06/Aug/2023

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Dear Dr. Kevin Shoemaker ,

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

**Documents Approved:**

| Document Name | Document Type | Document Date |
|---|---|---|
| 220915_SSAVE_protocol_Changes | Protocol | 26/Sep/2022 |
| 221005_SSAVE_LOI_Amend | Consent Form | 05/Oct/2022 |

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

**Electronically signed by:**

Karen Gopaul , Ethics Officer on behalf of Dr. Philip Jones, HSREB Chair, 07/Oct/2022 11:13

**Reason:** I am approving this document.

***Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).***

UT Southwestern Medical Center — eIRB System

Date: Thursday, June 16, 2022

To: Qi Fu, Belinda Sanchez,

CC:

From: Ahamed Idris

Chair, IRB 4

Protocol Number: STU-2022-0433

Title: The effect of Sex and Sex hormones on Autonomic and Vascular function during Exercise

Funding: Internal - Departmental

| Agency | Grant Number |
|---|---|
| There are no items to display | |

Review: New Study Review - ***Activated***

Review Type: Full Board

Documents: FormB-Personnel.docm, FormD-DSMP.docm, FormJ-Collection-Questionnaire.docm, FormJ-Collection-Screening.docm, FormE-Consent-Main.docm, FormI-Recruitment-Email.docm, FormI-Recruitment-PhoneScript.docm, FormA5-StudyTable.xlsm, FormA-ResearchProtocol.docm, FormA5-ClinCard-Waiver.pdf, FormC-Population.docm, FormG3-WaiveAlterConsentRecruit.docm, FormH-HIPAAWaiver-Partial.pdf, FormH-HIPAAWaiver-Partial.docm

Dear Principal Investigator,

Your New study was reviewed and APPROVED by the IRB on Wednesday, June 8, 2022

The IRB reviewed your submission and determined there is Minimal risk to subjects who will participate in this study. The IRB made determinations for approval for this study which may be found in eIRB in the Determinations tab.

As of , the study met all the required approvals and may begin at the performance sites below with a status of "Approved," **For those performance sites listed as "Pending," you may not begin research activities until approval has been issued.**

| Approval Component | Component Name | Status |
|---|---|---|
| Performance Site | UTSW | Not Applicable |
| Performance Site | Children's | Not Applicable |
| Performance Site | Parkland | Not Applicable |
| Performance Site | THR | Not Applicable |

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| Coverage Analysis | Coverage Analysis | Pending |
| Contract | Clinical Trial Agreement | Pending |

If the study is sponsored, sponsor activation may be required before study activities may begin.

The expiration date for the current approval is: Wednesday, June 7, 2023

*Please note, to continue your study, your Continuing Review/Annual Update submission must be received **30 days prior** to the expiration date.*



