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Title:
Sex differences in the role of sympathetic nerve activity in the development of hypertension in humans

SEX DIFFERENCES IN THE ROLE OF SYMPATHETIC NERVE ACTIVITY IN THE DEVELOPMENT OF HYPERTENSION IN HUMANS

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A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Life Sciences.

School of Physiology, Pharmacology & Neuroscience.

March 2022

Word count: 62389

Abstract

Hypertension is a prevalent condition with clear links to increased mortality and morbidity. The sympathetic nervous system is a key regulator of blood pressure and sex differences in sympathetic blood pressure regulation may contribute to the reduced hypertension prevalence in premenopausal women compared to young men. However, hypertension in premenopausal women exists and the underlying mechanisms are poorly understood. Furthermore, whilst female hypertension risk is greater after the menopause, the mechanisms promoting hypertension in some postmenopausal women but not others remain unclear. This thesis hypothesised that hypertensive women exhibit altered sympathetic regulation versus normotensive controls. Firstly, the transduction of sympathetic nerve activity into blood pressure was measured in hypertensive and normotensive pre- and postmenopausal women (and equivalent male groups), using an established method. Transduction was increased in hypertensive versus normotensive premenopausal, but not postmenopausal women. Additionally, sympathetic transduction was negatively associated with age in hypertensive women. These data indicate a role for increased sympathetic transduction in hypertensive premenopausal but not postmenopausal women. The hypothesised mechanism driving increased sympathetic transduction in hypertensive premenopausal women (poorer beta-adrenergic receptor function versus normotensive controls), was investigated, but data collection was difficult (due to the COVID-19 pandemic), and this question remains unanswered. Additionally, the thesis aimed to determine whether respiratory modulation of sympathetic activity was altered in postmenopausal versus premenopausal women, given that age does not appear to affect respiratory sympathetic modulation in men. The data showed that respiratory sympathetic modulation is reduced in healthy postmenopausal women compared to younger adults, but that hypertension had no additional effect. As such, poorer respiratory modulation may be a mechanism by which ageing is associated with increased sympathetic activity in women. Overall, these data have contributed to the understanding of two aspects of sympathetic blood pressure regulation in women, with important implications for the understanding of hypertension development in women.

Acknowledgements

I am grateful to so many people for their help in completing this PhD. Thanks first to all the research participants for their trust in me. Thanks to Dr Emma Hart for her endless support and patience, for gently encouraging me to push myself and for the opportunity to do this work, which I have loved. Thanks to Dr Angus Nightingale, Dr Ana Abdala Sheikh, Dr Adrian Kendrick, and Prof Julian Paton for all their advice and support. Thanks to my brilliant colleagues Hazel Blythe, Dr Ben Chant, Dr Tom Hinton, Dr Katrina Hope and Dr Richard Baker for being a wonderful team. Thanks Hazel and Ben for being the most supportive lab friends I could wish for. Thanks Tom, Katrina, and Richard for your clinical expertise, calming presence and for everything you have taught me. Thank you to all the research nurses that have supported my study over the years, Laura Gallego, Isobel Sullivan, Sheila Bell, Abby Gill, Laura Wilkinson, Edel Murphy, and Vincy John; without your support, especially in the early days, I would not have had the confidence to conduct research in humans. A huge thank you to my family, in particular my parents and Sam, without your endless support and belief in me I would not have finished this work. Thanks to John, and to my friends Alysha, Carissa, Becs, Jon, Tian, Beckie, Alfie, and Jamie for their support over the years and patience with me. Finally, I additionally want to thank Hazel, Ben, Emma, Angus, Katrina, Richard, and Ana for their support when I was having a tough time. I am grateful and proud to have worked in such a supportive group.

My PhD was funded by the British Heart Foundation (FS/17/38/32935).

COVID-19 Impact statement

The main impact of COVID-19 on the research activities associated with this thesis was closure of the research study to recruitment during lockdown periods. The study was closed between 24/03/2020 and 30/09/2020 during the first UK national lockdown. When the second lockdown was announced on 05/11/20 pre-arranged studies were carried out, but recruitment of new participants was halted until February/March 2021 upon advice of supervisors. In total the study was closed due to COVID-19 for approximately nine months, which made achieving recruitment targets more difficult. Given the COVID-19 related closure in addition to the time taken to receive ethical approval, the study was open for 20 months of my funded PhD time (39 months, including a 3-month funding extension related to COVID-19). Clinical research staff were redeployed during the pandemic and were not available to help with data collection (the study relied on clinical research staff for clinical procedures and safety), even when studies were permitted again. As a result, I have been unable to demonstrate as much novel data collection as would normally be expected of a doctoral student and data from previous studies has been used in this thesis in addition to data collected specifically for the thesis (data from previous studies was used in chapters 3 and 6). However, some of the microneurography data collected from these other studies was collected by me, as part of my wider work in the research group (detailed in chapter 3). Chapter 6 was not part of the original plan for the thesis and uses data collected entirely from previous studies. Additionally, restrictions allowing the conduct of research in human participants during COVID-19 limited my ability to collect certain types of data. Social distancing restrictions limited the number of people allowed into the study room, which prevented running of the study in chapter 4, given that this study needed more than two research staff present to run. Furthermore, the building in which the study took place changed ownership (from University to NHS Trust) during the pandemic, which resulted in loss of access to shared equipment (ultrasound machine), meaning that I could no longer collect echocardiography data. My PhD funding finished in July 2021.

Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ....Zoe Adams..... DATE:...18/02/2022.....

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Abbreviations

ABPM	Ambulatory blood pressure monitoring
ATP	Adenosine triphosphate
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CVLM	Caudal ventrolateral medulla
ECG	Electrocardiography
eNOS	Endothelial nitric oxide synthase
ER	Oestrogen receptor
GPER	G-protein coupled oestrogen receptor
GPRK	G-protein receptor kinase
HTN	Hypertensive
L-NMMA	N-monomethyl-L-arginine
LVOT	Left ventricular outflow tract
MMP	Matrix metalloproteinase
mRNA	messenger RNA
MSNA	Muscle sympathetic nerve activity
NPY	Neuropeptide-Y
NTN	Normotensive
NTS	Nucleus tractus solitarius
PVC	Premature ventricular contraction
RVLM	Rostral ventrolateral medulla
VTI	Velocity time integral

Chapter 1 Literature Review

1.1 Introduction

Hypertension is a risk factor for cardiovascular disease that affects a significant proportion of the global adult population (World Health Organisation, 2017). The autonomic nervous system is a key regulator of blood pressure and evidence suggests that altered autonomic regulation of blood pressure may be an important mechanism driving the development of hypertension (Koeners et al., 2016). Autonomic blood pressure regulation differs by sex in healthy adults and sex differences in blood pressure regulation may contribute to sex differences in hypertension prevalence (Hart et al., 2012). However, the mechanisms leading to hypertension in women remain incompletely understood. The overall aim of this thesis was to contribute to the understanding of autonomic blood pressure regulation in hypertensive women, in order to help identify factors that might precipitate hypertension in women.

1.2 Note on terminology

The term sex describes the biological traits (sex chromosomes, sex hormones, gonads, secondary sexual characteristics) by which an individual is identified as male or female. Additionally, some individuals have biological traits associated with more than one sex and may identify as intersex or as having differences in sexual development (National Academies of Sciences and Medicine, 2022). The term gender describes an individual's identity and is related to societal and cultural influences (National Academies of Sciences and Medicine, 2022, Connelly et al., 2021). National Institutes of Health recommendations indicate that biological sex data is collected in research only when relevant to the topic studied (National Academies of Sciences and Medicine, 2022). Given that this thesis focuses on the role of sex hormones in the development of hypertension, the thesis will discuss sex differences rather than gender differences. Some researchers use the terms male and female when referring to sex, and the terms men and women when referring to gender (Connelly et al., 2021). However, these terms are often not clearly distinguished (National Academies of Sciences and Medicine, 2022). Most of the core literature referenced in this thesis use the terms men and women, although some papers use male and female to describe research participants. When the terms men and women are used in the literature,

it is generally assumed, although often not explicitly stated, that the authors are referring to cisgender men and women (whose gender matches the sex they were assigned at birth (National Academies of Sciences and Medicine, 2022)). In the literature review chapter, the terms used by the authors of the referenced work have been used (usually ‘men’ and ‘women’). The research participants contributing data to the thesis were recruited as male or female, therefore ‘male’ and ‘female’ are used in the experimental chapters of the thesis (including when referring to the work of others, for consistency). The research could have been made more inclusive by collecting both sex and gender data, as is recommended (National Academies of Sciences and Medicine, 2022) and future work will follow these guidelines.

1.3 Overview of hypertension

1.3.1 Global prevalence of hypertension

Hypertension describes a state where average blood pressure is consistently above normal levels (Guyenet, 2006). Global hypertension prevalence in adults was estimated to be 22% in 2015 (World Health Organisation, 2017). Hypertension is prevalent across the world; for example, within the past decade prevalence estimates of adult hypertension were reported at 34% in the US (Benjamin et al., 2017), 29% in Brazil (Picon et al., 2012), 25% in Europe (Timmis et al., 2019), 25% in East and West Sub-Saharan Africa (Okello et al., 2020), 23% in China (Wang et al., 2018), and 19% in India (Gupta et al., 2021), although the methodology used to determine prevalence varied among these studies. When definition of hypertension and method of determining prevalence were controlled for in a meta-analysis across seven global regions, Europe and Central Asia was reported to have the highest prevalence among men (38.8%), whilst sub-Saharan Africa was reported to have the highest prevalence among women (36.9%) (Mills et al., 2016). Whilst hypertension prevalence varies within the geographical regions and countries listed above, these statistics illustrate that a substantial proportion of the global population is directly affected by hypertension. Furthermore, global hypertension prevalence was reported to rise by 5.2% between 2000 and 2010 (Mills et al., 2016) and global prevalence is predicted to be 29% in 2025 (Kearney et al., 2005). Therefore, hypertension will continue to be a major factor in global healthcare for years to come.

1.3.2 Global consequence of hypertension

There is considerable evidence that hypertension is associated with increased mortality and morbidity (Whelton, 1994). Hypertension is associated with increased risk of stroke and coronary heart disease (MacMahon et al., 1990), renal disease (Klag et al., 1996) and vascular dementia (Sharp et al., 2011, Gorelick et al., 2011). For example, for a resting systolic blood pressure above 115 mmHg, risk of death by stroke or ischaemic heart disease was reported to be approximately two-times greater for every 20 mmHg increase in systolic blood pressure in those aged 60-69 years (Lewington et al., 2002). The Global Burden of Disease study reported that in 2017, hypertension was the single risk factor, among 476 studied, that was associated with the greatest number of disability-adjusted life years (218 million, as well as 10.4 million deaths) (Global Burden of Disease 2017, 2018). Therefore, lowering blood pressure in individuals with hypertension is an important global healthcare goal.

1.3.3 Defining hypertension

The threshold at which blood pressure is considered to be high varies geographically and as new evidence emerges. Global, European and UK guidelines indicate that when measured in the clinic, a systolic blood pressure $\geq$ 140 mmHg and a diastolic blood pressure $\geq$ 90 mmHg is indicative of hypertension (Unger et al., 2020, Williams et al., 2018, National Institute for Health and Care Excellence, 2019). American guidelines now suggest that stage one hypertension occurs when systolic blood pressure is ≥130 mmHg or diastolic blood pressure is >80 mmHg, with pharmacological treatment offered from stage one depending on individual cardiovascular risk (Whelton et al., 2018). In this thesis, hypertension is defined according to the UK guidelines (National Institute for Health and Care Excellence, 2019), given that hypertensive patients taking part in the research would be treated according to these guidelines.

1.4 Sex differences in hypertension

1.4.1 Sex, age, and hypertension prevalence

There is clear evidence that hypertension prevalence increases with advancing age (Burt et al., 1995, Benjamin et al., 2017, Mills et al., 2016, Kearney et al., 2005, Gu et al., 2002, Lovic et al., 2013). In England, 54% of adults aged 65-74 years had hypertension in 2019, compared to 7% of those aged 25-34 years

(Lifestyles Team NHS Digital, 2020). Similarly, in America, 67% of those aged above 60 years had hypertension in 2011-2014 compared to 12% of those aged 20-39 years (Benjamin et al., 2017). A small number of studies have reported no relationship between age and hypertension prevalence, for example in a rural community in Panama (Hollenberg et al., 1997), but large scale studies from various countries, such as those listed above, generally support the idea of increased hypertension risk with increasing age.

When adults of all ages are considered, hypertension prevalence does not differ greatly between men and women (e.g., 32% of women versus 31% of men in America (Whelton et al., 2018)). However, hypertension prevalence is influenced by sex differently in younger and older adults. In general, hypertension prevalence is lower in young women versus young men, but greater in older women versus older men (Burt et al., 1995, Benjamin et al., 2017, Gu et al., 2002) (Benjamin et al. use the terms male and female). The age at which prevalence of hypertension in women overtakes hypertension in men varies between studies, from ~65 years (Gu et al., 2002, Benjamin et al., 2017) to 70 years (Burt et al., 1995), and depends on how the age groups are categorised. Overall, hypertension risk increases with age in both men and women, however it appears that young women are at reduced risk of hypertension compared to young men, whilst older women are at greater risk versus older men.

1.4.2 Hypertension in postmenopausal women

Given that hypertension risk is low in younger versus older women, it is thought that female sex hormones may offer some protection against cardiovascular disease (Deroo and Korach, 2006). There is some evidence that female sex hormones influence blood pressure via their effects on the vasculature (section 1.4.1.1) and the menopause is associated with declining levels of oestrogen and progesterone (Moreau, 2018). Across studies from various countries, the average age of menopause was reported as 50.5 years (InterLACE Study Team, 2019). However menopausal transition lasts multiple years, with sex hormone concentrations fluctuating over this time before reaching postmenopausal levels (Moreau, 2018). Despite this, studies have attempted to determine whether menopause is an independent risk factor for hypertension. Amigoni et al., reported that when age and body mass index (BMI) were controlled for, hypertension was still more prevalent in postmenopausal versus premenopausal women (Amigoni et al., 2000), whilst others have reported no difference in

hypertension prevalence between pre- and postmenopausal women (Staessen et al., 1997). However, women reaching menopause at an earlier age (within the normal menopausal age range, so not considered early-onset menopause) were found to be at greater risk of hypertension than those reaching menopause several years later (Song et al., 2018). Furthermore, postmenopausal women were found to have a greater increase in systolic blood pressure over five years compared to premenopausal women, whilst no group difference was seen in age-matched men (Staessen et al., 1997). Therefore, there is some evidence that menopause may contribute to the risk of hypertension, but this risk is difficult to study independently from the concurrent risk of increasing age (Staessen et al., 1998).

1.4.3 Hypertension in premenopausal women

Whilst older adults are at the most risk of developing hypertension, some young adults also develop the condition, with hypertension considered 'young-onset' if occurring in adults younger than 40 years (Chen et al., 2004). Young-onset hypertension is less common in women versus men, for example 4% of women compared to 11% of men aged 25-34 years in England (Lifestyles Team NHS Digital, 2020). In women aged 35-44 years, who may no longer be considered to have young-onset hypertension, but who are still of premenopausal age, hypertension prevalence rises to 9% (versus 13% in men) (Lifestyles Team NHS Digital, 2020). These statistics demonstrate that premenopausal hypertension does occur and may occur despite the potential protective effects of female sex hormones. Therefore, it is important to consider the mechanisms driving hypertension in premenopausal women, given that most premenopausal women do not experience hypertension. This thesis focuses on the regulation of blood pressure by the sympathetic nervous system, with the ultimate aim of determining whether altered sympathetic regulation contributes to hypertension in premenopausal women.

1.5 Blood pressure homeostasis

1.5.1 Overview

Blood pressure is a physiological variable under the control of several regulatory mechanisms. These include neural reflexes via the autonomic nervous system, which in turn receive additional regulation from higher brain regions (Dampney,

2016); properties of the vascular muscle and endothelium (Dampney, 2016); and hormonal signalling mechanisms including the renin-angiotensin-aldosterone system (Miller and Arnold, 2019) and natriuretic peptides (Rubattu and Gallo, 2022). In the short-term, control of arterial blood pressure ensures adequate tissue perfusion (Dampney, 2016). In the long-term, maintenance of blood pressure within a normal range avoids the additional risks associated with hypertension (Benjamin et al., 2017). This thesis focuses on sympathetic control of blood pressure, and in particular, the sympathetic control of vasoconstrictor tone.

1.5.2 The arterial baroreflex

Rapid buffering of changes in blood pressure occurs via the arterial baroreflex (Dampney, 2016). Arterial baroreceptor afferents in the carotid sinus and aortic arch (Guyenet, 2006) encode blood pressure by firing in response to stretch of the arterial wall, where increased pressure results in increased stretch (Dampney, 2016). Baroreceptor afferent activity is conveyed to the nucleus tractus solitarius (NTS) in the medulla via the glossopharyngeal (carotid baroreceptors) and vagus (aortic baroreceptors) nerves (Dampney, 2016). Projections from the NTS to other regions of the brainstem regulate the outflow of autonomic efferent activity in response to baroreceptor afferent input. Sympathetic nervous system activity originates in the pre-sympathetic neurones of the rostral ventrolateral medulla (RVLM) (Guyenet, 2006). The RVLM receives inhibitory input from the caudal ventrolateral medulla (CVLM), which in turn receives excitatory input from the NTS (Guyenet, 2006). As such, increased baroreceptor afferent activity results in increased CVLM inhibition of the pre-sympathetic neurones, which inhibits sympathetic outflow (Guyenet, 2006). Parasympathetic nervous system activity originates in the nucleus ambiguus, which receives excitatory input from the NTS. Therefore, increased baroreceptor afferent activity results in increased parasympathetic outflow (Dampney, 2016). Via this mechanism, a fall in blood pressure (reduced baroreceptor afferent activity) results in increased sympathetic and decreased parasympathetic activity, whilst increased baroreceptor afferent activity results in reduced sympathetic and increased vagal activity (Dampney, 2016).

Baroreflex effector mechanisms are brought about by the activity of the sympathetic and parasympathetic projections to the heart, blood vessels and adrenal medulla. Cardiac sympathetic efferents promote increases in heart rate

and contractile force, resulting in increased cardiac output, whereas cardiac parasympathetic efferents promote bradycardia (Dampney, 2016). Vascular sympathetic efferents promote vasoconstriction, which increases peripheral resistance (Dampney, 2016). Therefore, in response to falling blood pressure, baroreflex effector mechanisms bring about increased cardiac output and total peripheral resistance. Given that mean arterial blood pressure is equal to the product of cardiac output and peripheral resistance (Thomas, 2011), these effector mechanisms act to increase blood pressure.

1.5.3 Sympathetic control of long-term blood pressure

In addition to regulating blood pressure on a beat-to-beat basis, there is evidence that the arterial baroreflex contributes to regulation of long-term resting blood pressure. For example, patients with damaged baroreceptor afferents exhibit labile blood pressure that can become very high in association with high levels of sympathetic activity (Heusser et al., 2005). Furthermore, exacerbating baroreceptor stimulation (baroreflex activation therapy) can be an effective non-pharmacological treatment for hypertension (Spiering et al., 2017) that is associated with a reduction in sympathetic activity (Heusser et al., 2010). The role of the sympathetic nervous system in contributing to hypertension is supported by evidence showing higher levels of resting sympathetic nerve activity in hypertensive patients compared to normotensive controls (Hogarth et al., 2007b, Hogarth et al., 2011, Grassi et al., 2000). Although this finding is not common to all studies assessing sympathetic nerve activity in hypertension (Schobel et al., 1996), a meta-analysis of 63 studies concluded that hypertension is usually associated with increased sympathetic activation (Grassi et al., 2018). A key hypothesis for the cause of this sympathoexcitation is that afferent activity from tissues receiving inadequate blood supply triggers increased sympathetic activity in order to increase perfusion (Koeners et al., 2016). Examples of organs implicated in this hypothesis include brain, the carotid body (location of peripheral chemoreceptors), and exercising skeletal muscle (Koeners et al., 2016). For example, hypertensive individuals had lower cerebral blood flow and were more likely to have cerebrovascular variants (that could limit perfusion) compared to normotensive controls, with borderline hypertensive participants exhibiting reduced cerebral blood flow in the absence of excess sympathetic activity (indicating that cerebral hypoperfusion precedes chronic symapthoexcitation) (Warnert et al., 2016). Meanwhile, removal of carotid body afferents delayed the onset of hypertension in a rat model of hypertension (Abdala et al., 2012), whilst

carotid body excision was shown to be an effective treatment for hypertensive patients, but only in those who exhibited excess chemoreceptor sensitivity at baseline (Narkiewicz et al., 2016). As such, these data, among others, demonstrate a role for afferent activity in promoting chronic sympathoexcitation, which may underlie or contribute to the development of hypertension.

1.5.4 Regulation of vasoconstrictor tone

1.5.4.1 Overview

Sympathetic control of blood pressure depends not only on the amount of sympathetic activity directed towards the vasculature, but also the efficiency with which a given level of sympathetic activity triggers vasoconstriction (sympathetic transduction) (Hart and Charkoudian, 2014). This depends on several factors, including the relative level of innervation (Thomas, 2011), neurotransmitter release, receptor type and number on the innervated tissue (Tymko et al., 2021), circulating vasoactive substances, and structural properties of the arteries (Thomas, 2011). Furthermore, receptors for sympathetic neurotransmitters are located both pre- and post-junctionally (Thomas, 2011), as well as on the vascular endothelium (Guimarães and Moura, 2001).

1.5.4.2 Sympathetic control of vascular smooth muscle

Vascular sympathetic efferents control vasoconstrictor tone via the action of the neurotransmitter noradrenaline, and co-transmitters adenosine triphosphate (ATP) and neuropeptide-Y (NPY) (Huidobro-Toro and Donoso, 2004). Noradrenaline binds alpha and beta-adrenergic receptors which are present on the abluminal vascular smooth muscle (Michelotti et al., 2000). The vasoconstrictor response of noradrenaline is achieved by vascular smooth muscle contraction, secondary to alpha-adrenergic receptor activation, with the alpha-1 subtype (Gq-coupled) acting to increase intracellular calcium and the alpha-2 subtype (Gi-coupled) acting to reduce intracellular cAMP and the associated smooth muscle relaxation (Michelotti et al., 2000). Beta-adrenergic receptors (Gs-coupled) stimulate cAMP production when activated, and therefore promote vascular smooth muscle relaxation (Michelotti et al., 2000). Given that alpha-adrenergic receptors are more highly expressed than beta-adrenergic receptors in the arteries contributing to vascular tone, vasoconstriction is the net effect of sympathetic activity directed to the vasculature (Riedel et al., 2019). Co-transmitters ATP and NPY can contribute to the contractile effect of noradrenaline. Activation of P2X receptors (mainly $P2X_1$ (Martin-Aragon Baudel

et al., 2020)) on vascular smooth muscle by ATP promotes depolarisation (Huidobro-Toro and Donoso, 2004) via influx of cations into the smooth muscle cells (Burnstock and Williams, 2000). Meanwhile, NPY activation of the Y1 receptor is thought to enhance the vasoconstrictor effects of the other transmitters (Huidobro-Toro and Donoso, 2004). Additionally, receptors located on the sympathetic nerve terminals (alpha-2 (Michelotti et al., 2000) and Y2 (Huidobro-Toro and Donoso, 2004)) act to inhibit further release of neurotransmitters, whilst P2Y receptors located on vascular smooth muscle and vascular endothelium influence vasoconstrictor tone in both directions depending on subtype (Martin-Aragon Baudel et al., 2020).

1.5.4.3 Endothelial contribution to vasoconstrictor tone

The endothelium produces both vasodilation (nitric oxide, prostaglandin) and vasoconstriction-promoting factors (endothelin) (Orshal and Khalil, 2004), which can be released in response to mechanical stimuli (shear stress) (Zhao et al., 2015), or upregulation by other factors, including sex hormones (Zhao et al., 2015, Miller and Duckles, 2008). Nitric oxide promotes vasodilation through a cGMP-mediated relaxation of the vascular smooth muscle (Zhao et al., 2015). Protein kinase G activation by cGMP leads to reduced calcium entry into smooth muscle cells via voltage-gated membrane channels or sarcoplasmic reticulum channels, as well as promoting re-uptake of calcium into the sarcoplasmic reticulum (Zhao et al., 2015). Additionally, nitric oxide can promote s-nitrosylation of receptors, which encourages their downregulation (Zhao et al., 2015). Evidence from rat arterial tissue showed that nitric oxide promotes downregulation of alpha-adrenergic receptors via this mechanism (Nozik-Grayck et al., 2006). However, s-nitrosylation of G-protein receptor kinase (GPRK) acts to inhibit phosphorylation of beta-adrenergic receptors by GPRK, which reduces beta-adrenergic downregulation (Whalen et al., 2000). As such, nitric oxide may further promote vasodilation by discouraging loss of functional beta-adrenergic receptors to downregulatory mechanisms.

Adrenergic receptor subtypes alpha-2 and beta-2 are also found on vascular endothelium, where their activation may promote nitric oxide production (Guimarães and Moura, 2001). Ferro et al. demonstrated that the endothelium is crucial in beta-adrenergic vasodilatory mechanisms in human vein tissue. The vasodilator response to beta-adrenergic agonists was lost upon removal of the endothelium or inhibition of eNOS (Ferro et al., 1999). Therefore, both vascular

smooth muscle and endothelial adrenergic receptors appear to play an important role in regulating vasoconstrictor tone.

1.5.4.4 Arterial stiffness

Arterial stiffness is another factor contributing to blood pressure. Stiffer arteries are less compliant (Rossi et al., 2011) and are associated with increased systolic and pulse pressure (but decreased diastolic blood pressure) (Lakatta and Levy, 2003). Epidemiologically, increased arterial stiffness is associated with increased mortality and morbidity (Laurent et al., 2001), and is also associated with hypertension even in younger adults (Gokaslan et al., 2019). Arterial stiffness arises through alterations to the structure of the arterial wall, with increased collagen and reduced elastin characteristic of stiffer arteries (Lakatta and Levy, 2003). There is evidence that sympathetic nerve activity promotes arterial stiffening, for example alpha-adrenergic receptor activation was shown to promote vascular smooth muscle hypertrophy in rats (Zhang and Faber, 2001). Furthermore, in hypertensive patients, baroreflex activation therapy (which reduces blood pressure via a sympathoinhibitory mechanism (Spiering et al., 2017)) caused reductions in arterial stiffness (Wallbach et al., 2015). Furthermore, age and sex influence arterial stiffness (discussed below), which may contribute to the relative risk of hypertension in different patient groups.

1.6 Sex differences in blood pressure control

1.6.1 Younger adults

1.6.1.1 Sex hormones and the cardiovascular system

Oestrogen and androgens are produced in male and female adults (Maranon and Reckelhoff, 2013), although the relative circulating concentrations of each hormone vary by sex. In the absence of exogenous hormones or hormone blockers, young women produce more oestrogen than men, whilst young men produce more androgens (Salerni et al., 2015). In premenopausal women who experience regular menstrual cycles, oestrogen and progesterone levels vary across stages of the menstrual cycle, with their production in the ovaries regulated by gonadotropin hormones (follicle stimulating hormone and luteinising hormone) (Hawkinsa and Matzuk, 2008). Oestrogen and progesterone concentrations are both low during the early follicular phase of the menstrual cycle, with the late follicular phase associated with low progesterone but high

oestrogen. Meanwhile, both oestrogen and progesterone concentrations are high in the mid-luteal phase (Carter et al., 2013).

Receptors for oestrogen, progesterone and testosterone are found throughout the cardiovascular system, for example in cardiomyocytes (Salerni et al., 2015), vascular smooth muscle cells (Rossi et al., 2011, Orshal and Khalil, 2004), and vascular endothelial cells (Orshal and Khalil, 2004). Receptors for oestrogen, progesterone and testosterone all have roles regulating gene expression (Salerni et al., 2015). However, oestrogen and androgen receptors additionally have more rapid effects, for example triggering intracellular signalling cascades (Salerni et al., 2015) via activation of receptors at the plasma membrane, as opposed to nuclear receptors (Thompson and Khalil, 2003).

Oestrogen is known for its dilatory effect on the vasculature. Oestrogen upregulates endothelial nitric oxide synthase (eNOS) (Miller and Duckles, 2008) and nitric oxide is a key component of oestrogen-induced vasodilation. This has been demonstrated in experiments where the vasodilatory effect of oestrogen is lost in eNOS knockout mice (Guo et al., 2005). Several mechanisms by which oestrogen promotes nitric oxide production have been identified. eNOS mRNA was increased after oestrogen supplementation in mouse vessels (Stirone et al., 2003). However, data also show that phosphoinositide 3-kinase (PI3K) signalling and phosphorylation of eNOS is increased with oestrogen (Stirone et al., 2005) and kinase inhibitors reduce the vasodilatory response to oestrogen (Guo et al., 2005). Thus, nitric oxide production is increased with oestrogen by several mechanisms occurring over different time frames.

Given that progesterone does not occur in the absence of oestrogen in vivo, the vascular effects of progesterone are less well studied (Rossi et al., 2011) and existing evidence about progesterone's role in influencing vasoconstrictor tone is contradictory (Thompson and Khalil, 2003). Studies in rats suggest that progesterone also has a vasodilatory effect, as shown by an inhibition of contractile responses to exogenous vasoconstrictors in isolated tissue (tail artery and aorta) (Barbagallo et al., 2001). In agreement, oestrogen treatment was associated with an increase in eNOS production (You et al., 2020). However, others have shown that exogenous oestrogen but not progesterone reduced the vasoconstrictor response to noradrenaline in ovariectomised rats (Riedel et al., 2019).

Study of the vascular effects of testosterone in vivo are complicated by the conversion of androgens to oestrogens (Salerni et al., 2015). However, studies suggest that testosterone can enhance nitric oxide levels independently of oestrogen, given that this effect was observed in cultured vascular smooth muscle cells in the presence of blockers of aromatase enzymes (Campelo et al., 2012). Furthermore, in human studies, testosterone supplementation has been shown to enhance both endothelium-dependent and -independent vasodilation in oestrogen-supplemented older women (who therefore were not oestrogen-deficient). Although the authors could not rule out that this effect was mediated by aromatisation and subsequent excess circulating oestrogen (Worboys et al., 2001).

Furthermore, there is evidence for nitric oxide independent regulation of vascular tone by sex hormones. K^+ channels on vascular smooth muscle were stimulated by oestrogen application (White et al., 1995) and levels of the vasoconstrictor endothelin-1 were reduced with oestrogen (Best et al., 1998). Furthermore, oestrogen, progesterone and testosterone all reduced the contractile response to an endothelium-independent stimulus (KCl) in pig aorta which lacked endothelial tissue (Crews and Khalil, 1999).

Furthermore, sex hormones were shown to influence the composition of the arterial wall. In in vitro studies of human aortic cells, collagen was reduced in the presence of oestrogen, progesterone, and testosterone (independently), with elastin levels increased in the presence of oestrogen and progesterone. The mechanism behind these changes was linked to matrix metalloproteinase (MMP) activity, given that mRNA for matrix metalloproteinase-3 (MMP-3) was increased in the presence of progesterone and testosterone (but not oestrogen) (Natoli et al., 2005). These data provide evidence for a role of sex hormones in regulating arterial wall composition, and therefore has implications for the development of arterial stiffness in men and women.

In summary, oestrogen, progesterone, and testosterone appear to have vasodilatory effects on the vasculature, and a key mechanism is the upregulation of nitric oxide production. Furthermore, these sex hormones may interact directly with vascular smooth muscle and may also contribute to prevention of arterial stiffness. Given this, and the sex differences in hypertension prevalence in young

adults, previous research has focused on whether sex (or sex hormones) influence sympathetic control of blood pressure.

1.6.1.2 Sex differences in sympathetic control of blood pressure

There is conflicting evidence about whether there is a direct relationship between sex hormone concentration and resting blood pressure. Some reports suggest that blood pressure varies with phase of the menstrual cycle, with peak blood pressure seen at the start of the early follicular phase (low oestrogen and progesterone) (Dunne et al., 1991). However, other reports contradict this, reporting no association between menstrual cycle stage and blood pressure (Williamson et al., 1996, Carter et al., 2013).

However, there is evidence that sex hormones may influence the level of sympathetic activity. MSNA is increased during the mid-luteal versus the early follicular phase of the menstrual cycle in healthy women (Minson et al., 2000, Carter et al., 2013), but does not appear to differ between the early follicular and late follicular phase (Ettinger et al., 1998). Together, these studies suggest that both oestrogen and progesterone have some influence over sympathetic outflow. Oestrogen is low in the early follicular phase but increased in the late follicular and mid-luteal phases, whereas progesterone is low in early and late follicular but increased in the mid-luteal phase (Carter et al., 2013). As such, Carter et al. suggest that the increase in MSNA during the mid-luteal phase is related to progesterone rather than oestrogen (Carter et al., 2013). This is supported by evidence that application of oestrogen to the brainstem of rat models causes a reduction in sympathetic activity (Saleh et al., 2000), and that in a human model of low circulating sex hormones (use of a gonadotropin-hormone blocker), addition of exogenous oestrogen similarly reduces MSNA (Day et al., 2011). Day et al. were unable to show that addition of exogenous progesterone during gonadotropin-hormone block affected MSNA, although the sample size was very small (N=3) (Day et al., 2011). Despite this evidence that sex hormones can affect MSNA, there is contradictory evidence about whether resting MSNA differs between young women and men. A large cross-sectional study showed that among adults in their 20's, resting MSNA did not differ between healthy men and women. However, women in their 30's showed a reduced MSNA burst incidence compared to men of similar age, the cause of which is currently unclear (Keir et al., 2020). Thus, sympathetic activation may differ by sex at certain points of young adulthood, but whether this difference drives the sex difference in

hypertension prevalence ultimately depends on the influence of MSNA on vasoconstrictor tone. Narkiewicz et al. demonstrated that in both young men and women, individual resting blood pressure is not related to resting sympathetic activity. Thus, in young adults, blood pressure appears to be dissociated from resting sympathetic activation (Narkiewicz et al., 2005), and therefore any sex differences in the resting level of MSNA would be unlikely to underlie sex differences in hypertension prevalence.

Further research has demonstrated sex differences in vasoconstriction for a given level of sympathetic activity. Initial studies showed that infusing noradrenaline into the circulation of the forearm caused smaller vasoconstrictor responses in young women versus young men (Kneale et al., 1997). Subsequent research showed that this sex difference was abolished by antagonism of the beta-adrenergic receptors in the forearm vasculature (co-infusion of propranolol) (Kneale et al., 2000). These findings pointed to a clear role of the vascular beta-adrenergic receptors in regulating sympathetic control of vasoconstrictor tone in women but not in men. Later research assessing the relationships between MSNA and blood pressure on a systemic level supported this. Young women showed a dissociation between resting MSNA and resting total peripheral resistance, whereas young men showed a positive relationship between these variables (Hart et al., 2009). When the beta-adrenergic receptors were blocked by systemic infusion of propranolol, the relationship between MSNA and peripheral resistance was unchanged in young men but became positive in young women (Hart et al., 2011a). Additionally, later analysis of the same data showed that when the transfer of MSNA into vasoconstrictor tone (sympathetic transduction) is quantified, young women show lower sympathetic transduction compared to young men at rest, but similar levels during beta-adrenergic blockade (Briant et al., 2016). As such, these studies provide evidence that young women show reduced sympathetic control of the vasculature compared to young men, and that the vascular beta-adrenergic receptors underlie this effect. Lower sympathetic transduction in younger women, secondary to vascular beta-adrenergic activity, could therefore, at least partially, explain the sex differences in hypertension prevalence (Hart et al., 2011a). Whilst sympathetic transduction also depends on the vascular alpha-adrenergic receptors, they are not thought to contribute to the sex difference in sympathetic transduction, given that under beta-blockade, vasoconstrictor responses to noradrenaline are comparable in young men and women (Kneale et al., 2000). The mechanisms by which the

beta-adrenergic receptors might influence sympathetic transduction in women are discussed in section 1.5.

1.6.1.3 Sex differences in endothelial function

Given that endothelial production of vasodilatory factors (Orshal and Khalil, 2004) would affect the ability of sympathetic efferents to cause vasoconstriction, any sex differences in endothelial function could be important in explaining the reduced hypertension risk in younger women versus men. In healthy young women, endothelial function measured by flow mediated dilatation varied across the menstrual cycle, with endothelial function lower during the early luteal phase compared to early and late follicular, and late luteal (Williams et al., 2001) Furthermore, in a large cohort, women were shown to have increased endothelial function compared to men, until aged ~70 years (Benjamin et al., 2004). Given the role of oestrogen in upregulating eNOS (Miller and Duckles, 2008), enhanced endothelial function in women may be related to increased levels of nitric oxide. In agreement, Sudhir et al. showed that in peri-menopausal women vasoconstrictor responses to eNOS inhibition were enhanced after oestrogen supplementation, suggesting that nitric oxide made a greater contribution to resting vasoconstrictor tone in the presence of oestrogen (Sudhir et al., 1996). As such, enhanced endothelial function, secondary to oestrogen-stimulated production of nitric oxide, may also be a factor contributing to the lower hypertension prevalence in young women versus young men.

1.6.1.4 Sex differences in arterial stiffness

Arterial stiffness is increased in hypertension in young adults (Gokaslan et al., 2019, Isaykina et al., 2017) and increases considerably with age even in adults who exhibit a modest age-related rise in blood pressure (14% rise in blood pressure versus 100% rise in pulse wave velocity over ~70 years; (Vaitkevicius et al., 1993). Therefore, any sex differences in arterial stiffening could influence the sex-specific risk of hypertension. Research has shown that arterial stiffness varies across phases of the menstrual cycle, with lower stiffness observed during the luteal versus early-follicular phase (Aminuddin et al., 2018). However, cross-sectional studies tracking arterial stiffness with ageing showed no sex difference in arterial stiffness in younger adults, when sex differences in hypertension prevalence are already present (<40 years (Smulyan et al., 2001); <50 years (AlGhatrif et al., 2013)). This suggests that a sex difference in arterial stiffness does not underlie the sex difference in hypertension prevalence in younger

adults. These studies included hypertensive patients whose use of anti-hypertensive medication had been paused for a month prior to the study, thus the pulse wave velocity measurements would not have been confounded by medication use (Smulyan et al., 2001). However, the lack of an overall sex difference in arterial stiffness (Smulyan et al., 2001, AlGhatrif et al., 2013) suggests that female sex hormones do not contribute to a basal difference in arterial stiffness between men and women, and therefore are unlikely to underlie the sex differences in hypertension prevalence in young adults.

1.6.1.5 Mechanisms of hypertension in premenopausal women

As demonstrated above, there are several mechanisms that could contribute to the sex differences in hypertension prevalence in young adults. Reduced sympathetic transduction in younger women versus younger men, in association with enhanced beta-adrenergic vasodilation and possibly linked to increased nitric oxide production, could underlie the reduced risk of hypertension in premenopausal women. But despite these protective mechanisms, hypertension does occur in some premenopausal women, the underlying mechanism of which is not known. It is possible that the sympathetic regulation of blood pressure in hypertensive premenopausal women is altered versus normotensive younger women. Therefore, the first aim of this thesis was to determine whether sympathetic transduction is altered in hypertensive versus normotensive premenopausal female participants.

1.6.2 Older adults

1.6.2.1 Ageing, sex hormones and cardiovascular disease

Ageing is associated with increased risk of various forms of cardiovascular disease, including hypertension (Benjamin et al., 2017). This increased risk has been linked to multiple physiological changes, such as altered autonomic regulation of blood pressure (Barnes et al., 2014, Jones et al., 2001), increased arterial stiffness (Mitchell et al., 2004), and poorer endothelial function (Benjamin et al., 2004). Importantly, sex is a factor in the age-related increase in cardiovascular risk. Prevalence of hypertension in women exceeds that of men from around 65-70 years (Benjamin et al., 2017, Gu et al., 2002), and given the influence of sex hormones on the vasculature, age-related changes to circulating sex hormone concentrations are an important factor when considering cardiovascular risk in ageing adults.

Menopause occurs in middle age and is characterised by a decline in oestrogen and progesterone concentration, but an increase in follicle stimulating hormone concentration (Moreau, 2018). Women also show a fall in testosterone concentration with age (Moreau et al., 2020, Davison et al., 2005), but this decline is thought to be independent of menopause (Davison et al., 2005). Whilst the average age of menopause is reported as 51 years (InterLACE Study Team, 2019), the transition from premenopausal to postmenopausal status occurs over several years, and it can therefore be difficult to separate the effects of menopause and ageing on cardiovascular risk in women (Moreau, 2018). Men also exhibit age-related changes to sex hormones, with circulating testosterone concentration falling with age (Moreau et al., 2020). However, this decline occurs over a wider timeframe than the changes associated with menopause, with testosterone starting to decline in some men in their 20's (Matsumoto, 2002).

1.6.2.2 Autonomic control of blood pressure with ageing

Previous research has demonstrated that several changes to the autonomic regulation of blood pressure occur with age. Firstly, it is well-established that resting level of sympathetic nerve activity rises with age (Keir et al., 2020). By age 50 years, resting level of MSNA is similar again in men and women (having been lower in women versus men in their 30's) and increases with age similarly in both sexes thereafter (Keir et al., 2020). Additionally, there appears to be a shift towards blood pressure regulation being under greater sympathetic influence in older age. Studies where inhibition of the autonomic ganglia was induced pharmacologically (trimethaphan infusion) measured the heart rate and blood pressure response as indicators of resting parasympathetic and sympathetic activity respectively. Compared to young adults, older men and women showed smaller increases in heart rate but larger reductions in blood pressure from baseline during ganglionic blockade, indicating respectively that resting parasympathetic activity was reduced and resting sympathetic activity was increased in older adults compared to younger controls (Jones et al., 2001, Barnes et al., 2014). These changes were similar in older men compared to younger men (Jones et al., 2001) versus postmenopausal women compared to premenopausal women (Barnes et al., 2014). Therefore, an increased sympathetic contribution to blood pressure regulation appears to be a feature of ageing in both men and women. In support of this, blood pressure becomes related to resting MSNA in older adults, having been dissociated from MSNA in younger adults (Narkiewicz et al., 2005).

Furthermore, the dissociation of peripheral resistance from sympathetic activity observed in premenopausal women is not replicated in postmenopausal women, in whom greater resting MSNA is associated with greater peripheral resistance (Hart et al., 2011a). This also appears to be related to the vascular beta-adrenergic receptors, given that systemic beta-blockade does not affect the SNA-peripheral resistance relationship in postmenopausal women (Hart et al., 2011a). These data suggest that the beta-adrenergic driven dissociation of peripheral resistance from sympathetic activity, that potentially protects younger women from developing hypertension, is not present in postmenopausal women. As such, this change may to contribute to the greater risk of hypertension in postmenopausal women (Hart et al., 2011a).

1.6.2.3 Endothelial function and ageing

Endothelial function is known to decline with age (Benjamin et al., 2004, Celermajer et al., 1994). Some reports indicate a sex difference in this age-related decline, with Celermajer et al. showing that men exhibited endothelial function decline from a younger age versus women (in 40's compared to 50's) (Celermajer et al., 1994). However, others have found that the rate of decline is similar in men and women, given that there was no sex difference in endothelial function in adults until their 70's (Benjamin et al., 2004). Any sex difference in endothelial function may be related to menopausal decline in circulating sex hormones, given that oestrogen supplementation improves endothelial function in recently postmenopausal women (Sherwood et al., 2007). Endothelial function is poorer in hypertension (Routledge et al., 2012), so a menopause-related decline in female sex hormones may contribute to the increased hypertension risk in older women (Moreau, 2018). In a hypertensive cohort, women had poorer endothelial function compared to similarly-aged men (Routledge et al., 2012). The participants were aged 40-60 years, so likely included pre-, peri-, and postmenopausal women. However, these data still demonstrate a potential role for poor endothelial function in contributing to hypertension in older women.

1.6.2.4 Arterial stiffness and ageing

Similarly, ageing is associated with increased arterial stiffening (Mitchell et al., 2004, Vermeersch et al., 2008, Baldo et al., 2018, Vaitkevicius et al., 1993). Several reports suggest that the rate of age-related arterial stiffening is similar in men and women (Mitchell et al., 2004, Vermeersch et al., 2008, Baldo et al.,

2018, Vaitkevicius et al., 1993). The effect of menopause on arterial stiffness has been studied, with postmenopausal women likely to have stiffer arteries compared to peri- and premenopausal women (Hildreth et al., 2014, O'Neill et al., 2012). However, the effect of menopause is not statistically independent of the effect of age (Hildreth et al., 2014, O'Neill et al., 2012). As such, it is difficult to quantify the contribution of menopause to arterial stiffening (Moreau, 2018). However, the lack of sex difference in the rate of age-related arterial stiffening suggests that there is not an obvious contribution of menopause independent of ageing.

1.6.2.5 Mechanisms of hypertension in postmenopausal women

Overall, the sympathetic regulation of blood pressure is altered in several ways in postmenopausal versus premenopausal women. Level of sympathetic activation is increased (with age), and transduction of sympathetic nerve activity into vasoconstrictor tone appears to be enhanced. Additionally, endothelial function is poorer and arterial stiffness greater in older versus younger women. These factors are likely to underlie the increased risk of hypertension in postmenopausal versus premenopausal women. Despite this, not all postmenopausal women develop hypertension. Level of sympathetic nerve activity may be an important factor in hypertension in postmenopausal women, given that hypertensive middle-aged women (some of which were postmenopausal), had greater resting levels of sympathetic activity compared to normotensive controls (Hogarth et al., 2007b). Additionally, sympathetic transduction appears to be enhanced in hypertensive older women compared to hypertensive older men, given that a similar blood pressure was observed in these groups even though level of sympathetic activity was lower in the female versus male group (Hogarth et al., 2008). However, whether increased sympathetic transduction is an important factor in determining which postmenopausal women develop hypertension remains unclear. Therefore, a second aim of this thesis is to determine whether sympathetic transduction is altered in hypertensive versus normotensive postmenopausal female participants.

1.7 The role of the vascular beta-adrenergic receptors in sympathetic transduction in women

1.7.1 Overview

As discussed above, the vascular beta-adrenergic receptors play an important role in regulating sympathetic control of the vasculature in women. Sympathetic transduction is lower in younger women versus younger men at rest, but similar during beta-adrenergic receptor blockade (Kneale et al., 2000, Hart et al., 2011a, Briant et al., 2016). Therefore, the beta-adrenergic receptors may have a greater vasodilatory effect in women compared to men (Hart et al., 2011a). Given that the beta-adrenergic receptors appear to contribute less to regulating vasoconstrictor tone in postmenopausal versus premenopausal women, the underlying mechanism is more likely to involve female sex hormones than be related to female sex itself (Hart et al., 2011a).

1.7.2 Mechanisms of enhanced beta-adrenergic vasodilation in young women

1.7.2.1 Nitric oxide

Studies in both animals and humans support the idea that nitric oxide is important in beta-adrenergic vasodilation. In isolated human vascular tissue, both inhibition of nitric oxide synthase (L-NMMA) and removal of the endothelium reduced the vasodilator response to isoprenaline (Ferro et al., 1999). Additionally, in the intact human forearm, vasodilator responses to isoprenaline were smaller in the presence of L-NMMA (Cardillo et al., 1997). Given that oestrogen is known to increase nitric oxide synthase activity (Guo et al., 2005), it is possible that oestrogen-induced upregulation of nitric oxide is responsible for the enhanced beta-adrenergic vasodilation in young women (Hart et al., 2011a). In support of this, the vasodilator response to isoprenaline was enhanced in arteries from rats treated with oestrogen versus those from untreated controls (Ferrer et al., 1996). Enhanced beta-adrenergic vasodilation in the presence of oestrogen may be linked to an increase in basal nitric oxide concentrations, given that nitric oxide inhibition (L-NMMA) caused less vasoconstriction after treatment with oestrogen in postmenopausal women (Sudhir et al., 1996).

1.7.2.2 Beta-adrenergic receptor expression

Another possible explanation for increased beta-adrenergic vasodilation in young women is altered beta-adrenergic receptor expression in the vasculature relative to young men. Along these lines, beta-adrenergic receptor density in young women was greater than that of young men during the luteal phase, but not the early follicular phase of the menstrual cycle (Wheeldon et al., 1994).

1.7.3 Role of the vascular beta-adrenergic receptors in hypertensive premenopausal women

Given that in postmenopausal women, a decline in beta-adrenergic vasodilation is associated with increased sympathetic transduction and increased risk of hypertension (Hart et al., 2011a), a similar mechanism may contribute to hypertension in premenopausal women. Specifically, if hypertensive premenopausal women do show an increased sympathetic transduction compared to healthy controls, this may be associated with poorer beta-adrenergic vasodilation. Therefore, a third aim of this thesis was to assesses the role of the beta-adrenergic receptors in sympathetic transduction in hypertensive versus normotensive premenopausal female participants.

1.8 Sympathetic transduction in exercise

The haemodynamic and sympathetic responses to exercise have also been shown to exhibit differences with sex, age, and hypertension. This thesis considered the haemodynamic response to isometric handgrip exercise, given that this is compatible with microneurographic recordings.

Sex differences in the response to isometric handgrip exercise are apparent even in healthy young adults. The pressor response associated with handgrip exercise has been shown to be smaller in healthy young women versus young men (Ettinger et al., 1996, Jarvis et al., 2011), although in other cases no sex difference was found (Jones et al., 1996). Furthermore, the sympathoexcitation that occurs with handgrip exercise was also found to be greater in younger men versus younger women (Ettinger et al., 1996, Jarvis et al., 2011, Jones et al., 1996) (Jones et al. use the terms male and female). Previous work suggests that this sex difference is at least partially due to sex differences in the metaboreflex (Ettinger et al., 1996) and mechanoreflex (Ives et al., 2013). Multiple studies report smaller pressor responses to post-exercise ischaemia in young women

versus men (Ettinger et al., 1996, Jarvis et al., 2011), which may be linked to reduced production of muscle metabolites in women versus men, even when mass of the exercising limb is similar between groups (Ettinger et al., 1996). However, given that sympathetic transduction is lower in young women than in men (Hart et al., 2009, Briant et al., 2016), it is possible that lower sympathetic transduction during exercise also contributes to the smaller pressor responses in women versus men (Smith et al., 2019). If sympathetic transduction is enhanced in hypertensive versus normotensive younger women, this may have consequences for the pressor responses to exercise. Therefore, this thesis aimed to assess whether sympathetic transduction levels were maintained in sex-hypertension participant groups relative to other groups during isometric handgrip exercise.

1.9 Respiratory modulation of the cardiovascular system

1.9.1 Overview

Respiration is an important factor in the regulation of sympathetic nerve activity. Firing of sympathetic efferents increases towards the end of inspiration and decreases towards the end of expiration (Seals et al., 1993). Subsequently, blood pressure is modulated by respiration, with mean arterial pressure increasing with inspiration and decreasing with expiration (Traube-Hering waves) (Barnett et al., 2020). Multiple mechanisms underlie the respiratory modulation of heart rate, MSNA and mean arterial pressure. There is evidence for central respiratory regulation of MSNA in the brainstem (Zoccal et al., 2014). However, intrathoracic pressure changes with respiration also influence cardiovascular reflexes, for example through the cardiopulmonary baroreceptors, as well as influencing ventricular filling and subsequent loading of the arterial baroreceptors. Given that sympathetic control of blood pressure changes with ageing, it is possible that respiratory cardiovascular modulation also differs with age. Importantly, there may also be sex differences in the age-related changes in respiratory cardiovascular modulation.

1.9.2 Mechanisms of respiratory sympathetic modulation

MSNA is coupled to respiration such that sympathetic activity is lowest during and shortly after the peak of inspiration and greatest towards the end of expiration (Seals et al., 1993, Eckberg et al., 1985). Given that sympathetic

activity remains coupled to phrenic nerve activity in vagotomised animals (Barman and Gebber, 1976, Preiss et al., 1975), a central mechanism is thought to be the main source of respiratory sympathetic modulation (Zoccal et al., 2014). In agreement with this are data showing that recipients of lung transplants, who lack afferent input from the lungs to the brainstem, still exhibit respiratory modulation of MSNA (Seals et al., 1993). However, lung stretch receptor afferents have still been shown to influence respiratory sympathetic modulation for example, at increased tidal volumes, respiratory modulation of MSNA is enhanced (Seals et al., 1990). The effect of tidal volume on respiratory sympathetic modulation is absent in lung transplant patients, thus is thought to be mainly due to lung stretch receptor afferent activity (Seals et al., 1993).