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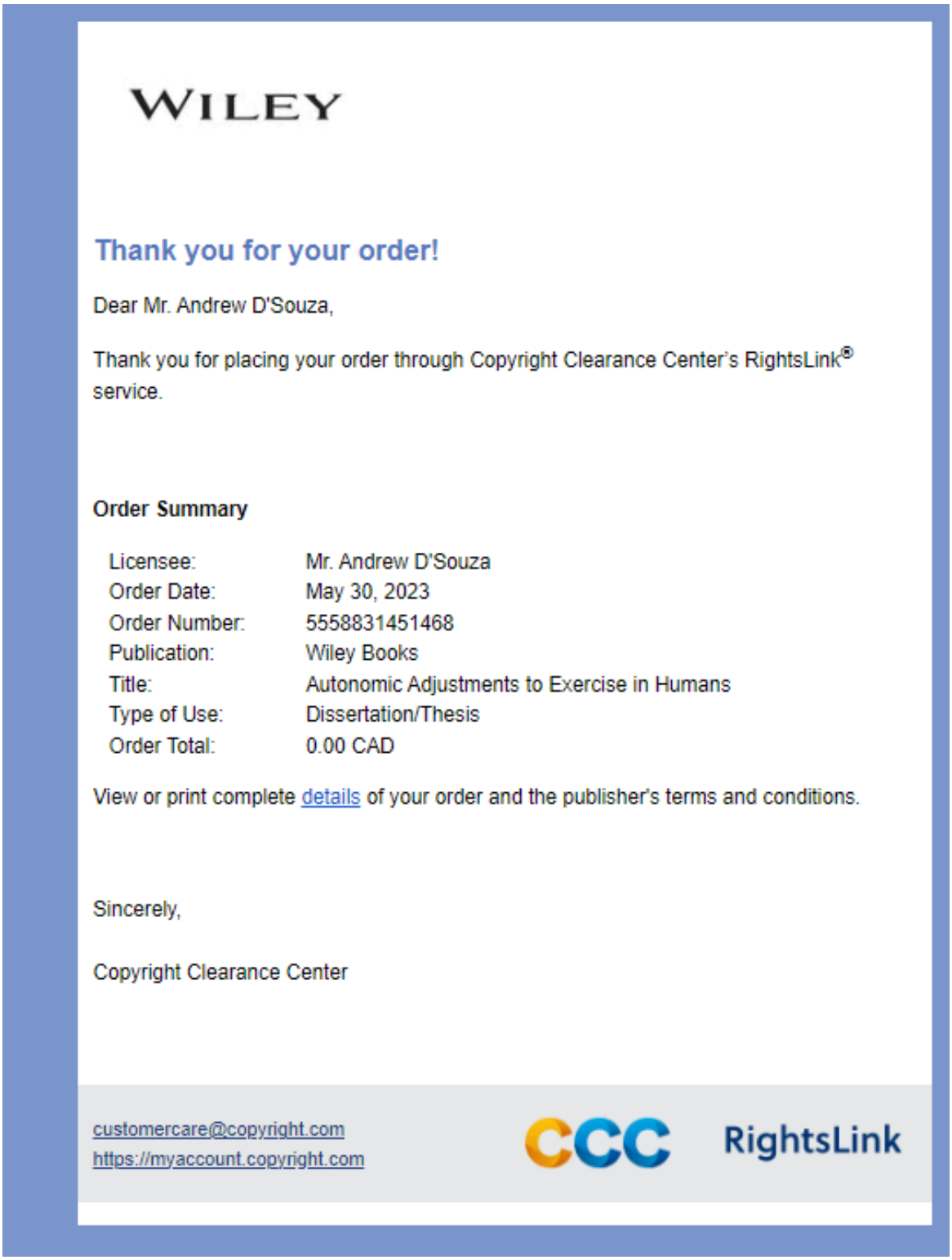
# Appendix B: Journal permissions



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# Curriculum Vitae

**Andrew W. D'Souza, PhD(c)**
**BSc (Hons.), MSc.**

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Bilingual: English and
French Citizenship:
Canadian

## CERTIFICATIONS AND MEMBERSHIPS

- Catheter Insertion and Venous Puncture Certification, University of Ottawa – June 2018
- Esophageal Probe Insertion Certification – July 2017
- Standard First Aid CPR C +AED – July 2020

## EDUCATION

2020 to 2023 – **Doctor of Philosophy in Integrative Biosciences**, Western University, London, ON. ***Thesis title:*** *The sympathetic neural control of the circulation at rest and during exercise: effects of age, biological sex, and sex hormones*

2017 to 2019– **Master of Science in Human Kinetics**, University of Ottawa, Ottawa, ON.

***Thesis title:*** *The interactive effects of sex and age on whole-body heat loss*

2012 to 2017- **Honours Bachelor of Science in Human Kinetics,** University of Ottawa, Ottawa, ON.

***Honours project title:*** *A Case Report: The Physiological Strain Incurred by Electrical Utility Workers During Consecutive Work Days*

## TRAINEE SCHOLARSHIPS/FELLOWSHIPS (total of $338,500)

2023-2025 – **NSERC Postdoctoral Fellowship award -** Natural Sciences and Engineering Research Council of Canada. Value of the award: $90,000

2023 – **Ontario Graduate Scholarship – Doctoral program**. Value of the award: $15,000

2022 – **Michael Smith Foreign Study Supplement –** Natural Sciences and Engineering Research Council of Canada. Value of Award: $6,000

2020 – **Ontario Graduate Scholarship – Doctoral program**. Value of the award: $15,000 (Declined)

2020-2023 – **Alexander Graham Bell Canada Graduate Scholarship – Doctoral (CGS-D) -** Natural Sciences and Engineering Research Council of Canada. Value of the award: $150,000

2018 – **Canada Graduate Scholarships - CGS-Master's -** Natural Sciences and Engineering Research Council. Value of the award: $17500

2018 – **University of Ottawa Excellence Scholarship**, Faculty of Health Sciences, School of Human Kinetics, University of Ottawa, Canada**.** Value of the award: up to $15000

2017 – **Queen Elizabeth II Graduate Scholarship in Science and Technology**, Carré Technologies Inc – Hexoskin, Montreal, Quebec, Canada**.** Value of the award: $15000

2017 – **University of Ottawa Excellence Scholarship**, Faculty of Health Sciences, School of Human Kinetics, University of Ottawa, Canada**.** Value of the award: up to $15000

## HONOURS AND AWARDS

2023 - **Lawson Health Lucille & Norton Wolf London Health Research Day Trainee Publication Award.** Western University. Value of the award: $1000

2023 – **School of Kinesiology Graduate Travel Award –** Western University. Value of the award: $500

2023 – **American Physiological Society Neural Control and Autonomic Regulation (NCAR) Research Recognition Award.** Value of the award: $500

2019 – **University of Ottawa Masters thesis Prize Nominee**, University of Ottawa, Canada**.**

2016 **– University of Ottawa Merit Scholarship,** Faculty of Health Sciences, School of Human Kinetics, University of Ottawa, Canada**.** Value of the award: $1000.

2016 - **Human and Environmental Physiology Research Unit Student Bursary,** Faculty of Health Sciences, School of Human Kinetics, University of Ottawa, Canada. Value of the award: 4000$.

2016- **Academic All-Canadian,** University of Ottawa, Canada. Value of award: $0

2015- **Ottawa Sports Award – Badminton Athlete of the Year,** Ottawa, Canada. Value of award: $0

2014- **Ottawa Sports Award – Badminton Athlete of the Year,** Ottawa, Canada. Value of award: $0

2012- **Ottawa Sports Award – Badminton Athlete of the Year,** Ottawa, Canada. Value of award: $0

2012 - **Undergraduate Admission Bursary,** Faculty of Health Sciences, School of Human Kinetics, University of Ottawa, Canada**.** Value of the award: $1000.

2010- **Ottawa Sports Award – Badminton Athlete of the Year,** Ottawa, Canada. Value of award: $0

## PEER-REVIEWED PUBLICATIONS (published, accepted, under review)

1. **D'Souza AW,** Hissen SL, Manabe K, Washio T, Sanchez B, Annis MC, Fu Q, Shoemaker JK. The effect of biological sex and oral contraception on the sympathetic neurocirculatory adjustments to static handgrip exercise in humans. *Under review at The American Journal of Physiology: Regulatory, integrative and comparative physiology*.

2. **D'Souza AW,** Hissen SL, Manabe K, Takeda R, Washio T, Coombs GB, Sanchez B, Fu Q, Shoemaker JK. Age- and sex-specific changes in sympathetic vascular transduction and neuro-hemodynamic balance in humans. *Under review at The American Journal of Physiology – Heart and Circulatory Physiology* (ID: H-00301-2023)

3. **D'Souza AW,** Yoo JK, Bhai S, Sarma S, Levine BD, Fu Q. Attenuated peripheral oxygen extraction and greater cardiac output in women with posttraumatic stress disorder during exercise. *Under review at the Journal of Applied Physiology* (ID: JAPPL-00161-2023)

4. **D'Souza AW,** Takeda R, Manabe K, Hissen SL, Washio T, Coombs GB, Sanchez B, Fu Q, Shoemaker JK. The interactive effects of age and sex on the neuro-cardiovascular responses during fatiguing rhythmic handgrip exercise. *The Journal of Physiology (*Online ahead of print: PMID: 37083007).