The arterial baroreflex has also been shown to influence respiratory sympathetic coupling. Respiratory modulation of MSNA is lost under certain conditions of baroreceptor unloading, such as strong (60-80 degree) head-up tilt stimuli (Cooke et al., 1999). However during shorter periods of baroreceptor unloading, sympathetic activity is still modulated by respiration, with smaller sympathoexcitatory responses to transient neck pressure observed during inspiration than expiration (Eckberg et al., 1985). Furthermore, during conditions of high baroreceptor loading (phenylephrine infusion), when sympathetic outflow is inhibited, there is little difference in MSNA during inspiration versus expiration (Eckberg et al., 1988). Along these lines, Eckberg suggests that the arterial baroreflex interacts with respiratory modulation of sympathetic activity such that arterial baroreflex control over MSNA is dominant only at high and low blood pressures (Eckberg, 2003). Despite this evidence for an interaction between the arterial baroreflex and respiratory sympathetic modulation, there is also evidence that the arterial baroreflex does not drive respiratory sympathetic coupling. Under anaesthesia with mechanical ventilation, respiratory modulation of blood pressure is altered such that blood pressure rises with inspiration rather than expiration. MSNA remains entrained to respiration in the same pattern as in conscious humans, therefore respiratory sympathetic modulation does not arise as a consequence of respiratory-related changes in blood pressure via the baroreflex (Macefield and Wallin, 1995b).

Another reflex influencing the respiratory modulation of sympathetic nerve activity is the peripheral chemoreflex, which promotes respiration in response to falling partial pressure of oxygen detected at the carotid body chemoreceptors (Dampney, 2016). Chemoreflex activation is associated with sympathoexcitation and increased respiration (Dampney, 2016).

Overall, it appears that respiratory sympathetic modulation occurs primarily as a result of a central regulatory mechanism, but that the lung stretch receptor reflex, arterial baroreflex and peripheral chemoreflex all influence the primary coupling mechanism. Zoccal et al. suggest that the interaction between these reflexes arises because of the location of reflex afferent termination points in the NTS (Zoccal et al., 2014). Slowly-adapting lung stretch receptor afferents and arterial baroreflex afferents both terminate in the intermediate NTS (Zoccal et al., 2014), whilst peripheral chemoreflex afferents and fast-adapting lung stretch receptor afferent terminate in the caudal NTS (Zoccal et al., 2014). Furthermore, connections between respiratory and sympathetic control regions elsewhere in the brainstem, such as input from Boztinger complex neurones to the RVLM facilitate the interactions between respiration and sympathetic outflow (Zoccal et al., 2014)

1.9.3 Sex and age differences in respiratory sympathetic modulation

1.9.3.1 In healthy adults

Previous work has studied the effect of ageing on respiratory sympathetic modulation. Shantsila et al. found that among healthy men, there was no effect of age on the degree of respiratory modulation of sympathetic activity, despite older men having a greater resting level of sympathetic activity (Shantsila et al., 2015). Whether respiration has a similar effect on sympathetic activation in older versus younger women is not fully understood. There is evidence that oestrogen is sympathoinhibitory in the brainstem (Saleh et al., 2000), therefore the decline in oestrogen with menopause is an additional factor influencing sympathetic outflow in older women versus older men. A further aim of this thesis was to determine whether age affected respiratory sympathetic modulation differently in healthy female participants (postmenopausal versus premenopausal), compared to male participants.

1.9.3.2 In disease states

Some research into respiratory sympathetic modulation in cardiovascular disease states exists. In hypertensive patients, respiratory sympathetic modulation did not appear to differ from normotensive controls (Fatouleh and Macefield, 2011). This cohort included some women, but the sample size was small, and it was not clear whether menopausal status was controlled for, therefore it is not fully clear whether hypertension has an effect on respiratory modulation in women. In

patients with heart failure, respiratory sympathetic modulation was linked to resting level of sympathetic activity, such that individuals with higher resting burst incidence had less respiratory modulation of MSNA (Goso et al., 2001). Whilst heart failure is a more severe pathophysiological state than hypertension (Drazner, 2011), some hypertensive individuals also display high resting levels of sympathetic activity (Abraham William et al., 2015). Therefore, a final aim of this thesis was to determine whether hypertension affects respiratory sympathetic modulation differently in older female versus male participants, and whether this was associated with resting burst incidence.

1.10 Hypothesis

The overall experimental and null hypotheses of this thesis are as follows:

H_1: There is a difference in sympathetic regulation of blood pressure between hypertensive and normotensive (1) premenopausal female participants and (2) postmenopausal female participants.

H_0: There is no difference in sympathetic regulation of blood pressure between hypertensive and normotensive (1) premenopausal female participants and (2) postmenopausal female participants.

1.11 Aims

Related to the experimental hypothesis, this thesis has a number of aims across the different chapters.

Chapter 3:

1. To determine whether sympathetic transduction is altered in hypertensive versus age-matched normotensive premenopausal female participants.
2. To determine whether sympathetic transduction is altered in hypertensive versus age-matched normotensive postmenopausal female participants.
3. To investigate whether there is an association between age and sympathetic transduction among pre-, peri- and postmenopausal female participants.

Chapter 4:

1. To determine whether hypertension in premenopausal female participants is associated with reduced beta-adrenergic sensitivity versus age-matched normotensive female participants.

Chapter 5:

1. To determine whether resting levels of sympathetic transduction are maintained during static exercise in hypertensive and normotensive younger and older male and female participants.

Chapter 6:

1. To determine whether respiratory sympathetic coupling is maintained in healthy postmenopausal versus premenopausal female participants.
2. To determine whether respiratory sympathetic coupling is altered in hypertensive versus normotensive (1) postmenopausal female participants and (2) older male participants.

Chapter 2 General Methods

2.1 Overview

The data in this thesis were collected both in previous research studies and in a novel research study. The sections below describe the methods related to data collection in the novel study, before discussing methods common to all of the studies.

2.2 Novel data collection

2.2.1 Ethical approval

The novel data presented in this thesis were collected in a study titled: Sex Differences in the Role of Sympathetic Nerve Activity in the Development of Hypertension in Humans (IRAS ID: 243054). The study sponsor was the University of Bristol and the research took place at the University Hospitals Bristol and Weston NHS Foundation Trust (UHBW) Clinical Research Facility (CRF) (formerly the Clinical Research and Imaging Centre, University of Bristol), in collaboration with the NHS Trust. The study was reviewed by the South West Frenchay NHS Research Ethics Committee (18/SW/0237) on 12/10/2018 and received approvals from the Research Ethics Committee, Health Research Authority, local NHS Research and Innovation Department (UHBW), study site and sponsor prior to commencement. The study was conducted in accordance with the Health Research Authority-approved study protocol and the principles of the UK Policy Framework for Health and Social Care Research, which is based in part on the Declaration of Helsinki (Health Research Authority, 2017). The study received support from the West of England Local Clinical Research Network. Additionally, the study was registered on the ISRCTN registry under the registration number ISRCTN13479086. The period from initial submission of the ethics application for Sponsor review (06/02/2018) to receipt of the Sponsor letter (22/02/2019) was over one year.

2.3 Data management

Study data were collected in accordance with UK law and General Data Protection Regulation (UK Government, 2018). Information on the use of confidential data was outlined to participants in section 13 of the participant

information sheet (Appendix 1). Identifiable information was anonymised using a study identification number, from which only the study team could identify individual participants. Study data (paper and electronic files) will be stored securely at the University of Bristol for 15 years from the date of the last participant study visit.

2.3.1 Participants

2.3.1.1 General criteria for inclusion

i. Aged 18 to 75 years.

2.3.1.2 Criteria for inclusion of postmenopausal female participants

i. Amenorrhoea for at least 12 months in the absence of hormonal contraception, as per national guidelines (National Institute for Health and Care Excellence, 2015).

2.3.1.3 General criteria for exclusion

i. Major illness (for example cancer, inflammatory disease), or in receipt of palliative care.

ii. Diagnosis of cardiovascular, respiratory (including asthma), psychiatric, renal or ophthalmic disease.

iii. Congenital or acquired neurological conditions (including dementia).

iv. Diabetes.

v. Chronic pain conditions (excluding menstrual pain and minor occasional headaches).

vi. Language disorders.

vii. Use of nitrate, steroid, anti-coagulant or immunosuppressant medication, or use of medication as part of a clinical trial.

viii. Body mass index (BMI) $\geq$ 30 kg/m^2.

ix. Regular consumption of > 28 units of alcohol per week or use of illicit drugs.

x. Symptoms of febrile illness less than a week before the experiment.

xi. Phobia of needles that would prevent participation.

xii. Inability to understand instructions given in English.

xiii. Secondary causes of hypertension, if applicable.

2.3.1.4 Criteria for exclusion of female participants

i. Current pregnancy or breastfeeding.

ii. Oophorectomy prior to natural menopause.

iii. Use of hormone replacement therapy.

2.3.2 Participant recruitment

Hypertensive patients were recruited through the Bristol Heart Institute Specialist Hypertension Clinic. Participants in all groups were recruited from the University of Bristol and surrounding community through emailed advertisements and newsletters, the CRIC Bristol website, and paper leaflets and posters left in University buildings and community buildings such as shops and libraries. Through the Primary Care department of the West of England CRN, it was arranged for study leaflets and posters to be displayed in GP waiting rooms across Bristol, North Somerset, and South Gloucestershire. Additionally, some participants were recruited through the CardioNomics Research Group participant database, which stores details of individuals who agree to be contacted about involvement in studies.

2.3.3 Recruitment monitoring

The study opened to recruitment on 06/03/2019. Participants who completed a telephone screening were assigned a screening identification number. Those who were enrolled onto the study were assigned a study identification number following receipt of informed consent. Participants were logged in a local portfolio management system (EDGE, Clinical Informatics Research Unit, c/o The National Blood and Transplant Service, Southampton, UK) following telephone screening. As part of inclusion on the NIHR portfolio, non-identifiable study recruitment data was recorded in the NIHR Clinical Research Network Central Portfolio Management System. The participant information sheet was sent to 295 individuals who had expressed interest in the study. Of these, 61 individuals were screened and 25 were recruited. The first participant was recruited on 22/07/2019.

2.3.4 Impact of COVID-19

Study recruitment paused between March 2020 and October 2020 during the first COVID-19 lockdown and again in December 2020/January 2021 during the

second and third lockdowns. Therefore, the study was closed for approximately eight months in total.

2.3.5 Written informed consent

Participants gave written informed consent to participate by signing a consent form (Appendix 2), having been informed of the potential risks of participating by reading the participant information sheet and receiving a verbal explanation from a researcher. Participants had the opportunity to ask questions of the researcher before giving consent to participate. Participants were reminded of their right to withdraw from the study at any time, without the need to provide an explanation.

2.3.6 Screening

2.3.6.1 Telephone screening

Individuals interested in participating completed a telephone screening questionnaire with a researcher to determine their eligibility (Appendix 3). Those eligible according to the telephone screening were then invited to take part.

2.3.6.2 In-person screening

After giving informed consent, participant eligibility on the day of the study was determined by the following screening procedures. Ineligible participants did not take part and were recorded in the recruitment records as a screen fail.

i. Medical history questionnaire to record details of any current or historic disease and family history of disease (Appendix 4).

ii. Height and weight measurements using stadiometer (Seca, Hamburg, Germany) and scales (Seca, Hamburg, Germany) for calculation of BMI.

iii. 12-lead electrocardiograph (ECG) (MAC 1600, GE Medical Systems, Chicago, USA) to confirm absence of any obvious cardiovascular disease.

iv. Clinic blood pressure measurements (705IT, Omron Healthcare, Kyoto, Japan) to determine hypertensive status. Readings were taken twice on each arm, ignoring first reading.

v. Urine dipstick (Multistix 8SG, Siemens Healthcare, Erlangen, Germany) to confirm absence of any obvious renal disease.

vi. Beta-human chorionic gonadotropin urine test (hCG Easy 25, Alere (now Abbott Laboratories), Illinois, USA) to confirm absence of pregnancy in premenopausal females.

2.3.6.3 Ambulatory blood pressure monitoring

At the end of the main study activities, participants were given an ambulatory blood pressure monitor (ABPM) (Mobil-o-graph, IEM GmbH, Stolberg, Germany; or 90217A, Spacelabs, Snoqualmie, USA) to wear at home over 24 hours. The monitors automatically measured blood pressure at 30-minute intervals during the day and at 60-minute intervals during the night. Participants were instructed in correct positioning and use of the monitor. Results of the ABP monitoring did not determine eligibility to participate but were used to categorise participants into hypertensive and normotensive subgroups.

2.3.7 Allocation to subgroups

Hypertension status was determined in accordance with NICE guidelines (National Institute for Health and Care Excellence, 2019), and the European Society for Hypertension, (Williams et al., 2018). Clinic blood pressure of ≥ 140 / 90 mmHg and daytime ambulatory blood pressure ≥ 135 / 85 mmHg was considered hypertensive. There were insufficient numbers of participants to have sub-groups of isolated systolic hypertension and white-coat hypertension. Therefore, where participants had hypertensive systolic but not diastolic blood pressure, they were classified as hypertensive. Where clinic but not ambulatory blood pressure was high, participants were classified as normotensive. Participants with hypertension that was unknown to them prior to their involvement in the study were included in the hypertensive group.

2.3.8 Incidental findings

In some participants, screening and study procedures identified abnormal results. Participants were notified of these results and advised to see their GP. If participants gave consent, participants' GPs were also directly informed of the incidental findings. Nine participants were identified as having hypertension that was previously unknown to them. In one participant, the study echocardiogram identified abnormalities that required the participant to have subsequent investigations and treatment, and the participant was excluded.

2.3.9 Blood sampling of oestradiol and progesterone

A sample of venous blood was collected into a serum separating tube (Vacutainer SST II Advance 3.5 ml, BD, New Jersey, USA). Samples were transported at room temperature to the Unit for the Support of Trials and Research, Department of Clinical Biochemistry, Bristol Royal Infirmary. Serum

was extracted and oestradiol and progesterone concentrations determined by electrochemiluminescence immunoassay using an Elecsys and Cobas e analyser and the Elecsys oestradiol II or Elecsys progesterone III immunoassay (all Roche, Basel, Switzerland). The electrochemiluminescence immunoassay technique combines antibody binding to the substrate of interest with the use of an electrochemiluminescent label in order to determine the concentration of the substrate. Most assays of this type use ruthenium as the label which, in the presence of a triphenylamine co-reactant, emits light at 620 nm following electrically induced reduction and oxidation (Muzyka, 2014). The Elecsys oestradiol and progesterone immunoassays use a competitive method, where the substrate competes with an analogue for binding to the antibody (Muzyka, 2014). In this case the substrate is endogenous oestradiol or progesterone, and the analogue is ruthenium-labelled oestradiol or progesterone derivatives (Morgan and Witham, 2015, Roche Diagnostics, 2016). The signal produced by the electrochemiluminescent reaction reflects the concentration of the remaining antibody-bound analogue (Muzyka, 2014), thus endogenous oestradiol or progesterone concentration can be calculated from two-point calibration data (Morgan and Witham, 2015, Roche Diagnostics, 2016). Samples were processed anonymously, and results were uploaded to the UHBW internal system. Reference ranges for oestradiol and progesterone are shown in tables 2.1 and 2.2.

2.3.10 Echocardiography

2.3.10.1 Overview of the technique

Echocardiography is the technique of imaging the heart using ultrasound and was used here to obtain a value of stroke volume, which was used to calculate resting cardiac output. Stroke volume is calculated by multiplying the cross-sectional area of the left ventricular outflow tract (LVOT) by the velocity time integral (VTI) of blood flow in the LVOT (equation 2) (Tan et al., 2017). LVOT cross-sectional area is determined by equation 1 (Robson et al., 1988), using LVOT diameter measured in mid-systole from a 2D image of the LVOT in the parasternal long axis view (Wharton et al., 2015). LVOT VTI is measured by pulse wave doppler in the apical 5 chamber view. Both LVOT diameter and VTI are measured < 1 cm from the annulus of the aortic valve (Wharton et al., 2015). Cardiac output is then calculated using equation 3 (Robson et al., 1988) and cardiac index (cardiac output normalised for body surface area) by equation 4 (Tibby et al., 1997).

Equation 1. LVOT cross-sectional area = π(LVOT diameter/2)^2

Equation 2. Stroke volume = LVOT cross-sectional area x LVOT VTI

Equation 3. Cardiac output = stroke volume x heart rate

Equation 4. Cardiac index = cardiac output / body surface area

2.3.10.2 Accuracy and reliability of the method

Doppler ultrasound measurement of cardiac output has been shown to generate values similar to those generated by other techniques. Among cardiology patients, Doppler ultrasound estimates of cardiac output correlated well with estimates by the Fick method (r = 0.90) (Gola et al., 1996) and thermodilution method (r = 0.95) (Lewis et al., 1984). Much of the work comparing Doppler ultrasound to other techniques of measuring cardiac output has been done in patients rather than healthy volunteers, due to the invasiveness of the Fick and thermodilution methods which require catheterisation of the right side of the heart (Lewis et al., 1984). Studies in both heart failure patients (Pozzoli et al., 1995) and healthy participants (Robson et al., 1988) have demonstrated little variation in repeat measurements using the Doppler technique over the short-term (minutes) and longer-term (months) (Robson et al., 1988). For example, repeat measurements in healthy individuals showed an error of 4.1 ± 2.9 % (mean ± SD) when for measurements repeated within an hour, and 6.6 ± 2.0 % for repeats conducted between one and three months of the original measurement (Robson et al., 1988).

2.3.10.3 Study echocardiography protocol

Cardiac output measurements by echocardiography were obtained with an Aplio 500 ultrasound machine and PST-30BT cardiac transducer (Canon, Tokyo, Japan) by an experienced operator (not the author). Participants rested semi-supine whilst the operator obtained the parasternal long axis and apical 5-chamber views, and measured LVOT diameter and VTI. Continuous blood pressure and heart rate were monitored simultaneously (see 2.11). Cardiac output was calculated according to the above equation after the study. Where more than one measurement of LVOT or VTI was taken, these were averaged before cardiac output was calculated.

2.4 Microneurography

2.4.1 The technique of microneurography

Microneurography is a technique by which the activity of nerve fibres is directly measured by an electrode inserted into a peripheral nerve of a conscious participant. The method was developed in Sweden by Karl-Erik Hagbarth and Åke Vallbo, who first presented work on the technique in 1966 (Vallbo, 2018, Vallbo and Hagbarth, 1967). It was quickly demonstrated that the method could be used to record activity in a variety of fibres, including skin sensory afferents (Vallbo and Hagbarth, 1968), muscle afferents (Hagbarth and Vallbo, 1968b), sympathetic c-fibres (Hagbarth and Vallbo, 1968a) and nociceptive c-fibres (Hallin and Torebjörk, 1973). The electrodes used are made of tungsten, which is sufficiently strong but not liable to snapping (Vallbo, 2018). The nerves selected for the procedure are superficial, for example the peroneal, tibial, radial and ulnar nerves (Hart et al., 2017). The recording electrode, which has impedance as a result of an epoxy coating, is inserted into the nerve, whilst a reference electrode with no impedance is inserted subcutaneously 1-2 cm away (Hart et al., 2017). The recording electrode is then manipulated to enter a fascicle (Vallbo, 2018). In post-mortem studies, human peroneal nerve slices were found to contain 25-38 fascicles, the median size of which was 88 μm^2 (Tompkins et al., 2013). The tip of the recording electrode is ~3-5 μm in width, however the electrode does not always pierce a fascicle upon entering the nerve and has to be manipulated until it does (Vallbo, 2018). Once the electrode tip has entered a fascicle, it acts as a capacitor, accumulating charge relative to the charge of the area immediately surrounding the electrode tip (Vallbo, 2018). As such, the depolarisation of fibres close to the electrode tip can be detected and recorded (Hart et al., 2017).

2.4.2 Microneurographic recording of muscle sympathetic nerve activity

For the study of sympathetic blood pressure regulation, muscle sympathetic nerve activity (MSNA) rather than skin sympathetic nerve activity (SSNA) is recorded (Hart et al., 2017). The action potentials of muscle sympathetic fibres are triphasic in shape, with a negative element that corresponds to the depolarising current of the nerve fibre (Vallbo, 2018). Given that MSNA is baroreflex-controlled (Vallbo et al., 2004), the firing of muscle sympathetic fibres is inhibited at every systole (Hart et al., 2017). As such, MSNA occurs in pulse-synchronous ‘bursts’ (Hart et al., 2017), triggered during diastole (White et al.,

2015). However, activity may not occur with every diastole, depending on baroreflex afferent input (Vallbo et al., 2004). Given that the conduction velocity of unmyelinated c-fibres is ~1 m/s (Hagbarth and Vallbo, 1968a) there is a short lag between the detection of a burst of MSNA at the electrode and the cardiac cycle to which the burst is related. This is termed burst latency and is usually ~ 1.3 s in duration (Hart et al., 2017). A signal is therefore confirmed as MSNA by the presence of pulse-synchronous bursts, occurring ~1.3 ± 0.5 s after an R wave (Hart et al., 2017). An increase in activity at the end of an end-expiratory apnoea, which elicits chemoreflex-driven sympathetic activation, can be used as further confirmation of an MSNA signal. To rule out the presence of SSNA, the absence of a signal change in response to a startle stimulus (shout or loud clap) should also be confirmed (Hart et al., 2017).

The most common method of quantifying sympathetic activity from MSNA recordings is determining the number of cardiac cycles in which some level of MSNA occurs. This is done by identifying bursts in the signal that are large enough to be seen above the noise, usually at a 3:1 signal to noise ratio (White et al., 2015). The number of bursts can be quantified over a period time (burst frequency; bursts/min) or can be normalised to heart rate (burst incidence, bursts/100 heartbeats) (White et al., 2015). The amplitude of integrated bursts of MSNA depends on both the number of active fibres and the amplitude of the action potentials recorded (Shoemaker, 2017), which in turn depends on fibre proximity to the electrode (Hart et al., 2017). Therefore, direct comparison of burst amplitude or area between recordings is invalid. However, any change in burst amplitude or area within the same recording, for example in response to a stimulus, is possible (Hart et al., 2017). For this measure, burst amplitude is normally expressed as a percentage of the amplitude of the tallest burst in the recording (White et al., 2015).

As a technique for measuring sympathetic activation, microneurography is advantageous over methods such as noradrenaline spill over, in that the recordings are continuous (Hart et al., 2017). Although the participant must keep the recording limb very still, exercise of the other limbs is possible (White et al., 2015). Furthermore, research has confirmed that MSNA is highly reproducible within the same individual over different days at least 3 weeks apart (Sundlof and Wallin, 1977). A recording of MSNA in one limb is generally thought to be representative of sympathetic activity in other limbs (Sundlof and Wallin, 1977), allowing a choice of recording sites. Disadvantages of the method include the potential for side effects (section 2.9.3), however these are usually mild and

relatively uncommon (Meah et al., 2019). Microneurography is mentally demanding for the operator (Vallbo et al., 2004) and can take one year to learn (Hart et al., 2017) but despite this, the technique is incredibly useful in a human integrative physiology laboratory.

2.4.3 Safety of the technique

In general, the potential side effects associated with microneurography occur infrequently. During the search for a recording site, participants often experience temporary sensations in the leg, ankle and foot, including paraesthesia, cramping sensations and sensations of hot or cold. These sensations are an expected consequence of microneurography and are tolerable for the vast majority of participants (Meah et al., 2019). Other side effects associated with the search for a site are pain and pre-syncopal symptoms. A recent review of microneurographic studies reported incidences of 3.8 %, 1.4 % and 8.9 % for moderate to severe sensations, pain and pre-syncope, respectively (Meah et al., 2019). Following microneurography, side effects of persistent paraesthesia, pain and muscle weakness can occur. These symptoms occur within hours to days after the procedure and can last for several weeks (Meah et al., 2019). Across the studies assessed by Meah et al., persistent paraesthesia was experienced by 4.6% of participants, pain by 2.8% and muscle weakness by 1.5% (Meah et al., 2019). There are occasional reports of longer-term complications following microneurography, such as long-lasting paraesthesia, pain and/or muscle weakness lasting 6 months or more. However, these cases represent 0.2% of the participants in the studies included in the review by Meah et al. In addition, infection is a possible risk of microneurography, although data on the incidences of infection are infrequently reported (Meah et al., 2019). In the experiments presented in this thesis, risk of infection was reduced by using sterile electrodes, cleaning the skin with alcohol before placing the electrodes, and using a ‘no touch’ method, where the region of the electrode held by the researcher did not pass through the skin.

Eckberg et al. reported that the risk of long-term complications was reduced when a recording site was found within 45 minutes. As a result, they suggested that microneurographic searching time should be limited to 60 minutes. In addition, they recommended that the operator should be sufficiently trained and that the procedure is not repeated in the same limb within one month (Eckberg et al., 1989). To reduce the risk of long-term side effects, these guidelines were followed in the experiments discussed in this thesis.

2.4.4 Microneurography protocol for novel data collection

Tungsten microelectrodes (length 35 or 40 mm, diameter 200 μm, impedance 2 ± 0.4 MΩ with an epoxy-coated tip (active electrodes) or no impedance (reference electrodes); FHC, Bowdoin, USA), were sterilised by ethylene oxide (Andersen Caledonia, Bellshill, UK). Participants were asked to sit in a semi-supine position with their leg elevated such that the region between the back of the knee and fibula head was easily accessible. This position was adjusted until participants were comfortable and able to release all tension from their leg. The common peroneal nerve was located by palpation at the fibula head and the region immediately proximal. The location of the nerve was confirmed by induction of dorsiflexion or eversion of the foot with an electrical stimulus applied to the skin above the suspected location of the nerve (stimulus isolator and stimulator rod (AD Instruments, Dunedin, New Zealand); 1-4 mA pulses applied at a rate of 1 Hz lasting 1 ms). The site for the placement of the electrodes was chosen according to ease of identification of the nerve by palpation, the strength of the twitch elicited by stimulation and ease of access with the electrodes. The active electrode was placed across the skin at the suspected location of the nerve, and the reference electrode was placed across the skin ~1-2 cm away. Both electrodes were attached to a preamplifier and the signal was fed from the preamplifier to an amplifier (662C-4 Nerve Traffic Analyzer, University of Iowa Bioengineering, Iowa, USA), where the signal was processed (see 2.9.5). The audio was played for the operator to hear.

The active electrode was angled and advanced in a systematic fashion until either the participant reported sensations in their leg, or the researcher identified an appropriate sound associated with the location of the electrode. The signal was then assessed by the researcher and either the signal was recorded, or the electrode was moved again. This process was repeated until MSNA was identified, the 60-minute search time expired, or the participant requested that the procedure be stopped. MSNA was identified by the presence of narrow, pulse-synchronous bursts of activity, in the absence of activity elicited by light touching of the skin (activates sensory afferents in the skin and suggests that SSNA could be present (White et al., 2015)) or a startle stimulus. The MSNA signal was confirmed by increased activity during an end-expiratory apnoea.

2.4.5 Data acquisition and real time processing

The raw signal was processed in real time by the amplifier and fed into a Powerlab (see 2.14). A total amplification of 80,000 times was achieved by the preamplifier and amplifier. The signal was bandpass filtered between 0.7-2 kHz and displayed as an amplified, filtered signal. Additionally, the signal was rectified and integrated (0.1 s time constant) and displayed separately as an integrated signal. The amplified, filtered signal was sampled by the Powerlab at 10 kHz and the integrated signal sampled at 1 kHz.

2.5 Physiological monitoring

2.5.1 ECG monitoring

ECG was monitored in all studies contributing data to this thesis. In the novel study, Lead II ECG was monitored throughout the study using a 3-lead system and Bioamp (both AD Instruments, Dunedin, New Zealand). The ECG was sampled at a rate of 1 kHz and a bandpass filter of 0.3 to 1000 Hz was applied.

2.5.2 Continuous blood pressure monitoring

In the studies contributing data to this thesis, blood pressure was measured continuously by either photoplethysmography or intra-arterial pressure transducer. Photoplethysmography (Finometer PRO, Finapres Medical Systems, Enschede, the Netherlands) uses an infrared emitter and receiver inside a finger cuff to monitor the volume of arterial blood in the finger. Cuff pressure is rapidly adjusted so that the same finger arterial blood volume (measured by a constant infrared signal) is maintained throughout the arterial pressure waveform, (Stokes et al., 1991). Therefore, changes in cuff pressure are representative of changes in finger arterial pressure (Langewouters et al., 1998). When cuff pressure is such that there is zero transmural pressure in the finger artery, cuff pressure is equal to arterial pressure, thus allowing the continuous monitoring of finger arterial pressure (Langewouters et al., 1998). Finger pressure can be used to derive brachial pressure, once corrections for differences in the waveforms and pressure gradients are made (Bos et al., 1996). In this thesis, derived brachial blood pressure was used in the analyses unless otherwise stated. The absolute brachial blood pressure estimates determined by this method are of limited accuracy compared to invasive methods of measuring continuous blood pressure. For example, in patients receiving cardiac or neurological surgery,

correlations between systolic and diastolic measurements made via Finometer and arterial line had correlation coefficients of 0.82 and 0.68, respectively (Stokes et al., 1991). However, the Finometer correctly detected the direction of blood pressure changes compared to arterial line in the majority of measurements (overall 83 % correct direction detection across 30-s measurement intervals) (Stokes et al., 1991). The primary outcome for the analyses in this thesis is sympathetic transduction slope, which is quantified using changes in diastolic blood pressure, rather than absolute blood pressure values. Where blood pressure was monitored by intra-arterial pressure transducer, this method is discussed in the relevant chapter.

2.5.3 Respiratory monitoring

Respiration was monitored using a respiratory belt (capacitive sensor, AD Instruments, Dunedin, New Zealand) that was fitted around the participant's thorax at the level of the diaphragm. The change in position of the belt with breathing was recorded and used to identify inspiratory and expiratory phases of the respiratory cycle. The data were sampled at 200 Hz.

2.6 Handgrip

Participants performed isometric handgrip exercise using a hand-held force transducer (AD Instruments, Dunedin, New Zealand). Force transducer output was fed into the Powerlab and sampled at 10 kHz. Force was normalised to the greatest value generated by the participant over three maximal voluntary contractions via a two-point calibration. Normalised force was expressed in % of maximum. Participants were asked to maintain two minutes of isometric handgrip exercise at 40% of their maximum and were aided in this by receiving visual feedback via Labchart.

2.7 Data acquisition

Data collected by microneurography and physiological monitoring were fed into a Powerlab 16/30 (AD Instruments, Dunedin, New Zealand). Data were displayed in real time through LabChart software (v7, AD Instruments, Dunedin, New Zealand).

2.8 Data analysis

2.8.1 Analysis of muscle sympathetic nerve activity

2.8.1.1 Overview

MSNA and physiological monitoring data from all studies were analysed in Spike2 (version 8, Cambridge Electronic Design, Cambridge, UK). MSNA, ECG and blood pressure data were analysed using a custom semi-automated script (E. Hart 2013, edited by Z. Adams & H. Blythe 2020). Peaks in the integrated neurogram above the signal noise were marked and confirmed as bursts of MSNA by checking that they occurred within ~0.9-1.4 s of an R wave, depending on individual average burst latency. A negative relationship between burst amplitude and burst latency was confirmed in each individual before burst identification was finalised. MSNA bursts associated with premature ventricular contractions (PVC) were removed from the analyses by discounting the period in which they occurred (three R waves before to two R waves after the PVC). The marking of MSNA bursts was conducted blind to participant group in all analyses. In beta-blockade studies, MSNA bursts were marked blind to experimental condition as well as participant group.

2.8.1.2 Intra-observer reliability of MSNA analysis

Intra-observer reliability of MSNA burst identification (quantified as burst incidence) by the researcher (Z. Adams) was measured across recordings from four individuals, which were each analysed three times on separate days. The average coefficient of variation for the repeat analyses was 2.8 ± 2.1 % (mean ± SD), ranging from 6.1 % in one individual to 0.29 % in another individual.

2.8.2 Analysis of haemodynamic variables

Using the script mentioned above, R waves in the ECG signal were detected and beat to beat heart rate was calculated from the time difference between consecutive R waves. Beat to beat systolic and diastolic blood pressure was calculated at the peak and trough of each blood pressure waveform, respectively. Further details about specific data analyses are included in each results chapter.

2.8.3 Analysis of sympathetic transduction

Sympathetic transduction analysis aims to quantify the vasoconstrictor effect that a given amount of sympathetic nerve activity has on skeletal muscle vasculature

(Hart and Charkoudian, 2014). As a concept, its importance lies in the fact that simply measuring activity of the sympathetic nervous system does not provide information on the actual effect of that sympathetic activity on the vasculature (and hence on blood pressure). This is because research has shown that sympathetic nerve activity is known to be uncoupled from blood pressure in young adults (Narkiewicz et al., 2005) and from peripheral resistance in young women (Hart et al., 2009). Sympathetic transduction is the primary outcome for most of the chapters in this thesis.

Measures of sympathetic transduction have been reported for a number of years. The simplest way to quantify sympathetic transduction is to measure the average blood pressure for a given level of sympathetic nerve activity (Jarvis et al., 2014), or the ratio of the changes in these variables (Notarius et al., 2012). When this method uses only single values (e.g. (Jarvis et al., 2014)), it provides information on the overall relationship between sympathetic activity and blood pressure but provides little detail about the acute effect that individual MSNA bursts have on the vasculature. Vianna et al. developed a technique that tracked the vascular response to single bursts of MSNA over a series of subsequent 15 cardiac cycles (Vianna et al., 2012), with vascular conductance quantified using blood pressure (Vianna et al., 2012) or vascular conductance (Fairfax et al., 2013b). This analysis was later extended to include series of bursts occurring in consecutive cardiac cycles (i.e., sets of two, three or four bursts), in order to assess the impact of burst series on the vasculature (Fairfax et al., 2013a), with comparison between experimental conditions or individuals achieved by generating a slope of MSNA area against the peak of the vascular response over the series of cardiac cycles (Fairfax et al., 2013a). Whilst informative, this technique becomes difficult when participants have high levels of MSNA, given that there are few non-bursting periods to compare the bursting periods to (Briant et al., 2016). Briant et al. developed a separate technique that overcomes this by relating the activity in the integrated neurogram across (sampled at every cardiac cycle) to subsequent changes in blood pressure. The area under the curve of the integrated neurogram is sampled across two-cardiac cycles and associated with subsequent diastolic blood pressures (Briant et al., 2016). Using MSNA area accounts for the size of any MSNA bursts, so when burst incidence is high, the variation in burst size is still accounted for. Diastolic blood pressure was chosen as the dependent variable in this analysis, given that it can be measured using a Finometer and is representative of vascular tone (Briant et al., 2016).

This thesis quantified sympathetic transduction using Briant et al.'s method, given that recording of vascular conductance with ultrasound was not possible and that high levels of MSNA burst incidence were observed in some participants. The analysis was conducted using a custom script (Spike2, Cambridge Electronic Design, Cambridge, UK, script by Z. Adams). Bursts of MSNA were identified prior to starting the analysis. The script normalised the integrated neurogram to 100 % in a two-point calibration using user-defined values, where 0 % was the amplitude of a period of signal noise (no bursts) and 100 % was the amplitude of the tallest burst in the analysis window. The script then identified individual diastolic blood pressures and measured the area under the curve (modulus) of the normalised integrated neurogram over two cardiac cycles at a pre-determined lag behind the diastolic blood pressure. In each individual, the analysis was repeated for eight different lags, ranging from 1-3 cardiac cycles to 8-10 cardiac cycles behind the diastolic blood pressure, where cardiac cycle position one was the assigned to the R wave triggering the diastolic blood pressure (checked using recordings containing PVCs or arrhythmias, and assumes the same electrical delay in data acquisition system in all individuals).

2.8.4 Statistical analysis

Statistical analysis was conducted in IBM SPSS Statistics 24 (IBM, New York, USA). Details of specific statistical analyses are given in each chapter, however the majority of the analyses in this thesis aimed to compare variables between participant groups (e.g., T tests or ANOVA, or non-parametric equivalents). For each analysis, whether the data met the assumptions of the statistical test was checked in SPSS. Where data violated assumptions, this is discussed and either the data were transformed, or a non-parametric test was used. Throughout, *P* value <0.05 was considered significant. Data are displayed as mean ± standard deviation (SD) or median ± interquartile range, unless otherwise stated in the chapter. Throughout, data were graphed using GraphPad Prism (version 8, GraphPad Software, San Diego, USA).

2.9 Tables and figures

Table 2.1 Reference ranges for serum oestradiol concentration.

Group	Reference range (pmol/l)
Premenopausal female (follicular phase)	46 – 607
Premenopausal female (luteal phase)	161 – 774
Postmenopausal female	<200
Male (13 – 120 years)	28 – 156

Table 2.2 Reference ranges for serum progesterone concentration.

Group	Reference range (nmol/l)
Premenopausal female (follicular phase)	0.588 (0.181 – 2.84)
Premenopausal female (ovulation phase)	1.60 (0.385 – 38.1)
Premenopausal female (luteal phase)	31.9 (5.82 – 75.9)
Postmenopausal female	<0.159 (<0.159 – 0.401)
Male	<0.159 (<0.159 – 0.474)

Median (5^{th} percentile – 95^{th} percentile).

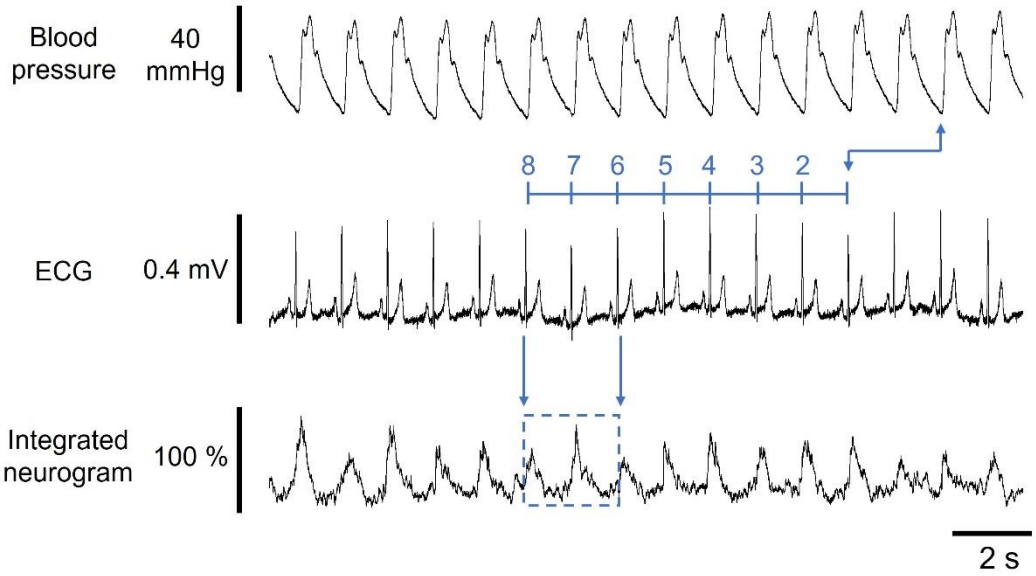

Figure 2.1 Sympathetic transduction analysis. Modulus of the normalised integrated neurogram across two cardiac cycles (dashed box) was measured between the R waves corresponding to cardiac cycles six and eight behind the relevant diastolic blood pressure (arrow). The R wave assigned to cardiac cycle number one (arrow) is the R wave associated with the diastolic blood pressure wave.

Chapter 3 Sympathetic transduction in hypertensive premenopausal and postmenopausal females.

3.1 Note on use of existing data

Some of the analysis in this chapter used data collected in previous studies. The data may have been published before but the analysis presented here is novel and unpublished. The analysis was conducted solely by the author using analysis scripts written by the author unless stated otherwise. In Table 3.1, all the MSNA data collection in the first study and some of the data collection in the second study (7/12 files) was done by the author.

3.2 Background

3.2.1 Introduction

There are sex differences in hypertension prevalence, such that females are less likely than males to develop hypertension in early adulthood, but more likely to be hypertensive than males in later life (Burt et al., 1995, Benjamin et al., 2017, Gu et al., 2002). Research has demonstrated that there are sex differences in sympathetic regulation of blood pressure that could act to reduce hypertension risk in premenopausal females relative to young males (Hart et al., 2011a). However, current understanding does not explain cases of hypertension in premenopausal females or explain why some postmenopausal females develop hypertension whilst others do not. This chapter considers whether sympathetic regulation of blood pressure is altered in hypertensive versus normotensive younger and older female participants.

3.2.2 Sympathetic blood pressure regulation in young adults

In both younger males and females without hypertension, blood pressure is dissociated from the resting level of sympathetic nervous system activity (Narkiewicz et al., 2005). In premenopausal females, this dissociation extends to sympathetic control of the vasculature, such that resting total peripheral resistance is not related to resting MSNA (Hart et al., 2009). As such, when the influence of MSNA on the vasculature (sympathetic transduction) is quantified, healthy premenopausal females display a lower transduction of MSNA into diastolic blood pressure compared to healthy young males (Briant et al., 2016).

These data are supported by work from other groups showing that vasoconstrictor response to a sympathoexcitatory stimulus (isometric handgrip exercise) was lower in healthy young females versus males, even though the groups showed similar sympathetic responses to the stimulus (Hogarth et al., 2007a). However, others have reported contradictory findings, with the ratio between diastolic blood pressure and sympathetic responses to handgrip exercise found to be similar in young males and females (Jarvis et al., 2011). Additionally, when sympathetic transduction was assessed as the blood pressure or vascular resistance change following individual bursts of MSNA, some groups have reported no sex difference among healthy adults (Vianna et al., 2012, Robinson et al., 2019, Hissen et al., 2019).

Level of sympathetic transduction could influence hypertension risk, such that the relatively low level of transduction in premenopausal females could reduce hypertension risk in this group (Hart et al., 2011a). However, hypertension is not non-existent in premenopausal females. Health Survey for England data show that 9 % of 35-44 year old females had hypertension in 2019, two-thirds of which were untreated (Lifestyles Team NHS Digital, 2020). Whether sympathetic transduction is altered in hypertensive premenopausal females is unknown. This chapter aims to address this question by comparing sympathetic transduction in hypertensive and normotensive premenopausal female participants (Aim 1).

In contrast, resting total peripheral resistance correlates positively with resting MSNA in healthy younger males (Hart et al., 2009) and their level of sympathetic transduction into diastolic blood pressure is higher than that of similarly-aged healthy females (Briant et al., 2016). The dissociation of resting blood pressure from resting MSNA is instead explained by a reciprocal relationship between MSNA and cardiac output, such that males with higher MSNA have higher vasoconstrictor tone, but lower cardiac output (Hart et al., 2009). This mechanism does not appear to prevent the development of hypertension in some younger males, however. When sympathetic transduction was quantified in untreated hypertensive and normotensive younger males, the hypertensive group were found to exhibit a lower level of transduction than healthy controls (Kobetic et al., 2022). Thus, increased sympathetic transduction does not appear to explain hypertension in younger males, given that hypertension occurred despite a reduced sympathetic transduction. Whilst this chapter is focused on hypertensive female participants, sympathetic transduction was also measured in hypertensive

and normotensive younger male participants, as a comparison to the female groups (Aim 2). It was anticipated that including a younger male group in the analysis would provide information on whether the effect of hypertension on sympathetic transduction was different in males and females. As such, this could indicate whether altered sympathetic transduction is a mechanism of hypertension development of particular importance in premenopausal female hypertension.

3.2.3 Sympathetic blood pressure regulation in older adults

Hypertension prevalence increases with age in males and females but, in contrast to early adulthood, prevalence of hypertension in females exceeds that of males in later adulthood (Burt et al., 1995, Gu et al., 2002, Benjamin et al., 2017). Additionally, sympathetic regulation of blood pressure is altered in ageing. Healthy postmenopausal females have been shown to exhibit an increased sympathetic transduction versus younger females (Briant et al., 2016), which may contribute to the elevated hypertension risk in postmenopausal females (Hart et al., 2011a). However, given that not all postmenopausal females develop a blood pressure within the hypertensive range, it is not clear whether sympathetic transduction is different in postmenopausal females with hypertension versus those without. This chapter aims to address this question by quantifying sympathetic transduction in age-matched hypertensive and normotensive postmenopausal female participants (Aim 1). In healthy ageing males, a reduction in sympathetic transduction has been observed (Briant et al., 2016), possibly as a result of alpha-adrenergic desensitisation that has been demonstrated in older males (Dinenno et al., 2002). Whether hypertensive older males display reduced sympathetic transduction similarly to normotensive older males is unclear. Therefore, this chapter additionally aims to measure sympathetic transduction in hypertensive and normotensive older male participants (Aim 2). Given the sex differences in the effect of age on sympathetic transduction in normotensive adults, it was anticipated that the effect of hypertension on sympathetic transduction may differ between postmenopausal female and older male participants, thus an older male group was included in the analysis to address this question.

3.2.4 Sympathetic transduction with ageing and menopause

Ageing in males and females is associated with a variety of changes to the sympathetic nervous system and the vasculature, including increased resting

MSNA (Keir et al., 2020), increased arterial stiffness (Mitchell et al., 2004) and poorer endothelial function (Benjamin et al., 2004). In ageing females, it is difficult to attribute these changes specifically to ageing, the effect of menopause, or both (Moreau et al., 2020), and similar difficulties exist with research into sympathetic transduction. Existing research has compared sympathetic transduction in postmenopausal females with that of young premenopausal females (30 years difference in group mean age) (Briant et al., 2016), and the level of sympathetic transduction in older premenopausal females is not known. Therefore, this chapter additionally aimed to correlate sympathetic transduction with age in pre- and postmenopausal female participants over a larger age-range than previous analyses (Aim 3). Given that ageing appears to affect sympathetic transduction differently in healthy males and females, sympathetic transduction was also correlated with age in younger and older male participants (Aim 3). This was done to determine whether the relationship between age and sympathetic transduction differed by sex, which could indicate sex-specific age-related changes in sympathetic blood pressure regulation.

3.2.5 Aims and hypotheses

Aim 1: To determine whether sympathetic transduction is altered in hypertensive versus normotensive premenopausal females and postmenopausal females.

> H_0: There will be no difference in sympathetic transduction between hypertensive and normotensive (1) premenopausal females and (2) postmenopausal females
>
> H_1: There will be a difference in sympathetic transduction between hypertensive and normotensive (1) premenopausal females and (2) postmenopausal females

Aim 2: To determine whether sympathetic transduction is altered in hypertensive versus normotensive younger and older males.

> H_0: There will be no difference in sympathetic transduction between hypertensive and normotensive (1) younger males and (2) older males.
>
> H_1: There will be a difference in sympathetic transduction between hypertensive and normotensive (1) younger males and (2) older males.

Aim 3: To determine whether a relationship exists between age and sympathetic transduction in (1) females and (2) males.

H_0: There will be no relationship between age and sympathetic transduction in females or males.

H_1: There will be a relationship between age and sympathetic transduction in females and males.

3.3 Methods

3.3.1 Participants

Sympathetic transduction was quantified in eight participant groups: (1) hypertensive premenopausal female (N=8), (2) normotensive younger premenopausal female (N=10), (3) hypertensive younger male (N=13), (4) normotensive younger male (N=15), (5) hypertensive postmenopausal female (N=11), (6) normotensive postmenopausal female (N=15), (7) hypertensive older male (N=15), and (8) normotensive older male (N=13). A combination of existing data and newly collected data were analysed. Participants were considered hypertensive if at least one of the following criteria was met: (i) participants had received a diagnosis of hypertension, (ii) participants used anti-hypertensive medication, (iii) daytime average ambulatory systolic blood pressure measured as part of the study was ≥135 mmHg. Female participants were considered postmenopausal if they reported at least one year of amenorrhoea in the absence of hormone replacement therapy (National Institute for Health and Care Excellence, 2015), with the exception of one normotensive postmenopausal woman who reported use of local hormone replacement therapy (for vaginal symptoms; this form of hormone replacement was not found to increase circulating oestrogen concentration above the normal level for postmenopausal females (Krause et al., 2010)). Premenopausal female participants took part during the early follicular phase of the menstrual cycle (identified by self-reported timing of menses), or at any time if they used hormonal contraception that delivers a constant level of hormones (e.g., progesterone-only pill, intrauterine device).

3.3.1.1 Use of existing data

Existing data from seven previously conducted studies were included in the analysis. One study was conducted at the Mayo Clinic and was given ethical approval by the Mayo Clinic Institutional Review Board. The remaining six studies were conducted at the University of Bristol and the University Hospitals Bristol and Weston NHS Foundation Trust, and all received ethical approval from an NHS Research Ethics Committee. One of the Bristol studies had a second site at the Medical University of Gdansk and ethical approval was received for that site from the Medical University of Gdansk Independent Bioethics Commission for Research. Details of these studies are included in Table 3.1. The data collected

in these previous studies have in some cases been published elsewhere, but the analysis presented in this chapter has not been conducted or published previously.

3.3.1.2 Novel data collection

Novel data analysed in this chapter was collected as part of a study at the University of Bristol and University Hospitals Bristol and Weston NHS Foundation Trust (Sex Differences in the Study of SNA in Hypertension in Humans). The study received ethical approval from an NHS Research Ethics Committee in October 2018 (REC reference 18/SW/0237).

3.3.2 Procedures

In all studies, resting MSNA was measured by microneurography in the common peroneal nerve (detailed description in section 2.10; (Hart et al., 2017)). All studies used the same system to record MSNA (Nerve Traffic Analyser, University of Iowa Bioengineering, Iowa, USA) and followed a standardised procedure (Hart et al., 2017). Simultaneous ECG was recorded by 3-lead ECG (AD Instruments, Dunedin, New Zealand). In most participants, blood pressure measurements were made by Finometer (Finapres Medical Systems, the Netherlands). In four participants (all normotensive older males) who took part in the study at the Mayo Clinic, blood pressure was measured by a pressure transducer inserted into the brachial artery. MSNA, ECG and blood pressure were recorded at rest.

3.3.3 Data analysis

A 5-10-minute period of recording during quiet rest was extracted and used in the analysis. MSNA bursts were marked using a custom script (Spike2, Cambridge Electronic Design, Cambridge UK; by E. Hart, edited by H. Blythe and Z. Adams). Resting burst incidence and burst frequency were calculated across the entire analysis window. Burst latency was calculated for every burst and averaged across the analysis window. Heart rate was calculated beat to beat from the ECG and systolic, diastolic, pulse pressure and mean arterial blood pressure were calculated for every blood pressure waveform. Heart rate and blood pressure measures were then averaged across the analysis window.

3.3.4 Analysis of sympathetic transduction

Sympathetic transduction was analysed using a custom script (Spike2, Cambridge Electronic Design; written by Z. Adams) according to a previously established method (section 2.14.3) (Briant et al., 2016). Following identification of bursts of MSNA, the integrated neurogram was normalised (units of percent) in a two-point calibration, where 100 % was the peak amplitude of the tallest burst and 0 % was the amplitude of a representative period of signal noise within a non-bursting region. Each diastolic blood pressure during the analysis window was matched to a two-cardiac cycle window occurring before the diastolic blood pressure at a set lag. The modulus of the normalised integrated neurogram was measured during this cardiac cycle window. Thus, each diastolic blood pressure measurement was associated with a two-cardiac cycle period of MSNA area. This method was repeated across eight different lags (1-3 cardiac cycles to 8-10 cardiac cycles prior to the diastolic blood pressure measurement) (Figure 2.1). Cardiac cycle windows containing artifacts in the neurogram or MSNA bursts associated with premature ventricular contractions were removed from the analysis.

Data for each lag were binned by MSNA area in 1 %.s bins, providing a mean diastolic blood pressure for every 1 %.s bin of MSNA area. Mean diastolic blood pressure was plotted against MSNA area and a weighted linear regression was applied, the slope of which was taken as the measure of sympathetic transduction. Sympathetic transduction slope was calculated for each lag in each individual, and the largest slope was used as each individual's measure of sympathetic transduction in subsequent analyses (Figure 3.1).

3.3.5 Statistical analysis

Statistical analyses were conducted in IBM SPSS statistics 24 (IBM, NY, USA). Sympathetic transduction, participant demographics and haemodynamic variables were compared between hypertensive and normotensive participants within each age-sex group by independent samples T test or Mann-Whitney U test of medians or mean ranks where appropriate. Effect sizes for these comparisons (Cohen's D) were calculated as: the difference in the group means/pooled standard deviation (independent samples T test); the standardised test statistic/square root of number of datapoints in comparison (Mann-Whitney U) (Field, 2018). Correlations between haemodynamic variables were tested by

Pearson's correlation or Spearman's rank correlation where appropriate. The effect of anti-hypertensive medication on sympathetic transduction slope in treated hypertensive, untreated hypertensive and normotensive participants was tested by Kruskal-Wallis test of mean ranks. Throughout, results are presented as mean ± SD or median [interquartile range] unless otherwise stated. For correlations, r indicates the Pearson's correlation coefficient whilst ρ indicates the Spearman's rank correlation coefficient.