5. Manabe K, **D'Souza AW**, Washio T, Takeda R, Hissen SL, Akins JD, Fu Q. Sympathetic and hemodynamic responses to exercise in heart failure with preserved ejection fraction. *Frontiers in Cardiovascular Medicine 2023 Apr 17;10:1148324.*

6. Washio T, Hissen SL, Takeda R, Manabe K, Akins JD, Sanchez B, **D'Souza AW**, Nelson DB, Khan S, Tomlinson AR, Babb TG, Fu Q. Effects of posture changes on dynamic cerebral autoregulation during early pregnancy in women with normal-weight and obesity. *Clinical Autonomic Research. 2023 Apr;33(2):121-131.*

7. Badrov MB**,** Yoo JK, Hissen SL, **D'Souza AW,** Nelson DB, Shoemaker JK, Fu Q. Muscle Sympathetic Action Potential Firing Patterns During Normotensive and

Hypertensive Pregnancy: A Longitudinal Assessment. *Circulation (ID: CIRCULATIONAHA/2022/062192R1)*

8. **D'Souza AW,** Hissen SL, Okada Y, Jarvis SS, Washio T, Akins JD, Nelson DB, Fu Q. Differential regulation of sympathetic neural burst frequency and amplitude throughout normal pregnancy: a longitudinal study. *The American Journal of Physiology: Regulatory, integrative and comparative physiology*

9. **D'Souza AW**, Yoo JK, Takeda R, Manabe K, Badrov MB, Parker RS, Anderson EH, Wiblin JI, North CS, Suris A, Shoemaker JK, Fu Q. Neuro-cardiovascular dysregulation during orthostasis in women with post-traumatic stress disorder. *Circulation. Nov 8;146(19):1483-1485. doi: 10.1161/CIRCULATIONAHA.122.061705.*

10. **D'Souza AW**, Klassen SA, Badrov MB, Lalande S, Shoemaker JK. The spontaneous baroreflex control of sympathetic action potential subpopulations is enhanced with human aging. *J Appl Physiol (1985). 2022 Jun 23. doi: 10.1152/japplphysiol.00045.2022.*

11. **D'Souza AW**, Yoo JK, Takeda R, Manabe K, Badrov MB, Parker RS, Anderson EH, Wiblin JI, North CS, Suris A, Shoemaker JK, Fu Q. Impaired sympathetic neural recruitment during skeletal muscle metaboreflex activation in women with posttraumatic stress disorder. *Clin Auton Res. 2022 Feb 28. doi: 10.1007/s10286-022-00858-1.*

12. **D'Souza AW**, Badrov MB, Wood KN, Lalande S, Suskin N, Shoemaker JK. The impact of 6 months of exercise-based cardiac rehabilitation on sympathetic neural recruitment during apneic stress. Am J Physiol Regul Integr Comp Physiol. 2021 Aug 1;321(2):R176-R185. doi: 10.1152/ajpregu.00080.2021. PMID: 34133229

13. Notley SR, **D'Souza AW**, Meade RD, Richards BJ, Kenny GP. Whole-body heat exchange in women during constant- and variable-intensity work in the heat. Eur J Appl Physiol. 2020 Dec; 120(12):2665- 2675. doi: 10.1007/s00421-020-04486-3. Epub 2020 Sep 9. PMID: 32902693

14. Notley SR, Meade RD, **D'Souza AW**, Rutherford MM, Kim JH, Kenny GP. Heat exchange in young and older men during constant- and variable-intensity work. Med Sci Sports Exerc. 2020 May 18. PMID: 32433432.
15. **D'Souza AW**, Notley SR, Kenny GP. The relation between age and sex on whole-body heat loss during exercise-heat stress. Med Sci Sports Exerc. 2020 Apr 20. doi: 10.1249/MSS.0000000000002373. PMID: 32496737

16. Muia CM, Notley SR, Saci S, **D'Souza AW**, and Kenny GP. Whole-body heat exchange in black-African and Caucasian men during exercise eliciting matched heat-loss requirements in dry heat. *Experimental Physiology* (*IF=2.91).* 2020 Jan;105(1):7-12. doi: 10.1113/EP088091. PMID: 31628699

17. Notley SR, Poirier MP, Sigal RJ, **D'Souza A**, Flouris AD, Fujii N, Kenny GP. Exercise Heat Stress in Patients With and Without Type 2 Diabetes. *JAMA* (*IF=51.3),* DOI: 0.1001/jama.2019.10943. PMID: 31593261.