3.4 Results

3.4.1 Data selection

Data from previous studies (Table 3.1) were used in addition to data collected specifically for this study. Across nine studies, 167 MSNA files of at least five minutes were identified for analysis. Of these, 26 were rejected based on quality of the MSNA signal or another issue with the recording; 23 were rejected because the participant met one or more exclusion criteria; 13 were not used because sufficient participant numbers had been reached in that group (normotensive); two were rejected to balance the age of participant groups; two were rejected because they were conducted in the same individual; and two were rejected for other reasons e.g., missing demographic data. 100 files across the eight participant groups remained and were used in the analysis. The data selection process is shown in Figure 3.12.

3.4.2 Participant characteristics

3.4.2.1 Premenopausal females

Hypertensive and normotensive premenopausal female participants were matched for age, height, weight, and BMI (statistical test data and effect sizes in Table 3.2). Hormonal contraception use at the time of the study was reported in two of eight hypertensive female and three of 10 normotensive female participants. Clinic blood pressure was greater in the hypertensive versus normotensive premenopausal female group (systolic 198 [49] versus 121 [10] mmHg (median [interquartile range]), $P<0.0005$; diastolic 106 ± 12 versus 79 ± 9 mmHg (mean ± SD), $P<0.0005$). Similarly, daytime average ambulatory blood pressure was greater in the hypertensive versus normotensive premenopausal female group (systolic 151 ± 20 versus 118 ± 6 mmHg, $P=0.004$; diastolic 94 ± 8 versus 76 ± 3 mmHg, $P=0.001$). Neither clinic nor daytime average ambulatory

heart rate differed between hypertensive and normotensive premenopausal female groups (Table 3.2). Among the 10 hypertensive premenopausal females, three were untreated, one was treated with controlled blood pressure, and four were treated with uncontrolled blood pressure. The anti-hypertensive medication used by hypertensive participants is shown in Table 3.3.

3.4.2.2 Postmenopausal females

The hypertensive and normotensive postmenopausal female groups were matched for age and height (Table 3.5). Hypertensive postmenopausal females weighed more and had a greater BMI versus normotensive postmenopausal females (81.6 [26.5] versus 62.2 [15.1] kg, P=0.016; 30.4 ± 4.7 versus 24.6 ± 3.9 kg/m^2, P=0.002; Table 3.5). Clinic and daytime average ambulatory blood pressure was greater in hypertensive versus normotensive postmenopausal females (clinic systolic 154 ± 23 versus 119 ± 10 mmHg, P<0.0005; clinic diastolic 92 ± 13 versus 74 ± 8 mmHg, P<0.0005; ambulatory systolic 136 [9] versus 118 [15] mmHg, P<0.0005; ambulatory diastolic 86 [9] versus 73 [25] mmHg, P=0.0007; Table 3.5). Neither clinic nor ambulatory heart rate differed between the hypertensive and normotensive postmenopausal female groups (Table 3.5). Data about anti-hypertensive treatment are available for 9/11 hypertensive postmenopausal female participants. Among these, two females were untreated, four were treated with controlled blood pressure and three were treated with uncontrolled blood pressure (Table 3.6). The classes of anti-hypertensive medication used by hypertensive postmenopausal female participants are shown in Table 3.6.

3.4.2.3 Younger males

Hypertensive and normotensive younger males were matched for age and height (Table 3.8). Hypertensive younger males weighed more and had a greater BMI versus normotensive younger males (92.0 ± 7.9 versus 77.6 ± 9.2 kg, P<0.0005; 29.0 [2.6] versus 23.4 [4.9] kg/m^2, P<0.0005; Table 3.8). Clinic blood pressure was greater in hypertensive versus normotensive younger males (systolic 147 [23] versus 124 [11] mmHg, P<0.0005; diastolic 93 ± 11 versus 76 ± 10 mmHg, P<0.0005; Table 3.8). Similarly, daytime average ambulatory blood pressure was greater in hypertensive versus normotensive younger males (systolic 137 [12] versus 121 [12] mmHg, P<0.0005; diastolic 86 [11] versus 72 [18] mmHg, P=0.006; Table 3.8). Clinic heart rate was similar between the groups, however daytime ambulatory heart rate was greater in hypertensive versus normotensive

younger males (80 [15] versus 62 [7] beats/min, *P*=0.003, Table 3.8). Of the 13 hypertensive younger males, six were untreated, two were treated with controlled blood pressure, and five were treated with uncontrolled blood pressure (Table 3.9). The anti-hypertensive medications used by the treated hypertensive younger male participants are displayed by class in Table 3.9.

3.4.2.4 Older males

Hypertensive and normotensive older males were matched for age, height, weight and BMI (Table 3.11). Clinic systolic blood pressure was greater in the hypertensive versus normotensive older male group (140 [32] versus 119 [19] mmHg, *P*=0.005; Table 3.11), but clinic diastolic blood pressure was similar between the groups (84 [12] versus 73 [10] mmHg, *P*=0.108; Table 3.11). Daytime average ambulatory systolic and diastolic blood pressure were greater in the hypertensive versus normotensive older male group (systolic 143 ± 8 versus 125 ± 5 mmHg, *P*<0.0005; diastolic 86 ± 5 versus 72 ± 11, *P*=0.005; Table 3.11). Neither clinic nor ambulatory heart rate differed between the groups (Table 3.11). Among the 15 hypertensive older male participants, six were untreated, three were treated with controlled blood pressure and six were treated with uncontrolled blood pressure. The anti-hypertensive medications used by the treated hypertensive older male participants are listed by class in Table 3.12.

3.4.3 Sympathetic transduction in hypertensive and normotensive females

3.4.3.1 Premenopausal females

The maximal transduction slope of each individual was greater in hypertensive versus normotensive premenopausal females (0.221 [0.16] versus 0.086 [0.03] mmHg/%.s, *P*=0.027, Figure 3.2A). The average cardiac cycle lag producing the maximal transduction slope in each individual did not differ between groups (Figure 3.2B). The range of 1 %.s bins of MSNA burst area over which individual maximal transduction slopes were calculated did not differ between hypertensive and normotensive groups (30 [6] versus 33 [6] %.s for hypertensive versus normotensive, *P*=0.274).

Although sympathetic transduction was greater in the hypertensive versus normotensive premenopausal female group, the groups had similar levels of resting sympathetic nerve activity. Resting MSNA burst incidence and frequency were 57 [22] versus 59 [15] bursts/100 heartbeats (*P*=0.763), and 39 [13] versus 35 [5] bursts/min (*P*=0.203) in hypertensive and normotensive groups

respectively. Mean burst latency did not differ between groups (1.31 [0.07] versus 1.31 [0.14] s, *P*=0.696). Resting heart rate during the analysis window was similar in hypertensive and normotensive premenopausal females (67 [2] versus 58 [10] beats/min, *P*=0.068). Systolic blood pressure, pulse pressure and mean arterial pressure during the analysis window were greater in hypertensive versus normotensive premenopausal females, however diastolic blood pressure was similar between groups. Statistical test data and effect sizes for the above variables are shown in Table 3.4.

When resting MSNA burst incidence was correlated with concurrent blood pressure, there were no significant correlations between burst incidence and either systolic, diastolic, or mean arterial pressure in either hypertensive or normotensive younger females (Table 3.16). Furthermore, neither clinic nor ambulatory systolic or diastolic blood pressure was significantly correlated with resting burst incidence in either group of younger females (Table 3.16).

3.4.3.2 Postmenopausal females

The individual maximum sympathetic transduction slope did not differ between hypertensive and normotensive postmenopausal females (0.078 [0.12] versus 0.085 [0.06] mmHg/%.s, *P*=0.683, Figure 3.3A). The average cardiac cycle lag producing the greatest transduction slope did not differ between groups (Figure 3.3B). The range of 1 %.s MSNA area bins over which individual maximum transduction slopes were calculated did not differ between groups (33 [10] versus 30 [12] %.s for hypertensive versus normotensive, *P*=0.443). Given that BMI was greater in the hypertensive group, the analysis was repeated with BMI included as a covariate (ANCOVA). Transduction slope remained similar in hypertensive and normotensive groups (*P*=0.701), however ANCOVA may not be a suitable analysis for this data, given that the some of the transduction slopes in the normotensive group were considered outliers.

The hypertensive and normotensive postmenopausal female groups had a similar resting MSNA burst incidence (80 ± 13 versus 77 ± 9 bursts/100 heartbeats, T(25)=0.922, *P*=0.522), although MSNA burst frequency was greater in hypertensive versus normotensive postmenopausal females (51 ± 8 versus 44 ± 7 bursts/min, *P*=0.032, Table 3.7). There was no group difference in individual mean burst latency (Table 3.7). Resting heart rate across the analysis window was greater in hypertensive versus normotensive postmenopausal females (65

[12] versus 56 [10] beats/min, *P*=0.027, Table 3.7). Systolic, diastolic, pulse pressure and mean arterial blood pressure were similar in hypertensive and normotensive female groups during the analysis window (Table 3.7).

Concurrent blood pressure was not significantly correlated with resting MSNA burst incidence in hypertensive postmenopausal females (Table 3.16). In contrast, normotensive postmenopausal females showed a significant positive correlation between burst incidence and concurrent systolic blood pressure (ρ=0.541, *P*=0.037, Table 3.16). However, concurrent diastolic and mean arterial pressure were not significantly correlated with burst incidence (diastolic r=0.358, *P*=0.191; mean arterial pressure r=0.443, *P*=0.098; Table 3.16). When clinic and ambulatory blood pressure was correlated with resting burst incidence, hypertensive postmenopausal females showed a significant negative correlation between burst incidence and daytime ambulatory diastolic blood pressure (ρ=-0.738, *P*=0.015). However, neither daytime ambulatory systolic, nor systolic or diastolic clinic blood pressure was significantly correlated with resting burst incidence in hypertensive postmenopausal females (Table 3.16). Normotensive postmenopausal females showed no significant relationship between resting burst incidence and either ambulatory or clinic systolic or diastolic blood pressure (Table 3.16).

3.4.4 Sympathetic transduction in hypertensive and normotensive males

3.4.4.1 Younger males

There was no difference in individual maximal sympathetic transduction slope between the hypertensive and normotensive younger male groups (0.115 [0.03] versus 0.131 [0.08] mmHg/%.s, *P*=0.254, Figure 3.4A). Additionally, there was no difference in the average cardiac cycle lag producing the maximum slope between hypertensive and normotensive younger males (Figure 3.4B). The range of 1 %.s MSNA area bins over which individual maximum transduction slopes were calculated did not differ between the hypertensive and normotensive groups (35 [20] versus 40 [12] %.s for hypertensive versus normotensive, *P*=0.618). BMI was greater in the hypertensive young males, so analysis was repeated with BMI added as a covariate (ANCOVA). There remained no group difference in transduction slope (*P*=0.552), but the data may not be suitable for ANCOVA, given that transduction slopes in hypertensive young males were not normally distributed.

Resting MSNA burst incidence was similar in hypertensive and normotensive younger males (67 ± 10 versus 67 ± 10 bursts/100 heartbeats, *P*=0.897, Table 3.10), but burst frequency was greater in hypertensive versus normotensive younger males (45 ± 8 versus 36 ± 6 bursts/min, *P*=0.003, Table 3.10). Individual mean burst latency did not differ between the groups (Table 3.10). Mean heart rate during the analysis window was greater in hypertensive versus normotensive younger males (67 ± 9 versus 55 ± 7 beats/min, *P*=0.001, Table 3.10). Mean systolic and mean arterial blood pressure across the analysis window were greater in hypertensive versus normotensive younger males (systolic 143 ± 17 versus 127 ± 16 mmHg, *P*=0.013; mean arterial pressure 97 ± 12 versus 86 ± 12, *P*=0.020). Mean diastolic and pulse pressure during the analysis window did not differ between the groups (diastolic 74 ± 11 versus 66 ± 11, *P*=0.061; pulse pressure 68 [18] versus 60 [18], *P*=0.142; Table 3.10).

Hypertensive younger males showed a significant positive correlation between resting MSNA burst incidence and daytime ambulatory systolic blood pressure (ρ=0.582, *P*=0.037), but not between burst incidence and any other measure of resting blood pressure (Table 3.17). Normotensive younger males showed significant positive correlations between resting MSNA burst incidence and clinic systolic blood pressure (ρ=0.580, *P*=0.023) and clinic diastolic blood pressure (r=0.545, *P*=0.036), but not between burst incidence and daytime ambulatory measures of blood pressure (Table 3.17).

3.4.4.2 Older males

Maximum sympathetic transduction slope was similar in hypertensive and normotensive older males (0.111 [0.07] versus 0.115 [0.20] mmHg/%.s, *P*=0.892, Figure 3.5A). The average cardiac cycle lag that produced the maximum slope was not different between the groups (Figure 3.5B). The range of 1 %.s MSNA area bins over which individual maximum transduction slopes were calculated did not differ between groups (39 ± 11 versus 39 ± 9 %.s for hypertensive versus normotensive, *P*=0.992).

Resting MSNA burst incidence and frequency did not differ between hypertensive and normotensive older male groups (82 ± 12 versus 76 ± 10 bursts/100 heartbeats, *P*=0.210; 45 [9] versus 43 [7] bursts/min, *P*=0.316; Table 3.11). Similarly, mean burst latency did not differ between the groups (Table 3.11). Resting heart rate during the analysis window was similar in the hypertensive and

normotensive older males (58 ± 10 versus 57 ± 8 beats/min, *P*=0.883). Systolic, diastolic, pulse pressure and mean arterial pressure during the analysis window were greater in hypertensive versus normotensive older males (comparison of Finometer data only, Table 3.11).

Neither hypertensive, nor normotensive older males showed any significant correlation between resting MSNA burst incidence and any measure of resting blood pressure (Table 3.17).

3.4.5 Sympathetic transduction across the lifespan in hypertensive and normotensive adults

3.4.5.1 Correlations between sympathetic transduction slope and age

The existence of a relationship between age and sympathetic transduction was assessed separately for each sex. In the female groups, there was no significant correlation between age and individual maximum sympathetic transduction slope (*P*=0.115, Figure 3.9A). When hypertensive and normotensive females were considered separately, there remained no significant correlation between age and maximum transduction slope in normotensive females (Figure 3.10, Spearman's rank correlation *P*=0.868). However, there was a significant negative correlation between age and transduction slope in hypertensive females (Figure 3.10, Spearman's rank correlation ρ=-0.551, *P*=0.014). There was no correlation between age and transduction slope in males when the hypertensive and normotensive groups were considered together (*P*=0.927, Figure 3.9B). When hypertensive and normotensive males were considered separately, there was no correlation between age and transduction slope in either the hypertensive (Figure 3.10, *P*=0.890) or normotensive groups (Figure 3.10, *P*=0.708).

3.4.5.2 Correlations between sympathetic transduction slope and resting MSNA

Transduction slope was not correlated with MSNA burst incidence in either females or males when hypertensive and normotensive participants were considered together (Figure 3.8A and B, ρ=-0.121, *P*=0.436, N=44; and ρ=-0.126, *P*=0.355, N=56; for females and males, respectively). When considered separately, there remained no relationship between burst incidence and transduction slope in hypertensive females (r=-0.286, *P*=0.234, N=19) or normotensive females (ρ= 0.182, *P*=0.385, N=25), nor in hypertensive males (ρ=-0.033, *P*=0.867, N=28) or normotensive males (ρ=-0.251, *P*=0.197, N=28).

3.4.6 Effect of treatment on transduction slope

There was no apparent effect of anti-hypertensive treatment on transduction slope in any participant group, when untreated hypertensives, treated hypertensives, and normotensive participants were all considered (Figure 3.11). Furthermore, when only hypertensive participants were considered, there remained no effect of anti-hypertensive treatment on sympathetic transduction slope in an age-sex group (Table 3.18).

3.5 Discussion

3.5.1 Summary of findings

The main findings of this study are: A) In pre-menopausal females, average sympathetic transduction slope was greater in hypertensive participants versus normotensive controls. B) However, there was no difference in transduction slopes between hypertensive and normotensive younger males, hypertensive and normotensive postmenopausal females, or hypertensive and normotensive older males. C) Sympathetic transduction slope was inversely related to age in hypertensive females, but no age-transduction relationship was observed in normotensive females, hypertensive males, or normotensive males. D) Sympathetic transduction was not related to resting MSNA burst incidence in females or males.

3.5.2 Sympathetic transduction in hypertensive females

3.5.2.1 Sympathetic transduction is increased in hypertensive premenopausal females

A greater sympathetic transduction slope in hypertensive premenopausal female participants versus normotensive controls lends support to the idea that hypertension in young females is associated with increased conversion of sympathetic activity into diastolic blood pressure. Increased sympathetic transduction could result in greater vasoconstriction for a given level of sympathetic activation, and this may promote hypertension. In healthy premenopausal females, blunted sympathetic transduction relative to healthy males of similar age is thought to explain, at least partially, the lower risk of hypertension in this group (Hart et al., 2011a). As such, it appears that the potential protective effect of low sympathetic transduction may be absent in premenopausal hypertensive females. The current data show no difference in the level of sympathetic activation among hypertensive and normotensive premenopausal females. Therefore, increased sympathetic activity does not account for hypertension in these young females. However, the current data are contradictory of previous reports where hypertensive premenopausal females were found to have increased MSNA burst incidence versus normotensive controls (Hogarth et al., 2011).

Although hypertensive premenopausal females exhibited an enhanced sympathetic transduction compared to normotensive controls, both groups displayed a dissociation of blood pressure from resting sympathetic activity. Average resting blood pressure (Finometer, daytime ambulatory, or clinic) was not correlated with resting MSNA burst incidence in either the hypertensive or normotensive premenopausal female groups. This agrees with previous reports that show a dissociation of resting blood pressure from resting MSNA in healthy young females (Narkiewicz et al., 2005, Hart et al., 2009). However, the lack of relationship between MSNA and blood pressure in hypertensive premenopausal females despite an increased sympathetic transduction appears counterintuitive. It may be that hypertensive premenopausal females show greater sympathetic control over beat-to-beat blood pressure, but that sympathetic control over longer-term blood pressure remains poor (relative to age-matched males). However, it is important to note that these methods quantify sympathetic blood pressure regulation differently, given that the transduction slope method uses individual blood pressures rather than the average over an entire analysis period. Furthermore, clinic and daytime ambulatory blood pressure readings were not measured at the same time as MSNA, thus the data are not directly linked. As such, the methods are not directly comparable, and these methodological differences may account for the disagreement in the results.

When treated and untreated hypertensive premenopausal females were compared, no difference in sympathetic transduction slope was found (Table 3.18). Therefore, sympathetic transduction was greater in hypertensive premenopausal females despite the use of anti-hypertensive medication in five of the eight participants in this group. However, no data is available to confirm the presence of anti-hypertensive urinary metabolites in these patients.

3.5.2.2 Sympathetic transduction is not increased in hypertensive postmenopausal females

In this study, no difference in sympathetic transduction slope was observed in hypertensive and normotensive postmenopausal females. Previous research has demonstrated that sympathetic transduction increases in healthy females after the menopause (Hart et al., 2011a, Briant et al., 2016) and that this enhanced transduction may contribute to the rise in hypertension prevalence after menopause (Hart et al., 2011a). This is supported by work demonstrating that hypertensive older females exhibit the same blood pressure as hypertensive

older males whilst having a lower resting level of sympathetic nerve activity (Hogarth et al., 2008). Therefore, it is possible that sympathetic transduction may be greater in those postmenopausal females who become hypertensive, versus those who do not. However, the current findings do not support this. There was no group difference in level of sympathetic activation, thus high sympathetic nerve activity does not underlie the hypertension in the postmenopausal participants of the current study. Previous studies have showed an increased level of sympathetic nerve activity in hypertensive versus normotensive older females (Hogarth et al., 2007b), including a group comprised entirely of postmenopausal female participants (Hogarth et al., 2011). As such, the current data disagree with previous reports. The similar level of sympathetic activity in the current hypertensive and normotensive postmenopausal groups is despite a greater BMI in the hypertensive group, which is associated with increased levels of MSNA in the absence of hypertension (Grassi et al., 2000). Furthermore, some hypertensive participants used anti-hypertensive medication, which can also lead to increased sympathetic activation (Fu et al., 2005, de Champlain et al., 1998). However, there is inter-individual variability in resting level of MSNA (Sundlof and Wallin, 1977) and the current cohort was fairly small.

Previous research has reported that postmenopausal female participants show a positive relationship between resting level of sympathetic activation and resting blood pressure, which is absent in premenopausal participants (Narkiewicz et al., 2005, Hart et al., 2011a). In agreement, the current data showed a positive correlation between MSNA burst incidence and concurrent systolic blood pressure (Finometer) in the normotensive postmenopausal female group. However, the other measures of blood pressure were not correlated with MSNA in the normotensive group. In contrast, hypertensive postmenopausal females showed a negative correlation between MSNA burst incidence and daytime ambulatory diastolic blood pressure, meaning that those with a higher resting burst incidence had a lower resting diastolic blood pressure. This is perhaps related to anti-hypertensive treatment, given that some anti-hypertensive treatment has been linked to an increase in MSNA (Fu et al., 2005, de Champlain et al., 1998). However, the sample size was relatively small (N=10) and there was heterogeneity in the hypertensive group in terms of blood pressure control.

3.5.2.3 Mechanisms underlying enhanced sympathetic transduction in hypertensive premenopausal females

Several mechanisms could be responsible for the increased sympathetic transduction in hypertensive premenopausal females. The vascular beta-adrenergic receptors are important in maintaining low sympathetic transduction in healthy premenopausal females relative to males. Sex differences in vasoconstrictor responses in healthy young adults can be abolished by both local (Kneale et al., 2000) and systemic beta-blockade (Hart et al., 2011a). Thus, beta-adrenergic vasodilation may buffer alpha-adrenergic vasoconstriction in healthy premenopausal females, leading to lower sympathetic transduction (Hart et al., 2011a). Poorer beta-adrenergic buffering of vasoconstriction in hypertensive relative to normotensive premenopausal females could therefore explain increased sympathetic transduction in this group. This question is addressed further in chapter 4. Alternatively, hypertensive premenopausal females may show similar beta-adrenergic sensitivity to normotensive females but may have increased alpha-adrenergic sensitivity. Existing evidence on alpha-adrenergic sensitivity in hypertension is contradictory. Some results indicate enhanced sensitivity with hypertension (Sherwood et al., 2017, Jie et al., 1986), whilst others report reduced sensitivity (Kotchen et al., 1982), or no difference (Egan et al., 1987). In healthy adults, no sex difference in vasoconstrictor response to alpha-adrenergic agonists was shown in the absence of noradrenaline (ganglionic blockade), thus removing any beta-adrenergic effect (Christou et al., 2005). In contrast, in a larger study group of hypertensive and normotensive adults, alpha-adrenergic sensitivity to phenylephrine was enhanced in females versus males, although there was no additional effect of hypertension in combination with female sex (Sherwood et al., 2017). As such, enhanced alpha-adrenergic sensitivity cannot be discounted as a mechanism contributing to increased sympathetic transduction in hypertensive younger females, although there is little data available that directly addresses this question.

Nitric oxide is a key factor in the control of vasoconstrictor tone in premenopausal females. Oestrogen enhances endothelial nitric oxide synthase activity (Miller and Duckles, 2008). Additionally, poor endothelial function has been associated with hypertension in several young-hypertensive cohorts (Gokaslan et al., 2020, Taddei et al., 1997), both of which included females. Furthermore, endothelial function has been shown to be poorer in female versus male hypertensive patients (Routledge et al., 2012), although this cohort ranged from age 40 to 60 years, so likely included both pre- and postmenopausal hypertensive females.

Increased arterial stiffness may also contribute to enhanced sympathetic transduction and hypertension in young females, given that hypertension is associated with stiffer arteries in hypertensive cohorts containing both young males and females (Gokaslan et al., 2019, McEniery et al., 2005). Overall, a number of mechanisms could contribute to the increase in sympathetic transduction in hypertensive premenopausal females.

3.5.3 Sympathetic transduction in hypertensive males

(Kobetic et al., 2022) demonstrated that untreated hypertensive younger males had a lower level of sympathetic transduction compared to normotensive controls. As such, hypertension in these individuals appears to occur despite, not because of, their level of sympathetic transduction. The current work failed to replicate these findings, although the hypertensive group in the current study contained both treated and untreated hypertensive participants, whereas Kobetic et al. studied only untreated hypertensive individuals.

Briant et al. showed that as healthy males age, sympathetic transduction decreases. This supports previous work indicating that alpha-adrenergic receptor desensitisation occurs in the vasculature of older males (Dinenno et al., 2002). It is unclear whether hypertensive and normotensive older males show similar alpha-adrenergic desensitisation. It is possible that hypertension in older males may be associated with less desensitisation, and therefore an increased sympathetic transduction, which would drive increased vasoconstrictor tone for a given level of MSNA. Alternatively, hypertensive older males may show reduced sympathetic transduction versus normotensive controls, given that younger untreated hypertensive males have a lower sympathetic transduction compared to healthy males of a similar age (Kobetic et al., 2022). The current study found no difference in the sympathetic transduction slopes of hypertensive and normotensive older male participants. However, the hypertensive group was mixed, with some participants treated and some untreated. In addition, the current study showed similar levels of resting sympathetic nerve activity in hypertensive and normotensive males, which contrasted with previous reports of increased MSNA in hypertension (Hogarth et al., 2007b). Hogarth et al.'s cohort were younger than the current group of older males (early 50's compared to late 50's-early 60's), but whether the age difference fully accounts for these contradictory results is unclear.

When haemodynamic variables were correlated, positive relationships were found between MSNA and ambulatory systolic blood pressure in hypertensive younger males, and between MSNA and clinic systolic and diastolic blood pressure in normotensive younger males. These results appear to contradict previous work showing that MSNA does not correlate with mean arterial blood pressure in young males (Narkiewicz et al., 2005). However, similar relationships between daytime ambulatory blood pressure and MSNA have been shown previously in hypertensive and normotensive males (Kobetic et al., 2022). Neither clinic nor ambulatory blood pressure correlated with MSNA in older male participants.

3.5.4 Age and sympathetic transduction

The current study assessed whether a relationship exists between sympathetic transduction and age. The inclusion of older premenopausal females into the correlation was done with the aim of providing insight into whether sympathetic transduction is related solely to menopause or might be an effect of ageing. Perimenopausal females were not included in the analysis, as sex hormone concentrations are known to fluctuate during perimenopause (Moreau, 2018), and the available data in perimenopausal females did not have associated hormone concentration data. No significant relationship between age and transduction slope was found in either females or males, in contrast to previous reports (Briant et al., 2016, Kobetic et al., 2022). When the analysis was repeated in sex-hypertension groups, the only significant correlation was in hypertensive females, who showed a negative relationship between age and transduction slope. The negative relationship in hypertensive females may support the idea that high sympathetic transduction is an important factor in hypertension in young females, but less important in older females, in whom other hypertension risk factors are more likely to be present (e.g., increased sympathetic activity (Keir et al., 2020), greater arterial stiffness (Mitchell et al., 2004) and poorer endothelial function (Benjamin et al., 2004)). The lack of relationship between age and transduction in the other groups is unexpected and may be a consequence of the study being underpowered.

3.5.5 Limitations

3.5.5.1 Heterogeneity of participant groups

An important limitation of this study is the heterogeneity of the hypertensive participants within each age-sex group. It is possible that anti-hypertensive

medication alters sympathetic transduction, given that many classes of these drugs aim to reduce vasoconstrictor tone (see 3.4.5.2). Furthermore, if hypertensive patients are treated with uncontrolled blood pressure, this may represent poor efficacy of the anti-hypertensive medications. Thus, the effect of these drugs on sympathetic transduction may differ between patients with treated controlled and treated uncontrolled blood pressure. Additionally, some hypertensive participants in this study were taking multiple anti-hypertensive medications and were recruited to studies investigating non-pharmacological treatments for hypertension such as renal denervation and carotid body excision. Therefore, these patients likely represent a more severe hypertension phenotype than other participants in this study, for example those with mild undiagnosed hypertension that was detected in the course of the study. The current study groups these participants together, but it may be beneficial to sub-group participants in future larger studies, for example by hypertension stage (National Institute for Health and Care Excellence, 2019).

In younger males and postmenopausal females, BMI was greater in the hypertensive group versus the normotensive control group. Given that BMI is associated with sympathetic activation (Grassi et al., 2000), this is a potential confounding variable. When group differences in sympathetic transduction were tested with BMI included as a covariate (ANCOVA), there remained no difference in transduction slopes between the hypertensive and normotensive groups of younger males and postmenopausal females. Despite this, the sympathetic and vascular effects of high BMI should not be discounted, and future work should aim to match groups for BMI.

3.5.5.2 Effect of antihypertensive treatment

Sympathetic transduction slope did not appear to differ between treated and untreated hypertensive patients in any age-sex group. However, there was a larger degree of variability among the treated hypertensive patients in terms of the number and drug class of anti-hypertensive medications used. It could be expected that anti-hypertensive medication may alter sympathetic transduction, given that some anti-hypertensive medications, for example calcium channel antagonists, act by reducing vasoconstrictor tone (de Champlain et al., 1998, Frishman, 2007). Furthermore, some participants reported use of centrally-acting anti-hypertensives (moxonidine), which reduces sympathetic outflow from the brainstem via imidazoline receptor stimulation (Fenton et al., 2006). Although the

current analysis is limited, some participants reporting use of multiple anti-hypertensive medication had relatively high transduction slopes within their age-sex group, and as such, anti-hypertensive medication use is not necessarily associated with lower sympathetic transduction. A further limitation is that no urinary data were available to confirm the presence of anti-hypertensive medication metabolites in the hypertensive patients (Lawson et al., 2020).

3.5.5.3 Negative transduction slopes

Several participants from different groups demonstrated transduction slopes that were negative. It is likely that these slopes demonstrate a poor relationship between MSNA and blood pressure. It may be that these individuals exhibit better cardiac coupling to blood pressure than those that demonstrated greater MSNA coupling to blood pressure, however the current data are unable to answer this. Given the lack of relationships found between resting MSNA and sympathetic transduction, it does not appear that individuals with very high resting MSNA are more likely to show poor sympathetic transduction. It is possible that for some individuals, the range of MSNA area over which sympathetic transduction was assessed was smaller compared to others, which may give a reduced transduction slope. However, the participants with negative transduction slopes included both normotensive and hypertensive individuals, and within each age-sex group there was no effect of hypertension status on MSNA area range included in the analysis.

3.5.6 Implications of results for females

The results presented in this chapter may be important for the understanding of sympathetic blood pressure control in hypertensive younger females. Hypertensive premenopausal females had enhanced sympathetic transduction compared to normotensive controls, despite some hypertensive females using anti-hypertensive medication. As such, these hypertensive females do not appear to exhibit the dissociation of blood pressure from sympathetic nerve activity, that in healthy premenopausal females is considered protective (Hart et al., 2011a). Furthermore, given the negative correlation between sympathetic transduction and age in hypertensive females, raised sympathetic transduction may be an important mechanism in the development of hypertension in particular in premenopausal females. Whilst the mechanism behind increased sympathetic transduction in premenopausal hypertension is currently unclear, investigating

the cause may provide opportunities to target treatment specifically at reducing sympathetic transduction in hypertensive premenopausal females.

3.6 Tables and figures

Table 3.1 Studies contributing data to Chapter 3.

Study short name	Ethical approval board	Number of participants
Sex Differences in the Role of SNA in Hypertension in Humans	NHS REC 18/SW/0237	23
Carotid Body Activity in Young Onset Hypertension	NHS REC 17/SW/0171	12
Blunting Blood Pressure During Exercise in Humans with Hypertension	NHS REC 17/NI/0097	1
Hypertension, Brain Blood Flow and Nerve Activity.	NHS REC 11/SW/0207	38
Peripheral Chemosensitivity in Hypertensive and Normotensive Humans.	NHS REC 14/SW/0054	14
Renal Denervation for Resistant Hypertension	NHS REC 11/SW/0254	6
Carotid Body Removal for the Treatment of Resistant Hypertension	Bristol site: NHS REC 12/SW/0277 Gdansk site: Medical University of Gdansk Independent Bioethics Commission for Research	2
Mayo Clinic study	Mayo Clinic Institutional Review Board	4

NHS REC; National Health Service Research Ethics Committee.

Table 3.2 Participant characteristics in premenopausal females.

	HTN YF	NTN YF	Test statistic	*P* value (Effect size)
Age (years)	44 [9.25] (11.56)	34 [21.25] (7.85)	z=-1.468	0.146 (0.245)
Height (m)	1.65 ± 0.07	1.72 ± 0.08	T(15)=1.651	0.120 (0.813)
Weight (kg)	67.2 [34.4]	62.7 [17.8]	z=-0.390	0.740 (0.067)
BMI (kg/m^2)	26.3 [5.9] (11.75)	21.1 [4.2] (7.70)	z=-1.599	0.122 (0.267)
Clinic systolic BP (mmHg)	198 [49] (14.38)	121 [10] (5.60)	z=-3.469	***<0.0005*** (0.578)
Clinic diastolic BP (mmHg)	106 ± 12	79 ± 9	T(16)=5.615	***<0.0005*** (2.664)
Clinic heart rate (beats/min)	73 [18] (8.40)	66 [4.5] (5.14)	z=-1.543	0.149 (0.315)
Ambulatory systolic BP (mmHg)	151 ± 20	118 ± 6	T(14)=4.281	***0.004*** (2.413)
Ambulatory diastolic BP (mmHg)	94 ± 8	76 ± 3	T(14)=5.322	***0.001*** (2.984)
Ambulatory heart rate (beats/min)	75 ± 8	71 ± 7	T(12)=1.018	0.339 (0.577)
Hormonal contraceptive use (number of participants)				
Progesterone-only pill	1	0		
IUD	1	3		
Total	2	3		

HTN; hypertension, NTN; normotension, YF; premenopausal females, BMI; body mass index, BP; blood pressure, IUD; intrauterine device, z; standardised test statistic for Mann-Whitney U. Data are mean ± SD or median [interquartile range] with (mean rank) where appropriate. Group differences were tested by independent samples T-test or Mann-Whitney U test. Effect size is Cohen's D. N=8 HTN YF versus N=10 NTN YF. Ambulatory blood pressure available for 7/8 hypertensive females and 9/10 normotensive females.

Table 3.3 Anti-hypertensive medication in hypertensive premenopausal females.

Number of anti-hypertensive medications reported	Number of hypertensive participants
0	3
2	1
3	1
5	1
6	2
Anti-hypertensive medication subtype	**Number of hypertensive participants**
ACE inhibitor	3
Angiotensin receptor antagonist	2
Beta-adrenergic receptor antagonist	4
Alpha-adrenergic receptor antagonist	1
Calcium channel antagonist	2
Diuretic	4
Renin inhibitor	1
Aldosterone receptor antagonist	1
Alpha-adrenergic/Imidazoline receptor antagonist	3

ACE; angiotensin-converting enzyme. Medication use was reported by participants during screening.

Table 3.4 Resting haemodynamic variables in premenopausal females.

	HTN YF	NTN YF	Test statistic	*P* value (Effect size)
MSNA (bursts/100 HB)	57 [22] (9.06)	59 [15] (9.85)	z=0.311	0.762 (0.052)
MSNA (bursts/min)	39 [13] (11.31)	35 [5] (8.05)	z=-1.293	0.203 (0.216)
Mean burst latency (s)	1.31 [0.07] (10.12)	1.31 [0.14] (9.0)	z=-0.444	0.696 (0.074)
Heart rate (beats/min)	67 [2] (12.12)	58 [10] (7.40)	z=-1.866	0.068 (0.311)
Systolic BP (mmHg)	160 ± 24	127 ± 17	T(15)=3.342	***0.004*** (1.647)
Diastolic BP (mmHg)	80 [24] (11.38)	73 [10] (8.0)	z=-1.333	0.203 (0.222)
Pulse pressure (mmHg)	85 ± 24	54 ± 13	T(15)=3.467	***0.003*** (1.709)
Mean arterial pressure (mmHg)	104 ± 13	91 ± 14	T(15)=1.951	0.070 (0.961)

HTN; hypertension, NTN; normotension, YF; premenopausal females, MSNA; muscle sympathetic nerve activity, HB; heartbeats, BP; blood pressure, z; standardised test statistic for Mann-Whitney U. Data are mean ± SD or median [interquartile range] with (mean rank) where appropriate. Group differences were tested by unpaired T-test or Mann-Whitney U test. N=8 HTN YF versus N=10 NTN YF.

Table 3.5 Participant characteristics in postmenopausal females.

	HTN PMF	NTN PMF	Test statistic	*P* value (Effect size)
Age (years)	56 [9] (10.95)	62 [11] (15.37)	z=1.457	0.148 (0.202)
Height (m)	1.59 [0.12]	1.63 [0.09]	z=0.293	0.796 (0.042)
Weight (kg)	81.6 [26.5] (16.55)	62.2 [15.1] (9.61)	z=-2.372	***0.016*** (0.342)
BMI (kg/m^2)	30.4 ± 4.7	24.6 ± 3.9	T(24)=3.421	***0.002*** (1.358)
Clinic systolic BP (mmHg)	154 ± 23	119 ± 10	T(24)=4.788	***<0.0005*** (2.115)
Clinic diastolic BP (mmHg)	92 ± 13	74 ± 8	T(24)=4.604	***<0.0005*** (1.828)
Clinic heart rate (beats/min)	69 ± 9	69 ± 8	T(22)=0.208	0.837 (0.086)
Ambulatory systolic BP (mmHg)	136 [9] (20.10)	118 [15] (8.27)	z=-3.947	***<0.0005*** (0.558)
Ambulatory diastolic BP (mmHg)	86 [9] (18.75)	73 [25] (9.17)	z=-3.196	***0.0007*** (0.452)
Ambulatory heart rate (beats/min)	73 ± 10	69 ± 8	T(19)=1.229	0.837 (0.569)

HTN; hypertension, NTN; normotension, PMF; postmenopausal females, BMI; body mass index, BP; blood pressure. Data are mean ± SD or median [interquartile range] with (mean rank) where appropriate. Group differences were tested by unpaired T-test or Mann-Whitney U test. Effect size is Cohen's D. N=11 HTN PMF; N=15 NTN PMF.

Table 3.6 Anti-hypertensive medication in hypertensive postmenopausal females.

Number of anti-hypertensive medications reported	Number of hypertensive participants
0	2
1	4
2	2
3	1
7	1
Anti-hypertensive medication subtype	**Number of hypertensive participants**
ACE inhibitor	5
Angiotensin receptor antagonist	2
Beta-adrenergic receptor antagonist	1
Alpha-adrenergic receptor antagonist	1
Calcium channel antagonist	3
Diuretic	1
Renin inhibitor	1
Aldosterone receptor antagonist	1
Alpha-adrenergic/Imidazoline receptor antagonist	1

ACE; angiotensin converting enzyme. Data are available for 9/11 HTN participants.

Table 3.7 Resting haemodynamic variables in postmenopausal females.

	HTN PMF	NTN PMF	Test statistic	*P* value (Effect size)
MSNA (bursts/100 HB)	80 ± 13	77 + 9	T(24)=0.650	0.522 (0.258)
MSNA (bursts/min)	51 ± 8	44 ± 7	T(24)=2.276	***0.032*** (0.904)
Mean burst latency (s)	1.20 ± 0.08	1.25 ± 0.07	T(24)=1.620	0.118 (0.642)
Heart rate (beats/min)	65 [12] (17.36)	56 [10] (10.67)	z=-2.206	***0.027*** (0.306)
Systolic BP (mmHg)	146 [38] (15.18)	130 [15] (12.27)	z=-0.960	0.357 (0.133)
Diastolic BP (mmHg)	69 ± 13	62 ± 12	T(24)=1.324	0.198 (0.525)
Pulse pressure (mmHg)	72 ± 21	70 ± 8	T(24)=0.322	0.753 (0.144)
Mean arterial pressure (mmHg)	93 ± 16	85 ± 12	T(24)=1.345	0.191 (0.534)

HTN; hypertensive, NTN; normotensive, PMF; postmenopausal females, MSNA; muscle sympathetic nerve activity, HB; heartbeats, BP; blood pressure. Data are mean ± SD or median [interquartile range] with (mean rank) where appropriate. Group differences were tested by unpaired T-test or Mann-Whitney U test. Effect size is Cohen's D. N=11 HTN PMF versus N=15 NTN PMF.

Table 3.8 Participant characteristics in younger males.

	HTN YM	NTN YM	Test statistic	*P* value (Effect size)
Age (years)	41 [12] (17.15)	35 [24] (12.20)	z=-1.591	0.118 (0.213)
Height (m)	1.79 ± 0.05	1.80 ± 0.07	T(25)=0.230	0.820 (0.001)
Weight (kg)	92.0 ± 7.9	77.6 ± 9.2	T(25)=4.303	***<0.0005*** (1.666)
BMI (kg/m^2)	29.0 [2.6] (20.46)	23.4 [4.9] (9.33)	z=-3.570	***<0.0005*** (0.477)
Clinic systolic BP (mmHg)	147 [23] (21.0)	124 [11] (8.87)	z=-3.893	***<0.0005*** (0.520)
Clinic diastolic BP (mmHg)	93 ± 11	76 ± 10	T(26)=4.194	***<0.0005*** (1.589)
Clinic heart rate (beats/min)	74 ± 15	59 ± 9	T(19)=2.775	0.120 (1.213)
Ambulatory systolic BP (mmHg)	137 [12] (16.65)	121 [12] (5.95)	z=-0.520	***<0.0005*** (0.555)
Ambulatory diastolic BP (mmHg)	86 [11]	72 [18]	z=-2.671	***0.006*** (0.394)
Ambulatory heart rate (beats/min)	80 [15]	62 [7]	z=-2.809	***0.003*** (0.423)

HTN; hypertension, NTN; normotension, YM; younger males, BMI; body mass index, BP; blood pressure. Data are mean ± SD or median [interquartile range] with (mean rank) where appropriate. Group differences were tested by unpaired T-test or Mann-Whitney U test. Effect size is Cohen's D. N=13 HTN YM; N=15 NTN YM. Ambulatory blood pressure data available in 10/15 NTN YM.

Table 3.9 Anti-hypertensive medication in hypertensive younger males.

Number of anti-hypertensive medications reported	Number of hypertensive participants
0	6
1	2
2	3
3	1
5	1
Anti-hypertensive medication subtype	**Number of hypertensive participants**
ACE inhibitor	3
Angiotensin receptor antagonist	2
Beta-adrenergic receptor antagonist	2
Alpha-adrenergic receptor antagonist	0
Calcium channel antagonist	4
Diuretic	4
Renin inhibitor	0
Aldosterone receptor antagonist	1
Alpha-adrenergic/Imidazoline receptor antagonist	0

ACE; angiotensin-converting enzyme.

Table 3.10 Resting haemodynamic variables in younger males.

	HTN YM	NTN YM	Test statistic	*P* value (Effect size)
MSNA (bursts/100 HB)	67 ± 10	67 ± 10	T(26)=0.130	0.897 (0.049)
MSNA (bursts/min)	45 ± 8	36 ± 6	T(26)=3.222	***0.003*** (1.221)
Mean burst latency (s)	1.35 [0.13] (15.85)	1.30 [0.07] (13.33)	z=-0.806	0.440 (0.108)
Heart rate (beats/min)	67 ± 9	55 ± 7	T(26)=3.939	***0.001*** (1.492)
Systolic BP (mmHg)	143 ± 17	127 ± 16	T(26)=2.658	***0.013*** (1.007)
Diastolic BP (mmHg)	74 ± 11	66 ± 11	T(26)=1.957	0.061 (0.742)
Pulse pressure (mmHg)	68 [18] (17.00)	60 [18] (12.33)	z=-1.497	0.142 (0.200)
Mean arterial pressure (mmHg)	97 ± 12	86 ± 12	T(26)=2.483	***0.020*** (0.941)

HTN; hypertension, NTN; normotension, YM; younger males, MSNA; muscle sympathetic nerve activity, HB; heartbeats, BP; blood pressure. Data are mean ± SD or median [interquartile range] with (mean rank) where appropriate. Group differences were tested by unpaired T-test or Mann-Whitney U test. Effect size is Cohen's D. N=13 HTN YM versus N=15 NTN YM.

Table 3.11 Participant characteristics in older males.

	HTN OM	NTN OM	Test statistic	*P* value (Effect size)
Age (years)	63 [5] (17.27)	59 [18] (11.31)	z=-1.920	0.058 (0.257)
Height (m)	1.76 ± 0.07	1.77 ± 0.06	T(16)=0.330	0.745 (0.156)
Weight (kg)	78.5 [23.1] (10.10)	77.7 [16.4] (8.75)	z=-0.533	0.633 (0.089)
BMI (kg/m^2)	27.0 [5.5] (16.33)	25.6 [5.0] (12.38)	z=-1.267	0.217 (0.169)
Clinic systolic BP (mmHg)	140 [32]	119 [19]	z=-2.713	***0.005*** (0.392)
Clinic diastolic BP (mmHg)	84 [12] (14.30)	73 [10] (9.50)	z=-1.610	0.108 (0.232)
Clinic heart rate (beats/min)	60 ± 8	60 ± 10	T(22)=0.192	0.850 (0.081)
Ambulatory systolic BP (mmHg)	143 ± 8	125 ± 5	T(21)=5.598	***<0.0005*** (2.451)
Ambulatory diastolic BP (mmHg)	86 ± 5	72 ± 11	T(21)=5.598	***0.005*** (1.373)
Ambulatory heart rate (beats/min)	72 ± 11	72 ± 14	T(11)=0.03	0.976 (0.020)

HTN; hypertension, NTN; normotension, OM; older males, BMI; body mass index, BP; blood pressure, z; standardised test statistic for Mann-Whitney U. Data are mean ± SD or median [interquartile range] with (mean rank) where appropriate. Group differences were tested by unpaired T-test or Mann-Whitney U test. Effect size is Cohen's D. N=15 HTN OM; N=13 NTN OM.

Table 3.12 Anti-hypertensive medication in hypertensive older males.

Number of anti-hypertensive medications reported	Number of hypertensive participants
0	6
1	2
2	4
3	3
Anti-hypertensive medication subtype	**Number of hypertensive participants**
ACE inhibitor	6
Angiotensin receptor antagonist	2
Beta-adrenergic receptor antagonist	0
Alpha-adrenergic receptor antagonist	1
Calcium channel antagonist	3
Diuretic	4
Renin inhibitor	1
Aldosterone receptor antagonist	1
Alpha-adrenergic/Imidazoline receptor antagonist	0

ACE; angiotensin converting enzyme. N=15 older males.

Table 3.13 Resting haemodynamic data in older males.

	HTN OM	NTN OM	Test statistic	*P* value (Effect size)
MSNA (bursts/100 HB)	82 ± 12	76 ± 10	T(26)=1.286	0.210 (0.487)
MSNA (bursts/min)	45 [9] (15.97)	43 [7] (12.81)	z=-1.015	0.316 (0.136)
Mean burst latency (s)	1.26 [0.07] (11.80)	1.31 [0.15] (17.62)	z=1.866	0.065 (0.249)
Heart rate (beats/min)	58 ± 10	57 ± 8	T(26)=0.148	0.883 (0.056)
Finometer blood pressure				
Systolic BP (mmHg)	155 [30] (14.27)	112 [22] (5.89)	z=-3.153	***0.001*** (0.705)
Diastolic BP (mmHg)	66 [17] (13.45)	56 [14] (6.89)	z=-2.469	***0.012*** (0.552)
Pulse pressure (mmHg)	87 ± 22	61 ± 16	T(18)=2.954	***0.008***
Mean arterial pressure (mmHg)	97 [22] (14.27)	72 [11] (5.89)		***0.001*** (0.705)
Brachial artery pressure transducer				
Systolic BP (mmHg)		140 [35]		
Diastolic BP (mmHg)		72 [14]		
Pulse pressure (mmHg)		68 [22]		
Mean arterial pressure (mmHg)		95 [21]		

HTN; hypertensive, NTN; normotensive, OM; older males, MSNA; muscle sympathetic nerve activity, HB; heartbeats, BP; blood pressure. Data are mean ± SD or median [interquartile range] with (mean rank) where appropriate. Group differences were tested by unpaired T-test or Mann-Whitney U test. Effect size is Cohen's D. N=15 HTN OM versus N=13 NTN OM.

Table 3.14 Statistical test information for comparisons of transduction slope.

HTN vs. NTN comparison (group)	Test statsitic	*P* value	Effect size (Cohen's D)
Transduction slope (YF)	z=-2.221	***0.027***	0.370
Lag producing maximal slope (YF)	T(16)=1.501	0.153	0.712
Transduction slope (PMF)	z=0.659	0.683	0.091
Lag producing maximal slope (PMF)	T(24)=0.922	0.366	0.366
Transduction slope (YM)	z=1.175	0.254	0.021
Lag producing maximal slope (YM)	z=-0.960	0.360	0.128
Transduction slope (OM)	z=-0.161	0.892	0.022
Lag producing maximal slope (OM)	T(26)=0.539	0.595	0.204

HTN; hypertensive, NTN; normotensive, YF; premenopausal females, PMF; postmenopausal females, YM; younger males, OM; older males, z; standardised test statistic for Mann-Whitney U test, T; test statistic for independent samples T-test.

Table 3.15 Statistical test data for correlations between age, burst incidence, and transduction slope.

Correlation (Group)	N	Correlation coefficient	*P* value
Age vs. transduction slope (all females)	44	ρ=-0.241	0.155
Age vs. transduction slope (all males)	56	ρ=-0.013	0.927
Age vs. transduction slope (HTN females)	19	ρ=-0.551	***0.014***
Age vs. transduction slope (NTN females)	25	ρ=-0.035	0.868
Age vs. transduction slope (HTN males)	28	ρ=-0.027	0.890
Age vs. transduction slope (NTN males)	28	ρ=0.074	0.708
Burst incidence vs. transduction slope (all females)	44	r=-0.121	0.436
Burst incidence vs. transduction slope (all males)	56	ρ=-0.126	0.355
Burst incidence vs. transduction slope (HTN females)	19	r=-0.286	0.234
Burst incidence vs. transduction slope (NTN females)	25	ρ=0.182	0.385
Burst incidence vs. transduction slope (HTN males)	28	ρ=-0.033	0.867
Burst incidence vs. transduction slope (NTN males)	28	ρ=-0.251	0.197

HTN; hypertensive, NTN; normotensive, ρ; Spearman's rho correlation coefficient, r; Pearson's correlation coefficient.

Table 3.16 Statistical test data for correlations between burst incidence and blood pressure in premenopausal and postmenopausal females.

Group	MSNA burst incidence vs.	n	Correlation coefficient	*P* value
	Premenopausal females			
HTN	Concurrent SBP	7	r=0.433	0.332
	Concurrent DBP	8	ρ=-0.410	0.313
	Concurrent MAP	7	r=0.041	0.931
	Daytime ambulatory SBP	7	r=0.176	0.706
	Daytime ambulatory DBP	7	r=0.055	0.907
	Clinic SBP	8	r=0.212	0.615
	Clinic DBP	8	r=0.409	0.315
NTN	Concurrent SBP	10	ρ=0.006	0.332
	Concurrent DBP	10	ρ=0.127	0.726
	Concurrent MAP	10	ρ=0.224	0.533
	Daytime ambulatory SBP	9	ρ=-0.245	0.526
	Daytime ambulatory DBP	9	ρ=-0.328	0.389
	Clinic SBP	10	ρ=0.212	0.615
	Clinic DBP	10	ρ=0.409	0.315
	Postmenopausal females			
HTN	Concurrent SBP	11	r=-0.055	0.873
	Concurrent DBP	11	r=0.347	0.295
	Concurrent MAP	11	r=0.156	0.646
	Daytime ambulatory SBP	10	ρ=-0.328	0.354
	Daytime ambulatory DBP	10	ρ=-0.738	***0.015***
	Clinic SBP	11	r=-0.334	0.315
	Clinic DBP	11	r=0.060	0.862
NTN	Concurrent SBP	15	ρ=0.541	***0.037***
	Concurrent DBP	15	r=0.358	0.191
	Concurrent MAP	15	r=0.156	0.646
	Daytime ambulatory SBP	15	r=0.042	0.880
	Daytime ambulatory DBP	15	r=-0.303	0.273
	Clinic SBP	15	r=-0.124	0.659
	Clinic DBP	15	ρ=-0.127	0.651

MSNA; muscle sympathetic nerve activity, HTN; hypertensive, NTN; normotensive, SBP; systolic blood pressure, DBP; diastolic blood pressure, n;

sample size, r; Pearson's correlation coefficient, ρ; Spearman's rho correlation coefficient. Daytime ambulatory values are the mean of at least 15 daytime readings over a 24 hour period. Clinic values are the mean of at least two readings taken on the day of the study.

Table 3.17 Statistical test data for correlations between burst incidence and blood pressure in younger and older males.

Group	MSNA burst incidence vs.	n	Correlation coefficient	*P* value
	Younger males			
HTN	Concurrent SBP	13	r=0.369	0.214
	Concurrent DBP	13	r=-0.043	0.890
	Concurrent MAP	13	r=0.149	0.627
	Daytime ambulatory SBP	13	ρ=0.582	***0.037***
	Daytime ambulatory DBP	13	ρ=-0.097	0.754
	Clinic SBP	13	ρ=0.137	0.655
	Clinic DBP	13	r=0.166	0.588
NTN	Concurrent SBP	15	r=0.255	0.360
	Concurrent DBP	15	r=0.084	0.766
	Concurrent MAP	15	r=0.167	0.551
	Daytime ambulatory SBP	10	r=0.498	0.143
	Daytime ambulatory DBP	10	r=0.430	0.215
	Clinic SBP	15	ρ=0.580	***0.023***
	Clinic DBP	15	r=0.545	***0.036***
	Older males			
HTN	Concurrent SBP	15	r=0.115	0.683
	Concurrent DBP	15	ρ=0.484	0.067
	Concurrent MAP	15	r=0.410	0.129
	Daytime ambulatory SBP	15	r=0.327	0.234
	Daytime ambulatory DBP	15	r=-0.249	0.371
	Clinic SBP	15	r=0.021	0.941
	Clinic DBP	15	ρ=-0.011	0.970
NTN	Concurrent SBP	13	r=-0.281	0.353
	Concurrent DBP	13	r=-0.110	0.720
	Concurrent MAP	13	r=-0.200	0.513
	Daytime ambulatory SBP	8	r=0.226	0.591
	Daytime ambulatory DBP	8	r=-0.020	0.962
	Clinic SBP	9	ρ=0.377	0.318
	Clinic DBP	9	ρ=0.033	0.932

MSNA; muscle sympathetic nerve activity, HTN; hypertensive, NTN; normotensive, SBP; systolic blood pressure, DBP; diastolic blood pressure, n;

sample size, r; Pearson's correlation coefficient, ρ; Spearman's rho correlation coefficient. Daytime ambulatory values are the mean of at least 15 daytime readings over a 24 hour period. Clinic values are the mean of at least two readings taken on the day of the study.

Table 3.18 Effect of anti-hypertensive treatment on sympathetic transduction slope.

Group (N treated vs. N untreated)	Test statistic	*P* value	Effect size
Younger females (5 vs. 3)	z=-0.447	0.786	0.112
Postmenopausal females (7 vs. 2)	z=0.242	0.333	0.057
Younger males (7 vs. 6)	z=0.429	0.731	0.084
Older males (10 vs. 5)	z=0.367	0.768	0.067

N; sample size, z; standardised test statistic for Mann-Whitney U test. Effect of treatment in each group assessed by Mann-Whitnry U test of mean ranks. Effect size is Cohen's D.

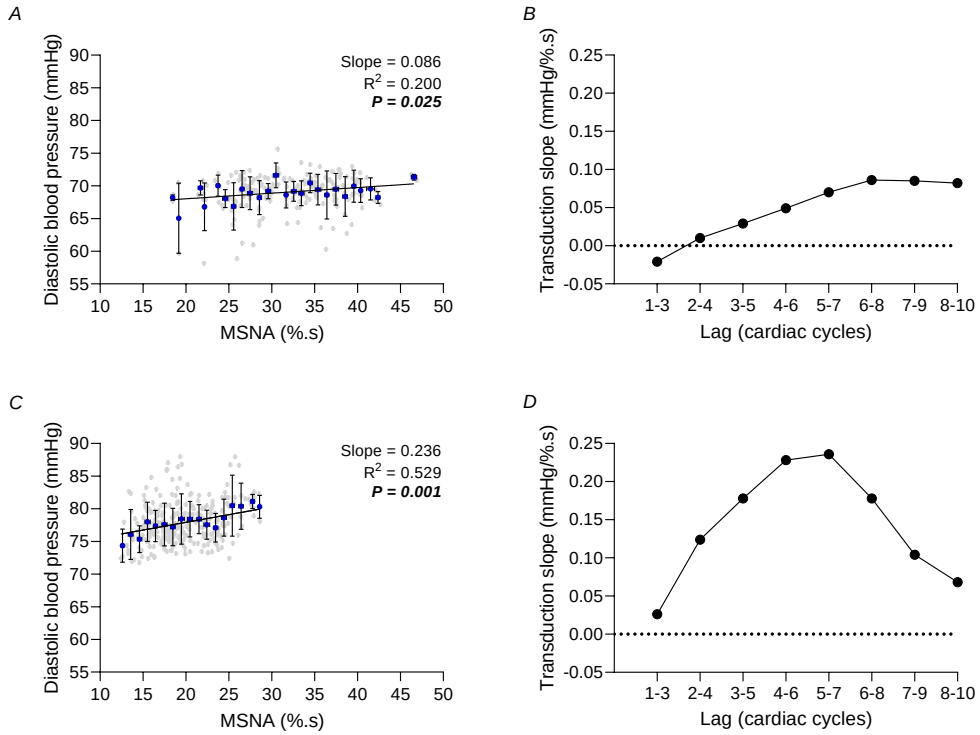

Figure 3.1 Example sympathetic transduction slopes in individuals with relatively low and high maximal slopes. (A) Maximal transduction slope in a normotensive premenopausal female participant with a relatively low slope (6-8 cardiac cycle lag) and (B) the range of transduction slopes for the same individual across all cardiac cycle lags. (C) Maximal transduction slope in a hypertensive postmenopausal female participant with a relatively high slope (5-7 cardiac cycle lag) and (D) the range of transduction slopes for the same individual across all cardiac cycle lags. In A and C, data for individual cardiac cycles are shown in grey, whilst mean ± SD data across 1 %.s bins are shown in blue. Slopes are weighted for data points per bin.

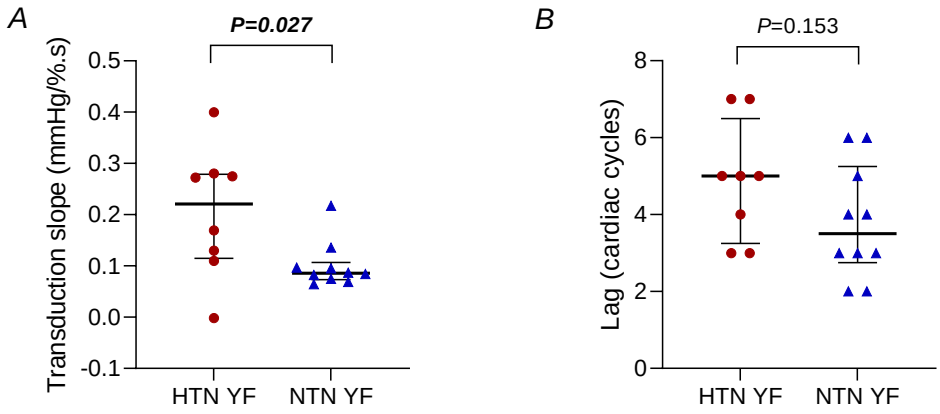

Figure 3.2 Maximum individual sympathetic transduction slope (A) and the cardiac cycle lag producing that slope (B) in hypertensive and normotensive premenopausal females. HTN; hypertensive, NTN; normotensive, YF; premenopausal females. Data median ± interquartile range (A) or mean ± SD (B). Group differences were tested by Mann-Whitney U test of mean ranks (A) or independent samples T-test (B). Mean ranks for transduction slope were 12.62 versus 7.0 (HTN versus NTN). N=8 HTN versus N=10 NTN.

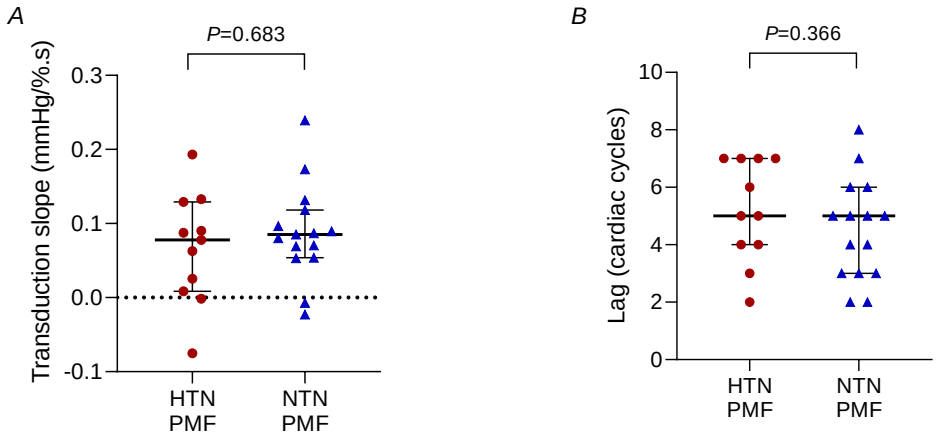

Figure 3.3 Maximum individual sympathetic transduction slope (A) and the cardiac cycle lag producing that slope (B) in hypertensive and normotensive postmenopausal females. HTN; hypertensive, NTN; normotensive, PMF; postmenopausal females. Data median ± interquartile range (A) or mean ± SD (B). Group differences were tested by Mann-Whitney U test of medians (A) or independent samples T test (B). N=11 HTN versus N=15 NTN.

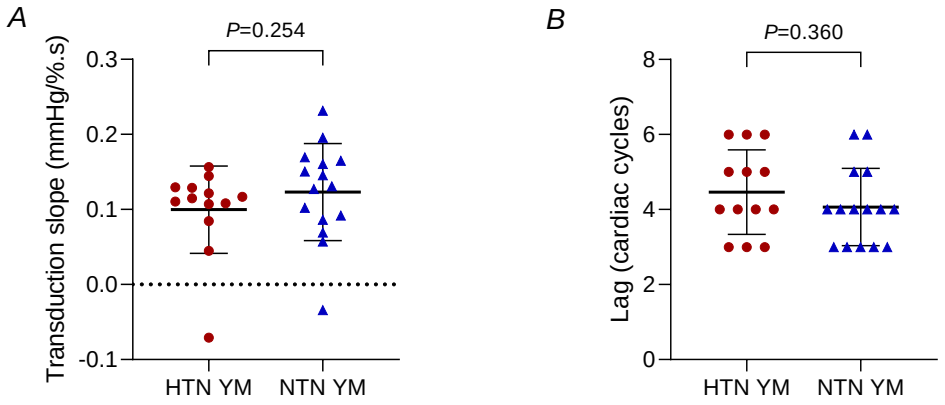

Figure 3.4 Maximum individual sympathetic transduction slope (A) and the cardiac cycle lag producing that slope (B) in hypertensive and normotensive younger males. HTN; hypertensive, NTN; normotensive, YM; younger males. Data are median ± interquartile range. Group differences were tested by Mann-Whitney U test of medians (A) or mean ranks (B). Mean ranks for cardiac cycle lag were 16.04 versus 13.17 (HTN versus NTN). N=13 HTN versus N=15 NTN.

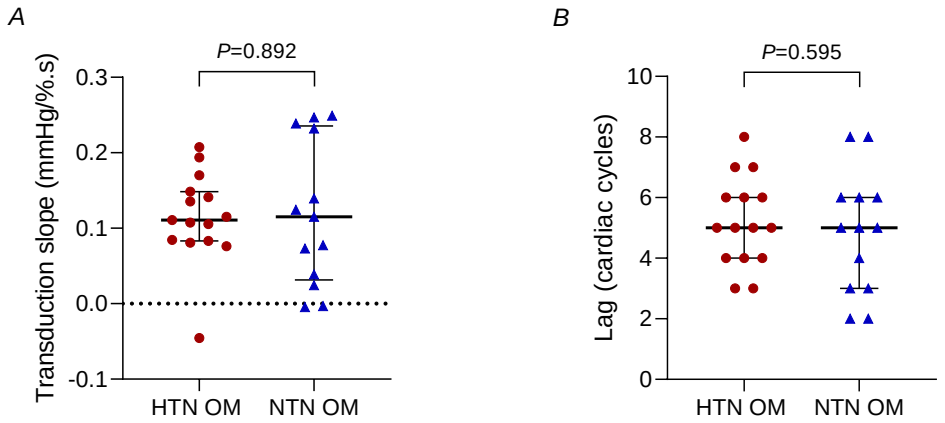

Figure 3.5 Maximum individual sympathetic transduction slope (A) and the cardiac cycle lag producing that slope (B) in hypertensive and normotensive older males. HTN; hypertensive, NTN; normotensive, OM; older males. Data median ± interquartile range (A) or mean ± SD (B). Group differences were tested by Mann-Whitney U test of mean ranks (A) or independent samples T test (B). Mean ranks for transduction slope were 14.73 versus 14.23 (HTN versus NTN). N=15 HTN versus N=13 NTN.

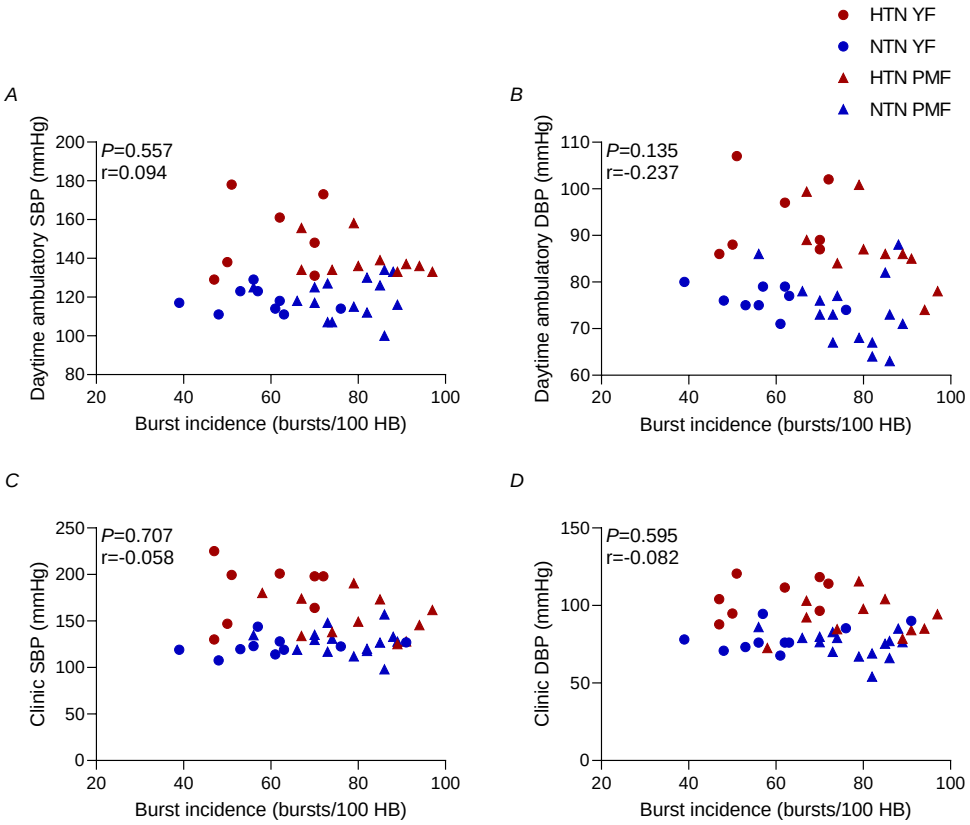

Figure 3.6 Relationships between resting MSNA and resting blood pressure in hypertensive and normotensive females. HTN; hypertensive, NTN; normotensive, YF; premenopausal females, PMF; postmenopausal females, SBP; systolic blood pressure, DBP; diastolic blood pressure, HB; heartbeats r; Pearson's correlation coefficient. (A) Burst incidence versus daytime ambulatory SBP (N=41), (B) Burst incidence versus daytime ambulatory DBP (N=41), (C) Burst incidence versus clinic SBP (N=44), (D) Burst incidence versus clinic DBP (N=44).

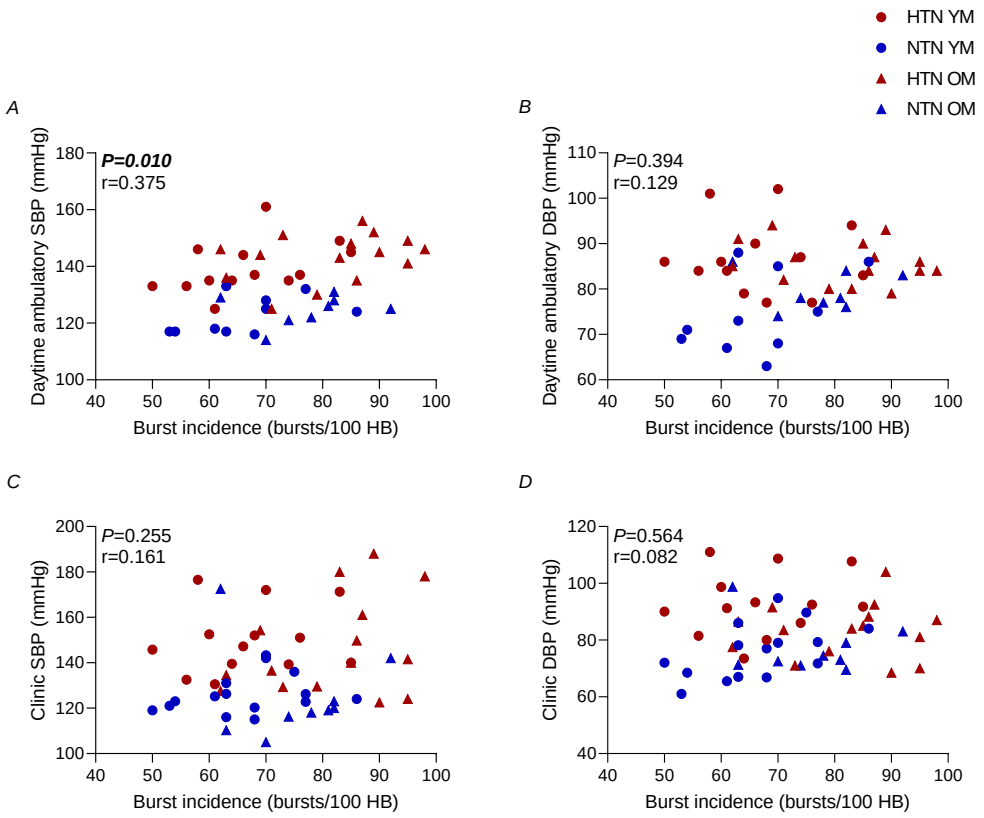

Figure 3.7 Relationships between resting MSNA and resting blood pressure in hypertensive and normotensive males. HTN; hypertensive, NTN; normotensive, YM; younger males, OM; older males, SBP; systolic blood pressure, DBP; diastolic blood pressure, HB; heartbeats r; Pearson's correlation coefficient. (A) Burst incidence versus daytime ambulatory SBP (N=46), (B) Burst incidence versus daytime ambulatory DBP (N=46), (C) Burst incidence versus clinic SBP (N=52), (D) Burst incidence versus clinic DBP (N=52).

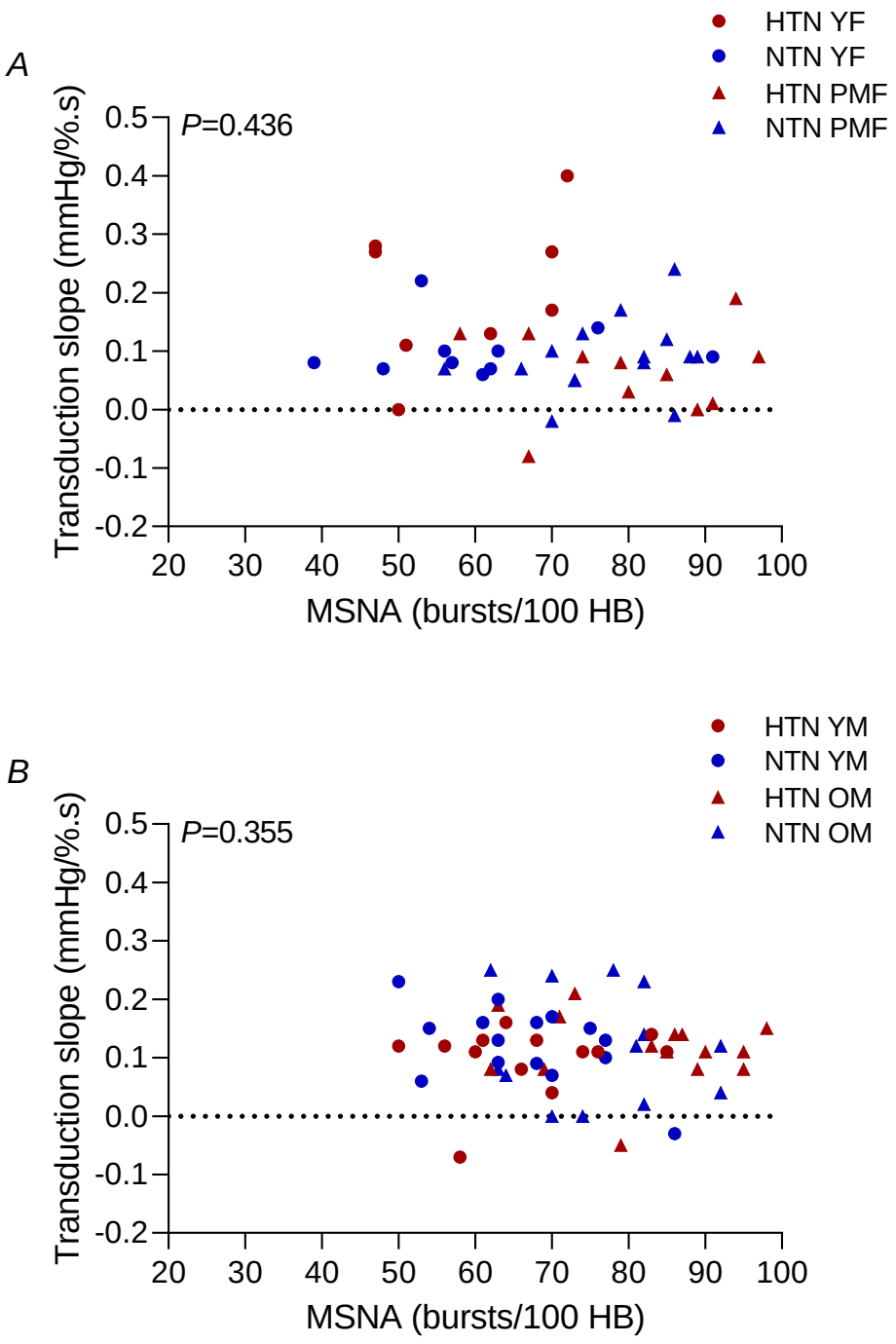

Figure 3.8 The relationship between individual resting MSNA burst incidence and individual maximum sympathetic transduction slope in (A) females (N=44) and (B) males (N=56). HTN; hypertensive, NTN; normotensive, YF; premenopausal females, PMF; postmenopausal females, YM; younger males, OM; older males, MSNA; muscle sympathetic nerve activity, HB; heartbeats. Spearman's rank correlation (A) ρ=-0.121 and (B) ρ=-0.126.

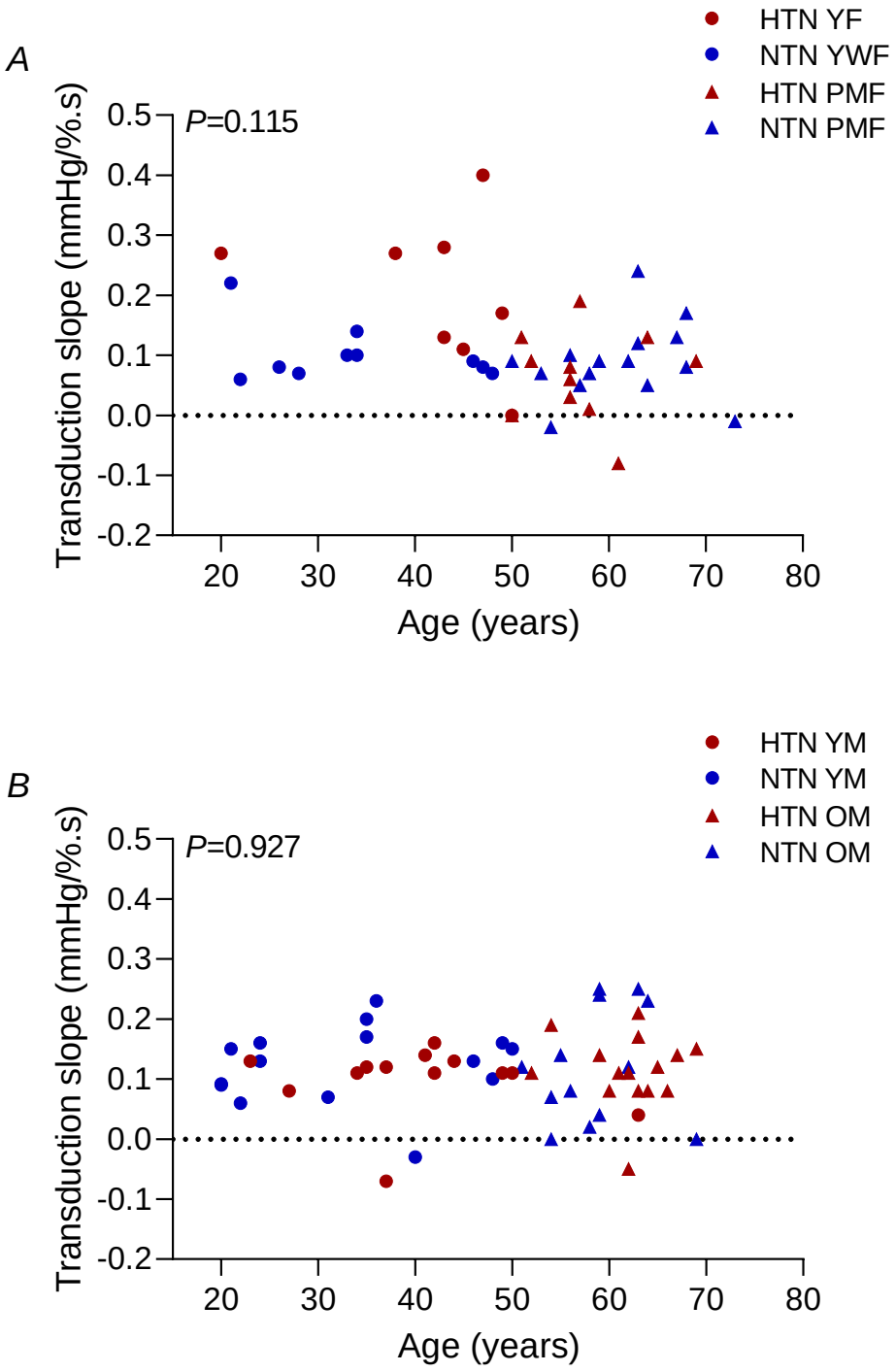

Figure 3.9 The relationship between age and individual maximum sympathetic transduction slope in (A) females (N=44) and (B) males (N=56). HTN; hypertensive, NTN; normotensive, YF; premenopausal females, PMF; postmenopausal females, YM; younger males, OM; older males. Spearman's rank correlation (A) ρ=-0.241 and (B) ρ=-0.013.

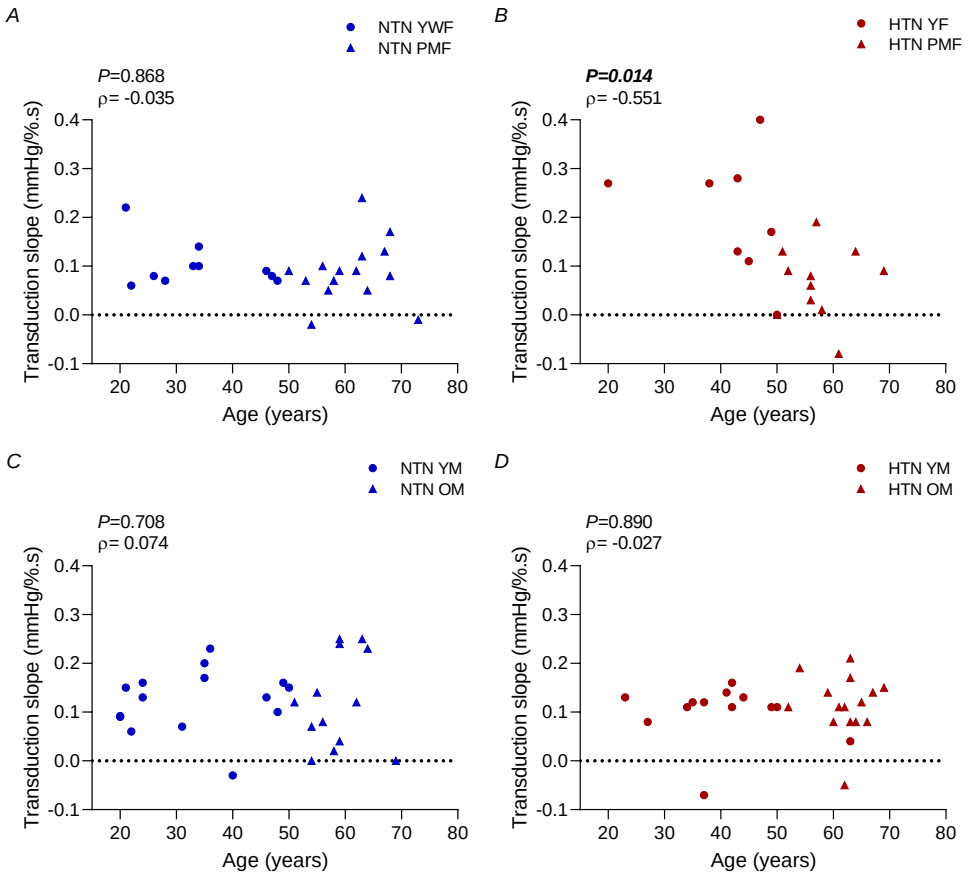

Figure 3.10 The relationship between age and sympathetic transduction slope by participant group. NTN; normotensive, HTN; hypertensive, YF, premenopausal females, PMF; postmenopausal females, YM; younger males, OM; older males, ρ; Spearman's rank correlation coefficient (A) normotensive females (N=25), (B) hypertensive females (N=19), (C) normotensive males(N=28), (D) hypertensive males (N=28).

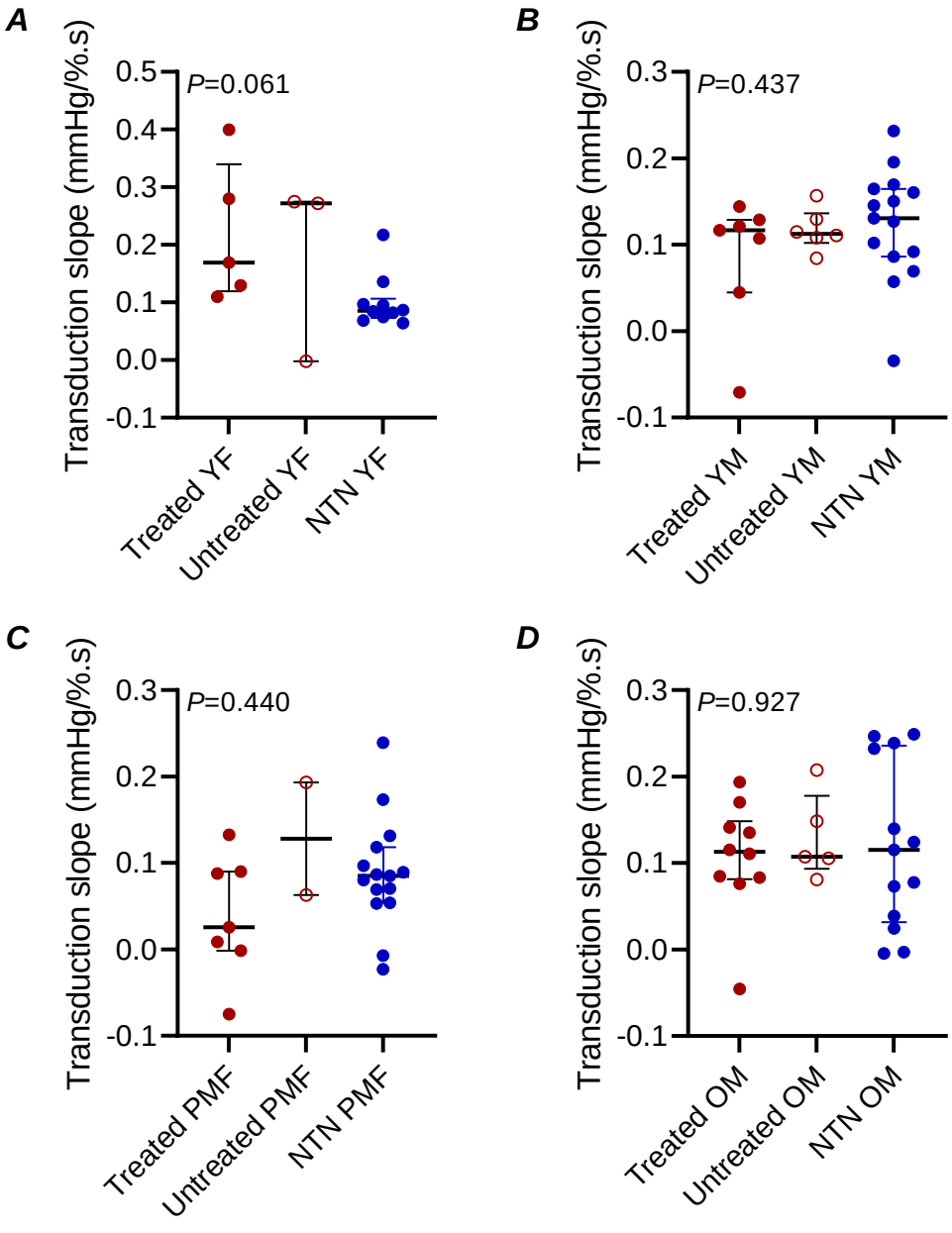

Figure 3.11 Maximum sympathetic transduction slope in treated hypertensive, untreated hypertensive, and normotensive (A) premenopausal females, (B) younger males, (C) postmenopausal females, (D) older males. YF; premenopausal females, YM; younger males, PMF; postmenopausal females, OM; older males, NTN; normotensive. Data are median ± interquartile range. Group difference was tested by Kruskal-Wallis test of mean ranks.

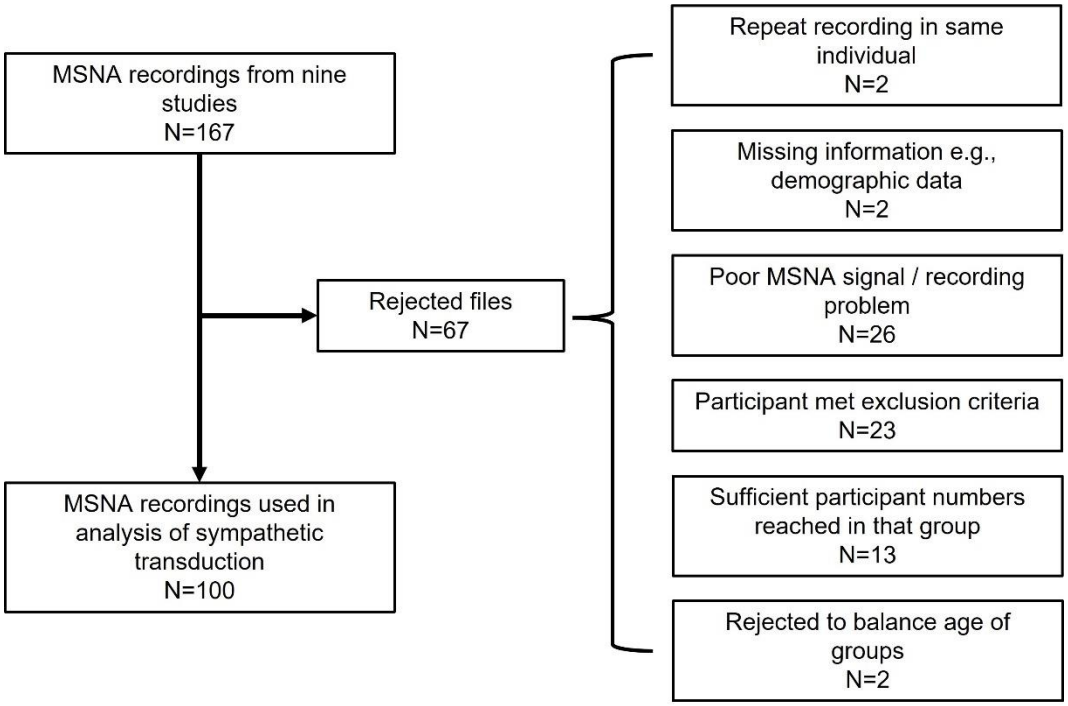

Figure 3.12 Data selection for Chapter 3.

N; sample size, MSNA; muscle sympathetic nerve activity.

Chapter 4 Sex Differences in the Effect of Systemic Beta-Adrenergic Blockade on Sympathetic Vascular Transduction in Hypertension.

4.1 Background

4.1.1 Introduction

Healthy premenopausal females show reduced transduction of sympathetic nerve activity into vasoconstrictor tone compared to age-matched males (Hart et al., 2009, Hogarth et al., 2007a). This sex difference in sympathetic vascular control may contribute to the relatively low prevalence of hypertension in young females (Hart et al., 2011a). However, hypertension occurs in some premenopausal females, and the data in chapter 3 suggest that sympathetic transduction is increased in hypertensive premenopausal females. The mechanism driving increased sympathetic transduction in hypertensive premenopausal females is unknown, but it might be related to the mechanisms by which low sympathetic transduction is maintained in normotensive premenopausal females. This chapter aimed to investigate one of these potential mechanisms, vascular beta-adrenergic receptor vasodilation, in contributing to hypertension in premenopausal females.

4.1.2 Vascular beta-adrenergic receptors in normotensive premenopausal females

In healthy adults, low sympathetic transduction in premenopausal females relative to male controls could occur through a variety of mechanisms, some of which are discussed in chapter 3. However, there is increasing evidence that the vascular beta-adrenergic receptors are important in regulating sympathetic transduction in young females.

Initial work by Kneale et al. demonstrated that the forearm vasculature of healthy young females was less responsive to infusion of noradrenaline compared to male controls (Kneale et al., 1997). This sex difference appeared to be driven by the beta-adrenergic, rather than alpha-adrenergic, receptors in the forearm, given that 1) an agonist selective for beta-2 adrenergic receptors caused greater

vasodilation in females versus males, and (2) antagonism of the beta-adrenergic receptors increased vascular responsiveness to noradrenaline in females but not males (Kneale et al., 2000). Later work supported this argument by showing that in the absence of noradrenaline (ganglionic blockade), alpha-adrenergic sensitivity was similar in young males and females (Christou et al., 2005). The role of the beta-adrenergic receptors in global blood pressure regulation in females was confirmed by Hart et al., who demonstrated that systemic beta-adrenergic antagonism (intravenous propranolol infusion) effectively re-coupled peripheral resistance to sympathetic nerve activity in young females, who exhibited no relationship between these variables prior to beta-blockade (Hart et al., 2011a). There was no effect of beta-blockade on SNA-peripheral resistance coupling in young males, however (Hart et al., 2011a). In the same cohort, it was later shown that beta-blockade increased the transduction of MSNA into diastolic blood pressure in the young females but had no effect in the young males (Briant et al., 2016). These studies suggest that the vascular beta-adrenergic receptors act to restrain the vasoconstrictor effect of sympathetic nerve activity in young females, possibly by causing greater vasodilation compared to males (Hart et al., 2011a). Importantly, there was no effect of beta-blockade on SNA-peripheral resistance coupling (Hart et al., 2011a) or transduction of MSNA into diastolic blood pressure in postmenopausal females (Briant et al., 2016). Furthermore, beta-blockade had no effect on the vascular response to noradrenaline in postmenopausal females (Hart et al., 2011a). Given that sympathetic blood pressure control appears to be modulated more heavily by beta-adrenergic vasodilation in young females versus young males and postmenopausal females, and that prevalence of hypertension is lower in young females versus both young males and postmenopausal females, it is thought that increased beta-adrenergic sensitivity could contribute to reduced hypertension risk in young females (Hart et al., 2011a).

Given that beta-adrenergic vasodilation appears to influence sympathetic control of the vasculature less in postmenopausal versus premenopausal females, it appears that female sex hormones, rather than female sex itself, underlie the mechanism of enhanced beta-adrenergic sensitivity in young females (Hart et al., 2011a). Beta-adrenergic receptor vasodilation is reliant on nitric oxide production, given that beta-adrenergic agonists have smaller vasodilatory effects in vasculature lacking an endothelium, or when nitric oxide production is inhibited (Ferro et al., 1999). Oestrogen is known to increase nitric oxide production (Miller

and Duckles, 2008), with eNOS mRNA increasing in the presence of oestrogen (Stirone et al., 2003) and eNOS knockout mice less able to exhibit a vasodilatory response to oestrogen compared to controls (Guo et al., 2005). Furthermore, human studies suggest that exogenous oestrogen increases the baseline level of nitric oxide (Sudhir et al., 1996). Additionally, oestrogen may influence expression of beta-adrenergic receptors, given that human artery tissue from females contained more mRNA for beta-1 and beta-3 (but not beta-2) adrenergic receptors than those from males (Riedel et al., 2019)

4.1.3 Vascular beta-adrenergic receptors in hypertensive young females

Chapter 3 showed that hypertensive premenopausal females have increased transduction of MSNA into diastolic blood pressure compared to normotensive controls. It is possible that this is linked to sensitivity of the beta-adrenergic receptors. If some premenopausal females exhibit reduced beta-adrenergic sensitivity compared to other premenopausal females, this may reduce their ability to modulate sympathetic vasoconstriction, leading to increased sympathetic transduction. As such, this could possibly explain cases of hypertension in premenopausal females. There is little existing data on beta-adrenergic sensitivity in hypertensive premenopausal females. Therefore, this chapter aimed to address this by assessing the contribution of the vascular beta-adrenergic receptors to sympathetic transduction in hypertensive compared to normotensive premenopausal females, using systemic beta-adrenergic blockade (propranolol infusion). It was hypothesised that if the beta-adrenergic receptors had little influence over sympathetic vasoconstriction in hypertensive premenopausal females, no significant change in sympathetic transduction would be seen in this group under beta-blockade.

4.1.4 Aims and hypotheses

Aim 1. To determine whether the effect of systemic beta-adrenergic receptor blockade on sympathetic transduction is different in hypertensive and normotensive premenopausal females.

> H_0: There will be no difference in the effect of systemic beta-adrenergic receptor blockade on sympathetic transduction between hypertensive and normotensive premenopausal females.

H_1: There will be a difference in the effect of systemic beta-adrenergic receptor blockade on sympathetic transduction between hypertensive and normotensive premenopausal females.

4.1.5 Impact of COVID-19.

Data were collected in five participants prior to the COVID-19 pandemic. The beta-blockade studies could not be conducted during COVID-19, as clinical staff were redeployed and there were limits on the number of people allowed in the laboratory. Therefore, the data in this chapter will instead act as pilot data, focusing on (1) the effect of systemic beta-blockade on sympathetic vascular transduction in the five participants recruited (comparison to previously collected data) and (2) the effect of systemic beta-blockade on sympathetic response to isometric handgrip exercise. The aims of this chapter adapted for COVID-19 are as follows:

Aim 1. To determine whether sympathetic transduction is altered by systemic beta-adrenergic receptor blockade in a pilot group of five participants.

Aim 2. To determine whether the sympathetic response to isometric handgrip exercise is altered by systemic beta-adrenergic receptor blockade in a pilot group of five participants.

4.2 Methods

4.2.1 Ethical approval

The data presented in this chapter were collected as part of the study ‘Sex Differences in the Role of SNA in Hypertension in Humans’, which received ethical approval as detailed in section 2.2.

4.2.2 Study design

The study was planned as a case-control study, with normotensive controls matched to hypertensive participants by age and sex. Participants attended the study site for approximately five hours. The study protocol is shown in Figure 4.1

4.2.3 Inclusion criteria for participation

ii. Aged 18 to approximately 40 years (female participants were premenopausal and male participants were age-matched accordingly).

4.2.4 Exclusion criteria for participation (in addition to general exclusion criteria)

i. Contraindications to propranolol, or circumstances where caution in using propranolol is advised, according to the instructions for use sheet (appendix).

ii. Use of medication that interacts with propranolol, according to the instructions for use sheet (appendix).

4.2.5 Classification of hypertensive participants

Participants were considered hypertensive if daytime average systolic ambulatory blood pressure was ≥135 mmHg (National Institute for Health and Care Excellence, 2019).

4.2.6 Induction of systemic beta-adrenergic blockade

Complete systemic antagonism of the beta-adrenergic receptors was achieved by intravenous infusion of propranolol. 1 mg/ml ampoules of propranolol hydrochloride (Dociton Solution for Injection, mibe GmbH Pharmaceuticals, Brehna, Germany) were sourced by the University Hospitals Bristol and Weston Trials Pharmacy from Germany, where marketing authorisation for the drug is held by mibe GmbH Pharmaceuticals.

The propranolol was diluted with normal 0.9 % saline to a concentration of 0.5 mg/ml and administered as a bolus of 0.15 mg/kg, followed by a maintenance infusion of 0.004 mg/kg/min, through a canula inserted into a vein at the antecubital fossa. Previous work informed the choice of dose for the bolus (Pellinger and Halliwill, 2007, Epstein et al., 1965, Gullestad et al., 1996, La Mura et al., 2009) and maintenance infusion (Hart et al., 2011a). The bolus was administered manually over 10 minutes (approximately one-tenth of the bolus volume injected per minute) and the maintenance infusion was administered

using an infusion pump (Injectomat MC Agilia, Fresenius Kabi, Malaysia), set to a pre-determined rate based on the participant's weight.

4.2.7 Safety considerations

The propranolol was prescribed to participants by one of the study doctors. A spreadsheet automatically calculated the volumes of propranolol and saline required according to participant weight (Appendix 1). These volumes were checked, and the infusions drawn up by clinical members of the team. The full list of potential side effects was presented to the participant in the Participant Information Sheet (appendix), and participants were told to expect a slowing of their heart rate in response to the infusion. It was explained to participants that the dose of propranolol they would receive could be above the maximum dose used clinically in the UK (10 mg) (National Institute for Health and Care Excellence, 2021b). For example, a 70 kg participant would receive 18.9 mg propranolol during the study. However, it was explained to participants that similar and higher doses had been previously used in research settings without leading to serious adverse events (Hart et al., 2011a).

The slow administration of the bolus (over 10 minutes) was done to allow monitoring of the participant's response to the infusion. Heart rate was monitored via two independent systems (3-lead ECG and pulse oximeter), and continuous blood pressure was monitored by finger photoplethysmography (Finometer). The infusion was stopped at the request of the participant or at the decision of the study doctor, for example in response to a large fall in heart rate. In addition, atropine and glucagon were available for emergency use as per the Advanced Life Support guidelines for bradycardia (Soar et al., 2021) and British National Formulary guidelines for glucagon (National Institute for Health and Care Excellence, 2021a). After the infusion, participants remained recumbent for 30 minutes with continuous heart rate and blood pressure monitoring, then remained with the study team for an additional 30 minutes before leaving the study site. Participants were asked to report any symptoms of orthostatic intolerance, such as dizziness, and clinic blood pressure was measured and confirmed to be within the normal range before participants left.

4.2.8 Recording of muscle sympathetic nerve activity

MSNA was recorded by microneurography in the common peroneal nerve at the fibula head, as described in section 2.10.

4.2.9 Physiological monitoring

Heart rate was monitored continuously by 3-lead ECG (AD Instruments, Dunedin, New Zealand) and by pulse oximeter. Continuous blood pressure was measured by finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Enschede, the Netherlands) (section 2.9.2).

4.2.10 Echocardiography

Echocardiography was used to measure cardiac output as described in section 2.9.3. Left ventricular outflow tract (LVOT) diameter and LVOT velocity time integral (VTI) measurements were taken before and during beta-blockade, whilst the participant rested semi-supine. Simultaneous beat to beat blood pressure was recorded by plethysmography and heart rate monitored by 3-lead ECG. Stroke volume was calculated as the product of LVOT VTI and LVOT cross-sectional area (section 2.9.1). If multiple measurements of LVOT diameter or VTI were taken within each measurement session, these were averaged, and the mean value used to calculate stroke volume. Cardiac output was calculated as the echocardiography derived stroke volume multiplied by the mean heart rate over the 10 cardiac cycles proceeding the end of VTI sampling (thus the VTI and heart rate measurements were sampled over approximately the same cardiac cycles). As the ultrasound and Powerlab systems were not integrated, it was not possible to match heart rate and VTI to the same cardiac cycle.

4.2.11 Isometric handgrip exercise

Participants completed two minutes of isometric handgrip exercise at 40 % of their maximal voluntary contraction (force transducer, AD Instruments, Dunedin, New Zealand). Visual feedback on strength of contraction was supplied to participants via LabChart, with guidelines set at 30 and 50 % of maximum voluntary contraction.

4.2.12 Study protocol

Figure 4.1 demonstrates the study protocol. Following baseline echocardiography, microneurography and handgrip exercise, the propranolol bolus was administered under careful observation of the participant. After 10 minutes, the maintenance infusion was started and MSNA and haemodynamic data were collected over a five-minute period of rest under beta-blockade. Then participant then repeated the handgrip exercise. Finally, the microneurography electrodes were removed and echocardiographic measurement of cardiac output was repeated under beta-blockade. The maintenance infusion was then stopped, and post-study monitoring of the participant began.

4.2.13 Data acquisition and analysis

Data were acquired using a Powerlab system as previously described (section 2.7). Bursts of muscle sympathetic nerve activity were identified using a custom script (Spike 2, Cambridge Electronic Design, UK, edited by Z. Adams), confirmed by an experienced investigator, and quantified as bursts/100 heartbeats and bursts/min. Burst amplitude was calculated as the peak of each burst, after all bursts had been normalised to the amplitude of the tallest burst in the analysis window, and the median burst amplitude was used to compare across experimental conditions (Sverrisdottir et al., 1998). Heart rate was calculated beat to beat and averaged across the analysis window. Systolic and diastolic blood pressure were measured respectively as the peak and trough of each blood pressure waveform and averaged across an analysis window. Beat to beat pulse pressure and mean arterial pressure were calculated from systolic and diastolic blood pressure values and similarly averaged across an analysis window. Beat to beat Finometer stroke volume was sampled at every blood pressure waveform and averaged across each analysis window. Sympathetic baroreflex sensitivity was quantified as previously described (Hart et al., 2011b). Briefly, the number of MSNA bursts (either 0 or 1) occurring in the cardiac cycle following each diastolic blood pressure was counted, and the percentage of cardiac cycles containing MSNA bursts was calculated for 1 mmHg bins of diastolic blood pressure. The weighted slope of the linear regression between percentage cardiac cycles with bursts and diastolic blood pressure was taken as a measure of sympathetic baroreflex sensitivity. Both time domain and frequency domain measures of heart rate variability were quantified using commercially

available software (LabChart 8, AD Instruments, Dunedin, New Zealand). Measures of sympathetic nerve activity, haemodynamic variables, sympathetic baroreflex sensitivity and heart rate variability were calculated over short periods of quiet rest (5 or 10 minutes) at baseline and during systemic beta-adrenergic receptor blockade.

4.2.13.1 Sympathetic transduction

The transduction of sympathetic nerve activity into vasoconstrictor tone was quantified over short periods of rest (5 or 10 minutes) at baseline and during systemic beta-adrenergic receptor blockade using a custom script (Spike2, Cambridge Electronic Design, Cambridge, UK, by Z. Adams) as previously described (section 2.8.3), (Briant et al., 2016). Briefly, for each diastolic pressure, the preceding MSNA within a two cardiac cycle window occurring at a fixed lag was calculated (modulus of the integrated neurogram). Data were averaged into 1 %.s bins of MSNA area, and the weighted slope of the linear regression between MSNA area and diastolic blood pressure was taken as a measure of sympathetic transduction. Within individuals, this was repeated for eight lags, ranging from 1-3 to 8-10 cardiac cycles.