18. **D'Souza AW**, Notley SR, Meade RD, Kenny GP. Intermittent sequential pneumatic compression does not enhance whole-body heat loss in elderly adults during extreme heat

exposure. *Appl Physiol Nutr Metab* (*IF=3.50),* 2019 Dec;44(12):1383-1386. doi: 10.1139/apnm-2019-0364. Epub. PMID: 31398291

19. Meade RD, Notley SR, **D'Souza AW**, Dervis S, Boulay P, Sigal RJ, Kenny GP. Interactive effects of age and hydration state on human thermoregulatory function during exercise in hot-dry conditions. *Acta Physiologica (IF=5.97),* doi: 10.1111/apha.13226. PMID: 30480873.

20. **D'Souza AW**, Notley SR, Brown EK, Poirier MP, Kenny GP. The Hexoskin® wearable body metrics vest does not impair whole-body heat loss during exercise in a hot, dry environment. *Appl Physiol Nutr Metab* (*IF=3.50),* DOI: 10.1139/apnm-2018-0370. [Epub ahead of print]. PMID:30336069.

21. Notley SR, Meade RD, **D'Souza AW**, McGarr GW, Kenny GP. Cumulative effects of successive workdays in the heat on thermoregulatory function in the aging worker. *Temperature,* DOI: 10.1080/23328940.2018.1512830.

22. Notley SR, Meade RD, **D'Souza AW**, Friesen BJ, Kenny GP. Heat loss is impaired in older men on the day following prolonged work in the heat. *Med Sci Sports Exerc.* (*IF=4.459), 50(9):1859-1867.* PMID:29683926

23. Friesen BJ, Poirier MP, Lamarche DT, **D'Souza AW**, Kim JH, Notely SR, Kenny GP. Postexercise whole-body sweating increases during muscle metaboreceptor activation. *Appl Physiol Nutr Metab.* (*IF=3.50), 43(4):423-426.* PMID: 29316406

24. Notley SR, Meade RD, Friesen BJ, **D'Souza AW**, Kenny GP. Does a Prolonged Work Day in the Heat Impair Heat Loss on the Next Day in Young Men? *Med Sci Sports Exerc*. (*IF=4.459),* 50(2):318-326. PMID:28991046

25. Meade RD, **D'Souza AW**, Krishen, L, Kenny GP. A Case Report: The Physiological Strain Incurred by Electrical Utility Workers During Consecutive Work Days. *J Occup Environ Hyg*. (*IF*=1.200), 14(12): 986-994. PMID:28825865

26. Lamarche DT, Meade RD, **D'Souza AW**, Flouris AD, Sigal RJ, Boulay P, Kenny GP. The Threshold Limit Values Fail to Maintain Body Core Temperature Within Safe Limits in Older Adults During Work in the Heat. *J Occup Environ Hyg*. (*IF*=1.200), 14(9): 703-711. PMID:28609164.

## PUBLISHED (OR SUBMITTED) CONFERENCE ABSTRACTS

1. **D'Souza AW**, Takeda R, Manabe K, Hissen SL, Coombs GB, Washio T, Sanchez B, Fu Q, Shoemaker JK. Sex-specific impact of aging on muscle sympathetic neural discharge patterns during incremental rhythmic handgrip exercise. *American Physiological Summit 2023 – **Oral and Poster presentation***
2. **D'Souza AW**, Manabe K, Hissen SL, Coombs GB, Washio T, Annis MC, Sanchez B, Fu Q, Shoemaker JK. Sex differences in sympathetic neurovascular transduction of action potential discharge during fatiguing handgrip exercise in young adults. *American Physiological Summit 2023 – **Poster presentation***

**3.** Washio T., Takeda R, Hissen SL, Akins JD, **D'Souza AW,** Manabe K, Hearon CM jr., MacNamara J, Sarma S, Levine BD, Fadel PJ, Fu Q. Normal sympathetic neural but blunted cardiovascular responses during static handgrip exercise in heart failure with preserved ejection fraction. *American Physiological Summit 2023* – ***Poster presentation***

**4.** Annis MC, **D'Souza AW**, Coombs GB, Manabe K, Sanchez B, Fu Q, Shoemaker JK. Brachial artery flow-mediated dilation during incremental handgrip exercise is not impacted by sex or female sex hormones. *American Physiological Summit 2023* – ***Poster presentation***

**5.** **D'Souza AW**, Yoo JK, Takeda R, Manabe K, Badrov MB, Parker RS, Anderson EH, Wiblin JI, North CS, Suris A, Shoemaker JK, Fu Q. Impaired sympathetic neural recruitment during skeletal muscle metaboreflex activation in women with posttraumatic stress disorder. ***ORAL AND POSTER PRESENTATION –*** *Experimental Biology 2022.* The FASEB Journal 36(1)R2284.