4.2.13.2 Sympathetic and haemodynamic response to handgrip exercise

Measures of MSNA (bursts/100 heartbeats and bursts/min) and beat to beat haemodynamic variables (heart rate, Finometer stoke volume and blood pressure) were measured over six minutes of the handgrip protocol (2 minutes of rest, 2 minutes of isometric handgrip exercise at 40 % MVC, and 2 minutes of recovery), during baseline and systemic beta-adrenergic receptor blockade. Data were averaged over 30 s intervals and the last 90 s of baseline was compared to the last 30 s of handgrip, and the last 30 s of recovery.

4.2.13.3 Sympathetic action potential response to handgrip exercise

In three of the five participants, the filtered MSNA signal was of sufficient quality to identify sympathetic action potentials (AP). Over the same six minutes of the handgrip protocol, APs were detected by custom software as previously described (Action Potential Detector, (Salmanpour et al., 2010). For each individual, APs were detected separately for baseline and systemic beta-adrenergic receptor blockade and automatically sorted into bins by the software using Scott's rule (Salmanpour et al., 2010). Given that this initially generated two separate sets of bin parameters (one for the APs at baseline and one for the APs

during beta-blockade), the bin parameters were adjusted so that all APs were binned according to the same parameters, allowing direct comparison between AP firing at baseline and during beta-blockade in the same individual. Of the two original sets of bin parameters per individual, the largest number of bins, the smallest bin width, and the largest maximum bin mean amplitude were chosen as the new parameters (Badrov et al., 2016a). These were then further adjusted if the histogram of APs in each bin deviated widely from a normal distribution in either dataset. Once re-binned, AP data were quantified as APs/100 heartbeats, APs/min, APs/burst (of muscle sympathetic nerve activity), and bins/burst over 30 s intervals of the handgrip protocol. The last 90 s of baseline was compared to the last 30 s of handgrip, and the last 30 s of recovery.

4.2.13.4 Statistical analysis

Statistical analyses were conducted using SPSS Statistics 24 (IBM, New York, USA). The effect of beta-blockade on resting echocardiographic measurements, sympathetic nerve activity, haemodynamic variables and heart rate variability were assessed by Wilcoxon signed rank test, given the small sample size. Effect size was calculated as the Wilcoxon standardised test statistic / the square root of the number of datapoints (Field, 2018). The effect of beta-blockade on sympathetic, haemodynamic and AP response to isometric handgrip exercise was tested by two-way repeated measures ANOVA, with effect size given as partial η^2. Bonferroni correction was applied to post-hoc tests to account for multiple comparisons. Data are presented throughout as median [interquartile range] or mean difference (95 % confidence intervals), unless otherwise stated.

4.3 Results

4.3.1 Participant characteristics

Demographic data are available in Table 4.1. Beta-blockade data are available in five participants (one female, four male). Four participants were considered normotensive, and one participant (male) was considered hypertensive (daytime ambulatory systolic blood pressure >135 mmHg). None of the participants had received a diagnosis of hypertension or took anti-hypertensive medication. The female participant reported using a continuous method of hormonal contraception (Mirena coil), so did not take part during a particular menstrual cycle phase.

However, plasma oestradiol and progesterone concentrations of the female participant (Table 4.2) were within the laboratory reference range for premenopausal females during the follicular phase.

4.3.2 Safety reporting

No adverse events relating to the systemic infusion of propranolol occurred in the study and the infusion did not need to be stopped early in any participant. Several participants reported feeling tired after the study, but none experienced symptoms of orthostatic intolerance.

4.3.3 Resting sympathetic and haemodynamic response to beta-blockade

4.3.3.1 Echocardiography

Echocardiographic measurements are shown in Table 4.3. There was no significant effect of beta-blockade on LVOT diameter (median [interquartile range] 2.29 [0.35] versus 2.26 [0.47] cm, *P*=0.715; mean difference (95 % confidence intervals) 0.02 (-0.19 to 0.23)), or on LVOT VTI (24.4 [8.9] versus 22.3 [12.7] cm, *P*=0.144; 3.66 (-5.05 to 12.37)). Similarly, stroke volume was not significantly affected by beta-blockade (98.2 [14.2] versus 93.4 [53.2] ml, *P*=0.465; 10.65 (-26.73 to 48.03)). Median heart rate and cardiac output during echocardiography were reduced from baseline with beta-blockade, although neither difference reached significance (heart rate 65 [20] versus 56 [15] beats/min, *P*=0.068, 7.22 (0.78 to 13.66); cardiac output 6.3 [2.6] versus 5.4 [3.4] l/min, *P*=0.068, 1.14 (0.11 to 2.17)). Statistical test data and effect sizes are shown in Table 4.3.

4.3.3.2 Haemodynamic data

Resting heart rate (average across the baseline period) was significantly reduced from baseline with beta-blockade (61 [17] versus 56 [13] beats/min, *P*=0.043, 6.53 (2.62 to 10.44); Figure 4.3B). Stroke volume assessed by Modelflow (Finometer) and corresponding cardiac output did not differ between baseline and beta-blockade (stroke volume 98.4 [38.6] versus 85.6 [33.3] ml, *P*=0.273, 5.88 (-8.78 to 20.54), Figure 4.3C; cardiac output 6.3 [2.0] versus 4.9 [1.9] l/min, *P*=0.068, 0.98 (-0.05 to 2.01), Figure 4.3D). Systolic, diastolic, pulse and mean arterial pressures (Figures 4.3E-H) were similar at baseline and during beta-blockade (systolic 119 [45] versus 122 [49] mmHg, *P*=0.686, 1.67 (-7.08 to 10.41); diastolic 72 [27] versus 75 [32] mmHg, *P*=0.345, -1.10 (-4.71 to 2.51);

pulse pressure 54 [24] versus 51 [23] mmHg, *P*=0.225, 2.77 (-2.86 to 8.40); mean arterial pressure 88 [32] versus 93 [36] mmHg, *P*=0.893, -0.18 (-5.39 to 5.03)). Statistical test data and effect sizes are shown in Table 4.4.

4.3.3.3 Heart rate variability

Measures of heart rate variability during baseline and beta-blockade are shown in Table 4.5. Frequency domain analysis of heart rate variability revealed that the ratio of low to high frequency domain was reduced from baseline during beta-blockade, thus low frequency domain power was reduced with beta-blockade for a given high frequency domain power (1.09 [1.16] versus 0.83 [0.46], *P*=0.043, 0.51 (-0.17 to 1.19)). Similarly, normalised low frequency domain power was reduced from baseline during beta-blockade (51.56 [23.80] versus 44.76 [8.50] nu, *P*=0.043, 11.51 (2.53 to 20.49)). Normalised high frequency power increased from baseline to beta-blockade, but the change did not reach significance (47.07 [15.65] versus 49.82 [8.32] nu, *P*=0.080, -7.72 (-17.10 to 1.66)). Time domain analysis of heart rate variability demonstrated significant increases in heart rate variability with beta-blockade versus baseline (Table 4.5). Standard deviation of RR intervals increased from 55.59 [82.05] during baseline to 55.92 [101.91] ms (*P*=0.043, -13.62 (-34.67 to 7.43)). Root mean square of RR intervals increased from 34.32 [87.55] to 51.99 [130.77] ms (*P*=0.043, -27.12 (-56.34 to 2.09)). The number of RR intervals longer than 50 ms as a percentage of all RR intervals increased from 12 [58] to 26 [65] % (*P*=0.043, -0.09 (-0.15 to -0.03)). Statistical test data and effect sizes are shown in Table 4.5.

4.3.3.4 Sympathetic nerve activity

The effect of beta-adrenergic receptor blockade on resting sympathetic nerve activity is shown in Figure 4.2. Across the five participants, there was no significant change in resting MSNA between baseline and beta-blockade (53 [21] versus 68 [31] bursts/100 heartbeats, *P*=0.138; 30 [12] versus 28 [18] bursts/min, *P*=0.686). However, individual mean MSNA burst latency was significantly reduced from baseline with beta-blockade (1.34 [0.07] versus 1.29 [0.08] s, *P*=0.043). Median burst amplitude (normalised to tallest burst in the analysis window) was not significantly altered from baseline by beta-blockade (33 [4] versus 39 [6] %, *P*=0.080). Sympathetic baroreflex sensitivity did not differ between baseline and beta-blockade (-3.99 [2.19] versus -3.57 [3.11] %/mmHg, *P*=0.686). Statistical test data and effect sizes are shown in Table 4.4.

4.3.3.5 Sympathetic transduction

Individual transduction slopes across all lags are shown in Figures 4.4A-E, with the single largest slope produced among all lags shown in Figure 4.4F. Overall, there was no difference in the largest transduction slope for each individual between baseline and beta-blockade (0.092 [0.111] versus 0.090 [0.098] mmHg/%.s, *P*=0.225). One participant (male) had a smaller transduction slope during beta-blockade versus baseline, whilst the other four participants (three male, one female) showed a larger transduction slope during beta-blockade. The difference in the lag number that produced the maximum transduction slope during baseline versus beta-blockade was not significantly different from zero (*P*=0.414), thus the lag producing the maximum slope did not appear to be affected by beta-blockade overall. Statistical test data and effect sizes are shown in Table 4.4.

4.3.4 Effect of beta-blockade on sympathetic and haemodynamic response to isometric handgrip exercise

The time course of the sympathetic and haemodynamic response to isometric handgrip exercise is shown in Figures 4.5 and 4.10.

4.3.4.1 Sympathetic nerve activity during isometric handgrip exercise

There was no significant blockade x time interaction for burst incidence (*P*=0.308, partial η^2=0.255), nor was there a significant main effect of blockade (*P*=0.127, partial η^2=0.127) or of time (*P*=0.321, partial η^2=0.321) (Figure 4.6A, statistical test data shown in Table 4.6). In agreement, the absolute and percentage change in burst incidence between baseline and handgrip was similar in the baseline and beta-blockade conditions (Figure 4.7, Table 4.8). Similarly, there was no significant blockade x time interaction (*P*=0.454, partial η^2=0.179) or significant main effect of blockade (*P*=0.844, partial η^2=0.011) for burst frequency. However, there was a significant main effect of time for burst frequency (*P*=0.012, partial η^2=0.671), with post-hoc analysis showing a significant pairwise comparison between rest and handgrip (*P*=0.047), but not between handgrip and recovery (*P*=0.321) or between rest and recovery (*P*=0.298) (Figure 4.6B). In agreement, the absolute and percentage change in burst frequency from rest to handgrip did not differ significantly in the baseline and beta-blockade conditions (Figure 4.7, Table 4.8). For burst latency, there was no significant blockade x time interaction (P=0.394, partial η2=0.208), main effect

of time (P=0.651 (Greenhouse-Geisser corrected), partial η2=0.059), or main effect of blockade (P=0.075, partial η2=0.588) (Figure 4.6C, statistical test data in Table 4.6). Absolute and percentage change in burst latency from rest to handgrip was similar in the baseline and beta-blockade conditions (Figure 4.7, Table 4.8).

4.3.4.2 Sympathetic action potentials during isometric handgrip exercise

The response of sympathetic action potentials to handgrip exercise before and during beta-blockade was assessed in three participants. The time course of the action potential response is shown in Figure 4.8. Statistical analysis compared the last 90 s rest to the last 30 s of handgrip and the last 30 s of a two-minute recovery period (Figure 4.9 A-D). There was no significant blockade x time interaction for any measure of sympathetic action potentials (action potential incidence *P*=0.323, partial η^2=0.431; action potential frequency *P*=0.533, partial η^2=0.270; action potentials per burst *P*=0.755, partial η^2=0.131; action potential clusters per burst *P*=0.275, partial η^2=0.475) (statistical test data in Table 4.7). Similarly, there was no simple main effect of either blockade or time for any measure of sympathetic action potentials (Table 4.7).

4.3.4.3 Haemodynamic response to isometric handgrip exercise

There was no significant blockade x time interaction for heart rate (*P*=0.091, partial η^2=0.450). However, there was a significant main effect of blockade (*P*=0.003, partial η^2=0.911) and a significant main effect of time (*P*=0.005, partial η^2=0.890). Post-hoc analysis for time showed significant pairwise comparisons between rest and handgrip (*P*=0.015), and between handgrip and recovery (*P*=0.013), but not between rest and recovery (*P*=0.166) (Figure 4.11, statistical test data in Table 4.6). In agreement with the lack of significant interaction, the absolute and percentage change in heart rate between rest and handgrip was similar during baseline and beta-blockade (Figure 4.12, Table 4.8).

For stroke volume, there was no significant blockade x time interaction (*P*=0.627, partial η^2=0.110), or significant main effect of blockade (*P*=0.189, partial η^2=0.385), but there was a significant main effect of time (*P*=0.016, partial η^2=0.642). However, there were no significant pairwise comparisons after correction for multiple comparisons (Figure 4.11B and Table 4.6). The absolute and percentage change in stroke volume between rest and handgrip did not differ between the baseline and beta-blockade conditions (Figure 4.12, Table 4.8).

For cardiac output, there was no significant blockade x time interaction (P=0.325, partial η^2=0.245), or significant main effect of time (P=0.066, partial η^2=0.492), but there was a significant main effect of blockade (P=0.017, partial η^2=0.793) (Figure 4.11C, Table 4.6). Absolute and percentage change in cardiac output from rest to handgrip was similar during baseline and beta-blockade (Figure 4.12, Table 4.8).

4.3.4.4 Effect of beta-blockade on the blood pressure response to isometric handgrip exercise

No measure of blood pressure showed a significant blockade x time interaction (systolic P=0.951, partial η^2=0.013; diastolic P=0.975, partial η^2=0.006; pulse pressure P=0.661, partial η^2=0.098; mean arterial pressure P=0.986, partial η^2=0.003; Figures 4.11, statistical test data in Table 4.6). There was no significant main effect of blockade for systolic, diastolic, or mean arterial pressure (P=0.154, partial η^2=0.435; P=0.779, partial η^2=0.022; P=0.705, partial η^2=0.040 respectively). However, there was a significant main effect of blockade for pulse pressure (P=0.008, partial η^2=0.861). Systolic, diastolic, and mean arterial pressure all showed a significant main effect of time (P=0.045 (Greenhouse-Geisser corrected), partial η^2=0.670; P=0.007, partial η^2=0.863; P=0.015, partial η^2=0.805). For systolic blood pressure, no pairwise comparisons reached significance after correction for multiple comparisons. For diastolic blood pressure, there were significant pairwise comparison between both rest and handgrip (P=0.029), and handgrip and recovery (P=0.013), but not between rest and recovery (P=1.0). For mean arterial pressure, there was a significant pairwise comparison between handgrip and recovery (P=0.034), but not between rest and handgrip (P=0.054) or between rest and recovery (P=0.585). There was no significant main effect of time for pulse pressure (P=0.244, partial η^2=0.297). For all measures of blood pressure, the absolute and percentage change between rest and handgrip was similar during the baseline and beta-blockade conditions (Figure 4.13, Table 4.8).

4.3.5 Handgrip force during baseline and beta-blockade

There was no significant difference in the force generated during the last 30 s of handgrip exercise at baseline and during beta-blockade (35 ± 2 versus 33 ± 3 %

maximal voluntary contraction, *P*=0.133, Cohen's D=0.841; statistical test data in Table 4.4).

4.4 Discussion

4.4.1 Overview

The original aim of this study was to investigate the influence of vascular beta-adrenergic receptors on sympathetic transduction in hypertensive versus normotensive premenopausal females. The study aimed to address this by quantifying sympathetic transduction in terms of the relationship between MSNA and subsequent diastolic blood pressure response (Briant et al., 2016), at baseline and during systemic beta-adrenergic receptor blockade. It was hypothesised that blocking the vascular beta-adrenergic receptors would have a smaller influence on sympathetic transduction in hypertensive versus normotensive premenopausal females. Data collection was limited by COVID-19 and the current data cannot address the original hypothesis. In this discussion, the pilot data presented in the chapter will be addressed first, followed by discussion about beta-adrenergic receptors in hypertension in females.

4.4.2 Discussion of current pilot data

4.4.2.1 Sympathetic transduction under systemic beta-adrenergic receptor blockade

The primary outcome of the study was the transduction slope of MSNA into diastolic blood pressure at baseline and during beta-blockade, quantified using an established method (Briant et al., 2016). Briant et al. have previously conducted this analysis in healthy adults during systemic beta-blockade and showed that there was little effect of beta-blockade on transduction slope in males or postmenopausal females, but a reduction in transduction slope in premenopausal females. In the current data (one normotensive premenopausal female, three normotensive young males, and one hypertensive young male), there was no overall effect of beta-blockade on transduction slope, and the individual effect was variable. The female participant showed a decrease in transduction slope under beta-blockade, in contrast to Briant et al.'s previous results where every young female participant showed an increase in transduction slope under beta-blockade (Briant et al., 2016). This discrepancy is difficult to explain. Whilst the dose of propranolol used in the current study was lower than that used in the studies analysed by Briant et al., complete beta-adrenergic blockade was still expected with the lower dose (section 4.4.4.1). The variable

response of transduction slope to beta-blockade in the younger males is less unexpected, given that a variable response was similarly reported in younger males previously (Briant et al., 2016). The study is not powered to provide useful information on the effect of beta-blockade on sympathetic transduction in hypertensive young males, although the single hypertensive male participant in the current study showed a reduction in transduction slope with beta-blockade.

4.4.2.2 Resting sympathetic nerve activity under beta-adrenergic blockade

Several previous studies have conducted complete beta-adrenergic blockade in healthy adults, so the current data can be compared to those previously reported. In the current data, beta-blockade did not significantly change resting sympathetic nerve activity. This agrees with previous reports, where systemic beta-blockade did not alter sympathetic activity in young healthy participants (Hart et al., 2011a). There was little change in resting blood pressure under beta-blockade (see below), and therefore there may have been little demand to raise sympathetic activity.

There was a significant reduction in mean MSNA burst latency with beta-blockade. Burst latency correlates negatively with burst amplitude (Wallin et al., 1994) and so bursts with shorter latencies are typically of higher amplitude. Whilst median burst amplitude in the current study was slightly higher during beta-blockade versus baseline, the difference was not significant (P=0.080), although the effect size was 0.554. Individual sympathetic fibres are more likely to be active during longer RR intervals (Macefield et al., 2002), and as burst amplitude depends in part on the number of active sympathetic fibres (Shoemaker, 2017), it is possible that firing of sympathetic fibres increased as heart rate fell with beta-blockade, resulting in taller bursts in the integrated neurogram, and reduced burst latency. Whilst resting action potential firing was not assessed in the current data, action potentials were identified during the handgrip protocol in three participants. However, there did not appear to be an effect of beta-blockade on the number of action potentials firing within each burst (Figure 4.9). This could indicate that reduced burst latency with beta-blockade was not associated with taller bursts due to more action potentials per burst, however the sample size was very small. The latency of individual sympathetic action potentials has been reported to shorten with some stimuli, although these tend to be interventions involving some conscious input (e.g., handgrip exercise but not post-exercise ischaemia is associated with reduced sympathetic action

potential latency (Shoemaker, 2017)). However, beta-blockade is not a stimulus that involves obvious conscious input. Given that propranolol may cross the blood brain barrier (Laurens et al., 2019), there may be a central mechanism by which propranolol alters action potential latency, especially as beta-adrenergic receptors have been identified in the RVLM (Oshima et al., 2014). Overall, the shortening of the latency of individual action potentials cannot be ruled out as a possible cause of the shortened burst latency under beta-blockade. Although sympathetic action potentials were studied during the handgrip part of the current protocol, they were not studied at baseline because it could not be guaranteed that the electrode did not shift position between the baseline and beta-blockade rest recordings.

4.4.2.3 Resting heart rate, cardiac output, and blood pressure under beta-adrenergic receptor blockade

The primary effect of beta-blockade on resting haemodynamic variables was a reduction in resting heart rate. Under normal conditions, cardiac sympathetic activity has a positive chronotropic and inotropic effect on the heart via noradrenergic activation of cardiac beta-adrenergic receptors (Gordan et al., 2015). Therefore, bradycardia is an expected effect of sytemic beta-blockade. The reduction in mean heart rate in this study (7 beats/min) is comparable to that of previous studies where systemic beta-blockade was similarly used (8 and 6 beats/min reduction in mean heart rate in young males and young females respectively, (Hart et al., 2011a). This reduction in heart rate with beta-blockade in young adults occurs despite previous reports that young adults exhibit more vagal control of blood pressure than older adults (Jones et al., 2001, Barnes et al., 2014). Thus, resting heart rate still appears to be under some sympathetic control in healthy young adults, despite the importance of vagal regulation of blood pressure in this group. Heart rate associated with the echocardiographic measurement window was lower during beta-blockade versus baseline, but the difference did not reach significance (P=0.068). The discrepancy between the effect of beta-blockade on heart rate during the echocardiography and overall is likely due to the much smaller sampling of heart rate during echocardiography (10 cardiac cycles were used to estimate heart rate during echocardiography, whereas the average of all cardiac cycles over 5 five minutes was used to determine overall heart rate).

No significant reduction was seen in LVOT diameter or LVOT VTI, and therefore no reduction in stroke volume. Given that neither stroke volume nor heart rate during echocardiography were reduced with beta-blockade, cardiac output was not reduced. Similarly, no reduction in stroke volume measured by Modelfow (Finometer) was observed, thus cardiac output calculated by this method was similarly unchanged by beta-blockade. Previous studies using systemic beta-blockade found a reduction in resting cardiac output in young healthy adults, which was mediated by a reduction in heart rate rather than Modelflow stroke volume (Hart et al., 2011a). Thus, the lack of a reduction in cardiac output observed in the current data may be a function of the study being underpowered. The current data showed no effect of beta-adrenergic blockade on resting systolic, diastolic, pulse pressure or mean arterial blood pressure. This agrees with previous reports that beta-blockade did not alter systolic or mean arterial pressure in young adults (Hart et al., 2011a). However, Hart et al., showed small increases in diastolic blood pressure with beta-blockade in both young males and females.

4.4.2.4 Heart rate variability under beta-adrenergic receptor blockade

Time domain measures of heart rate variability were increased during beta-blockade, with standard deviation and root mean square of RR intervals increasing versus baseline, in addition to an increase in the percentage of RR intervals greater than 50 ms. Given the significant reduction in heart rate with beta-blockade, the increase in percentage of RR intervals greater than 50 ms may be a function of the overall slowing of heart rate. However, the other time domain measures indicate an increase in heart rate variability independent of change in overall heart rate. Frequency domain analysis of heart rate variability revealed a reduction in the ratio of low-frequency to high-frequency domain, suggesting a decrease in low-frequency heart rate variability relative to high-frequency variability. Additionally, there was a reduction in the normalised low-frequency domain power. Given that low-frequency variability may be indicative of sympathetic influence on heart rate (Camm et al., 1996) a reduction in low-frequency domain power is not unexpected with beta-blockade. Normalised high-frequency domain power was unchanged with beta-blockade. As high-frequency domain is indicative of cardiac parasympathetic activity (Camm et al., 1996), it appears that an increase in parasympathetic modulation of heart rate with beta-blockade did not contribute to the increase in heart rate variability.

4.4.3 Effect of beta-adrenergic receptor blockade on sympathetic and haemodynamic response to isometric handgrip exercise

4.4.3.1 Sympathetic response to handgrip exercise under beta-blockade

The current data show that beta-blockade did not alter the sympathetic response to handgrip exercise, as there was no beta-blockade x time interaction for any measure of sympathetic nerve activity. Pairwise comparisons showed that burst frequency was increased during handgrip versus baseline, but this was similar in control and beta-blockade conditions. Pellinger and Halliwill conducted a study of the haemodynamic response to handgrip exercise under beta-blockade. Whilst they did not measure sympathetic nerve activity directly, they showed that diameter of the femoral artery was reduced with handgrip exercise similarly under control and beta-blockade conditions in young males (Pellinger and Halliwill, 2007). This is in agreement with the current data, if it is assumed that the femoral vasoconstrictor response is reflective of the sympathetic response to handgrip. In the current data, when the sympathetic response was considered in terms of sympathetic action potentials, there remained no effect of beta-blockade on the action potential response to handgrip exercise. There was no significant main effect of time on any measure of sympathetic action potentials, indicating that in the current data, handgrip did not appear to alter sympathetic action potential firing. This contradicts previous work (Badrov et al., 2016b), however the handgrip stimulus used by Badrov et al. was longer (to fatigue) compared to that used in the current study.

4.4.3.2 Haemodynamic response to handgrip exercise under beta-blockade

The current data showed a similar heart rate and blood pressure response to handgrip exercise during control and beta-blockade conditions (no beta-blockade x time interaction). This disagrees with previous reports that beta-blockade decreases the heart rate response to handgrip exercise in young males, although the same study found no effect of beta-blockade on the heart rate response in females (Pellinger and Halliwill, 2007). Additionally, Pellinger and Hallwill reported a larger increase in the mean arterial pressure response to handgrip exercise in females, but no effect of beta-blockade on the same response in males. The current analysis considered males and females together, given the small sample size, which may account for the discrepancy. Furthermore, the handgrip protocol used by Pellinger and Halliwill was longer-lasting than that of

the current study (handgrip until fatigue at 35 % MVC versus 2 minutes at 40 % MVC) and thus may have elicited a stronger haemodynamic response, allowing the effect of beta-blockade to be more obvious.

4.4.4 Limitations of the current study

4.4.4.1 Validation of complete beta-blockade

In order to determine the effect of vascular beta-adrenergic receptors on sympathetic transduction in hypertensive versus normotensive young females, complete systemic blockade of the beta-adrenergic receptors would need to be achieved. Whilst the propranolol dose used in the current study is similar to those used previously (Pellinger and Halliwill, 2007, La Mura et al., 2009, Gullestad et al., 1996, Epstein et al., 1965), validation of complete beta-blockade was not attempted. Previous validation of complete beta-blockade has been achieved by comparing the heart rate response to administration of the beta-adrenergic receptor agonist isoprenaline before and during propranolol infusion, in the expectation that the heart rate response to isoprenaline would be minimal during complete beta-blockade (Bell et al., 2001). Given the long protocol in the current study, it was deemed too logistically challenging to validate beta-blockade with isoprenaline in this instance. As propranolol is a competitive antagonist of isoprenaline at the beta-adrenergic receptors, the difference in heart rate response to isoprenaline before and during beta-blockade would depend on the dose of isoprenaline used (Coltart and Shand, 1970). Therefore, Coltart and Shand recommend assessing the effect of beta-blockade on physiological tachycardia, and showed that a plasma propranolol concentration of ~100 ng/ml was sufficient to prevent further propranolol-induced reductions in the heart rate response to maximal exercise (Coltart and Shand, 1970). Castleden and George reported that 0.15 mg/kg propranolol (the bolus dose used in the current study) produced a plasma concentration of ~50-60 ng/ml (Castleden and George, 1979), which is below the 100 ng/ml needed to achieve the maximum inhibitory effect on heart rate response to exercise (Coltart and Shand, 1970). Therefore, it is possible that the 0.15 mg/kg dose used did not produce plasma concentrations of 100 ng/ml in the current study. However, plasma propranolol concentrations were not measured, so this cannot be confirmed. Additionally, assessing the heart rate response to exercise or isoprenaline to determine efficacy of beta-blockade may not be appropriate for the current study, as the primary outcome of sympathetic transduction was measured in the resting state. Hansson et al. found

that when doses of propranolol were administered cumulatively, no additional significant reduction in cardiac index was achieved above a total dose of 0.05 mg/kg, with the propranolol-induced decline in cardiac index arising primarily from reductions in heart rate (Hansson et al., 1974). Therefore, smaller doses may be effective enough to achieve sufficient beta-blockade at rest. However, it is important to note that the above methods of assessing beta-blockade validity all rely on measuring block of the cardiac beta-adrenergic receptors. As the current study was primarily interested in blocking the vascular beta-adrenergic receptors, assessing cardiac beta-blockade may be of limited use. Overall, the most practical solution to address whether complete vascular beta-blockade was achieved in the current study would most likely have been to sample plasma propranolol concentration.

4.4.5 Beta-adrenergic receptors in hypertensive premenopausal females

This chapter was unable to answer whether hypertensive premenopausal females have reduced beta-adrenergic sensitivity compared to normotensive premenopausal females. However, the mechanisms behind a potential reduction in receptor sensitivity with hypertension can still be discussed in the light of existing evidence.

Reduced expression of beta-adrenergic receptors on the vasculature of hypertensive premenopausal females could reduce overall sensitivity. Evidence from animal models (Limas and Limas, 1979) and humans (Peng et al., 2000, Mills et al., 1995, Sherwood et al., 2017) shows that beta-adrenergic receptors can be downregulated in hypertension, secondary to high levels of MSNA (Sherwood et al., 2017). However, chapter 3 showed that hypertensive premenopausal females have increased sympathetic transduction compared to normotensive controls despite a similar level of resting MSNA. Therefore, there may not be sufficient sympathetic stimulus to cause significant receptor downregulation in hypertensive premenopausal females. Riedel et al. showed that oestrogen has a role in regulating expression of beta-adrenergic receptors in rats and humans, given that artery samples from females had greater levels of beta-adrenergic receptor mRNA compared to samples from male participants, and beta-adrenergic mRNA was reduced by ovariectomy in rats (Riedel et al., 2019). Therefore, early decline in oestrogen may influence beta-adrenergic receptor expression.

Another potential mechanism behind reduced beta-adrenergic sensitivity in hypertensive premenopausal females is variation in the beta-2 adrenergic receptor gene with effects on receptor function. Several single nucleotide polymorphisms (SNP) have been identified in the beta-2 adrenergic receptor gene and the functional implications of these have been studied in a variety of populations (Brodde, 2008). Of relevance to this chapter, some studies report that some SNPs are associated with enhanced vasodilator responses (particularly the Arg16Gly and Gln27Glu SNPs), although the variant associated with larger vasodilation is not consistent across studies (Brodde, 2008). For example, some studies reported that greater vasodilatory responses are associated with Arg16 (Gratze et al., 1999, Hoit et al., 2000), whilst others report greater vasodilation with Gly16 (Cockcroft et al., 2000, Garovic et al., 2003). Additional studies have studied the potential link between SNPs and hypertension, although the results are again contradictory. Hahntow et al. have collected studies that report associations between hypertension and both alleles of the Arg16Gly and Gln27Glu SNPs, as well studies that report no association (Hahntow et al., 2006), although these studies tend to be in a wide population. There is little data specifically in premenopausal females. A study of vascular responses to sympathoexcitatory stimuli in obese premenopausal females showed that greater vasodilatory responses were observed in those with the Gly16/Glu27 haplotype versus those with Arg16/Gln27 or Gly16/Gln27 (Trombetta et al., 2005). However, a study assessing sex x SNP interactions in a cohort of individuals with wide variation in blood pressure (top and bottom fifth percentiles) found that there was no effect of the Arg16Gly SNP on diastolic blood pressure in females, but there was an association in males (with those homozygous for Gly16 tended to have greater diastolic blood pressure) (Rana et al., 2007). Overall, there is not enough direct evidence to demonstrate whether SNPs in the beta-2 adrenergic receptor gene contribute to hypertension in premenopausal females, however the possibility of a genetic cause for reduced beta-adrenergic sensitivity in this group cannot be ruled out.

4.4.6 Conclusion

The original aim of this study remains unanswered. The pilot data in this chapter have shown that, similar to previous results, systemic beta-adrenergic blockade causes a reduction in heart rate with little change to cardiac output, blood

pressure of sympathetic activation. Beta-blockade also increased heart rate variability. Novel findings of this chapter are that the pilot data demonstrate a reduction in MSNA burst latency during beta-blockade versus baseline, however the mechanism behind this remains unknown. There was little effect of beta-blockade on the sympathetic and haemodynamic response to isometric handgrip exercise.

4.5 Tables and figures

Table 4.1 Participant characteristics for the beta-blockade study.

	NTN (N=4)	HTN (N=1)
N (female/male)	1/4	0/1
Age (years)	24 [13]	23
Height (m)	1.74 [0.15]	181
Weight (kg)	68.4 [13.7]	74.4
BMI (kg/m^2)	21.2 [3.9]	22.7
Clinic systolic BP (mmHg)	119 [4]	152
Clinic diastolic BP (mmHg)	69 [14]	80
Clinic heart rate (beats/min)	72 [19]	72
Daytime ambulatory systolic BP (mmHg)	117 [16]	137
Daytime ambulatory diastolic BP (mmHg)	80 [19]	77
Daytime ambulatory heart rate (beats/min)	64 [13]	73

N; sample size, BMI; body mass index, BP; blood pressure. Ambulatory blood pressure monitoring conducted in 4/5 participants. Median [interquartile range].

Table 4.2 Plasma oestradiol and progesterone concentrations on the day of the study.

	Plasma oestradiol concentration (pmol/l)	Plasma progesterone concentration (nmol/l)
Female (N=1)	156	<1
Male (N=4)	101 ± 60	<1 (N=2) 1 (N=2)

N; sample size. Data are median ± interquartile range where applicable. All values were within the laboratory reference ranges (Appendix 1).

Table 4.3 Resting echocardiographic data during baseline and beta-adrenergic receptor blockade.

	Baseline	Beta-blockade	*P* value	Test statistic	Effect size
LVOT diameter (cm)	2.29 [0.35]	2.26 [0.47]	0.715	4.0	0.129
LVOT VTI (cm)	24.4 [8.9]	22.3 [12.7]	0.144	1.0	0.517
Stroke volume (ml)	98.2 [14.2]	93.5 [53.2]	0.465	3.0	0.258
Heart rate (beats/min)	65 [20]	56 [15]	0.068	0.0	0.646
Cardiac output (l/min)	6.3 [2.6]	5.4 [3.4]	0.068	0.0	0.646

LVOT; left ventricular outflow tract, VTI; velocity time integral. N=4, median [interquartile range]. Wilcoxon signed rank test. Effect size is Cohen's D.

Table 4.4 Statistical test data for effect of beta- adrenergic receptor blockade on resting haemodynamic variables.

Variable	Test statistic	P value	Effect size
Heart rate	z=-2.023	***0.043***	0.640
Stroke volume (Modelflow)	z=-1.095	0.273	0.387
Cardiac output (Modelflow-derived)	z=-1.826	0.068	0.646
Systolic blood pressure	z=-0.405	0.686	0.128
Diastolic blood pressure	z=0.345	0.345	0.299
Pulse pressure	z=1.214	0.225	0.384
Mean arterial pressure	z=0.135	0.893	0.043
Burst incidence	z=1.483	0.138	0.469
Burst frequency	z=0.405	0.686	0.128
Mean burst latency	z=-2.023	***0.043***	0.640
Median burst amplitude	z=1.753	0.080	0.554
Sympathetic baroreflex sensitivity slope	z=-0.405	0.686	0.128
Maximum sympathetic transduction slope	z=-1.214	0.225	0.384
Lag number associated with maximum transduction slope	z=0.816	0.414	0.258
Force generated during last 30 s of isometric handgrip exercise	T(4) = 1.880	0.133	0.841

z; standardised test statistic for Wilcoxon signed rank test. Effect size is Cohen's D.

Table 4.5 Heart rate variability at baseline and during steady state beta-adrenergic receptor blockade.

	Baseline	Beta-blockade	z	*P* value	Effect size
LF/HF ratio	1.09 [1.16]	0.83 [0.46]	-2.023	***0.043***	-0.640
LF (nu)	51.56 [23.80]	44.76 [18.50]	-2.023	***0.043***	-0.640
HF (nu)	47.07 [15.65]	49.82 [8.32]	1.753	0.080	0.554
LF (ms^2)	555 [4182]	846 [4012]	-0.135	0.893	-0.043
HF (ms^2)	456 [5517]	830 [9806]	2.023	***0.043***	0.640
LF (%)	27.35 [13.67]	28.19 [2.35]	-0.674	0.500	-0.213
HF (%)	32.47 [21.79]	35.15 [16.39]	1.753	0.080	0.554
SDRR (ms)	55.59 [82.05]	55.92 [82.05]	2.023	***0.043***	0.640
RMSSD (ms)	34.32 [87.55]	51.99 [130.77]	2.023	***0.043***	0.640
pRR50 (%)	12.08 [57.70]	26.14 [64.99]	2.023	***0.043***	0.640

LF/HF ratio; ratio of high to low frequency, LF; low frequency domain, HF; high frequency domain, SDRR; standard deviation of RR intervals, RMSSD; root mean square of RR intervals, pRR50; RR intervals longer than 50 ms as a percentage of all RR intervals, z; standardised test statistic. N=5; median [interquartile range]. Wilcoxon signed rank test. Effect size is Cohen's D.

Table 4.6 Statistical test data for effect of beta-adrenergic receptor blockade on sympathetic and haemodynamic response to handgrip exercise.

Variable	Test statistic	*P* value	Partial η^2
Burst incidence			
Interaction	F(2, 8) = 1.369	0.308	0.255
Beta blockade	F(1, 4) = 3.697	0.127	0.480
Time	F(2, 8) = 1.313	0.321	0.27
Burst frequency			
Interaction	F(2, 8) = 0.873	0.454	0.179
Beta blockade	F(1, 4) = 0.044	0.844	0.011
Time	F(2, 8) = 8.168	***0.012***	0.671
Burst latency			
Interaction	F(2, 8) = 1.050	0.394	0.208
Beta blockade	F(1, 4) = 5.708	0.075	0.588
Time	F(2, 8) = 0.251	0.651	0.059
Heart rate			
Interaction	F(2, 8) = 3.278	0.091	0.450
Beta blockade	F(1, 4) = 40.964	***0.003***	0.911
Time	F(2, 8) = 0.005	***0.005***	0.890
Stroke volume			
Interaction	F(2, 8) = 0.494	0.627	0.110
Beta blockade	F(1, 4) = 2.505	0.189	0.385
Time	F(2, 8) = 7.166	***0.016***	0.642
Cardiac output			
Interaction	F(2, 8) = 1.296	0.325	0.245
Beta blockade	F(1, 4) = 15.32	***0.017***	0.793
Time	F(2, 8) = 3.879	0.066	0.492
Mean arterial pressure			
Interaction	F(2, 8) = 0.014	0.986	0.003
Beta blockade	F(1, 4) = 0.166	0.705	0.040
Time	F(2, 8) = 16.53	***0.015***	0.805
Systolic blood pressure			
Interaction	F(2, 8) = 0.051	0.951	0.013
Beta blockade	F(1, 4) = 3.079	0.154	0.435
Time	F(2, 8) = 8.129	***0.045*** (GG)	0.670

Diastolic blood pressure			
Interaction	F(2, 8) = 0.026	0.975	0.006
Beta blockade	F(1, 4) = 0.090	0.779	0.022
Time	F(2, 8) = 25.14	***0.007***	0.863
Pulse pressure			
Interaction	F(2, 8) = 0.437	0.661	0.098
Beta blockade	F(1, 4) = 24.736	***0.008***	0.861
Time	F(2, 8) = 1.691	0.244	0.297

F; ANOVA test statistic, GG; Greenhouse-Geisser correction. Two-way repeated measures ANOVA.

Table 4.7 Statistical test data for effect of beta-adrenergic receptor blockade on the sympathetic action potential response to handgrip exercise.

Variable	Test statistic	P value	Partial η^2
AP incidence			
Interaction	F(2, 4) = 1.518	0.323	0.431
Beta blockade	F(1, 2) = 0.016	0.911	0.008
Time	F(2, 4) = 0.400	0.694	0.167
AP frequency			
Interaction	F(2, 4) = 0.740	0.533	0.270
Beta blockade	F(1, 2) = 1.534	0.341	0.434
Time	F(2, 4) = 0.786	0.515	0.282
AP/burst			
Interaction	F(2, 4) = 0.301	0.755	0.131
Beta blockade	F(1, 2) = 0.494	0.555	0.198
Time	F(2, 4) = 0.455	0.664	0.185
Clusters/burst			
Interaction	F(2, 4) = 1.811	0.275	0.475
Beta blockade	F(1, 2) = 0.550	0.536	0.216
Time	F(2, 4) = 0.012	0.989	0.006

AP; action potential, F; ANOVA test statistic. Two-way repeated measures ANOVA.

Table 4.8 Statistical test data for the effect of beta-adrenergic receptor blockade on change in haemodynamic variables with isometric handgrip exercise.

Variable	Test statistic	*P* value	Effect size
Δ Burst incidence	T(4)=-1.762	0.153	-0.788
%Δ Burst incidence	T(4)=-2.370	0.077	-1.060
Δ Burst frequency	z=1.214	0.225	0.384
%Δ Burst frequency	z=1.483	0.138	0.469
Δ Burst latency	T(4)=-0.934	0.403	-0.418
%Δ Burst latency	T(4)=0.977	0.384	0.437
Δ Heart rate	z=-1.483	0.138	-0.469
%Δ Heart rate	z=-0.944	0.345	-0.299
Δ Stroke volume	T(4)=-0.516	0.633	-0.231
%Δ Stroke volume	z=-0.135	0.893	-0.043
Δ Cardiac output	T(4)=1.881	0.133	0.841
%Δ Cardiac output	T(4)=0.588	0.588	0.263
Δ Systolic blood pressure	z=0.135	0.893	0.043
%Δ Systolic blood pressure	T(4)=-0.177	0.868	-0.079
Δ Diastolic blood pressure	z=-0.135	0.893	-0.043
%Δ Diastolic blood pressure	T(4)=0.100	0.925	0.045
Δ Pulse pressure	T(4)=0.018	0.986	0.008
%Δ Pulse pressure	z=0.135	0.893	0.043
Δ Mean arterial pressure	z=-0.135	0.893	-0.043
%Δ Mean arterial pressure	T(4)=-0.045	0.967	-0.020

Δ; absolute change, Δ%; percentage change, z; standardised test statistic for Wilcoxon signed rank test. Effect of beta-blockade tested by paired T-test or Wilcoxon signed rank test. Effect size is Cohen's D.

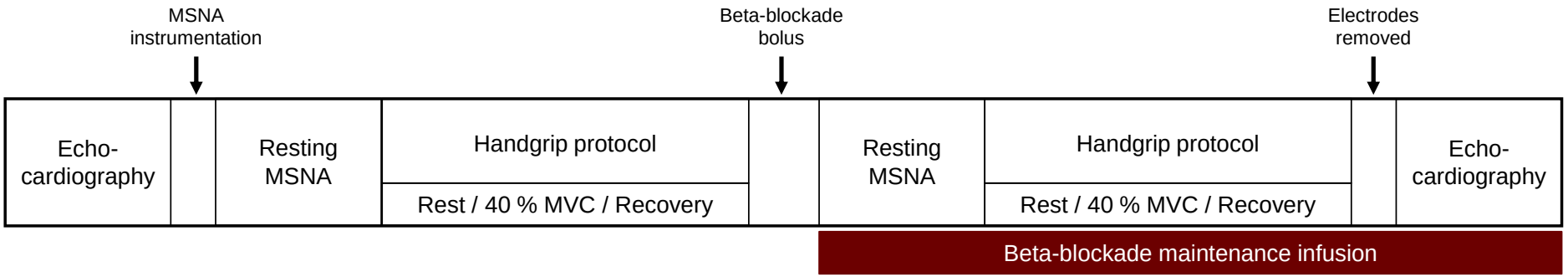

Figure 4.1 Experimental protocol for beta-blockade studies. MSNA; muscle sympathetic nerve activity, MVC; maximal voluntary contraction. MSNA instrumentation lasted up to 60 minutes; beta-blockade bolus administration lasted 10 minutes; beta-blockade maintenance infusion lasted up to 30 minutes.

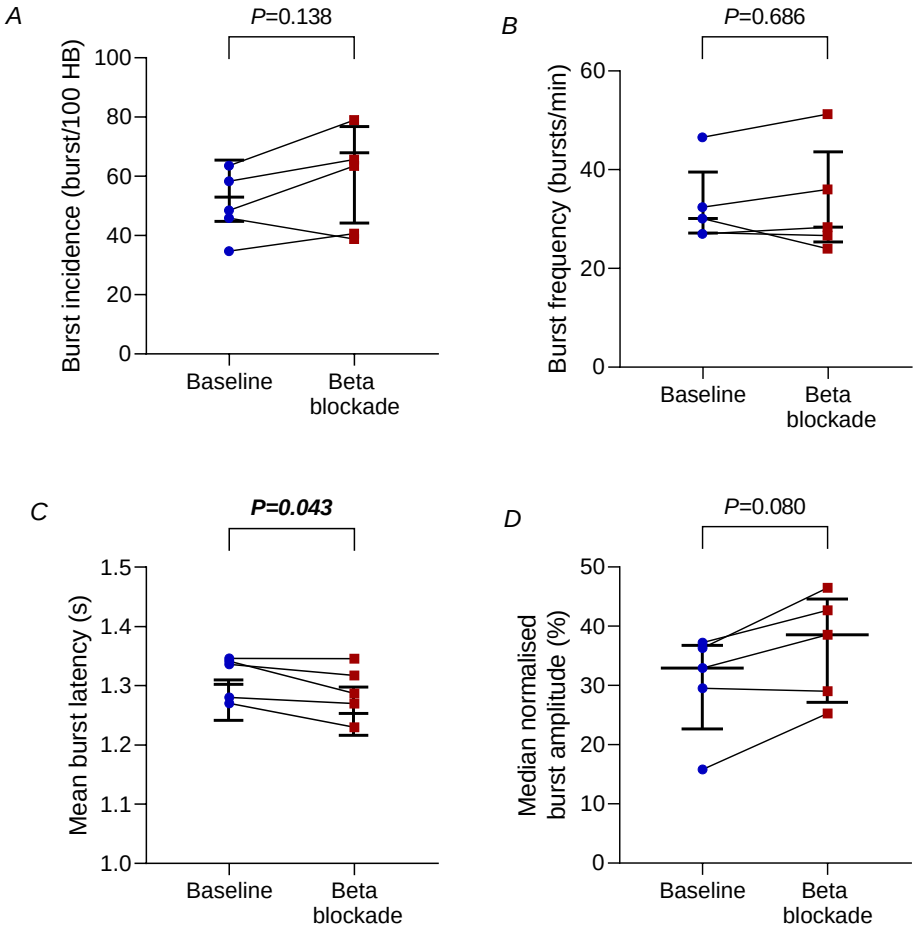

Figure 4.2 Sympathetic nerve activity during baseline and beta-adrenergic receptor blockade. HB; heartbeats. (A) burst incidence, (B) burst frequency, (C) burst latency, (D) burst amplitude normalised to amplitude of the tallest burst in the analysis window. N=5, median ± interquartile range. Wilcoxon signed rank test.

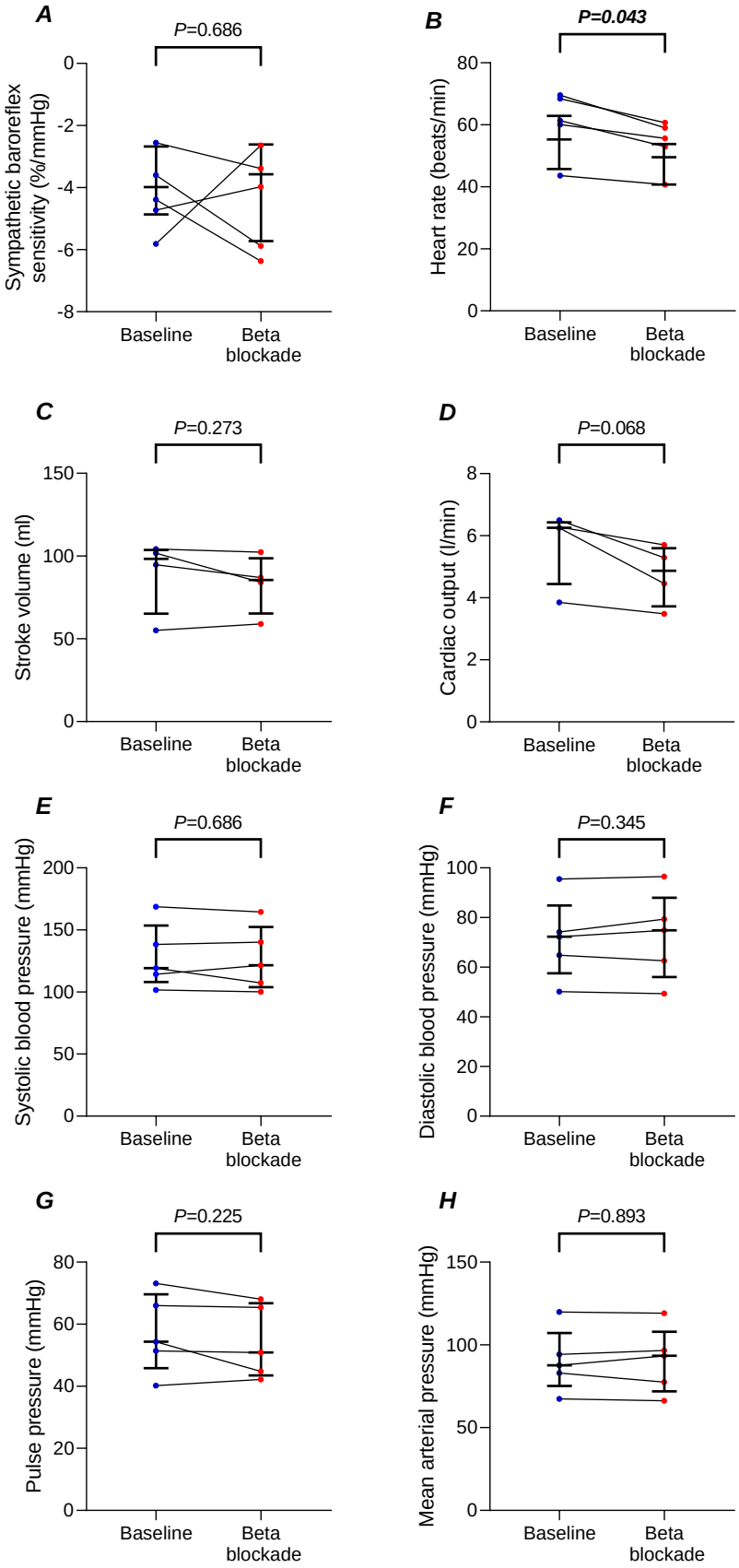

Figure 4.3 Resting haemodynamics during baseline and steady state beta-adrenergic receptor blockade. Blood pressure (E-H) measured by Finometer. Stroke volume (C) estimated by Modelflow (Finometer) and cardiac output (D)

derived from stroke volume and heart rate. N=5 except stroke volume and cardiac output (N=4), due to poor quality recording in one participant. For B-H, individual data are the mean over the analysis window (5-10 minutes). Group data are median ± interquartile range. Wilcoxon signed rank test.

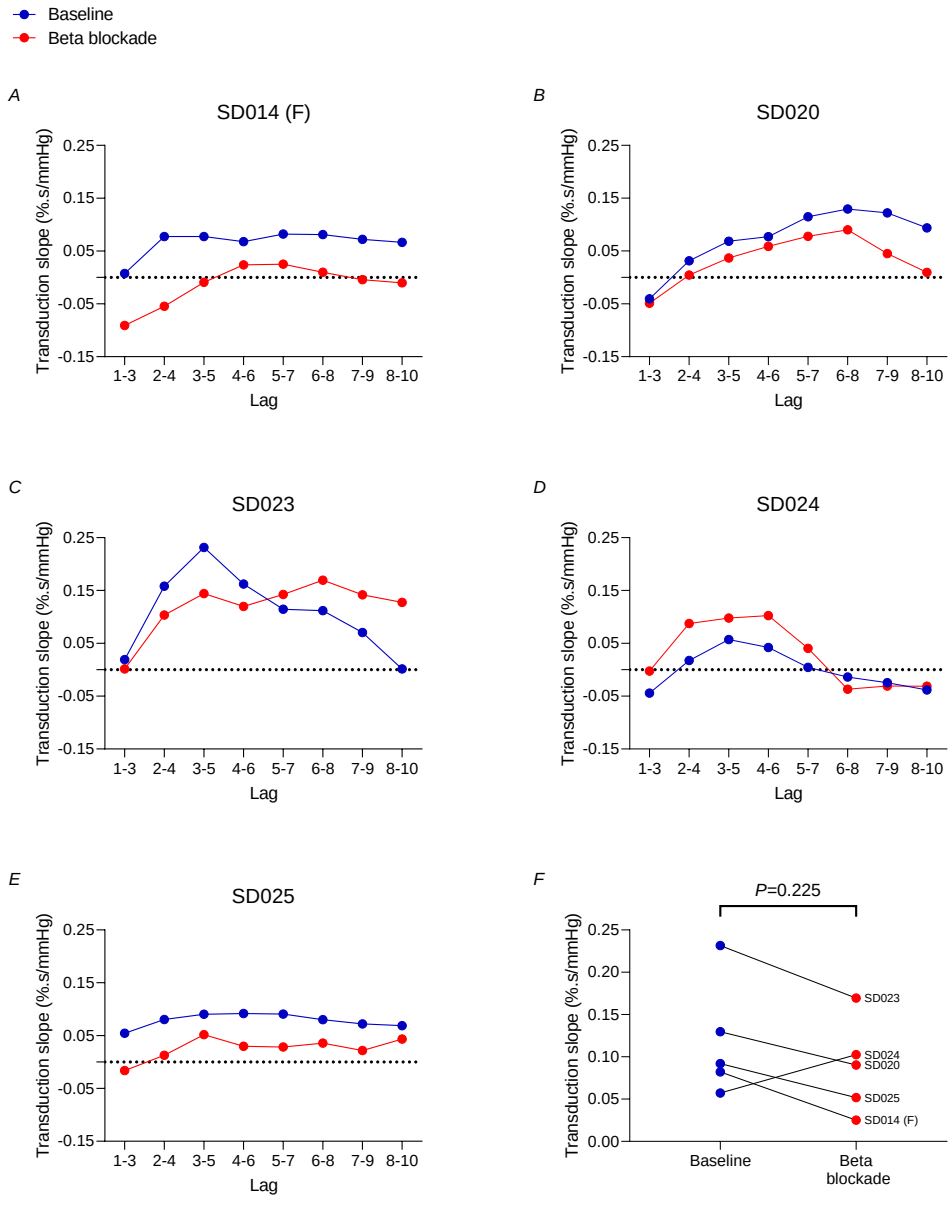

Figure 4.4 Sympathetic transduction during baseline and beta-adrenergic receptor blockade. Individual sympathetic transduction slopes across eight different cardiac cycle lags (A-E) and the single maximum transduction slope produced among all lags (F). Difference in maximum transduction slope (F)

tested by Wilcoxon signed rank test. SD014 was the female participant. SD020 was the hypertensive male participant.

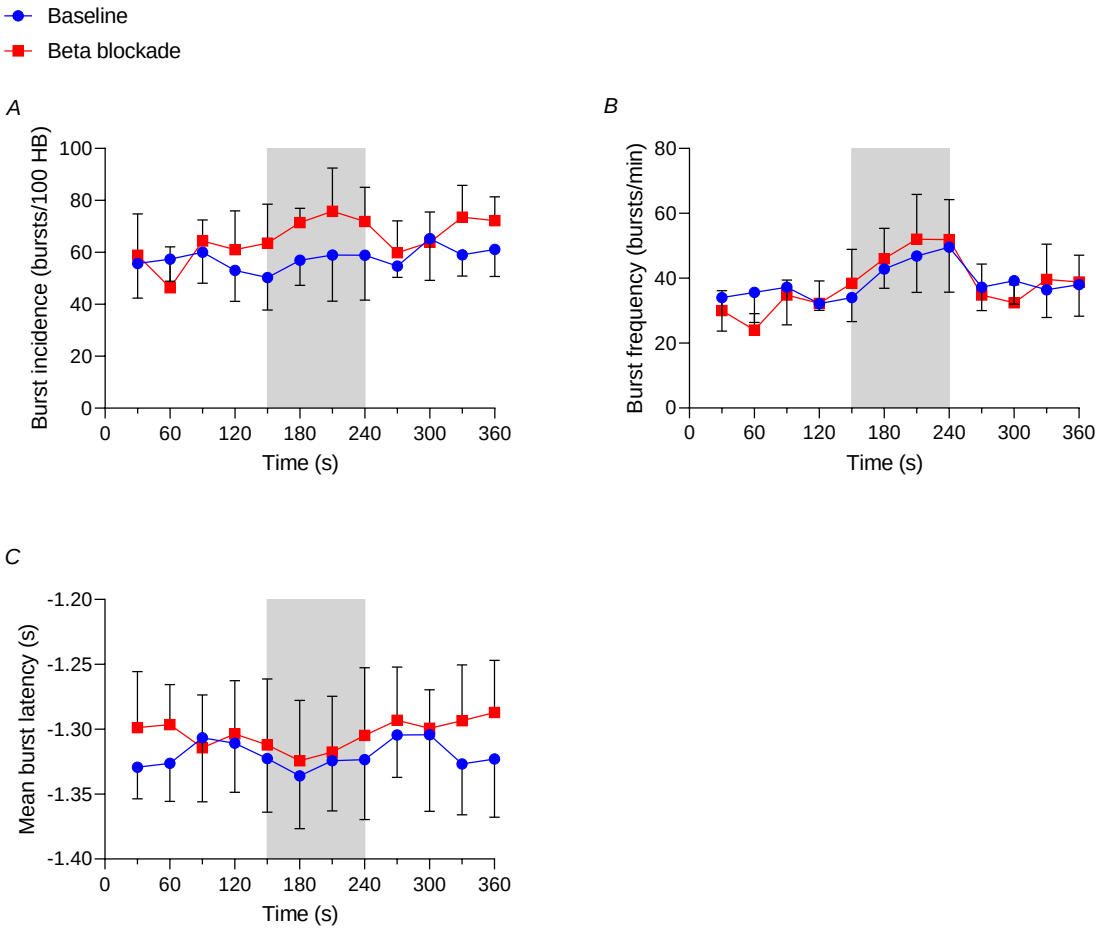

Figure 4.5 Time course of the sympathetic response to isometric handgrip exercise during baseline and beta-adrenergic receptor blockade. HB; heartbeats. (A) burst incidence, (B) burst frequency, (C) mean burst latency. Mean ± SD over 30 s intervals before, during (grey) and after two minutes of isometric handgrip exercise at 40 % maximal voluntary contraction. N=5.

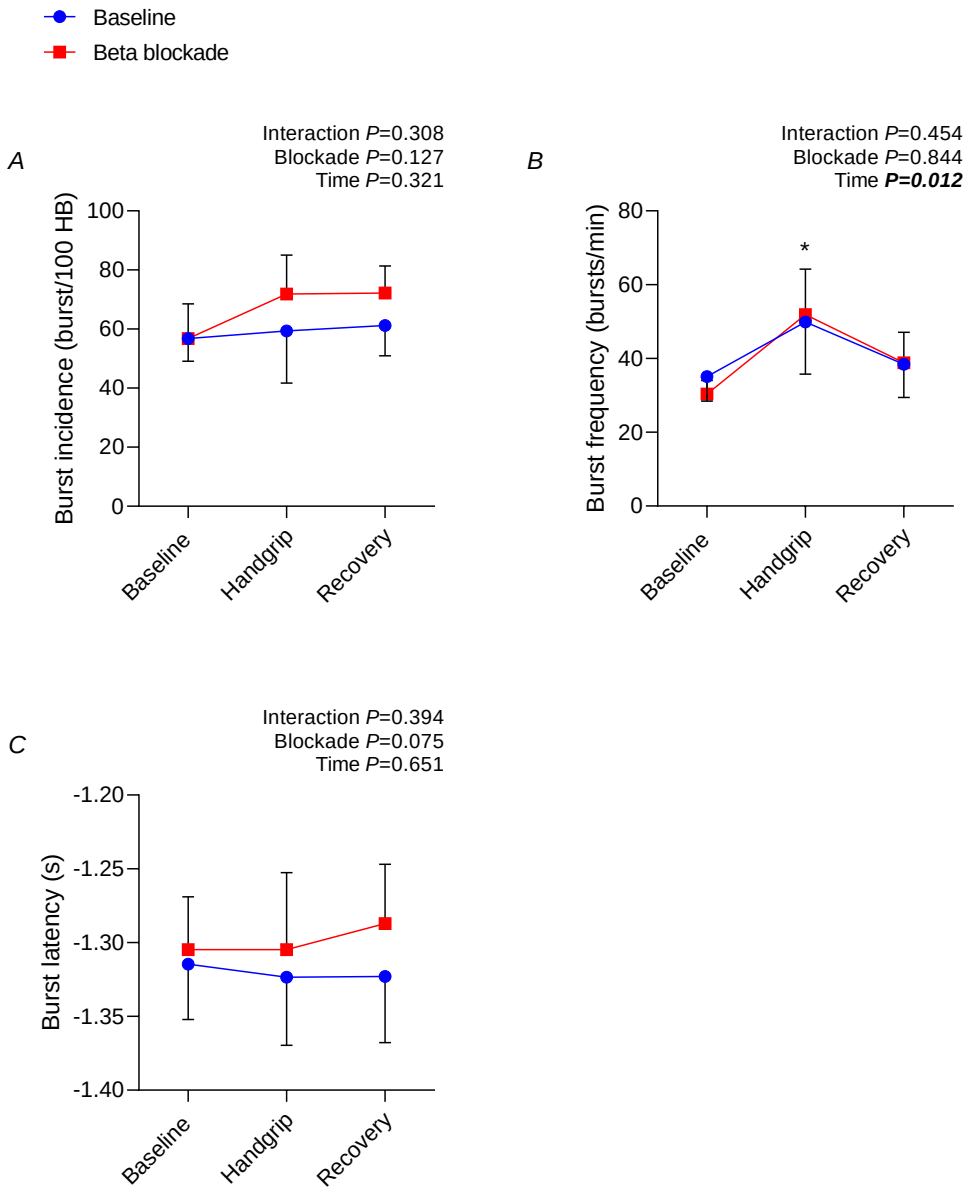

Figure 4.6 Sympathetic response to isometric handgrip exercise at baseline and during beta-adrenergic receptor blockade. HB; heartbeats. (A) burst incidence, (B) burst frequency, (C) mean burst latency. Mean ± SD last 90s baseline versus last 30 s handgrip versus last 30 s recovery. Two-way repeated measures ANOVA. *P<0.05 versus baseline (pairwise comparisons after significant main effect of time). N=5.

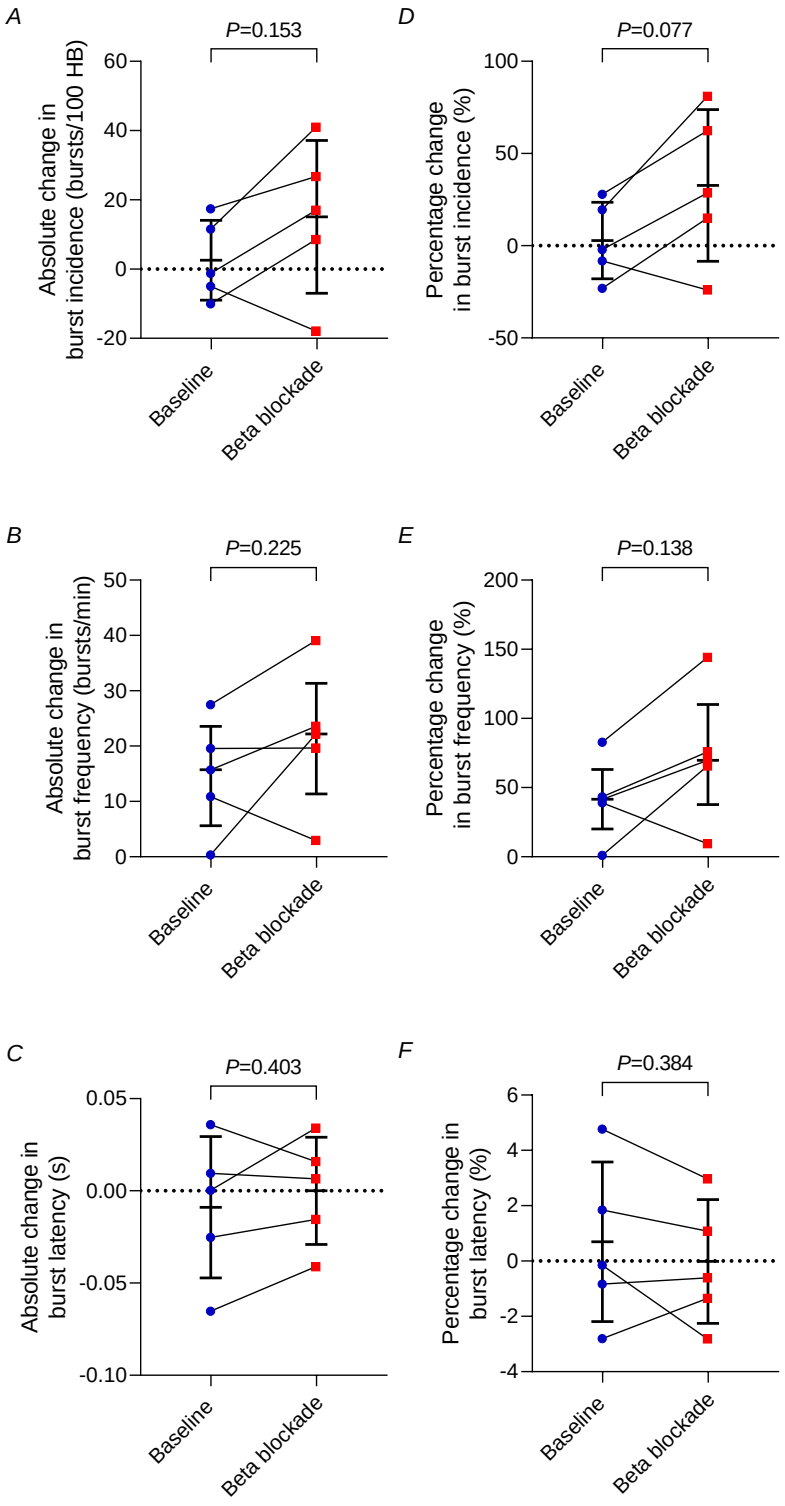

Figure 4.7 Absolute and percentage change in sympathetic variables with isometric handgrip exercise at baseline and during beta-adrenergic receptor blockade. HB; heartbeats. (A) and (D) burst incidence, (B) and (E) burst frequency, (C) and (F) mean burst latency. Absolute and percentage change from last 90 s baseline to last 30 s handgrip. Data are mean ± SD (A, D,

C, F) or median ± interquartile range (B, E). Effect of beta-blockade tested by paired T test (A, D, C, F) or Wilcoxon signed rank test (B, E). N=5.

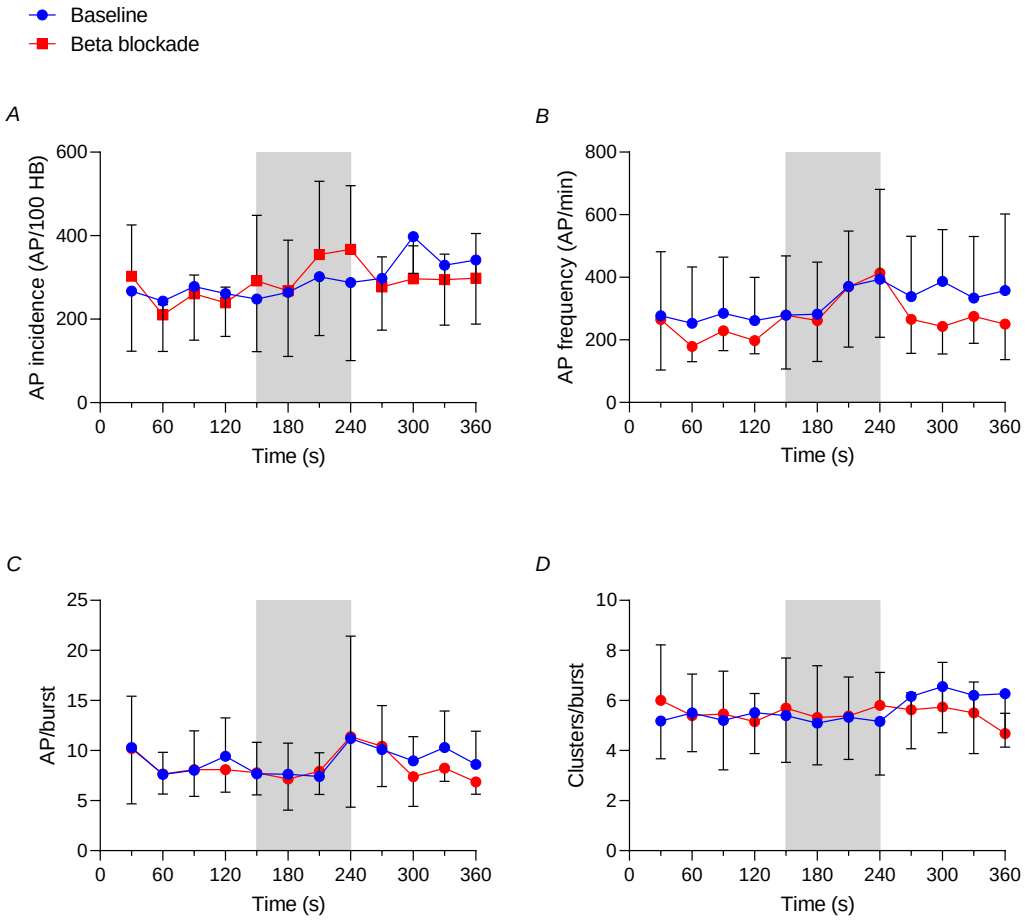

Figure 4.8 Time course of the sympathetic action potential response to isometric handgrip exercise at baseline and during beta-adrenergic receptor blockade. AP; action potentials, HB; heartbeats. (A) AP incidence, (B) AP frequency, (C) AP count/burst, (D) AP clusters (bins)/burst. Mean ± SD over 30 s intervals before, during (grey) and after two minutes of isometric handgrip exercise at 40 % maximal voluntary contraction. N=5.

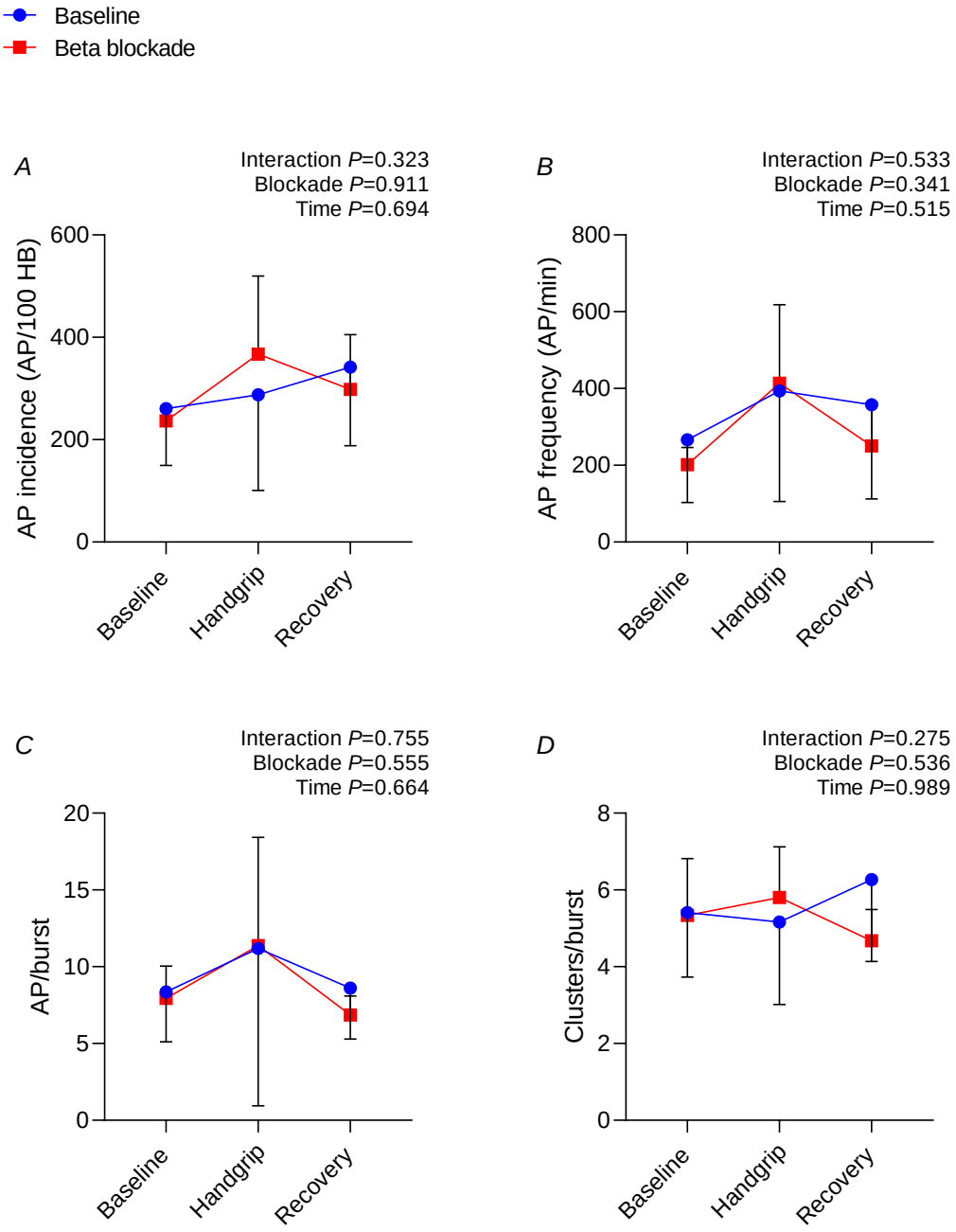

Figure 4.9 Sympathetic action potential response to isometric handgrip exercise at baseline and during beta-adrenergic receptor blockade. AP; action potentials, HB; heartbeats. (A) AP incidence, (B) AP frequency, (C) AP count/burst, (D) AP clusters (bins)/burst. Mean ± SD last 90s baseline versus last 30 s handgrip versus last 30 s recovery. Two-way repeated measures ANOVA. N=5.

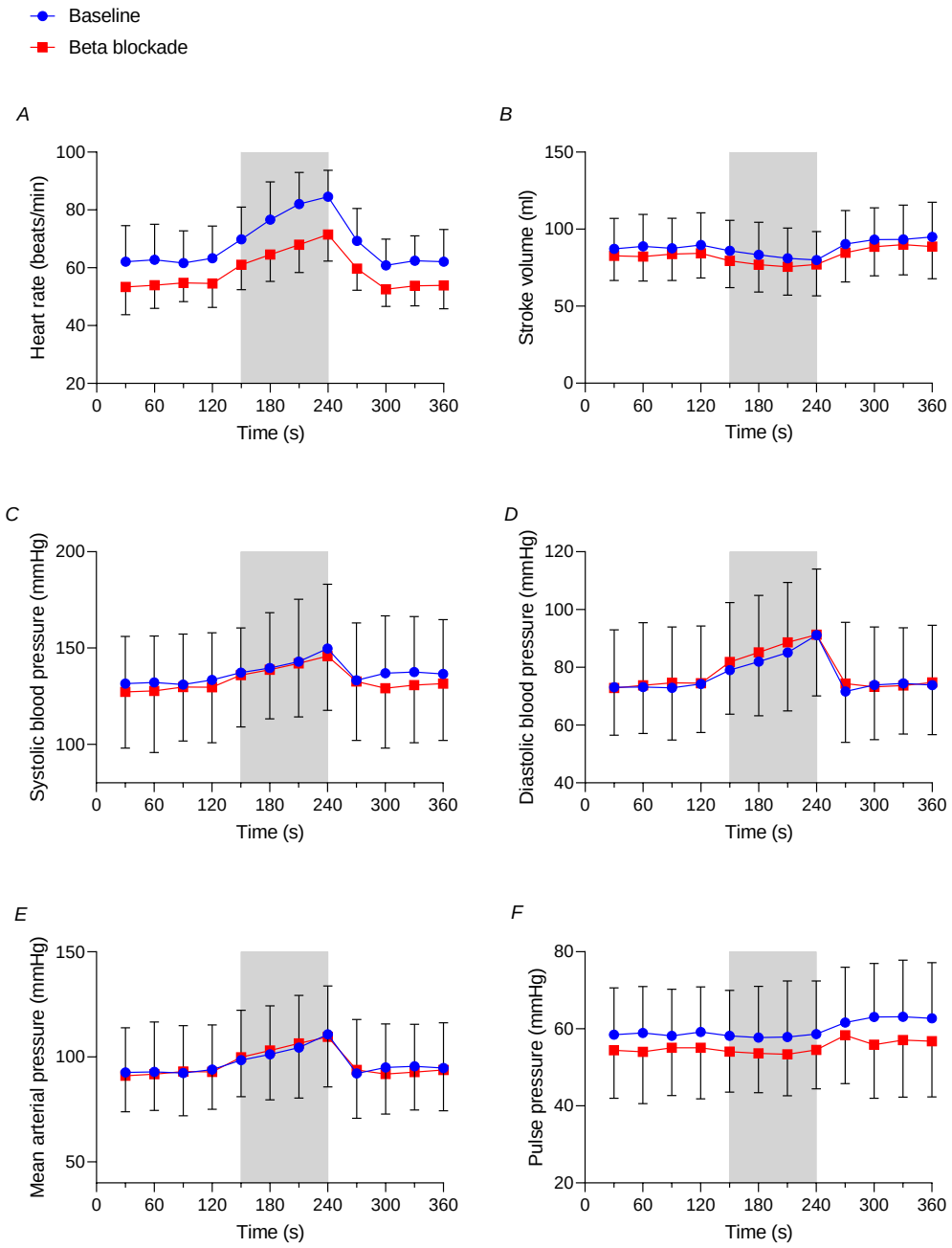

Figure 4.10 Time course of the haemodynamic response to isometric handgrip exercise during baseline and beta-adrenergic receptor blockade. Mean ± SD over 30 s intervals before, during (grey) and after two minutes of isometric handgrip exercise at 40 % maximal voluntary contraction. N=5.

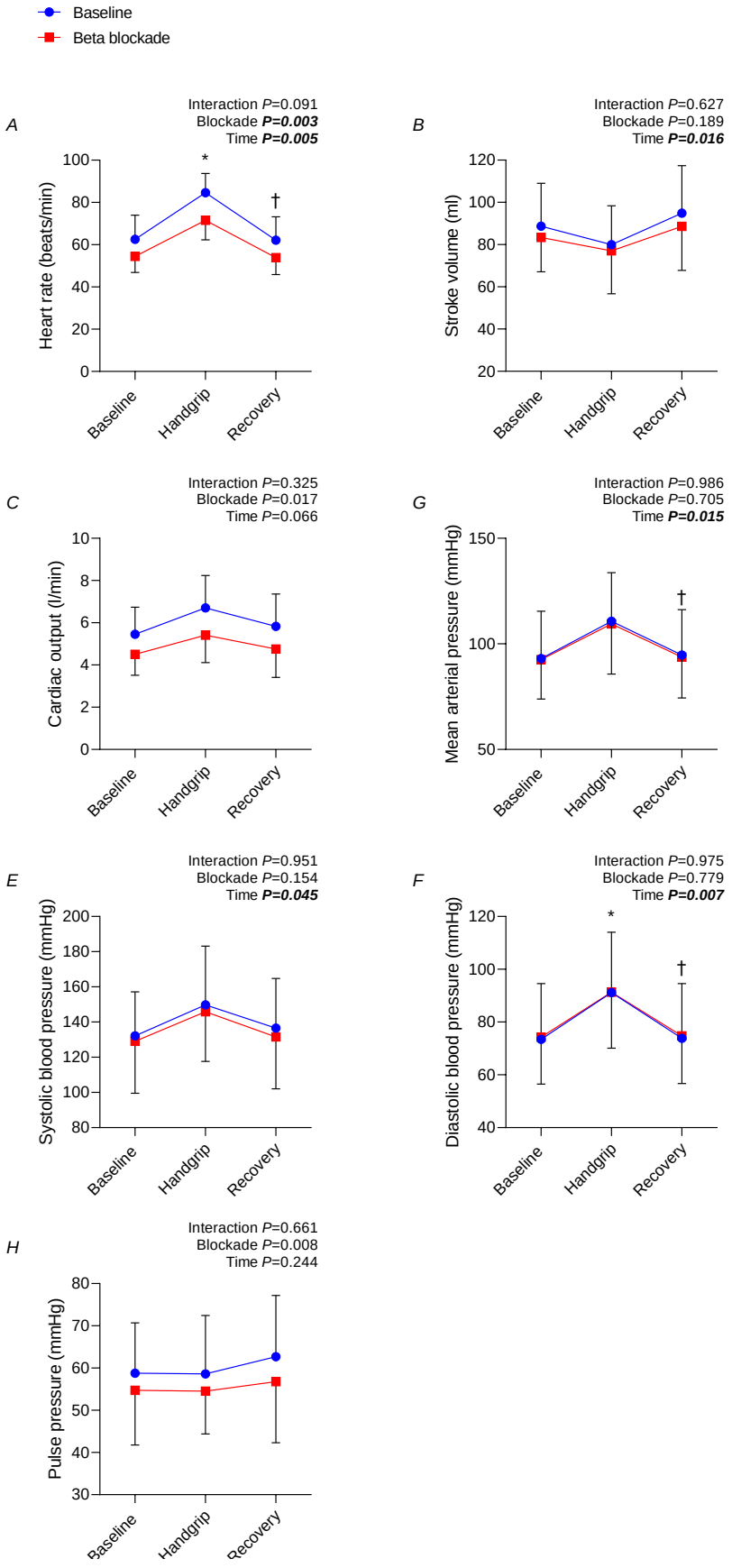

Figure 4.11 Haemodynamic response to isometric handgrip exercise at baseline and during beta-adrenergic receptor blockade. Mean ± SD last 90s

baseline versus last 30 s handgrip versus last 30 s recovery. Two-way repeated measures ANOVA. *P<0.05 versus baseline, †P<0.05 versus handgrip (pairwise comparisons following significant main effect of time). N=5.

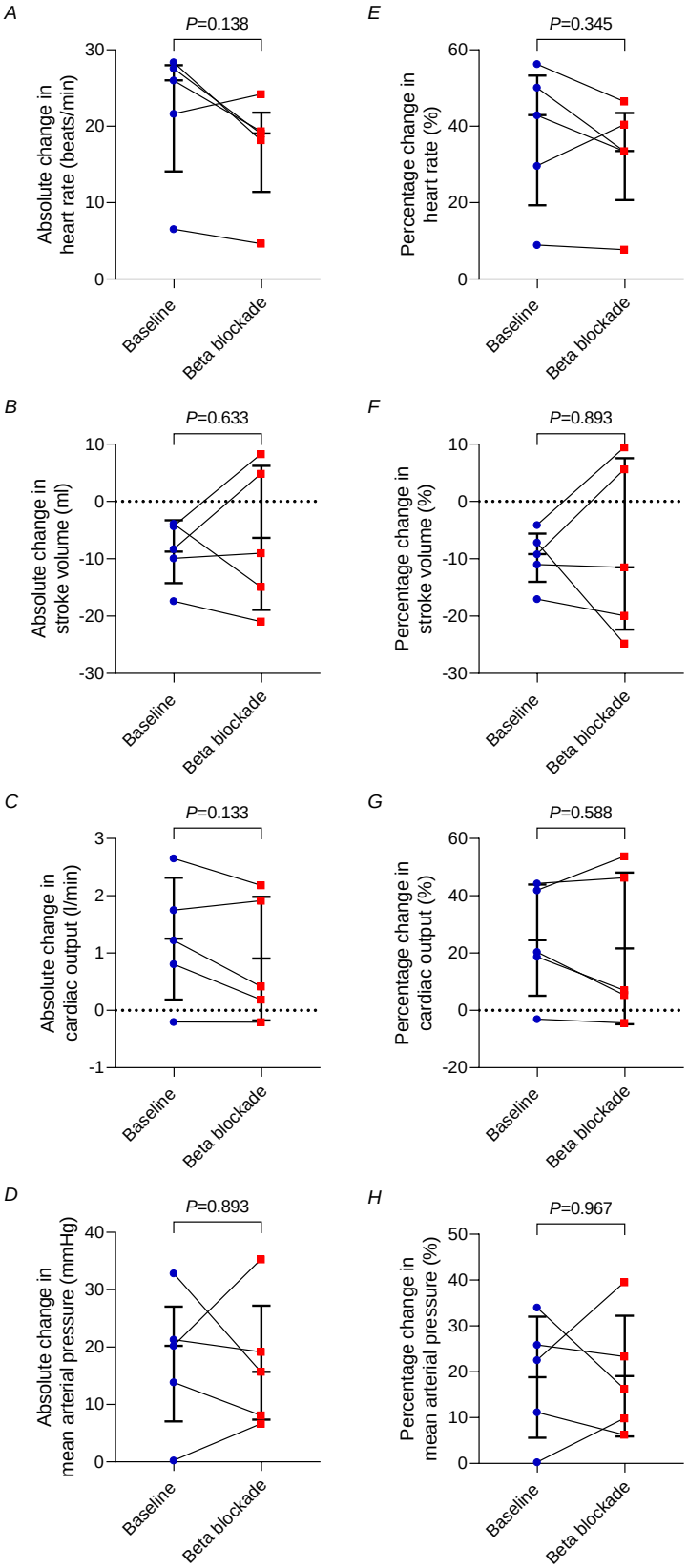

Figure 4.12 Absolute and percentage change in haemodynamic variables with isometric handgrip exercise at baseline and during beta-adrenergic receptor blockade. Absolute and percentage change from last 90 s baseline to last 30 s handgrip. Data are mean ± SD (B, C, G, H) or median ± interquartile

range (A, E, F, D). Effect of beta-blockade tested by paired T test (B, C, G, H) or Wilcoxon signed rank test (A, E, F, D). N=5.

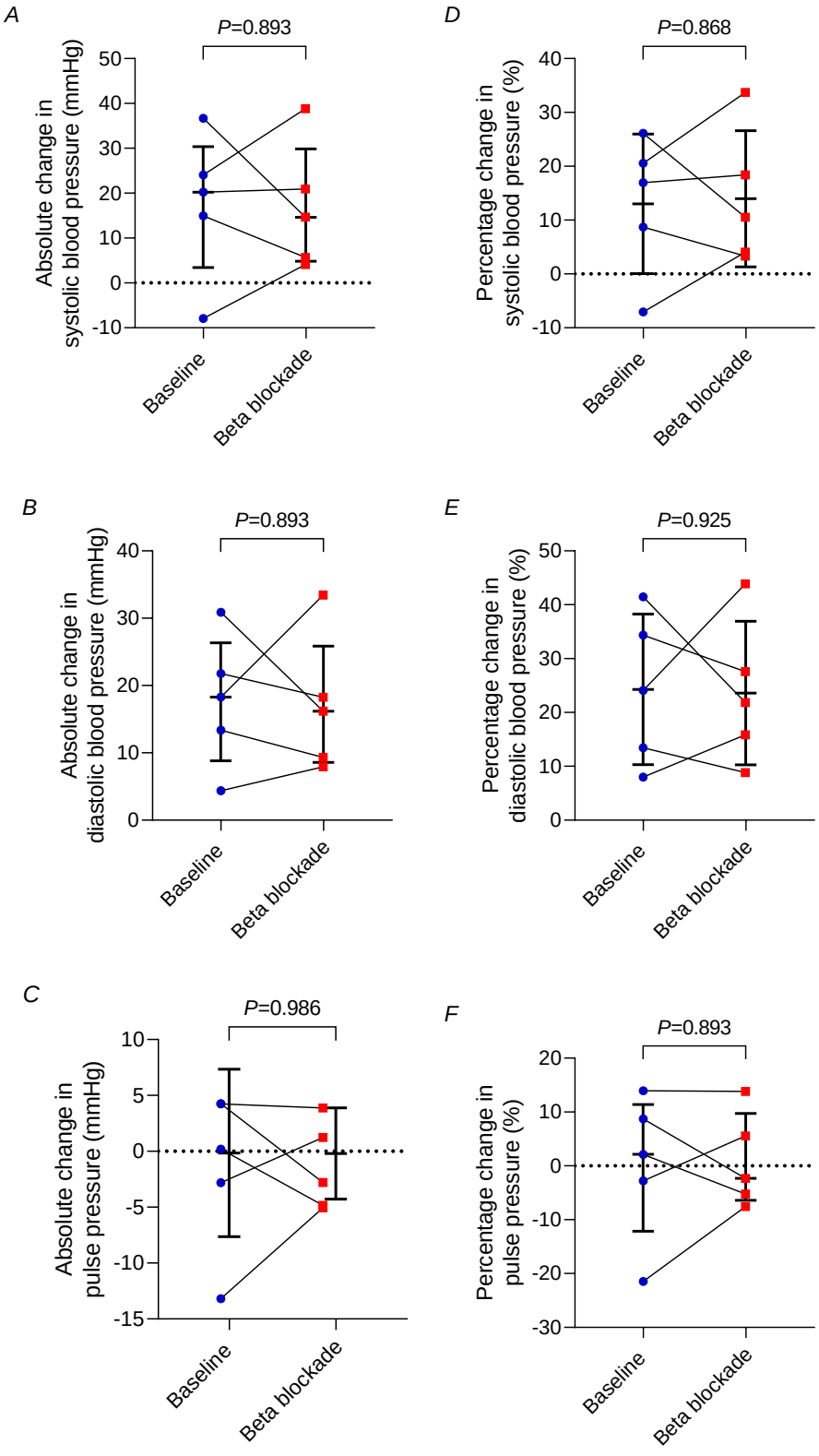

Figure 4.13 Absolute and percentage change in blood pressure with isometric handgrip exercise at baseline and during beta-adrenergic receptor blockade. Absolute and percentage change from last 90 s baseline to last 30 s handgrip. Data are mean ± SD (C, D, E) or median ± interquartile range

(A, B, F). Effect of beta-blockade tested by paired T test (C, D, E) or Wilcoxon signed rank test (A, B, F). N=5.

Chapter 5 Sex Differences in Sympathetic Vascular Transduction During Isometric Handgrip Exercise

5.1 Background

5.1.1 Introduction

Sex differences are observed in the regulation of blood pressure during exercise. The exercise pressor reflex elicits a rise in blood pressure with exercise, increasing blood flow to active skeletal muscle (Smith et al., 2019). The extent of this pressor response has been shown to vary by sex, with age, and with hypertension. Given that sympathetic transduction contributes to the differential blood pressure regulation at rest in females versus males, with and without hypertension, this chapter aimed to address the role of sympathetic transduction in regulating blood pressure during exercise. The chapter is focussed on static exercise in the upper limbs (isometric handgrip), as this is compatible with simultaneous microneurographic measurements.

5.1.2 Isometric handgrip exercise in young females

Several groups have demonstrated a smaller pressor response to isometric handgrip exercise in young females versus male controls (Ettinger et al., 1996, Jarvis et al., 2011), although others have reported no sex difference (Jones et al., 1996). The sympathetic response to handgrip exercise is greater in young males versus young females (Jones et al., 1996, Ettinger et al., 1996, Jarvis et al., 2011), and this may explain the larger pressor response in males. Both the metaboreflex (Jarvis et al., 2011) and mechanoreflex (Ives et al., 2013) have shown sex differences in sensitivity. Ettinger et al. measured a smaller change in muscle metabolites with handgrip exercise in young females versus young males, even though the groups were matched for forearm volume (Ettinger et al., 1996), which may explain the smaller pressor response to post-exercise ischaemia in females versus males (Jarvis et al., 2011). Meanwhile, the isolated mechanoreflex elicited a smaller cardiac output response in females versus males, but no sex difference in peripheral resistance or pressor response was seen (Ives et al., 2013).

Whilst there is evidence for reduced sensitivity of the metabo- and mechanoreflexes in females versus males, Smith et al. suggest that poor sympathetic transduction may also contribute to the smaller rise in blood pressure during exercise in females (Smith et al., 2019). Therefore, this chapter aimed to determine whether sympathetic transduction during isometric handgrip exercise could be quantified using the method used in this thesis (linear regression between MSNA area and subsequent diastolic blood pressure (Briant et al., 2016)), and whether sympathetic transduction remains lower in young females than young males during isometric handgrip exercise. If sympathetic transduction slope in premenopausal females and younger males is unaltered during isometric handgrip exercise (or altered similarly in both groups), sympathetic transduction in premenopausal females would remain low relative to younger males. This could therefore contribute to the smaller pressor responses reported in premenopausal females versus younger males. This was addressed in Aim 1.

5.1.3 Isometric handgrip exercise in postmenopausal females

Sympathetic and pressor responses to handgrip exercise are thought to be greater in older versus younger healthy adults. The MSNA and blood pressure response to beginning handgrip exercise (within the first 20 s) was found to be enhanced in healthy older males and females versus younger controls (Lalande et al., 2014). Furthermore, when healthy postmenopausal females are compared to premenopausal females, the MSNA and blood pressure responses to handgrip exercise are larger in the older group (Choi et al., 2012). Whilst these increased pressor responses to handgrip may be due to the greater increase in sympathetic activity, the increase in sympathetic transduction in postmenopausal versus premenopausal females (Hart et al., 2011a) may also be a contributing factor. Therefore, this chapter additionally aimed to determine whether increased sympathetic transduction in healthy postmenopausal versus premenopausal females was maintained during isometric handgrip exercise (Aim 2). Maintenance of a high sympathetic transduction slope in postmenopausal females (relative to premenopausal females) during isometric handgrip exercise could contribute to the larger pressor responses in postmenopausal females.

5.1.4 Isometric handgrip exercise in hypertension

Older hypertensive adults show greater blood pressure responses to isometric handgrip exercise versus normotensive controls (Aoki et al., 1983, Delaney et al., 2010), with some studies showing greater MSNA responses in hypertension (Delaney et al., 2010). In younger females, pre-hypertensive individuals showed enhanced blood pressure responses to handgrip exercise versus normotensive controls, however this was associated with enhanced heart rate rather than MSNA response (Bond et al., 2016). Chapter 3 demonstrated that hypertension was associated with an increased sympathetic transduction slope at rest in young females, but not in postmenopausal females. It is possible that the enhanced transduction slope in hypertensive young females may contribute to a greater pressor response to handgrip exercise. Therefore, this chapter further aimed to determine whether enhanced sympathetic transduction was maintained in hypertensive younger females during isometric handgrip exercise. COVID-19 impacted the recruitment of hypertensive patients from the local hypertension clinic and insufficient numbers of hypertensive participants were recruited to address this question. Therefore, the current data focus on sympathetic transduction during isometric handgrip exercise in normotensive younger and postmenopausal females.

5.1.5 Aims and hypotheses

Aim 1: To determine whether sympathetic transduction slope is affected by isometric handgrip exercise differently in normotensive premenopausal females versus younger males.

H_0: There will be no difference in the effect of isometric handgrip exercise on sympathetic transduction slope in normotensive premenopausal females and younger males.

H_1: There will be a difference in the effect of isometric handgrip exercise on sympathetic transduction slope in normotensive premenopausal females and younger males.

Aim 2: To determine whether sympathetic transduction slope is affected by isometric handgrip exercise differently in postmenopausal versus premenopausal normotensive females.

H_0: There will be no difference in the effect of isometric handgrip exercise on sympathetic transduction slope in postmenopausal normotensive females versus premenopausal normotensive females.

H_1: There will be a difference in the effect of isometric handgrip exercise on sympathetic transduction slope in postmenopausal normotensive females versus premenopausal normotensive females.

5.2 Methods

5.2.1 Participants

The data in this chapter were collected as part of a larger study at the University of Bristol (Sex Differences in the Role of SNA in Hypertension in Humans) which received ethical approval from an NHS Research Ethics Committee in 2018 (REC reference 18/SW/023), as in section 2.2. Data were available for seven normotensive premenopausal females, six normotensive younger males and eight normotensive postmenopausal females. One participant was removed due to a poor quality MSNA signal, two were removed due to signal quality changing across the analysis window (electrode moving), one was removed due to poor quality Finometer blood pressure data during handgrip exercise, and one was removed because bigeminy occurred during handgrip exercise. The final sample size was five normotensive premenopausal females, five normotensive younger males and six normotensive postmenopausal females. Postmenopausal and normotensive status were confirmed as described in section 2.3.7.

5.2.2 Procedures

MSNA was recorded by microneurography in a common peroneal nerve, as described in section 2.4.4 The signal was filtered and processed as described in section 2.4.5. Continuous blood pressure was measured by photoplethysmography on a finger of the non-handgrip arm (Finometer Pro, Finapres Medical Systems, The Netherlands). Continuous heart rate was

monitored by 3-lead ECG (AD Instruments, New Zealand). Stroke volume measurements were derived from the Finometer. Isometric contraction of the hand (handgrip exercise) was measured via a digital force transducer (AD Instruments, Dunedin, New Zealand). Maximal contractile force was taken as the greatest of three maximal contractions conducted by the participant and used to determine the required force for each participant to maintain during the handgrip protocol (40 ± 10 % maximal voluntary contraction). Participants were given real-time visual feedback to aid them in maintaining the required force (guidelines function in LabChart, showing ± 10 % of the required force in the handgrip channel).

5.2.3 Protocol

The handgrip protocol took place immediately after a baseline MSNA recording 5-10 minutes (data used in chapter 3). Participants were familiarised with the force transducer and the visual feedback they would receive during isometric handgrip. MSNA, ECG and blood pressure were recorded continuously during a two-minute rest period, two-minutes of isometric handgrip exercise at 40 % MVC, and a five-minute recovery period.

5.2.4 Data analysis

Analysis was conducted blind to participant group. Bursts of MSNA were identified across a 2-minute baseline, 2-minutes of handgrip and 2-minutes of recovery. Heart rate was calculated beat to beat across this analysis window. Systolic, diastolic, pulse pressure, mean arterial pressure and stroke volume were calculated for every cardiac cycle across the same analysis window. Cardiac output was calculated as the product of heart rate and stroke volume for corresponding cardiac cycles. Haemodynamic data were averaged over 30 s intervals from the start of the analysis window. Sympathetic transduction was calculated using a custom script (Z. Adams, Spike2, CED, Cambridge, UK), using a method previously described (section 2.8.3). A sympathetic transduction slope was calculated for the last 90 s of the rest period and the last 90 s of the handgrip period.

5.2.5 Statistical analysis

The haemodynamic response to isometric handgrip exercise was quantified as the change in variables from the average of the last 90 s intervals of the rest period to the last 30 s of handgrip exercise, and the last 30 s of recovery. The last 30 s of handgrip exercise was used, as the maximal change in blood pressure from baseline occurred within this period in all groups. Group differences in haemodynamic response were tested by one-way ANOVA or Kruskal-Wallis test. Group differences in sympathetic transduction slope were tested by two-way mixed model ANOVA. Group differences in the change in transduction slope between rest and handgrip were tested by one-way ANOVA or Kruskal-Wallis test. Statistical analysis was conducted in IBM SPSS for statistics 24 (IBM, New York, USA). Throughout, data are shown as mean ± SD or median [interquartile range].

5.3 Results

5.3.1 Participant characteristics

Participant characteristics are shown in Table 5.1, with statistical test data shown in Table 5.3. Postmenopausal females were older than both premenopausal females (66 [9] versus 28 [10] years, pairwise comparison *P*=0.023 and younger males (versus 27 [13] years, pairwise comparison *P*=0.012). The groups did not differ significantly in height or BMI (Table 5.1 and 5.3), but younger males weighed significantly more than postmenopausal females (76 [15] versus 59 [10] kg, pairwise comparison P=0.023). There was no significant group difference in clinic systolic or diastolic blood pressure, as well as in clinic heart rate (Table 5.1). Similarly, daytime average ambulatory blood pressure and heart rate did not differ between the groups (Table 5.1). Two of the five premenopausal females used hormonal contraception (intrauterine device for both).

5.3.2 Resting haemodynamic variables

Resting haemodynamic data was taken during the five to 10 minute baseline period of the study (reported in chapter 3) for all but two participants, in whom resting haemodynamic variables were averaged across the two-minute period immediately prior to handgrip exercise. These data are reported in Table 5.2, with full statistical data reported in Table 5.3. Resting burst incidence was greater in postmenopausal females versus both premenopausal females (78 [15] versus 62 [16] bursts/100 heartbeats, pairwise comparison *P*=0.027) and younger males (versus 54 [15] bursts/100 heartbeats, *P*=0.018), but there was no different between younger males and premenopausal females (*P*=1.0). Resting burst frequency was greater in postmenopausal females versus younger males (46 [14] versus 30 [8] bursts/min, *P*=0.026) but not versus premenopausal females (34 [7] bursts/min, *P*=0.107), and was not different between younger males and premenopausal females (*P*=1.0). Resting heart rate, stroke volume, cardiac output, systolic blood pressure, diastolic blood pressure, pulse pressure, and mean arterial pressure during the analysis window did not differ significantly between the groups (Table 5.2, statistical test data in Table S5.3).

5.3.3 Sympathetic response to isometric handgrip exercise

The overall time course of the sympathetic response to handgrip exercise is seen in Figure 5.2. Statistical analysis compared baseline (last 90 s) to the last 30 s of handgrip exercise, to the last 30 s of recovery.

5.3.3.1 Burst incidence

Overall, burst incidence was not altered by isometric handgrip exercise differently between the groups (Figure 5.3). Studentised residuals for burst incidence failed to meet the assumption of normality (QQ plots), which was not rectified by log transform. Therefore, z-scores were used in the analysis. There was no significant time x group interaction (P=0.882) or a significant main effect of time (P=0.993) for burst incidence z-scores, but there was a significant main effect of group (P=0.001). Post-hoc analysis showed significant pairwise comparisons between postmenopausal females and both premenopausal females (P=0.001) and younger males (P=0.023), but not between premenopausal females and younger males (P=0.355). Burst incidence increased by 1 ± 11 bursts/100 heartbeats (1 ± 23 %) in premenopausal females, 2 ± 7 bursts/100 heartbeats (4 ± 11 %) in younger males, and 4 ± 14 bursts/100 heartbeats (6 ± 19 %) in postmenopausal females (absolute change P=0.887; percentage change P=0.916; statistical test data in Table 5.5 and 5.7; Figure 5.6 and 5.7).

5.3.3.2 Burst frequency

Burst frequency increased with isometric handgrip exercise similarly among the groups. There was no significant time x group interaction for burst frequency (P=0.095), but there were significant main effects of both time (P<0.0005) and group (P=0.036) (Figure 5.3, statistical test data in Table 5.5). Post-hoc analysis showed significant pairwise comparisons in time between baseline and handgrip (P=0.001) and between handgrip and recovery (P=0.01), but not between baseline and recovery (P=0.960). There was a significant pairwise comparison in group between premenopausal females and postmenopausal females (P=0.035), but not between younger males and postmenopausal females (P=0.850), or between younger males and premenopausal females (P=0.266). In agreement, the absolute and percentage change in burst frequency from baseline to handgrip were similar between groups (absolute change 11 ± 13 bursts/min, 22 ± 8 bursts/min, and 11 ± 11 bursts/min in premenopausal females, younger males, and postmenopausal females respectively, P=0.226; percentage change 36 ± 42

%, 65 ± 25 %, and 27 ± 27 %, *P*=0.166; statistical test data in Table 5.7, Figure 5.6 and 5.7).

5.3.4 Haemodynamic response to isometric handgrip exercise

5.3.4.1 Heart rate

The heart rate response to isometric handgrip exercise differed between groups, with the main difference arising from a greater heart rate response in younger males compared to postmenopausal females (Figure 5.2). Studentised residuals for raw heart rate values were not normally distributed (as determined by QQ plot), therefore the analysis was repeated with log transformed heart rate values. There was a significant time x group interaction for heart rate (*P*=0.009; statistical test data in Table 5.5). Post-hoc tests revealed a significant simple main effect of time in each participant group (young females *P*=0.001; younger males *P*=0.003 (Greenhouse-Geisser corrected); postmenopausal females *P*=0.014 (Greenhouse-Geisser corrected); Table 5.6). Only younger males showed a significant difference in heart rate between baseline and handgrip (Bonferroni-corrected pairwise comparison *P*=0.006; versus *P*=0.052 for premenopausal females and *P*=0.067 for postmenopausal females; Table 5.6). All groups showed significant pairwise comparisons between handgrip and recovery, and none showed significant pairwise comparisons between baseline and recovery (Table 5.6). Additionally, there was a significant simple main effect of group during handgrip (*P*=0.049), but not during baseline (*P*=0.446) or recovery (*P*=0.856), with post-hoc analysis showing a significant pairwise comparison between younger males and postmenopausal females (*P*=0.041) but not between the other groups (Table 5.6). In agreement, absolute and percentage change in heart rate with handgrip exercise was smaller in postmenopausal females versus younger males (10 [19] beats/min (20 [33] %) versus 28 [17] beats/min (50 [35] %), pairwise comparisons *P*=0.013 and *P*=0.019 for absolute and percentage change respectively, Table 5.7), but not versus premenopausal females (20 [19] beats/min (30 [32] %), pairwise comparisons P=0.894 and P=1.0, Table 5.7), and did not differ between younger males and females (pairwise comparisons *P*=0.253 and *P*=0.189, Table 5.7, Figure 5.6 and 5.7).