6. **D'Souza AW**, Klassen SA, Badrov MB, Lalande S, Shoemaker JK. Aging Resets the Spontaneous Baroreflex Control of Sympathetic Action Potential Subpopulations in Humans. Experimental Biology 2021. The FASEB Journal. 35(1). *Virtual Conference* – ***Poster Presentation***

**7.** Notley SR*, Akerman AP, **D'Souza AW**, McCourt E, Kenny GP. (2020). Dose-dependent effects of lower-limb ischemia on whole-body heat loss during exercise-heat stress: preliminary observations. Physiology and Pharmacology of Temperature Regulation. ***Poster presentation. International.*** (MSc. Work).

8. **D'Souza AW**, Notley SR, Meade RD, Rutherford MM, Kenny GP. The Influence of Ingestion Time on the Validity of Gastrointestinal Pill Temperature as an Index of Body Core Temperature During Work in the Heat. Experimental Biology 2019. The FASEB Journal 33(1) 842.7. Orlando, Florida, USA April 21st to 25th 2019.– ***Poster Presentation.***

9. **D'Souza AW*,** Notley SR, Muia CM, Kenny GP. Preliminary evidence of sex-related differences in the effect of aging on whole-body heat loss during exercise in dry-heat. International Conference of Environmental Ergonomics. 2019. ***Oral presentation. International***. (MSc work).

10. Poirier MP*, Notley SR, **D'Souza AW**, Sigal RJ, Boulay P., Malcom J., Kenny G.P., Differential effects of short-term heat acclimation on whole-body heat loss in physically active older males with and without type 2 diabetes. International Conference of Environmental Ergonomics. 2019. ***Oral presentation. International***. (MSc work).

**11.** Meade RD, Notley SR, **D'Souza AW**, Rutherford MM, Kenny GP. On the effects of constant and variable work of equivalent average intensity on whole-body heat exchange. Experimental Biology 2019. The FASEB Journal 33(1) 842.4. Orlando, Florida, USA April 21st to 25th 2019.– ***Poster Presentation***

12. **D'Souza AW**, Notley SR, Poirier MP, Brown, EK, Kenny GP. The Influence of the Hexoskin® Wearable Body Metrics Vest on Whole-body Heat Loss During Exercise in the Heat. 2018: Health in Motion, Science in Exercise. Applied Physiology Nutrition and Metabolism. 43(10):S53. Niagara Falls, Ontario, Canada, October 31st -November 3rd,

2018 – ***Poster Presentation***

13. Poirier MP, **D'Souza AW**, Notley SR, Kenny GP. Heat Acclimation Enhances Whole-Body Heat Dissipation in Older Males with Type 2 Diabetes. Abstract submitted to CSEP 2018: Health in Motion, Science in Exercise. Applied Physiology Nutrition and Metabolism. 43(10):S88. Niagara Falls, Ontario, Canada, October 31st -November 3rd, 2018 – ***Poster Presentation***

14. Meade RD, Dervis S, **D'Souza AW**, Notley SR, Kenny GP. Regional Differences in the Influence of Hypohydration on Sweat Rate. Abstract submitted to CSEP 2018: Health in Motion, Science in Exercise. Applied Physiology Nutrition and Metabolism. 43(10):S79. Niagara Falls, Ontario, Canada, October 31st -November 3rd, 2018 – ***Poster Presentation***

**15. D'Souza AW**, Notley SR, Meade RD, Kenny GP. Do Graduated Compression Garments Enhance Whole-body Heat Loss During an Extreme Heat Exposure in Older Adults? Experimental Biology 2018. The FASEB Journal 32(1) 590.22. San Diego, California, USA April 21st to 25th 2018.– ***Poster Presentation***

16. Meade RD, Dervis S, **D'Souza AW**, Notley SR, Boulay P, Sigal RJ, Kenny GP. Age-related Impairments in Whole-body Evaporative Heat Loss During Exercise in the Heat are not Exacerbated by Hypohydration. Experimental Biology 2018. The FASEB Journal 32(1) 859.3. San Diego, California, USA April 21st to 25th 2018.– ***Poster Presentation***