5.3.4.2 Stroke volume and cardiac output

Stroke volume was reduced with handgrip exercise similarly across the groups (Figure 5.2). Stroke volume data for recovery violated the assumption of equality

of variances (Levene's test, *P*=0.049). This was not rectified by log transform, so the analysis was conducted using the raw data, but the violated assumption should be noted. There was no significant time x group interaction (*P*=0.411, Greenhouse-Geisser corrected), or significant main effect of group (*P*=0.429), but there was a significant main effect of time (*P*<0.0005, Greenhouse-Geisser corrected; statistical test data in Table 5.5). Post-hoc analysis showed significant pairwise comparisons between baseline and handgrip (*P*=0.005), handgrip and recovery (*P*=0.001), and baseline and recovery (*P*=0.002). In agreement, the change in stroke volume was similar between the groups (-5.2 ± 5.7 ml (-11.2 [20.9] %), -15.4 ± 9.2 ml (-17.0 [19.5] %), and -11.3 ± 14.3 ml (-9.3 [24.4] %); absolute change *P*=0.346; percentage change *P*=0.761; Table 5.7, Figure 5.6 and 5.7).

The cardiac output response to handgrip exercise differed between the groups (Figure 5.2). There was a significant time x group interaction for cardiac output (*P*=0.03, Greenhouse-Geisser corrected; Table 5.5). Post-hoc analysis showed a significant simple main effect of time for premenopausal females (*P*=0.014) and younger males (*P*=0.027), but not for postmenopausal females (*P*=0.105), however no pairwise comparisons of time reached significance for premenopausal females or younger males (Table 5.6). There was no significant simple main effect of group at any timepoint (Table 5.6). The absolute change in cardiac output was similar between the groups (0.5 ± 0.3 l/min, 1.9 ± 1.7 l/min, 0.3 ± 0.4 l/min for premenopausal females, younger males and postmenopausal females respectively, *P*=0.155). The *P* value for a group difference in percentage change in cardiac output approached but did not reach significance (*P*=0.05; 13 ± 5 %, 33 ± 27 %, 5 ± 6 %; Table 5.7, Figure 5.6 and 5.7).

5.3.4.3 Systolic, diastolic, pulse pressure and mean arterial pressure

Overall, the blood pressure response to isometric handgrip exercise was similar across the groups, with all groups showing increases in blood pressure with handgrip (Figure 5.2). There was no significant time x group interaction (*P*=0.541) or main effect of group (*P*=0.543) for systolic blood pressure (statistical test data in Table 5.5). However, there was a significant main effect of time (*P*<0.0005), with post-hoc analysis showing significant pairwise comparisons between baseline and handgrip (*P*<0.0005) and between handgrip and recovery (*P*=0.002), but not between baseline and recovery (*P*=1.0; Table 5.5). In agreement, systolic blood pressure increased similarly in premenopausal

females, younger males and postmenopausal females (absolute change 15 [32] mmHg, 20 [40] mmHg, 36 [28] mmHg respectively (mean ranks 7.20, 7.80, 10.17), *P*=0.544; percentage change 16 ± 14 %, 15 ± 15 %, 22 ± 11 %, *P*=0.651, Table 5.7, Figure 5.8 and 5.9).

There was no significant time x group interaction for diastolic blood pressure (log transformed, *P*=0.875, Table 5.5). There was a significant main effect of time (*P*<0.0005), with post-hoc analysis showing significant pairwise comparisons between baseline and handgrip (*P*<0.0005) and between handgrip and recovery (*P*<0.0005), but not between baseline and recovery (*P*=1.0). There was no significant main effect of group (*P*=0.158). Furthermore, absolute and percentage change in diastolic blood pressure was similar between groups (absolute change 14 [24], 23 [19], and 16 [16] mmHg (mean ranks 8.00, 10.40, 7.33), *P*=0.546; percentage change 15 [27] %, 34 [21] %, and 26 [25] % (mean ranks 7.60, 10.40, 7.67, *P*=0.560, Table 5.7; Figures 5.8 and 5.9).

There was no significant time x group interaction for mean arterial pressure (log transformed; *P*=0.913, Table 5.5), however there was a significant main effect of time (*P*<0.0005). Post-hoc analysis showed significant pairwise comparisons between baseline and handgrip (*P*<0.0005) and between handgrip and recovery (*P*<0.0005) but not between baseline and recovery (*P*=1.0). There was no significant main effect of group (*P*=0.449). In agreement, absolute and percentage change in mean arterial pressure were similar between groups (absolute change 15 [27], 21 [26] and 23 [20] mmHg (mean ranks 7.60, 9.60, 8.33), *P*=0.797; percentage change 12 [26] %, 26 [25] % and 25 [23] % (mean ranks 7.60, 9.40, 8.50), *P*=0.836, Table 5.7; Figures 5.8 and 5.9).

Absolute change in pulse pressure was greater in postmenopausal females versus both premenopausal females (16 ± 7 versus 3 ± 5 mmHg, pairwise comparison *P*=0.045) and younger males (versus -1 ± 11 mmHg, pairwise comparison *P*=0.008), but not between younger males and premenopausal females (pairwise comparison *P*=1.0, Table 5.7). Percentage change in pulse pressure was greater in postmenopausal females versus younger males (25 [16] versus -3 [35] %, pairwise comparison *P*=0.034) but not versus premenopausal females (5 [17] %, pairwise comparison *P*=0.169; Table 5.7; Figures 5.8 and 5.9).

5.3.5 Sympathetic transduction slope during isometric handgrip exercise

Sympathetic transduction slope at baseline and during handgrip exercise was compared in healthy premenopausal females, younger males, and postmenopausal females (Figure 5.10). There was no significant handgrip x group interaction of transduction slope (*P*=0.630, statistical test data in Table 5.4). Additionally, there was no significant main effect of either handgrip condition (P=0.169) or group (*P*=0.256). Studentised residuals for transduction slope during handgrip violated the assumption of normality (determined by QQ plot). However, there remained no significant interaction or main effects when the analysis was repeated with z-scores of transduction slopes (Table 5.4). There was no group difference in absolute change in sympathetic transduction slope (Figure 5.10A; 0.08 ± 0.16 versus 0.12 ± 0.27 versus 0.01 ± 0.14 mmHg/%.s for premenopausal females, younger males and postmenopausal females respectively; *P*=0.630, Table 5.7). Similarly, percentage change in transduction slope did not differ between groups (Figure 5.10B; -3 [344], 60 [346], 42 [263] % (mean ranks 6.40, 10.60 and 8.50), *P*=0.378; Table 5.7; Figure 5.11).

5.3.6 Relationship between change in sympathetic transduction slope and change in haemodynamic variables

Across all participants, correlation analysis was performed to determine whether there was a relationship between change in sympathetic transduction slope and change in MSNA or blood pressure during handgrip exercise. Absolute change in transduction slope was not significantly correlated with absolute change in either burst incidence, burst frequency, systolic blood pressure, diastolic blood pressure, or mean arterial pressure (Table 5.8). Similarly, percentage change in transduction slope was not significantly correlated with percentage change in burst incidence, burst frequency, systolic blood pressure, diastolic blood pressure, or mean arterial pressure (Table 5.8).

5.3.7 Maintenance of isometric handgrip exercise

The force generated during the last 30 s of isometric handgrip exercise was 36 ± 2, 36 ± 1, and 37 ± 3 % in premenopausal females, younger males, and postmenopausal females respectively. There was no significant group x time interaction for force maintained during isometric handgrip exercise (*P*=0.229).

Similarly, there was no main effect of either time (*P*=0.908) or group (*P*=0.285; statistical test data in Table 5.5). The studentised residuals for handgrip force during the last 30 s of handgrip violated the assumption of normality (QQ plots). However, there remained no significant interaction or main effects when the analysis was repeated with z-scores of handgrip force (Table 5.5).

5.4 Discussion

5.4.1 Sympathetic transduction during isometric handgrip exercise in females

The primary outcome of this chapter was sympathetic transduction slope before and during isometric handgrip exercise. Given the data demonstrating reduced resting transduction of MSNA into vasoconstrictor tone in younger females versus males (Kneale et al., 1997, Hart et al., 2009, Hart et al., 2011a), it has been suggested that poor transduction during exercise contributes to the smaller pressor responses seen in females (Smith et al., 2019). The current data indeed showed no overall effect of handgrip exercise on transduction slope in any group. However, the individual effect of exercise on transduction slope was variable. Additionally, no group differences in transduction slope were observed (at rest or during handgrip) and the group variance in transduction slope was higher than those previously reported at rest. For example, Briant et al. reported group SD for baseline transduction slope of 0.048, 0.042 and 0.045 mmHg/%.s for young males, young females and postmenopausal females respectively (Briant et al., 2016), compared to 0.087, 0.187 and 0.045 mmHg/%.s for the current data. As such, this raises concerns about whether the method used to quantify transduction slope is accurate during shorter periods.

There is some evidence that lower sympathetic transduction is maintained during exercise in young females. For example, Shoemaker et al. (2007) showed that handgrip exercise is associated with a rise in MSNA but no rise in total peripheral resistance in young females, supporting the idea of a dissociation between MSNA and vasoconstrictor tone that persists during exercise. They proposed that the associated pressor response in young females is due to diversion of blood away from the abdominal circulation and increased cardiac output, rather than peripheral vasoconstriction (Shoemaker et al., 2007). Furthermore, Hogarth et al. showed that a similar sympathetic response to handgrip exercise was associated with a smaller change in vascular resistance in females versus males (Hogarth et al., 2007a). Additionally, a non-exercise sympathoexcitatory stimulus (cold pressor test) was associated with smaller diastolic blood pressure changes in young females versus younger males, despite the groups showing similar changes in MSNA (Jarvis et al., 2011). This indicates that lower sympathetic

transduction may be maintained in young females versus males during a sympathoexcitatory stimuli.

Given that the vascular beta-adrenergic receptors contribute to maintaining lower sympathetic transduction in young females versus males (Kneale et al., 2000, Hart et al., 2011a), their role in the pressor response to exercise has also been studied. However, the vasoconstrictor response to isometric handgrip exercise was unchanged under systemic beta-blockade in both young females and males (Pellinger and Halliwill, 2007), suggesting that the beta-adrenergic receptors do not play a role in limiting sympathetic transduction during exercise in younger females (or younger males). This group did however show that the vascular beta-adrenergic receptors were involved in the post-exercise control of vasoconstrictor tone, with females showing poorer post-exercise vasodilation during beta-blockade but males showing no effect of beta-blockade (Pellinger and Halliwill, 2007). As such, the lack of evidence for a role of beta-adrenergic receptor involvement in regulation of vasoconstrictor tone during exercise may be confounded by other factors occurring during the exercise.

5.4.2 Haemodynamic response to isometric handgrip exercise in younger females

Previous studies in healthy young females showed that isometric handgrip elicits a rise in sympathetic nerve activity and blood pressure (Jarvis et al., 2011, Ettinger et al., 1996). In agreement, the current data showed an increase in MSNA burst frequency and systolic, diastolic, and mean arterial blood pressure with handgrip in younger females. Stroke volume estimated by Modelflow (Finometer) was reduced with handgrip exercise in the younger females. This agrees with previous reports that used a more direct assessment of stroke volume (doppler ultrasound) (Hisdal et al., 2004, Elstad et al., 2009, Toska, 2010), but contradicts others who found that handgrip exercise triggered a rise in stroke volume (Shoemaker et al., 2007). The current analysis of the heart rate response to handgrip exercise in young females revealed a pairwise comparison that did not reach significance (P=0.052), however previous studies have repeatedly shown that isometric handgrip exercise induces tachycardia versus baseline (Shoemaker et al., 2007, Ettinger et al., 1996, Jarvis et al., 2011). There was a significant change in cardiac output across the three timepoints in younger females (significant simple main effect of time), although none of the pairwise

comparisons reached significance. The pairwise comparison closest to significance was that of baseline versus handgrip (*P*=0.077), whilst the other comparisons were further from significance (handgrip versus recovery *P*=0.704; baseline versus recovery *P*=0.225). As such, the significant simple main effect of time may be explained by the change in cardiac output from baseline to handgrip, although correction for multiple comparisons may have prevented the pairwise comparison reaching significance. Overall, the current data broadly replicate previous findings of the sympathetic and haemodynamic response to handgrip exercise in younger females.

Several previous studies reported that the sympathetic response to handgrip was smaller in younger females versus age-matched males. For example, whilst burst frequency and total activity were similar in males and females at baseline, Jarvis et al. reported a larger increase in these variables in males performing handgrip exercise compared to females. They also reported a greater pressor response in males but no sex difference in the heart rate response (Jarvis et al., 2011). In agreement, others have also demonstrated that the pressor and sympathetic responses to isometric handgrip exercise are greater in young males than young females, but that the heart rate response is similar (Ettinger et al., 1996). The current data found no sex difference in the absolute or percentage change in MSNA, blood pressure or heart rate with handgrip exercise in young adults. It is possible that the sex difference in pressor response observed by Jarvis et al. was driven by the sex difference in the sympathetic response, given that neither was observed in the current data.

5.4.3 Haemodynamic response to isometric handgrip exercise in postmenopausal females

Previous research has also shown differences in the sympathetic and haemodynamic response to isometric handgrip exercise with age. Healthy older adults show a larger rise in blood pressure in response to the first 20 s of handgrip exercise versus younger adults (Lalande et al., 2014). Specifically in females, 3 minutes of isometric handgrip exercise (40 % MVC) was shown to have greater pressor and vasoconstrictor responses in postmenopausal females versus premenopausal females (Choi et al., 2012), suggesting a greater sympathetic response in the older group. In contrast, the current data showed no difference in the pressor or sympathetic response to handgrip in postmenopausal

females versus the younger groups (female or male). However, the current protocol used 2 minutes as opposed to 3 minutes of the handgrip stimulus. The current data did show that postmenopausal females had a greater change in pulse pressure with handgrip versus both premenopausal females and younger males. This agrees with previous work in a larger cohort of healthy adults which showed greater pulse pressure responses to handgrip exercise with increased age (Cauwenberghs et al., 2021).

Choi et al. showed that postmenopausal females showed a smaller increase in cardiac output with handgrip exercise versus premenopausal females. In agreement, the current data showed a significant main effect of time on cardiac output response to handgrip in premenopausal females and younger males, but not in postmenopausal females. Percentage change in cardiac output was close to being significantly different between the groups (P=0.050).

5.4.4 Limitations

5.4.4.1 The technique for measuring sympathetic transduction during exercise

The method used to quantify sympathetic transduction in this chapter involved relating MSNA area to subsequent changes in diastolic blood pressure. In this chapter this analysis was done over shorter time periods than those previously reported (90 s versus 5-10 minutes (Briant et al., 2016)). The last 90 s of handgrip exercise was chosen to avoid the initial changes in MSNA and blood pressure occurring within the first 30 s. However, this analysis still differs from previous analyses where sympathetic transduction was quantified during steady state (rest).

Additionally, MSNA bursts were normalised across the entire analysis period and the burst to which all others were normalised (the tallest) was likely to occur during the handgrip rather than baseline period. Therefore, sympathetic transduction slopes were generated over different x-axis ranges (different range of MSNA burst areas), and the range was likely to be larger during handgrip compared to baseline, given that the handgrip analysis window included the larger bursts towards the end of the exercise period. Whether this is a confounding factor in the analysis remains unclear. An alternative technique may be to assess the change in blood pressure across the 10-15 cardiac cycles following an individual MSNA burst, as used by some groups (Vianna et al., 2012,

Fairfax et al., 2013b). However, this method becomes difficult when burst frequency increases, and would be particularly difficult to interpret in participants who show very high levels of MSNA towards the end of handgrip exercise.
A further limitation of the technique is that the slope represented the conversion of MSNA area into diastolic blood pressure, not vascular resistance. Although diastolic blood pressure is used in this method as a surrogate for vascular tone (Briant et al., 2016), there are likely to be additional factors influencing diastolic blood pressure during handgrip than at rest. Indeed, the current data showed a significant main effect of the handgrip protocol on diastolic blood pressure across the groups. An alternative would be to measure changes in systemic vascular resistance, perhaps via vascular ultrasound detected changes in arterial diameter and blood flow in a non-exercising limb. This method was attempted during the current experiments, but COVID-19 and logistical issues prevented sufficient data collection.

5.4.5 Implications of results

The current data suggest that sympathetic transduction slope is unchanged during isometric handgrip exercise in healthy premenopausal females. As such, maintenance of lower transduction in premenopausal females versus younger males during exercise may limit the pressor response to exercise in premenopausal females (Smith et al., 2019). However, there are limitations to the current method of analysing transduction during exercise and the current results should be confirmed using an alternative method of quantifying transduction.

5.5 Tables and figures

Table 5.1 Participant characteristics for isometric handgrip protocol.

	YF	YM	PMF	*P* value
Age (years)	28 [10] (5.80)	27 [13] (5.20)	66 [9] (13.50)	***0.005***
Height (m)	170 ± 9	178 ± 6	160 ± 7	0.050
Weight (kg)	63.3 [20.5] (7.80)	75.5 [14.9] (13.00)	59.4 [10.2] (5.33)	***0.027***
BMI (kg/m^2)	21.6 [4.0] (8.00)	22.8 [6.3] (9.20)	23.7 [6.0] (8.33)	0.918
Clinic SBP (mmHg)	119 [7] (8.20)	121 [20] (9.4)	120 [25] (8.00)	0.873
Clinic DBP (mmHg)	76 [5] (9.30)	72 [14] (7.50)	74 [16] (8.67)	0.828
Clinic HR (beats/min)	65 [6] (8.90)	65 [18] (8.70)	65 [16] (8.00)	0.946
Daytime ambulatory SBP (mmHg)	119 ± 8	124 ± 7	116 ± 14	0.528
Daytime ambulatory DBP (mmHg)	78 ± 2	75 ± 9	70 ± 7	0.242
Daytime ambulatory HR (beats/min)	69 ± 7	64 ± 5	68 ± 9	0.656
Hormonal contraception use	2/5			
IUD	2			

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, HR; heart rate, IUD; intrauterine device. Data are mean ± SD or median [interquartile range] with (mean ranks) where appropriate. Group differences tested by one-way ANOVA or Kruskal-Wallis test. N=5 YF, N=5 YM, N=6 PMF.

Table 5.2 Resting haemodynamic variables for participants of the isometric handgrip protocol.

	YF	YM	PMF	*P* value
MSNA (bursts/100 HB)	62 [16] (5.80)	54 [15] (5.40)	78 [15] (13.33)	***0.007***
MSNA (bursts/min)	34 [7] (6.70)	30 [8] (5.20)	46 [14] (12.75)	***0.019***
Heart rate (beats/min)	57 [9] (8.80)	58 [10] (7.80)	58 [10] (8.83)	0.924
Systolic BP (mmHg)	128 [54] (8.80)	119 [74] (6.40)	134 [16] (10.00)	0.452
Diastolic BP (mmHg)	80 [48] (11.40)	72 [57] (8.60)	66 [14] (6.00)	0.173
Pulse pressure (mmHg)	58 [29] (6.60)	60 [28] (7.60)	68 [9] (10.83)	0.299
Mean arterial pressure (mmHg)	89 [37] (10.00)	83 [40] (7.00)	90 [15] (8.50)	0.609
Stroke volume (ml)	87 [79] (7.20)	104 [36] (8.80)	99 [51] (9.33)	0.750
Cardiac output (l/min)	4.8 [3.9] (7.80)	6.1 [4.4] (7.60)	5.6 [3.1] (9.83)	0.685

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, MSNA; muscle sympathetic nerve activity, HB; heartbeats, BP; blood pressure. Data are the average of a five to ten minute baseline period before the handgrip protocol, except in two participants (one YF, one YM) where the data are the average of the two-minute baseline periods immediately prior to handgrip. Data are median [interquartile range] with (mean ranks). Group differences tested by Kruskal-Wallis test. N=5 YF, N=5 YM, N=6 PMF.

Table 5.3 Statistical test data for demographic and resting haemodynamic variables.

Variable	Test statistic	*P* value	Effect size
Age	$X^2(2) = 10.628$	***0.005***	
YF vs. YM	z=0.199	1.0	0.063
YF vs. PMF	z=-2.671	***0.023***	0.805
YM vs. PMF	z=-2.879	***0.012***	0.868
Height	F(2, 13) = 8.366	***0.005***	0.563
YF vs. YM		0.191	
YF vs. PMF		0.120	
YM vs. PMF		***0.003***	
Weight	$X^2(2) = 7.229$	***0.027***	
YF vs. YM	z=-1.727	0.253	0.546
YF vs. PMF	z=0.856	1.0	0.258
YM vs. PMF	z=2.659	***0.023***	0.802
BMI	$X^2(2) =0.171$	0.918	
Clinic SBP	$X^2(2) =0.273$	0.873	
Clinic DBP	$X^2(2) =0.377$	0.828	
Clinic HR	$X^2(2) =0.110$	0.946	
Daytime ambulatory SBP	F(2, 11) = 0.678	0.528	0.110
Daytime ambulatory DBP	F(2, 11) = 1.621	0.242	0.228
Daytime ambulatory HR	F(2, 11) = 0.437	0.656	0.074
Burst incidence	$X^2(2) =9.926$	***0.007***	
YF vs. YM	z=0.133	1.0	0.042
YF vs. PMF	z=-2.615	***0.027***	0.830
YM vs. PMF	z=-2.754	***0.018***	0.788
Burst frequency	$X^2(2) =7.910$	***0.019***	
YF vs. YM	z=0.499	1.0	0.158
YF vs. PMF	z=-2.10	0.107	0.633
YM vs. PMF	z=--2.621	***0.026***	0.790
HR	$X^2(2) =0.157$	0.924	
SBP	$X^2(2) =1.588$	0.452	
DBP	$X^2(2) =3.512$	0.173	
Pulse pressure	$X^2(2) =2.416$	0.299	
Mean arterial pressure	$X^2(2) =0.993$	0.609	
Cardiac output	$X^2(2) =0.757$	0.685	

Stroke volume	$X^2(2) = 0.576$	0.750	

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, HR; heart rate, χ^2; Kruskal-Wallis test statistic; z; standardised test statistic for pairwise comparison following significant Kruskal-Wallis effect of group, F; ANOVA test statistic. Group differences tested by one-way ANOVA or Kruskal-Wallis test. ANOVA effect size is partial η^2. Effect size for Kruskal-Wallis was calculated for pairwise comparisons only, following a significant effect of group (calculated as z/(square root N)).

Table 5.4 Statistical test data for comparison of sympathetic transduction slope at baseline and during isometric handgrip exercise.

Variable	Test statistic	*P* value	Partial η^2
Sympathetic transduction slope			
Interaction	F(2, 13) = 0.480	0.630	0.069
Handgrip	F(1, 13) = 2.122	0.169	0.140
Group	F(2, 13) = 1.517	0.256	0.189
Sympathetic transduction slope Z-scores			
Interaction	F(2, 13) = 0.126	0.882	0.019
Handgrip	F(1, 13) = 0.001	0.980	0.0
Group	F(2, 13) = 1.455	0.269	0.183

F; ANOVA test statistic. Effect of isometric handgrip exercise on sympathetic transduction slope in the different groups was tested by two-way mixed model ANOVA. N=5 premenopausal females, N=5 younger males, N=6 postmenopausal females.

Table 5.5 Statistical test data for haemodynamic variables during isometric handgrip exercise.

Variable	Test statistic	*P* value	Partial η^2
Burst incidence z-scores			
Interaction	F(4, 24) = 0.289	0.882	0.046
Time	F(2, 24) = 0.007	0.993	0.001
Group	F(2, 12) = 12.056	***0.001***	0.668
Burst frequency			
Interaction	F(4, 24) = 2.241	0.095	0.272
Time	F(2, 24) = 15.528	***<0.0005***	0.564
Group	F(2, 12) = 4.448	***0.036***	0.426
Log_{10} heart rate			
Interaction	F(4, 26) = 1.202	***0.009***	0.393
Stroke volume			
Interaction	F(2.437, 15.841) = 0.983	0.411 (GG)	0.131
Time	F(1.219, 15.841) = 21.18	***<0.0005*** (GG)	0.620
Group	F(2, 13) = 0.903	0.429	0.122
Cardiac output			
Interaction	F(2.859, 18.581) = 3.781	***0.03*** (GG)	0.368
Log_{10} Mean arterial pressure			
Interaction	F(4, 26) = 0.241	0.913	0.036
Time	F(2, 26) = 27.405	***<0.0005***	0.678
Group	F(2, 13) = 0.853	0.449	0.116
Systolic blood pressure			
Interaction	F(4, 26) = 0.791	0.541	0.109
Time	F(2, 26) = 19.601	***<0.0005***	0.601
Group	F(2, 13) = 0.641	0.543	0.09
Log_{10} Diastolic blood pressure			
Interaction	F(4, 26) = 0.300	0.875	0.044
Time	F(2, 26) = 34.533	***<0.0005***	0.727
Group	F(2, 13) = 2.133	0.158	0.247
Handgrip force			
Interaction	F(4, 26) = 1.507	0.229	0.188
Time	F(2, 26) = 0.097	0.908	0.007
Group	F(2, 13) = 1.385	0.285	0.176

Handgrip force z-scores			
Interaction	F(4, 26) = 1.132	0.363	0.148
Time	F(2, 26) = 0.007	0.993	0.001
Group	F(2, 13) = 1.327	0.299	0.170

F; ANOVA test statistic, GG; Greenhouse-Geisser correction. Effect of isometric handgrip exercise on haemodynamic variables across the groups tested by two-way mixed model ANOVA. Where a significant interaction was found, further details are in Table 5.6. N=5 premenopausal females, N=5 younger males, N=6 postmenopausal females.

Table 5.6 Statistical test data where there was a significant interaction between group and handgrip.

Variable	Pairwise comparison	*P* value
Log_{10} Heart rate		
Simple main effect of group during baseline	Overall	0.446
Simple main effect of group during handgrip	Overall	***0.049***
	YF vs. YM	0.524
	YF vs. PMF	0.287
	YM vs. PMF	***0.041***
Simple main effect of group during recovery	Overall	0.856
Simple main effect of time in YF	Overall	***0.001***
	BL vs. HG	0.052
	HG vs. Rec	***0.029***
	BL vs. Rec	0.942
Simple main effect of time in YM	Overall	***0.003 (GG)***
	BL vs. HG	***0.006***
	HG vs. Rec	***<0.0005***
	BL vs. Rec	1.0
Simple main effect of time in PMF	Overall	***0.014 (GG)***
	BL vs. HG	0.067
	HG vs. Rec	***0.029***
	BL vs. Rec	1.0
Cardiac output		
Simple main effect of group during baseline	Overall	0.497
Simple main effect of group during handgrip	Overall	0.247
Simple main effect of group during recovery	Overall	0.389
Simple main effect of time in YF	Overall	***0.014***
	BL vs. HG	0.077
	HG vs. Rec	0.704
	BL vs. Rec	0.225
Simple main effect of time in YM	Overall	***0.027***
	BL vs. HG	0.183
	HG vs. Rec	0.566
	BL vs. Rec	0.171
Simple main effect of time in PMF	Overall	0.105

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, BL; baseline, HG; handgrip, Rec; recovery, GG; Greenhouse-Geisser correction.

Table 5.7 Statistical test data for group differences in absolute and percentage change in haemodynamic variables during isometric handgrip exercise.

Variable	Test statistic	*P* value
Δ Burst incidence	F(2, 13) = 0.121	0.887
%Δ Burst incidence	F(2, 13) = 0.088	0.916
Δ Burst frequency	F(2, 13) = 1.674	0.226
%Δ Burst frequency	F(2, 13) = 20.67	0.166
Δ Heart rate	$X^2(2) = 8.171$	***0.017***
%Δ Heart rate	$X^2(2) = 7.706$	***0.021***
Δ Stroke volume	F(2, 13) = 1.154	0.346
%Δ Stroke volume	$X^2(2) = 0.547$	0.761
Δ Cardiac output	F(2, 7.457) = 2.424	0.155 (Welch)
%Δ Cardiac output	F(2, 7.453) = 4.590	0.05 (Welch)
Δ Systolic blood pressure	$X^2(2) = 0.216$	0.544
%Δ Systolic blood pressure	F(2, 13) = 0.444	0.651
Δ Diastolic blood pressure	$X^2(2) = 1.212$	0.546
%Δ Diastolic blood pressure	$X^2(2) = 1.159$	0.560
Δ Pulse pressure	F(2, 13) = 7.619	***0.006***
%Δ Pulse pressure	F(2, 13) = 7.134	***0.028***
Δ Mean arterial pressure	$X^2(2) = 0.453$	0.797
%Δ Mean arterial pressure	$X^2(2) = 0.357$	0.836
Δ Transduction slope	F(2, 13) = 0.480	0.630
%Δ Transduction slope	$X^2(2) = 1.946$	0.378

Δ; absolute change, %Δ; percentage change, F; ANOVA test statistic, χ^2; Kruskal-Wallis test statistic.

Table 5.8 Correlation coefficients for the relationship between change in sympathetic transduction slope and change in haemodynamic variables with isometric handgrip exercise.

Correlation	Correlation coefficient	*P* value
Absolute change		
Δ transduction slope vs. Δ burst incidence	r=0.223	0.407
Δ transduction slope vs. Δ burst frequency	r=0.165	0.541
Δ transduction slope vs. Δ SBP	r=0.141	0.603
Δ transduction slope vs. Δ DBP	ρ=-0.041	0.880
Δ transduction slope vs. Δ MAP	ρ=0.212	0.430
Percentage change		
%Δ transduction slope vs. %Δ burst incidence	ρ=0.409	0.116
%Δ transduction slope vs. %Δ burst frequency	ρ=0.282	0.289
%Δ transduction slope vs. %Δ SBP	ρ=0.153	0.572
%Δ transduction slope vs. %Δ DBP	ρ=0.229	0.393
%Δ transduction slope vs. %Δ MAP	ρ=0.144	0.594

Δ; absolute change, %Δ; percentage change, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial blood pressure, r; Pearson's correlation coefficient, ρ; Spearman's rank correlation coefficient.

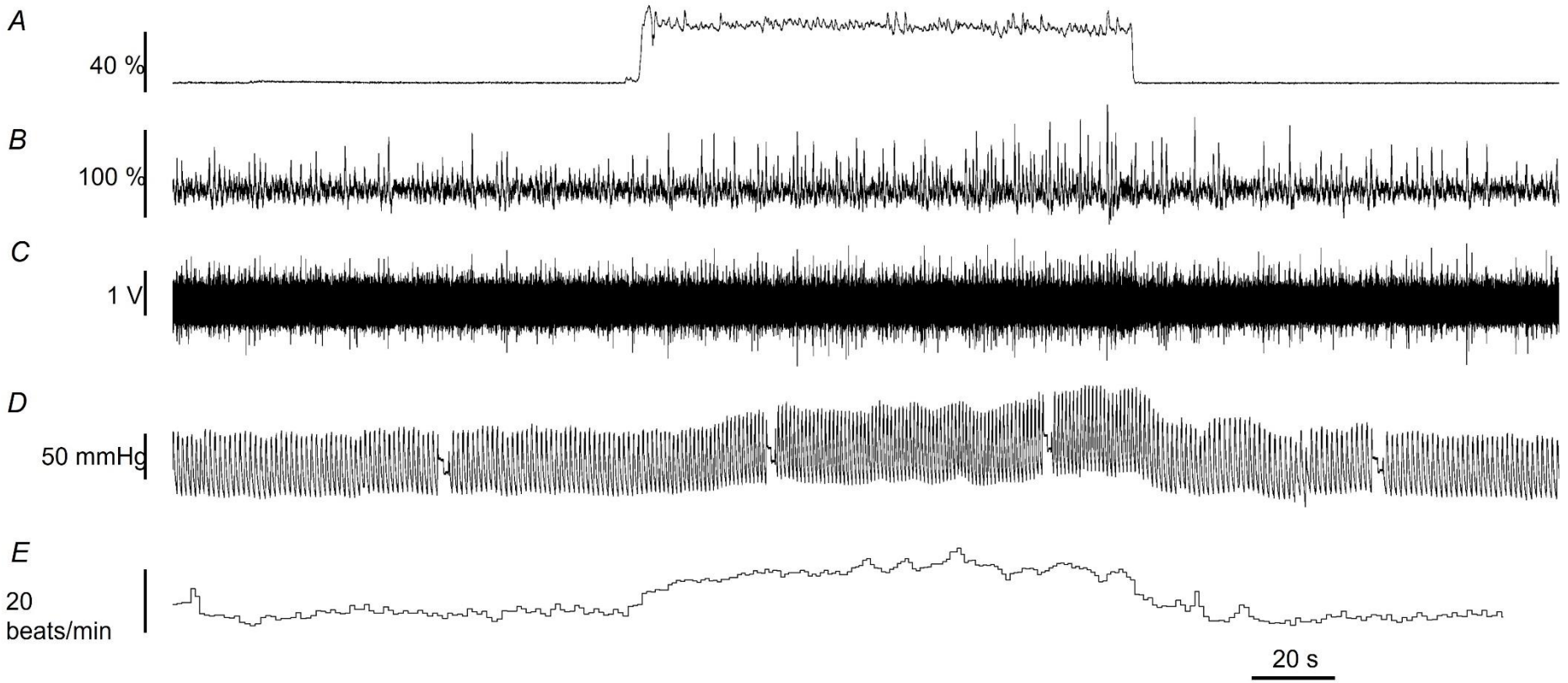

Figure 5.1 Example recording of the sympathetic response to isometric handgrip exercise in one individual. A; Handgrip force normalised to maximal voluntary contraction, B; Integrated neurogram normalised to the tallest burst, C; Raw neurogram, D; Finometer blood pressure , E; Beat-to-beat heart rate.

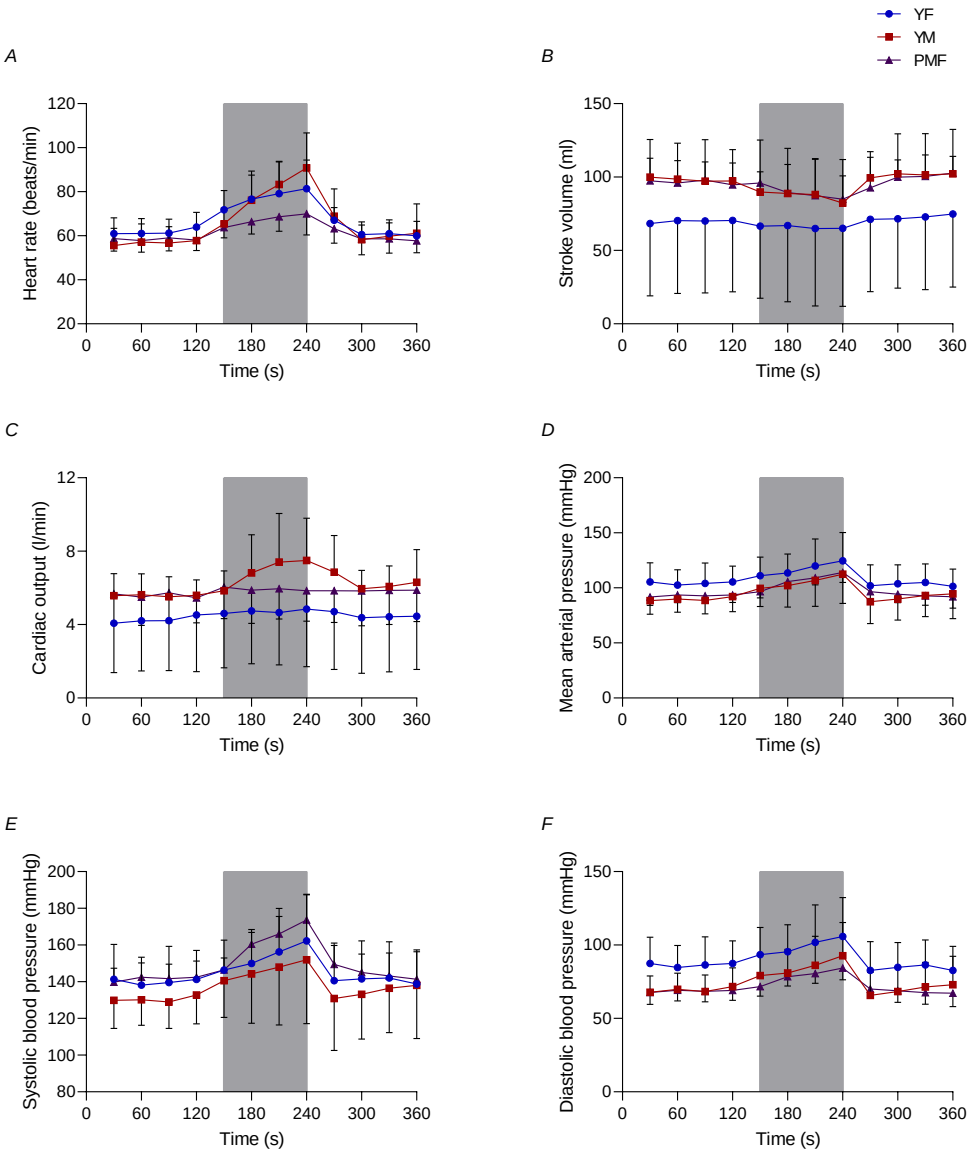

Figure 5.2 Time course of the haemodynamic response to isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females. Data are averaged over 30 s intervals and show mean ± SD. Grey indicates the handgrip period.

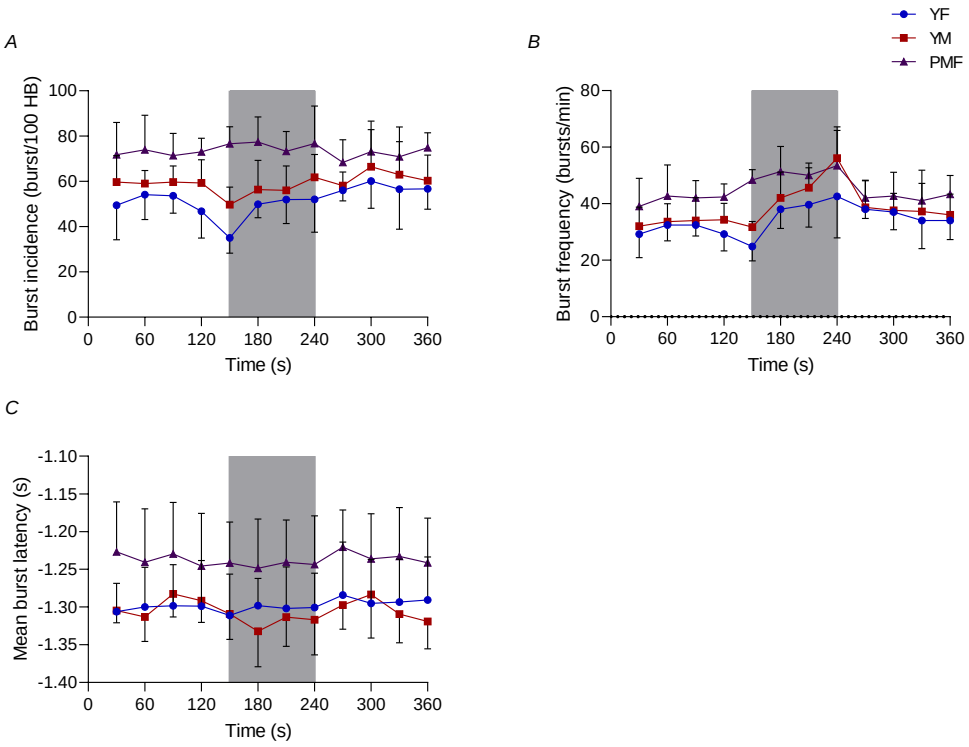

Figure 5.3 Time course of the sympathetic response to isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females, HB; heartbeats. Data are averaged over 30 s intervals and show mean ± SD. Grey indicates the handgrip period.

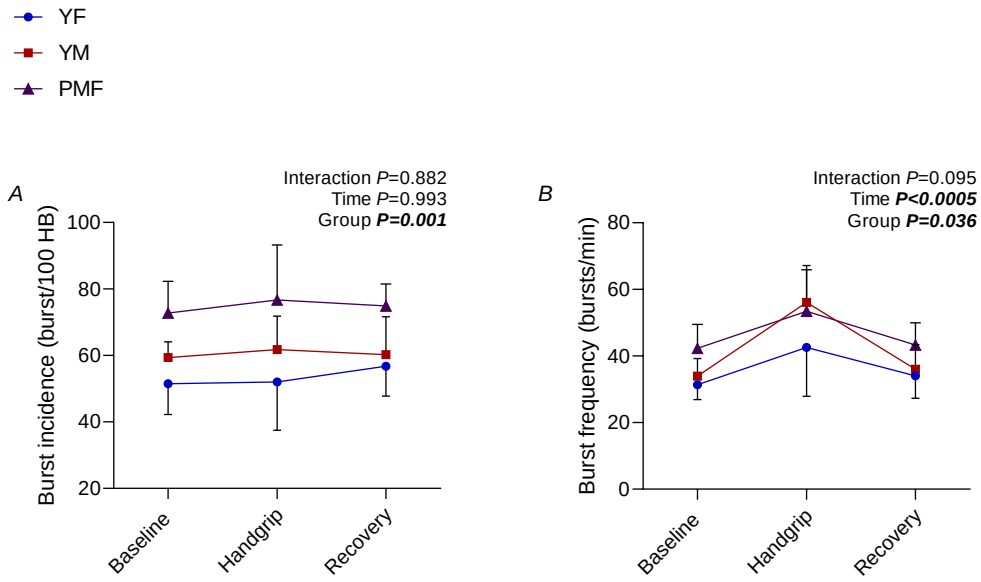

Figure 5.4 The sympathetic response to isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females, HB; heartbeats. Data are mean ± SD of the last 90 s rest versus the last 30 s handgrip versus the last 30 s recovery. Two-way mixed model ANOVA.

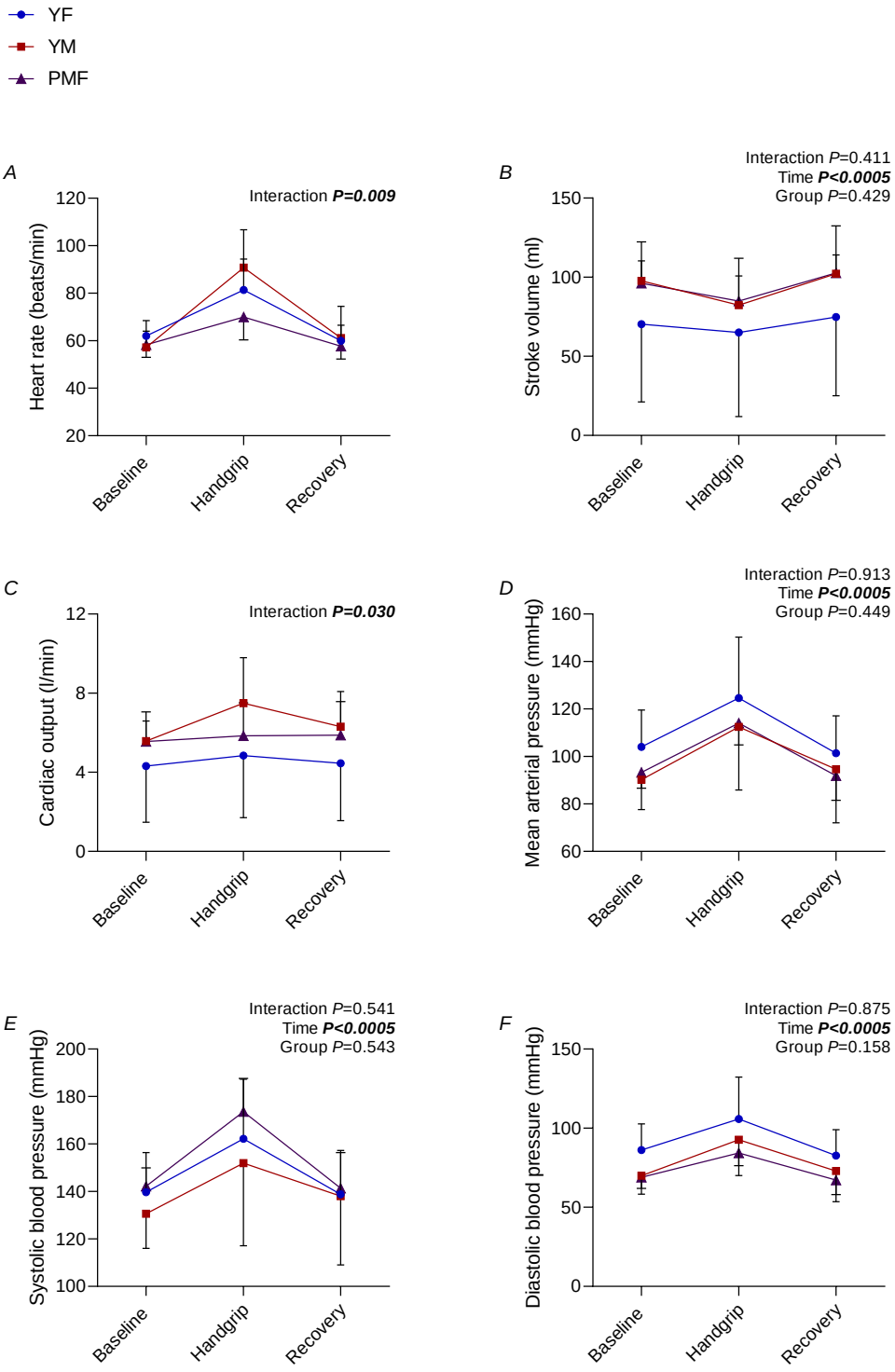

Figure 5.5 The haemodynamic response to isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females. Data are mean ± SD of the last 90 s rest versus the last 30 s handgrip versus the last 30 s recovery. Two-way mixed model ANOVA.

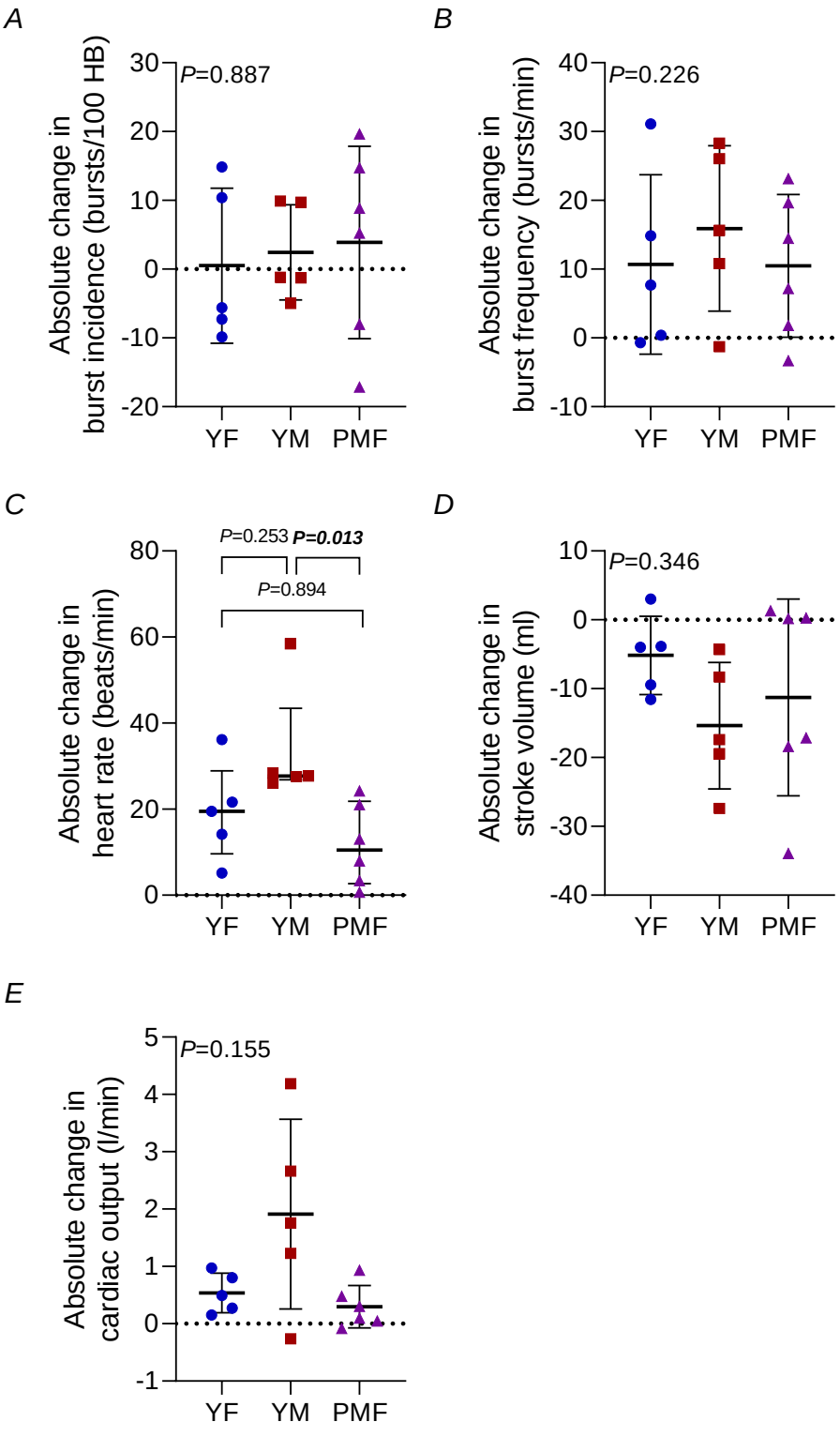

Figure 5.6 Group differences in the absolute change in haemodynamic variables with isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females, HB; heartbeats. Data are mean

± SD (A, B, D, E) or median [interquartile range] (C). Group differences tested by one-way ANOVA (A, B, D, E) or Kruskal-Wallis test (C).

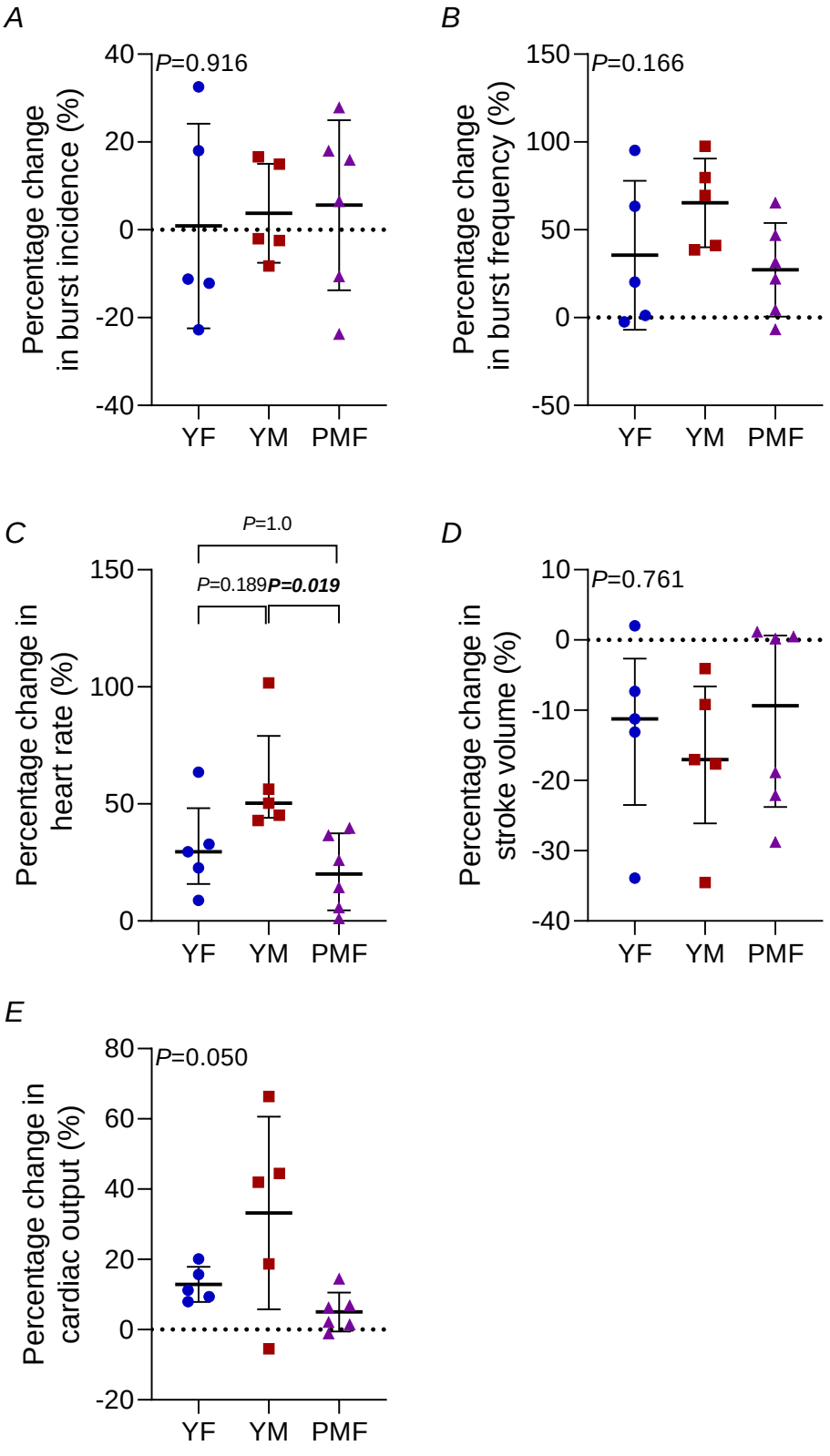

Figure 5.7 Group differences in the percentage change in haemodynamic variables with isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females, HB; heartbeats. Data are mean ± SD (A, B, E) or median [interquartile range] (C, D). Group differences tested by one-way ANOVA (A, B, E) or Kruskal-Wallis test (C, D).