17. Notley SR, Meade RD, Friesen BJ, **D'Souza AW**, Kenny GP. Does a Prolonged Work Day in the Heat Impair Heat Loss on the Next Day in Young Men? The American College of Sports Medicine's 65th

    Annual Meeting. *Med Sci Sports Exerc* 49(5): S517. Minneapolis, Minnesota, USA May 29th to June 2st 2018.– ***Poster Presentation***

18. Kenny GP, Lamarche DT, Meade RD, **D'Souza AW**. The Threshold Limit Values Fail to Maintain Body Core Temperature Within Safe Limits in Older Adults During Work in the Heat. *Med Sci Sports Exerc* 49:107-108. American College of Sports Medicine's 64th Annual Meeting held in Denver, Colorado, USA May 30th to June 1st 2017. – ***Poster Presentation***

19. Friesen BJ, Poirier MP**,** Lamarche DT, **D'Souza AW**, Kim JH, and Kenny GP. Postexercise Activation of Muscle Metaboreceptors Modulates Whole-Body Evaporative Heat Loss. *Med Sci Sports Exerc* 49:449. American College of Sports Medicine's 64th Annual Meeting held in Denver, Colorado, USA May 30th to June 1st 2017. – ***Poster Presentation***

20. **D'Souza AW**, Meade RD, Krishen L, Poirier MP, and Kenny GP. A case report: The physiological strain incurred by electrical utility workers during consecutive work days. *Med Sci Sports Exerc* 49:106-107. American College of Sports Medicine's 64th Annual Meeting held in Denver, Colorado, USA May 30th to June 1st 2017. – ***Poster Presentation***

## MENTORSHIP

1. Meghan Annis, Undergraduate thesis project. *The effects of sex and sympathetic activation on passive leg movement-induced vasodilation*.

## JOURNAL REVIEW ACTIVITIES

2021-PRESENT **Reviewer,** American Journal of Physiology Heart and Circulatory Physiology: completed reviews: 5
2021-PRESENT **Reviewer,** American Journal of Physiology regulatory, integrative and comparative physiology: completed reviews: 2
2021-PRESENT **Reviewer,** Journal of Applied Physiology: completed reviews: 6
2022-PRESENT **Reviewer,** Clinical Autonomic Research: completed reviews: 4

## WORK EXPERIENCE

2022-2023 **Teaching Assistant**

Western University, London ON

- *Correcting lab reports*
- *Providing constructive feedback to students via lab reports, and in lab presentations*
- *Teaching undergraduate students the scientific principles underlying the fundamental techniques and methods used in exercise physiology*
- *Teaching students how to conduct functional fitness assessments as per Canadian Society for Exercise Physiology (CSEP) guidelines.*
- *Assigned course(s):KINESIOL 2230, KINESIOL 3345*

2020 –2022 **Varsity Head Coach – Badminton**

Western University. London ON.

- *Organizing practices for varsity athletes (drills, matches, fitness)*
- *Organizing tournament schedules for the varsity athletic year.*
- *Teaching the fundamentals, techniques and strategies for singles, doubles and mixed doubles.*
- *Organizing team expenses and budgeting (holding fundraisers, financing attire, tournament costs, training costs).*
- *Working closely with Western University's athletic department to handle team logistics.*

Sept-Dec 2019 **Research Associate**

University of Ottawa, Ottawa ON

- *Assisting with data collection, analysis and report writing for Federal and provincial grant projects*
- *Writing and reviewing peer-reviewed journal articles*
- *Assisting masters students with Tri-council scholarship applications*
- *Mentoring undergraduate and Master's students on thermal physiology principles and techniques*
- *Working with a team of postdoctoral and doctoral students to design projects in human physiology including the effects of aging, sex, fitness, chronic disease and hydration on performance in extreme environments*
- *Organizing bi-monthly screening tests for ~40-60 adults between the ages of 60- 80 years.*
- *Coordinating a team of graduate students to recruit volunteers (e.g., organizing locations to speak about our work)*
- *Recruitment of study volunteers (i.e. lay presentations about our work to various groups ranging from 10-50 people)*

Jan-April 2019 **Teaching Assistant**

University of Ottawa, Ottawa ON

- *Correcting lab reports*
- *Providing constructive feedback to students via lab reports, and in lab presentations*
- *Teaching undergraduate students the scientific principles underlying the fundamental techniques and methods used in exercise physiology.*
- *Assigned course(s): APA 2314- Laboratory Techniques in Exercise physiology and Biomechanics*

Sept-Dec 2018 **Corrector**

University of Ottawa, Ottawa ON

- *Correcting exams, assignments and providing feedback to students*
- *Proctoring final exam*
- *Assigned course(s):APA 4313 – Exercise and Disease Prevention*

2008-2019 **Badminton Coach**

RA Centre, Ottawa ON

- *Teaching youth basic badminton techniques.*
- *Aid in physical testing and fitness assessments twice a year for all U19 national and provincial athletes.*
- *Teaching national and provincial level athletes techniques and strategies for singles, doubles and mixed doubles.*
- *Private and semi-private technical training sessions for national and provincial level athletes*

2008-2015 **Camp Counselor- Badminton Camp**

RA Centre, Ottawa ON

- *Responsible for 30+ kids, ages 8-12.*
- *Engaged campers in various activities (badminton, bowling, swimming).*
- *Instructed children on basic badminton techniques.*
- *Coordination with counselors from other camps to ensure safety of all the kids in the camps.*

## VOLUNTEER EXPERIENCE/COMMUNITY SERVICE

Sept 2022 – April 2023 **Undergraduate course guest lecturer**

- *Invited guest lecturer for the following courses and topics:*
- *Kinesiol 2230 (Western University) – Venous return and the Pulmonary Circulation*
- *Kinesiol 2230 (Western University) – The arterial circulation*
- *KIN 3W03 (McMaster University) – Sympathetic neural control of the circulation.*

June 2022 - Current **Topic coordinator and co-editor (Autonomic regulation of the cardiovascular system during exercise)**, *Frontiers in Physiology.*

- *Create a research topic and recruit expert co-editors*
- *Determine manuscript quality/significance, and send manuscripts for review to other experts in the field.*

October 2021 –2022 **Vice-president Social**, Exercise is Medicine Canada – Western University Branch, Western University, London, ON.

- *Responsible for organizing social events that encourage the Western University community to engage in regular physical activity*
- *Responsible for organizing a conference for members of the Western University community that focuses on the benefits of exercise, health and well-being, and methods of how to get involved in exercise*
- *Class presentations regarding upcoming events and opportunities*

August 2021 - Current **Junior reviewer**, American Journal of Physiology – Heart and Circulatory Physiology.

- *Conduct timely peer-reviews for manuscripts sent for publications in the field of cardiovascular physiology and autonomic neuroscience.*

July 2020- Current **Figure reviewer**, American Journal of Physiology – Heart and Circulatory Physiology.

- *Conduct timely peer-reviews for manuscripts sent for publications in the field of cardiovascular physiology and autonomic neuroscience.*
- *Reviewing figures to ensure that they abide by Journal policies*.

Sept 2017-2018 **General Council Member**, University of Ottawa, Ottawa, ON. Human Kinetics Graduate Student Association

- *Organizing fundraising events and an academic conference for graduate students and faculty*
- *Class presentations regarding upcoming graduate student events and opportunities*

Jan-May 2016 **Research Assistant -Participant**, University of Ottawa, Ottawa, ON. The Human and Environmental Physiology Research Unit

- *Assisted and participated with various projects in human physiology including the effects of aging, sex, fitness, and hydration on performance in extreme environments.*

Summer 2014 **Research Assistant-Participant**, The Ottawa Hospital Research Institute, Ottawa, ON. Institute for Rehabilitation Research and Development, Rehabilitation Technology Lab

- *Assisted in the analysis of foot pressure scan data and VICON motion capture data.*
- *Participated in a study assessing the benefits of wearable robotics in gait assistance for paraplegic individuals.*

Summer 2013 **Intern**

Peak Centre for Human Performance, Ottawa, ON.

- *Administrative duties including; scheduling appointments, answering questions via telephone and email*
- *Laboratory set up, Vo2max testing, Wingate testing, blood lactate analysis, test result analysis*
- *Carried out dryland training sessions with competitive, local U14 hockey teams*
- *Assisted in strength and endurance training program design for clients*
- *Designed promotional pamphlets for Peak Academy*
- *Daily maintenance of the training facilities*