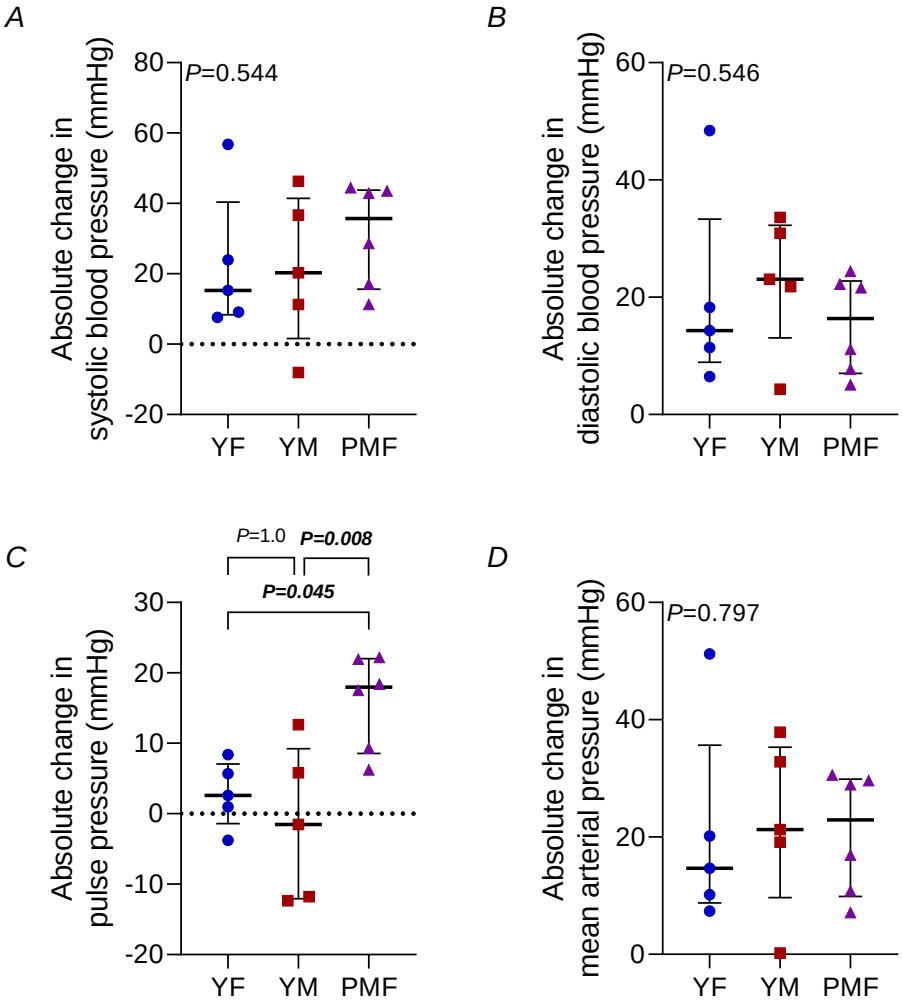

Figure 5.8 Group differences in the absolute change in blood pressure with isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females. Data are mean ± SD (C) or median [interquartile range] (A, B, D). Group differences tested by one-way ANOVA (C) or Kruskal-Wallis test (A, B, D).

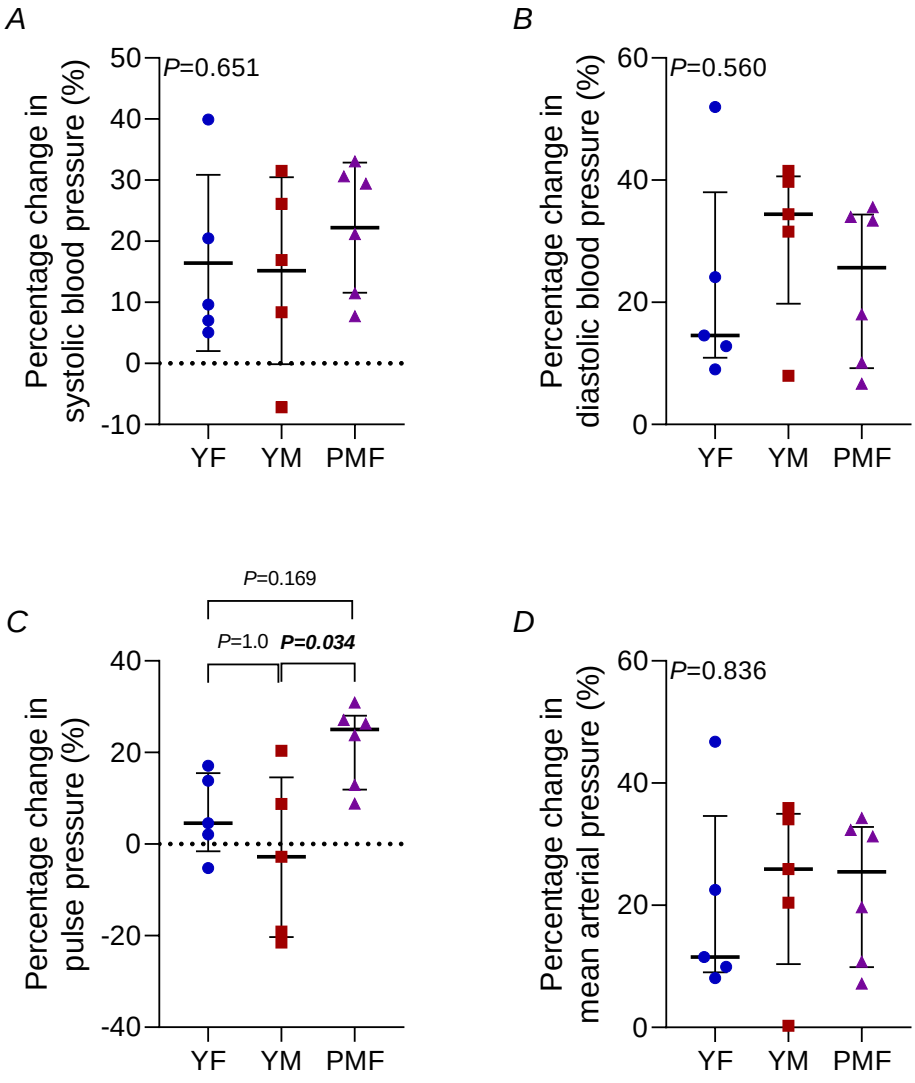

Figure 5.9 Group differences in the percentage change in haemodynamic variables with isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females. Data are mean ± SD (A, C) or median [interquartile range] (B, D). Group differences tested by one-way ANOVA (A, C) or Kruskal-Wallis test (B, D).

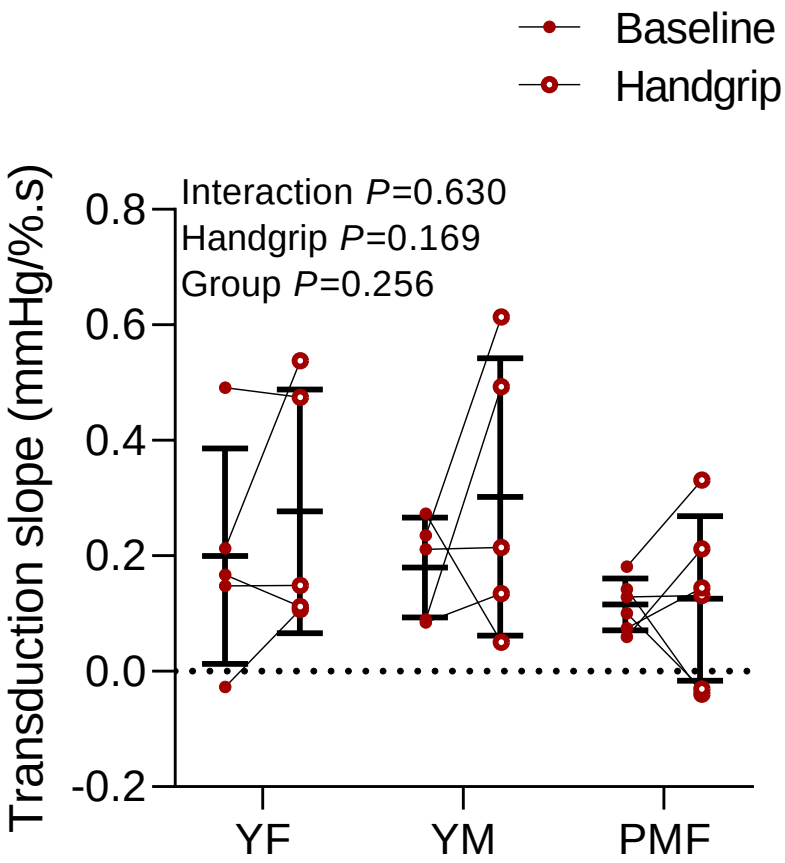

Figure 5.10 Sympathetic transduction slope at baseline and during isometric handgrip exercise. YF; premenopasual females, YM; younger males, PMF; postmenopausal females. Transduction slope sampled during the 90 s before start of handgrip exercise and the last 90 s of 2 min isometic handgrip at 40 % maximal voluntary contraction. Data are mean ± SD. Group differences tested by two-way mixed model ANOVA. N=5 YF, N=5 YM, N=6 PMF.

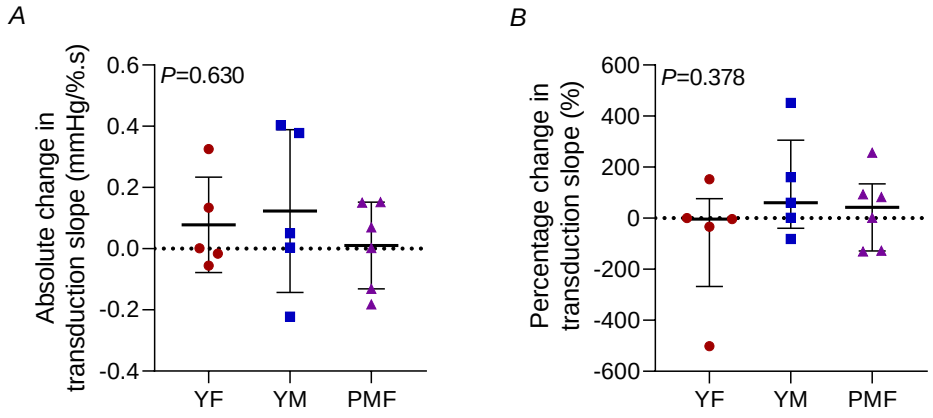

Figure 5.11 Absolute (A) and percentage (B) change in sympathetic transduction slope between baseline and isometric handgrip exercise. YF; premenopausal females, YM; younger males, PMF; postmenopausal females. Data are mean ± SD (A) or median ± interquartile range (B). Group differences tested by one-way ANOVA (A) or Kruskal-Wallis test (B). N=5 YF, N=5 YM, N=6 PMF.

Chapter 6 Sex and Age Differences in Respiratory Sympathetic Coupling in hypertension and normotension.

6.1 Note on use of existing data

The data presented in this chapter were collected in previous research studies conducted at the University of Bristol and the Mayo Clinic. The data have been published elsewhere, however the main analyses of this chapter have not been conducted or published before. Data analysis was conducted solely by the author, using scripts written by the author except where stated.

6.2 Background

6.2.1 Introduction

In healthy humans, blood pressure, heart rate and the activity of the sympathetic nervous system all exhibit respiratory modulation. Inspiration is associated with rising heart rate whilst expiration is associated with falling heart rate (respiratory sinus arrhythmia) (Elstad et al., 2018). Similarly, arterial blood pressure rises with inspiration and falls with expiration (Traube-Hering waves) (Barnett et al., 2020). In humans, sympathetic nerve activity increases towards the end of expiration and falls between peak inspiration to early expiration (Eckberg et al., 1985, Seals et al., 1990). As such, respiration is a key component of sympathetic blood pressure regulation. Research into respiratory sympathetic modulation with healthy ageing (Shantsila et al., 2015) and in disease states (Fatouleh and Macefield, 2011) has already been conducted. However, the influence of sex and menopause on respiratory sympathetic modulation remains unclear.

6.2.2 Mechanisms underlying respiratory sympathetic modulation

Sympathetic modulation by respiration is underpinned by a central coupling between respiratory and sympathetic centres in the brainstem, which can be further modulated by afferent input from arterial and cardiopulmonary baroreceptors, pulmonary stretch receptors and peripheral chemoreceptors. Respiratory coupling of sympathetic activity persists in the absence of afferent input, for example in vagotomised animal models (Barman and Gebber, 1976, Preiss et al., 1975) or human recipients of lung transplants (Seals et al., 1993), which is evidence for a primary central coupling mechanism. However, the

arterial baroreflex influences this mechanism, minimising respiratory modulation of MSNA when sympathetic drive is low, for example during arterial baroreceptor loading with pressor drugs (Eckberg et al., 1988). Similarly, when the arterial baroreceptors are unloaded and sympathetic drive is high, (e.g. during head-up tilt), respiratory modulation of MSNA is lost (Cooke et al., 1999). Independent of the arterial baroreflex, the cardiopulmonary baroreceptors are also thought to contribute to respiratory sympathetic modulation, given that during static lung inflation (end-inspiratory apnoea), MSNA remains high once arterial blood pressure has stabilised (Macefield and Wallin, 1995a). However, respiratory sympathetic modulation is not driven by either the arterial or cardiopulmonary baroreflex, as the entrainment of MSNA to respiration is unchanged when respiratory modulation of blood pressure is manipulated, for example with positive pressure ventilation (Macefield and Wallin, 1995b). In addition to the baroreflex, pulmonary stretch receptors influence respiratory sympathetic modulation, for example enhancing modulation with larger tidal volumes (Seals et al., 1990, Croix et al., 1999). Given that lung transplant recipients do not demonstrate a relationship between tidal volume and magnitude of respiratory sympathetic modulation, it is thought that the pulmonary stretch receptors underlie this mechanism (Seals et al., 1993). Furthermore, the peripheral chemoreflex may contribute to respiratory sympathetic modulation, particularly during periods of desaturation. Peripheral chemoreceptor stimulation by hypoxia (or hypoperfusion, hypercapnia, low pH, or hypoglycaemia (Iturriaga et al., 2016)), causes sympathoexcitation and stimulates respiration (Dampney, 2016), thus the peripheral chemoreflex contributes to respiratory and sympathetic regulation. The respiratory response to chemoreceptor activation can be enhanced by baroreceptor unloading in animals (Heistad et al., 1975) and humans (Hildebrandt et al., 2000, Koehle et al., 2010), whilst baroreceptor loading dampens the sympathoexcitatory response to chemoreceptor stimulation (Somers et al., 1991). Therefore, the peripheral chemoreflex is subject to regulation by the arterial baroreflex. As such, the contribution of the peripheral chemoreflex to respiratory sympathetic coupling may be important during periods of chemoreceptor activation.

6.2.3 Respiratory sympathetic modulation with age and sex

The influence of ageing on respiratory sympathetic modulation has been investigated in healthy males. Shantsila et al. found no difference in respiratory sympathetic modulation between older and younger male participants, despite the older group exhibiting the increased resting level of sympathetic activation that is common in older adults (Shantsila et al., 2015). This study did not, however, include females. In ageing females, there is the additional factor of menopause, which has been shown to influence some aspects of respiratory and autonomic ageing. In addition to the increase in MSNA with age (Keir et al., 2020), the sympathetic nervous system becomes a more important regulator of blood pressure in postmenopausal versus premenopausal females (Barnes et al., 2014). Furthermore, menopause appears to influence lung function, with poorer lung function observed in postmenopausal versus premenopausal females (Triebner et al., 2017). Furthermore, risk of sleep apnoea is increased after menopause (Young et al., 2003), with replacement female sex hormones able to reduce sleep apnoea risk in postmenopausal females (Shahar et al., 2003). Therefore, there may be sex hormone-related changes to respiratory sympathetic coupling in older females. This question is addressed in Aim 1, which aims to determine if respiratory sympathetic coupling is altered in postmenopausal females versus younger females. If these analyses show altered respiratory sympathetic coupling in postmenopausal versus premenopausal females, the results would contradict previous work in males (Shantsila et al., 2015) and therefore suggest a sex-specific effect of age on respiratory sympathetic modulation. In this case, it would be important to demonstrate whether sex differences in respiratory sympathetic modulation are apparent in young adults, or whether sex differences emerge with increasing age. As such, respiratory sympathetic modulation was additionally assessed in a group of younger males, compared to premenopausal females and postmenopausal females (Aim 2).

6.2.4 Respiratory sympathetic modulation in hypertension

Sympathetic regulation of blood pressure is altered in hypertension, with hypertensive patients demonstrating a greater resting level of sympathetic activation at a population level (Grassi et al., 2018). Additionally, there is evidence in animal models and humans that peripheral chemoreflex activity is

altered in hypertension. In a rat model of hypertension, denervation of the carotid bodies in young, not yet hypertensive rats delays the development and reduces the magnitude of hypertension versus controls (Abdala et al., 2012). Furthermore, hypertensive humans show a tonic contribution of the peripheral chemoreflex to level of sympathetic activity that is absent in normotensive controls (Sinski et al., 2012). As such, the contribution of the peripheral chemoreflex to respiratory sympathetic coupling may be altered in hypertension. Indeed, young spontaneously hypertensive rats show exaggerated respiratory modulation of sympathetic nerve activity versus control rats (Simms et al., 2009), for which the carotid body is at least partially responsible (McBryde et al., 2013).
Fatouleh and Macefield investigated respiratory sympathetic coupling in hypertensive and normotensive middle-aged adults and found no effect of hypertension on respiratory sympathetic modulation (Fatouleh and Macefield, 2011). Their participants included both males and females, however only three hypertensive females were studied and neither the hypertensive nor normotensive females were characterised by menopausal status. As such, the effect of hypertension on respiratory sympathetic coupling could be different in males and females. This is addressed in Aim 3, which aims to compare respiratory sympathetic modulation in hypertension versus normotension, in both postmenopausal females and older males.

6.2.5 Aims and hypotheses

Aim 1: To investigate whether respiratory sympathetic modulation is altered in healthy postmenopausal females versus premenopausal females.

> H_0: There will be no difference in the respiratory modulation of MSNA in healthy postmenopausal females versus premenopausal females.
>
> H_1: There will be a difference in the respiratory modulation of MSNA in healthy postmenopausal females versus premenopausal females.

Aim 2: To investigate whether respiratory sympathetic modulation is altered in healthy younger males versus premenopausal females and postmenopausal females.

H_0: There will be no difference in the respiratory modulation of MSNA in healthy younger males versus premenopausal females and postmenopausal females.

H_1: There will be a difference in the respiratory modulation of MSNA in healthy younger males versus premenopausal females and postmenopausal females.

Aim 3: To investigate whether respiratory sympathetic modulation is altered in hypertensive versus normotensive (1) postmenopausal females and (2) older males.

H_0: There will be no difference in the respiratory modulation of MSNA in hypertensive versus normotensive postmenopausal females and older males.

H_1: There will be a difference in the respiratory modulation of MSNA in hypertensive versus normotensive postmenopausal females and older males.

6.3 Methods

6.3.1 Participants

6.3.1.1 Healthy postmenopausal females and younger adults

To investigate respiratory sympathetic modulation in postmenopausal females, premenopausal females and younger males (Aim 1), existing data were analysed. The data were collected as part of a larger study (Hart et al., 2011a), conducted at the Mayo Clinic, which received ethical approval from the Mayo Clinic Institutional Review Board. Thirteen postmenopausal females, 12 premenopausal, premenopausal females and 12 young males gave written informed consent to participate. None reported a history of chronic illness or took regular medication except oral contraception. Postmenopausal status was confirmed by at least 12 months of reported amenorrhoea in the absence of hormone replacement therapy (National Institute for Health and Care Excellence, 2015). Premenopausal females participated during the early follicular phase of the menstrual cycle or the low hormone phase of oral contraceptive use.

6.3.1.2 Hypertensive and normotensive postmenopausal females and older males

Respiratory sympathetic coupling in hypertensive versus normotensive postmenopausal females and older males was investigated using existing data from four previous studies. Three were conducted at the University of Bristol and received ethical approval from an NHS Research Ethics Committee; one was conducted at the Mayo Clinic and received ethical approval from the Mayo Clinic Institutional Review Board (details of ethical approvals in Table 6.1). Participants were healthy and had no serious chronic health conditions except hypertension. Some of the hypertensive participants were treated with anti-hypertensive medication (Table 6.12 and 6.14).

6.3.2 Procedures

For both aims 1 and 2, MSNA was recorded by microneurography (Hart et al., 2017) using a tungsten microelectrode inserted into a common peroneal nerve (detailed description in chapter 2). MSNA was confirmed by the presence of pulse-synchronous bursts of activity that increased in response to end-expiratory

apnoea, and the absence of a response to startle or light tactile stimuli (Hart et al., 2017). The signal was processed and recorded as in section 2.4.5. Although recording of MSNA for aim 2 (hypertensive versus normotensive postmenopausal females and older males) was done at several sites by different investigators, all sites used an Iowa Nerve Traffic Analyser and obtained the recording in a standardised way by following widely accepted guidelines (Hart et al., 2017). For both aims 1 and 2, beat-to-beat heart rate was monitored by 3-lead ECG and respiration was recorded by a respiratory belt placed at the level of the diaphragm. For aim 1 (healthy postmenopausal females versus younger adults), continuous blood pressure was measured by a pressure transducer inserted into the brachial artery after administration of 2 % lidocaine. For aim 2, continuous blood pressure was recorded by brachial artery pressure transducer in 4/9 normotensive older males, and was recorded by photoplethysmography (Finometer Pro, Finapres Medical Systems, the Netherlands) in all other participants. Whilst these different methods of recording continuous blood pressure are not directly comparable, the main analysis involving continuous blood pressure for this chapter is within the same individual.

6.3.3 Data analyses

MSNA, blood pressure, heart rate and respiration were recorded over a period of quiet rest (5-10 minutes). Bursts of MSNA were identified by a trained experimenter using a custom script (Spike 2, Cambridge Electronic Design, Cambridge, UK; script by E. Hart, edited by Z. Adams and H. Blythe) and was conducted blind to participant group. Burst incidence and frequency were calculated and expressed as bursts/100 heartbeats and bursts/min, respectively. For measures of burst area, the integrated neurogram was smoothed to remove baseline drift (0.1 s time constant) and normalised in a two-point calibration to the neurogram baseline (0 %) and the tallest burst (100 %). Burst area was then calculated as the modulus of the normalised neurogram over a time period (specified below). Heart rate was calculated between consecutive R waves and averaged across the analysis window. Systolic and diastolic blood pressure were measured at every cardiac cycle and used to calculate beat to beat pulse pressure and mean arterial pressure. All blood pressure variables were then averaged across the analysis window.

6.3.3.1 Quantification of respiratory sympathetic coupling

Respiratory sympathetic coupling analysis was conducted using a custom script (Spike 2, Cambridge Electronic Design, Cambridge, UK; script by H. Blythe with edits and input from Z. Adams). Inspiratory and expiratory periods of the respiratory cycle were considered separately, with inspiration defined as trough to peak and expiration defined as peak to trough of the respiratory trace. The integrated neurogram was shifted backwards by individual mean burst latency (mean lag between each MSNA burst and the inhibiting R wave). Respiratory modulation of MSNA was measured by calculating burst incidence, frequency, and burst area for 20 % intervals within each inspiratory and expiratory period (Figure 6.2). MSNA burst and R wave event data were extracted using a publicly availably script (RasterDump, Spike2, Cambridge Electronic Design, Cambridge, UK) and burst area was measured as the modulus of the shifted, normalised neurogram within each 20 % respiratory interval, normalised for interval duration (expressed as burst area/s). MSNA data (burst incidence, burst frequency, total burst area/s and mean burst area/s) were analysed in two ways. Firstly, to determine whether MSNA was modulated between inspiration and expiration, the data were expressed as two respiratory phases, as previously described (Shantsila et al., 2015): inspiration/post-inspiration, represented by intervals 60-100 % of inspiration and 0-60 % of expiration; and mid-late expiration, represented by intervals 60-100 % of expiration and 0-60 % of inspiration. Burst incidence, burst frequency, and total bursts area/s data within the 20 % respiratory intervals were recalculated across these two respiratory phases, whist mean burst area/s data were re-averaged across the phases. These two respiratory phases were used, rather than simply inspiration and expiration, as the maximal reduction of MSNA with inspiration occurs at and shortly after peak inspiration (Eckberg et al., 1985), and thus is better captured using the phase inspiration/post-inspiration. Secondly, to more precisely determine where in the respiratory cycle any modulation of MSNA occurred, the 20 % interval inspiratory and expiratory data were considered together as an entire respiratory cycle, starting and ending at peak inspiration (0 and 100 %), with end expiration at 50 % (Shantsila et al., 2015). Therefore, the 20 % intervals of expiration represented 0-50 % of the entire respiratory cycle, whilst the 20 % intervals of inspiration represented 50-100 % of the entire respiratory cycle. Data for both aim one and aim two were analysed this way (Figure 6.2). Statistical analysis of respiratory sympathetic coupling data were analysed as described in section 6.3.4.

6.3.3.2 Quantification of Traube-Hering waves and respiratory-modulated heart rate

Traube-Hering waves and respiratory-modulated heart rate were measured using the same method as Shantsila et al. Beat to beat mean arterial pressure was calculated as the modulus of the blood pressure waveform (either Finometer or brachial artery transducer) between consecutive diastolic blood pressures. Heart rate was calculated on a beat to beat basis using RR interval. Respiratory-modulated heart rate was calculated as the largest absolute change in beat to beat heart rate across each respiratory cycle (end-expiration to end-expiration) (Shantsila et al., 2015). Traube-Hering wave amplitude was similarly calculated as largest absolute change in beat to beat mean arterial pressure across each respiratory cycle (Shantsila et al., 2015), however the sampling window for the absolute peak mean arterial blood pressure value was extended to one second after end-expiration, as the absolute largest value often occurred just after the end of expiration. Respiratory-modulated heart rate and Traube-Hering wave amplitude were averaged across the entire analysis window.

6.3.3.3 Correlations between respiratory trace amplitude and respiratory-modulated haemodynamic variables

To further quantify respiratory-sympathetic modulation, haemodynamic variables (Traube-Hering wave amplitude, respiratory-modulated heart rate and respiratory-modulated MSNA) were correlated with respiratory trace amplitude (Shantsila et al., 2015). Respiratory-modulated MSNA was taken as the modulus of the original, unshifted integrated neurogram, over 1.5 x the duration of each inspiration, from the start of inspiration (Shantsila et al., 2015). To address whether the arterial baroreflex played a role in determining Traube-Hering wave amplitude, respiratory-modulated MSNA was also correlated with the Traube-Hering wave of the previous respiratory cycle (Traube-Hering -1) and the following respiratory cycle (Traube-Hering +1) (Shantsila et al., 2015).

6.3.3.4 Sympathetic baroreflex sensitivity

Sympathetic baroreflex sensitivity (sBRS) was analysed using a custom script (Spike2, CED, Cambridge, UK; by Z. Adams) as previously described (Hart et al., 2011b). The integrated neurogram was shifted backwards by individual mean

burst latency. Each diastolic blood pressure was then associated with any MSNA bursts (either 1 or 0) falling within ± 0.4 s of the subsequent R wave. Diastolic blood pressure was averaged into 1 mmHg bins and the percentage of associated cardiac cycles containing bursts was calculated. sBRS was taken as the slope of the weighted linear regression between diastolic blood pressure bins and burst percentage.

6.3.3.5 Heart rate variability

Both time and frequency domain analysis of heart rate variability during quiet rest was obtained using commercially available software (LabChart version 8, AD Instruments, Dunedin, New Zealand).

6.3.4 Statistical analyses

Statistical analysis was conducted in SPSS Statistics (version 24, IBM). Analysis of respiratory sympathetic coupling data was conducted using a two-way mixed model ANOVA, where the repeated factor was respiratory phase (with either two or 10 levels, depending on the analysis), and the independent factor was group. The suitability of a mixed model ANOVA was determined by assessing whether the studentised residuals met the assumptions of normality, equality of variance and sphericity using Q-Q plots, Levene's test for equality of variance and Mauchly's test of sphericity, respectively. Where the assumption of sphericity was violated, the Greenhouse-Geisser correction was applied. Where the other assumptions were violated, transformation of the data was attempted, as described in the results. Where a significant respiratory phase x group interaction was found, the data were assessed for significant simple main effects of (1) group (univariate analysis of group at each level of respiratory phase), and (2) respiratory phase (univariate analysis of respiratory phase within each level of group). Where no significant interaction was found, the significance of main effects of respiratory phase and group are reported. Group differences in demographic, haemodynamic, respiratory-modulated haemodynamic variable data (e.g., Traube-Hering waves) were analysed by univariate ANOVA or Kruskal-Wallis test where there were more than two levels of the group factor (aim one), or independent sample T-test or Mann-Whitney U test where there were only two levels of the group factor (aim two). Correlations between respiratory and haemodynamic variables were assessed using Spearman's rank.

Group differences in the strength of these correlations was assessed by comparing the correlation coefficient Spearman's rho by univariate ANOVA, Kruskal-Wallis test, independent samples T-test, or Mann-Whitney U test, where appropriate.

6.4 Results

6.4.1 Aim 1: Respiratory sympathetic coupling in healthy postmenopausal females versus premenopausal females and younger males

6.4.1.1 Participant characteristics

Demographic data are available for 9/13 postmenopausal females, 11/12 premenopausal females and 12/12 younger males (Table 6.2), however all postmenopausal females reported at least one year of amenorrhoea in the absence of hormone replacement therapy and were therefore considered postmenopausal. Premenopausal females and younger males were matched for age (25 [5] and 26 [7] years, respectively, median [interquartile range], pairwise comparison P=1.0) and were younger than postmenopausal females (55 [9] years; pairwise comparison versus premenopausal females P<0.0005 and versus younger males P<0.0005). BMI did not differ significantly between groups (23.7 ± 1.0, 24.7 ± 2.2 and 24.2 ± 2.5 kg/m^2 for premenopausal females, younger males, and postmenopausal females, respectively, mean ± SD, P=0.320). Plasma noradrenaline concentration differed between groups (P=0.008), with greater plasma noradrenaline concentration observed in postmenopausal females versus both premenopausal females (P=0.016) and younger males (P=0.029), but no difference between premenopausal females and males (P=1.0). Group averages are shown in Table 6.2 with statistical test data in Table 6.5.

6.4.1.2 Resting sympathetic nerve activity

Resting MSNA differed between groups (burst incidence and burst frequency both P<0.0005, Table 6.2). Post-hoc analysis showed a greater resting burst incidence in postmenopausal females versus both premenopausal females (79 [1] versus 53 [13] bursts/100 heartbeats, P<0.0005) and younger males (51 [11] bursts/100 heartbeats, P<0.0005), but no difference between premenopausal females and younger males (P=1.0). Similarly, burst frequency was greater in postmenopausal females versus both premenopausal females (52 [13] versus 33 [10] bursts/min, P=0.001) and younger males (29 [6] bursts/min, P<0.0005), but did not differ between the younger groups (P=1.0). Statistical test data are in Table 6.5.

6.4.1.3 Resting haemodynamic variables

Resting heart rate, respiratory rate, diastolic blood pressure and mean arterial pressure were similar between groups (Table 6.3 with statistical test data in Table 6.5). Systolic blood pressure was greater in postmenopausal females versus younger males (146 [21] versus 125 [8] mmHg, *P*=0.003) but not versus premenopausal females (136 [17] mmHg, *P*=0.728). Systolic blood pressure did not differ between premenopausal females and younger males (*P*=0.119). Pulse pressure was lower in younger males versus postmenopausal females (52 ± 5 versus 71 ± 16 mmHg, *P*=0.002) and versus premenopausal females (59 ± 7 mmHg, *P*=0.023), but was not different between premenopausal females and postmenopausal females (*P*=0.050). Overall sBRS slopes were similar between groups (-3.4 [3.8], -5.3 [1.9], and -6.6 [4.7] %/mmHg for postmenopausal females, premenopausal females, and younger males, respectively, *P*=0.061).

6.4.1.4 Heart rate variability

Frequency domain analysis of heart rate variability revealed significant group differences in the absolute measures of heart rate variability (low frequency domain (ms^2) *P*<0.0005; high frequency domain (ms^2) *P*<0.0005), with smaller absolute low and high frequency domain power seen in postmenopausal females versus both premenopausal females and younger males (Table 6.4). However, there were no differences in low and high frequency domain power between premenopausal females and younger males. There were no group differences in the other frequency domain measures of heart rate variability (Table 6.4). Statistical test data are shown in Table 6.5.

There were also group differences in time domain measures of heart rate variability. SDRR, RMSSD and pRR50 were all reduced in postmenopausal females versus both premenopausal females and younger males (SDRR *P*=0.022 and *P*=0.001; RSD *P*=0.018 and *P*=0.001; pRR50 *P*=0.009 and P<0.0005 versus premenopausal females and younger males respectively, Table 6.4). However, neither SDRR, nor RSD, nor pRR50 was different between premenopausal females and younger males. As such, postmenopausal females showed reduced heart rate variability via all time domain measures and some frequency domain measures. Statistical test data are shown in Table 6.5.

6.4.1.5 Respiratory sympathetic modulation of burst incidence

Assessment of respiratory sympathetic coupling over two-respiratory phases showed that burst incidence was modulated by respiration differently across the participant groups. There was a significant respiratory phase x group interaction for burst incidence (*P*=0.004, Figure 6.3A). When the respiratory phases were compared separately within group (simple main effect of respiratory phase) burst incidence was reduced during inspiration/post-inspiration versus mid-late expiration in every group (premenopausal females 39 ± 11 versus 62 ± 17 bursts/100 heartbeats, *P*<0.0005; younger males 41 ± 8 versus 65 ± 11 bursts/100 heartbeats, *P*<0.0005; postmenopausal females 72 ± 12 versus 83 ± 12 bursts/100 heartbeats, *P*=0.001). Therefore, every group showed some respiratory modulation of burst incidence. However, postmenopausal females had a smaller reduction in burst incidence with inspiration/post-inspiration versus both premenopausal females and younger males (Figure 6.4; absolute change mean ranks 27.38 versus 15.58 and 13.33, *P*=0.019 and *P*=0.004; percentage change mean ranks 28.62 versus 14.33 and 13.25, *P*=0.003 and *P*=0.001; for postmenopausal females versus premenopausal females and younger males respectively). Absolute and percentage change in burst incidence did not differ between premenopausal females and younger males (*P*=1.0 and *P*=1.0 for absolute and percentage change respectively). Furthermore, when burst incidence was compared across groups during each respiratory phase (simple main effect of group), burst incidence was greater in postmenopausal females versus both premenopausal females and younger males during both respiratory phases (Table 6.6). Overall, these data show a higher level of MSNA burst incidence but smaller respiratory modulation of burst incidence in postmenopausal females compared to the younger groups.

6.4.1.6 Respiratory sympathetic modulation of burst frequency

MSNA burst frequency was also modulated by respiration differently across the groups. There was a significant respiratory phase x group interaction for burst frequency (*P*=0.029, Figure 6.3B). When respiratory phases were compared separately within each group (simple main effect of respiratory phase), burst frequency was reduced during inspiration/post-inspiration versus mid-late expiration in every group (premenopausal females 25 ± 7 versus 37 ± 10 bursts/min, *P*<0.0005; younger males 24 ± 7 versus 37 ± 9 bursts/min, *P*<0.0005; postmenopausal females 46 ± 9 versus 52 ± 9 bursts/min, *P*=0.005). Therefore,

every group showed respiratory modulation of burst frequency. However, percentage change in burst frequency was smaller in postmenopausal females versus both premenopausal females (-11 ± 11 versus -32 ± 15 %, *P*=0.001) and younger males (-25 ± 14 %, *P*=0.001) (Figure 6.4). Absolute change in burst frequency was smaller in postmenopausal females versus younger males (mean ranks 25.38 versus 14.58, *P*=0.038, but not versus premenopausal females (mean rank 16.5, *P*=0.121). Meanwhile, neither absolute nor percentage change in burst frequency differed between premenopausal females and younger males (*P*=1.0 and *P*=0.951). When burst frequency across groups was compared within each respiratory phase separately (simple main effect of group), postmenopausal females had a greater burst frequency versus both premenopausal females and younger males during both respiratory phases (Table 6.6). Overall, these data show a higher level of MSNA burst frequency but smaller respiratory modulation of burst frequency in postmenopausal females compared to the younger groups.

6.4.1.7 Respiratory sympathetic coupling of burst area.

MSNA burst area did appear to be modulated by respiration, but the modulation did not differ between the participant groups. There was no significant respiratory phase x group interaction for either total burst area/s (*P*=0.632) or mean burst area/s (*P*=0.503) (Figure 6.3C-D).

However, there was a significant main effect of respiratory phase for both total burst area/s (*P*<0.0005) and mean burst area/s (*P*<0.0005), with a smaller burst area seen during inspiration/post-inspiration versus mid-late expiration (total burst area/s 2778 ± 1007 versus 3417 ± 1065 %, *P*<0.0005; mean burst area/s 8.4 ± 2.2 versus 10.5 ± 2.5 %, *P*<0.0005). In agreement, absolute and percentage change in total burst area/s and mean burst area/s were similar across groups (Figure 6.4). Additionally, there was a significant main effect of group for both total burst area/s (*P*=0.009) and mean burst area/s (*P*<0.0005). Postmenopausal females showed a greater total burst area/s than premenopausal females (total burst area/s 3833 ± 989 versus 2626 ± 989 %, *P*=0.013) but not younger males (2835 ± 989 %, *P*=0.050). Although, postmenopausal females showed a greater mean burst area/s versus both premenopausal females (11.7 ± 2.1 versus 7.8 ± 2.1 %, *P*<0.0005) and younger males (8.8 ± 2.1 %, *P*=0.006). Overall, these data suggest that all groups showed similar respiratory modulation of burst area, with

inspiration associated with a reduced burst area. This occurred despite increased overall burst area in postmenopausal females compared to the younger groups.

6.4.1.8 Respiratory sympathetic modulation across 10 respiratory phases

To further investigate respiratory modulation of MSNA in healthy postmenopausal females and younger adults, data were considered as 10 phases of a complete respiratory cycle, starting and ending at peak inspiration (0 and 100 %), with end-expiration occurring at 50 % (Shantsila et al., 2015). Therefore, the original 20 % phases of expiration represented 0-50 % of the complete respiratory cycle, and the original 20 % phases of inspiration represented 50-100 % of the complete respiratory cycle (Figure 6.2). For each measure of MSNA, a two-way mixed model ANOVA was used to test for a respiratory phase x group interaction.
For bust incidence, there was a significant interaction between group and respiratory phase (P=0.021 (Greenhouse-Geisser corrected), Table 6.9; Figure 6.5). There was a simple main effect of respiratory phase for every group, indicating that burst incidence was modulated by respiration in each group (premenopausal females P<0.0005; younger males P<0.0005; postmenopausal females P=0.031; with statistical test data in Table 6.9). However, when group differences in burst incidence were compared within each respiratory phase, a simple main effect of group was found for every respiratory phase except 50-60 % and 60-70 %, representing end-expiration and shortly afterwards (Table 6.9). Post-hoc analysis showed that burst incidence was higher in postmenopausal females versus premenopausal females at every respiratory phase except 30-40 %, 50-60 % and 60-70 %; and versus younger males at every respiratory phase except 50-60 % and 60-70 % (Appendix 2). These data suggest that the groups have a similar burst incidence around end-expiration (50 % of the respiratory cycle) but postmenopausal females have a higher burst incidence compared to the younger groups towards peak inspiration (0 % and 100 %).

The 10-phase burst incidence data violated some of the assumptions associated with two-way mixed model ANOVA; studentised residuals were >3 SD for one postmenopausal woman at phase 60-70 % and one premenopausal woman at phase 40-50 %; and studentised residuals of phase 60-70 % deviated from normal (Q-Q plot). To address this the data were transformed using a reciprocal function after addition of a constant of 100 (to prevent loss of datapoints equal to

zero bursts/100 heartbeats). After reciprocal transform, there were still outlying studentised residuals for two participants (the same postmenopausal woman and premenopausal woman). When the data of these two participants was removed, no studentised residuals were considered outliers. This transformed dataset also produced a significant respiratory phase x group interaction (P=0.013, Appendix 2) and there was a significant simple main effect of group at all respiratory phases except 50-60 % and 60-70 %, with significant pairwise comparisons between postmenopausal females and premenopausal females, and postmenopausal females and younger males, but none between premenopausal females and younger males (Appendix 2). Therefore, the transformed data showed similar results to the untransformed data. However, reciprocal transform and removal of two outlying datasets was unable to correct for deviation from normality at one respiratory phase (60-70 %, assessed by Q-Q plot). Therefore, the outcome of the two-way mixed model ANOVA of 10-phase burst incidence data should be interpreted with caution.

There was no significant respiratory phase x group interaction for burst frequency (P=0.098), total burst area/s (P=0.330), or mean burst area/s (P=0.220); Figure 6.5. There was a significant main effect of respiratory phase for all three of these measures of MSNA (Table 6.9). This indicates that when all participants were considered together, there was respiratory modulation of burst frequency and burst area. There were numerous significant pairwise comparisons between individual respiratory phases.

Additionally, there was a significant main effect of group for burst frequency (P<0.0005), total burst area/s (P=0.009), and mean burst area/s (P<0.0005), indicating that burst frequency and burst area differed between groups across both respiratory phases. Post-hoc analysis revealed that postmenopausal females showed a greater burst frequency and burst area versus premenopausal females (49 ± 8 versus 31 ± 8 bursts/min, P<0.0005; 767 ± 198 versus 525 ± 198 %, P=0.013; 11.7 ± 2.1 versus 7.8 ± 2.1 %, P<0.0005; for burst frequency, total burst area/s and mean burst area/s respectively). Burst frequency and mean burst area/s were greater in postmenopausal females versus younger males (31 ± 8 bursts/min, P<0.0005; 8.8 ± 2.1 %, P=0.006). Total burst area/s was not significantly greater in postmenopausal females versus younger males (567 ± 198, P=0.05). Neither burst frequency, nor burst area differed between premenopausal females and younger males (Bonferroni-corrected pairwise

comparisons *P*=1.0, *P*=1.0 and *P*=0.759 for burst frequency, total burst area/s and mean burst area/s respectively). Q-Q plots revealed that the studentised residuals of some respiratory phases appeared to deviate from normal for burst frequency, total burst area/s and mean burst area/s. Additionally, some studentised residuals for total burst area/s and mean burst area/s were considered outliers (>3 SD), and one respiratory phase in the total burst area/s dataset violated the assumption of equality of variance (Levene's test). Therefore, the 10-phase data presented here may not be suitable for a mixed-model ANOVA and the results should be interpreted with caution.

6.4.1.9 Traube-Hering waves and respiratory modulation of heart rate

The effect of respiration on blood pressure, heart rate and MSNA was also compared in postmenopausal females, premenopausal females, and younger males. Traube-Hering wave amplitude was smaller in postmenopausal females versus both premenopausal females (mean ranks 9.54 versus 22.58, Bonferroni-corrected pairwise comparisons *P*=0.008) and younger males (mean rank 25.67, *P*=0.001). Furthermore, respiratory-modulated heart rate was smaller in postmenopausal females versus both premenopausal females (mean ranks 7.92 versus 23.5, *P*=0.001) and younger males (mean rank 26.5, *P*<0.0005). Neither Traube-Hering wave amplitude, nor respiratory-modulated heart rate was different between premenopausal females and younger males (*P*=1.0 and *P*=1.0, respectively). Statistical test data are shown in Table 6.9. Mean respiratory cycle duration did not differ between groups (*P*=0.702, Figure 6.6). To correlate respiratory and haemodynamic variables a different measure of respiratory-modulated MSNA area was used (the modulus of the unshifted, integrated neurogram across 1.5 x the duration of inspiration, from the start of inspiration (Shantsila et al., 2015)). This measure of MSNA area did not differ significantly between the groups (*P*=0.543, Table 6.10).

6.4.1.10 Correlations between respiratory-modulated haemodynamic variables

To assess whether respiratory modulation of haemodynamic variables is altered in postmenopausal females versus younger adults, correlations between respiratory amplitude and haemodynamic variables were generated by using Spearman's rank correlation (Figure 6.7). Group differences in the strength of correlations were then assessed by comparing Spearman's rho. Spearman's rho

did not differ significantly between groups for the correlations between (1) respiratory amplitude and Traube-Hering wave amplitude (*P*=0.06), (2) respiratory amplitude and respiratory-modulated MSNA (*P*=0.067), or (3) respiratory-modulated MSNA and Traube-Hering wave amplitude (*P*=0.338); statistical test data in Table 6.10. However, the correlation between respiratory amplitude and heart rate was smaller in postmenopausal females versus both premenopausal females (0.25 ± 0.20 versus 0.51 ± 0.18, *P*=0.009) and younger males (0.47 ± 0.24, *P*=0.030). However, there was no difference in Spearman's rho between premenopausal females and younger males (*P*=0.889). As such, the correlation between respiratory wave amplitude and respiratory-modulated heart rate was on average weaker in postmenopausal females versus the younger groups.

The relationship between respiratory-modulated MSNA and Traube-Hering wave amplitude in the different groups was further interrogated by comparing the correlation coefficients between (1) respiratory-modulated MSNA and the Traube-Hering wave of the following respiratory cycle (Traube-Hering wave plus 1), and (2) respiratory-modulated MSNA and the Traube-Hering wave of the preceding respiratory cycle (Traube-Hering wave minus 1) (Shantsila et al., 2015). There were no group differences in Spearman's rho for the correlation between respiratory-modulated MSNA and Traube-Hering wave plus 1 (*P*=0.198), or for the correlation between respiratory-modulated MSNA and Traube-Hering wave minus 1 (*P*=0.531), with statistical test data in Table 6.11.

6.4.1.11 Summary of results for aim 1

Study 1 has shown that, firstly, respiratory sympathetic coupling occurs in healthy postmenopausal females as well as younger adults. Secondly, that respiratory modulation of burst firing (incidence and frequency) is reduced in postmenopausal females versus younger adults. Thirdly, that respiratory sympathetic coupling does not appear to differ between healthy premenopausal females and younger males. Additionally, respiratory modulation of heart rate appears to be reduced in postmenopausal females versus younger adults, but respiratory modulation of blood pressure (Traube-Hering waves) does not appear to differ between these groups.

6.4.2 Aim 2: Respiratory sympathetic coupling in hypertensive versus normotensive postmenopausal females and older males

6.4.2.1 Participant characteristics for postmenopausal females

Seven hypertensive postmenopausal females, seven normotensive postmenopausal females, eight hypertensive older males, and nine normotensive older males were included in the analysis. Due to the small sample sizes, hypertensive and normotensive groups were compared within each sex. Hypertensive postmenopausal females did not differ from normotensive postmenopausal females in age, height, weight, or BMI (Table 6.12). Systolic and diastolic daytime ambulatory blood pressure were higher in the hypertensive versus normotensive postmenopausal females (systolic 136 [10] versus 125 [10] mmHg, *P*=0.001; diastolic 86 [7] versus 73 [11], *P*=0.011; Table 6.12). Clinic systolic blood pressure was not different between hypertensive and normotensive females (*P*=0.165, Table 6.12) but diastolic clinic blood pressure was higher in the hypertensive group (90 ± 6 versus 78 ± 6 mmHg for hypertension versus normotension, *P*=0.013, Table 6.12). Heart rate associated with the clinic and ambulatory blood pressure readings did not differ between the groups of postmenopausal females (Table 6.12).

Of the seven hypertensive postmenopausal females, one was untreated, two were treated with controlled blood pressure, and four were treated with uncontrolled blood pressure (Table 6.13). Among the six treated postmenopausal females, data on anti-hypertensive medication are available for five participants; three reported taking one anti-hypertensive medication, one reported taking two anti-hypertensive medications, and one reported taking three anti-hypertensive medications. The anti-hypertensive medications taken by the treated participants are listed by drug class in Table 6.13.

6.4.2.2 Resting sympathetic nerve activity and haemodynamic variables in postmenopausal females

Haemodynamic data during the period of quiet rest in which respiratory sympathetic coupling was measured are shown in Table 6.16. MSNA did not differ between hypertensive and normotensive postmenopausal females when quantified as burst incidence (80 ± 13 versus 75 ± 11 bursts/100 heartbeats,

P=0.465) or burst frequency (48 ± 8 versus 45 ± 5 bursts/min, *P*=0.190). Similarly, mean burst latency did not differ between the groups (*P*=0.259). Additionally, mean systolic and diastolic blood pressure, pulse pressure, mean arterial pressure, and heart rate were similar between the groups (Table 6.16). There was no difference in overall sympathetic baroreflex sensitivity slope between the hypertensive and normotensive postmenopausal females (Table 6.16) and no group difference in any frequency or time domain measure of heart rate variability (Table 6.17).

6.4.2.3 Respiratory sympathetic modulation of burst firing in postmenopausal females

Respiratory sympathetic modulation of burst firing was similar in hypertensive and normotensive postmenopausal females. There was no respiratory phase x group interaction for either burst incidence or burst frequency (*P*=0.273 and *P*=0.328 respectively; statistical test data in Table 6.18, with data shown in Figure 6.8). Respiratory sympathetic modulation of burst firing was present in both hypertensive and normotensive postmenopausal females, with a significant main effect of respiratory phase observed for burst incidence (*P*=0.004) and burst frequency (*P*=0.003) (Table 6.18). Post-hoc analysis showed that burst incidence and frequency were reduced during inspiration/post-inspiration versus mid-late expiration (72 ± 16 versus 83 ± 10 bursts/100 heartbeats; 42 ± 8 versus 49 ± 7 bursts/min, across both groups). The reduction in MSNA with inspiration was similar in hypertensive and normotensive postmenopausal females, with absolute and percentage change in burst incidence and frequency similar between the groups (Figure 6.9; burst incidence -15 ± 13 versus -8 ± 12 bursts/100 heartbeats, *P*=0.273, and -18 ± 15 versus -10 ± 15 %, *P*=0.332; burst frequency -8 ± 7 versus -5 ± 7 bursts/min, *P*=0.328, and -17 ± 13 versus -9 ± 15 %, *P*=0.331; for hypertensive versus normotensive, statistical test data in Table 6.19). There was no significant main effect of group for either burst incidence (*P*=0.417) or burst frequency (*P*=0.190, Table 6.18), indicating that MSNA was similar between the groups. Overall, these data show respiratory modulation of burst firing that is similar between hypertensive and normotensive postmenopausal females.

6.4.2.4 Respiratory sympathetic modulation of burst area in postmenopausal females

Respiratory modulation of burst area was present in both groups of postmenopausal females but may be greater in hypertensive versus normotensive females (Figure 6.8). There was no significant respiratory phase x group interaction for either total burst area/s (*P*=0.140) or mean burst area/s (*P*=0.082, statistical test data shown in Table 6.18). However, there was a significant main effect of respiratory phase on total burst area/s (*P*<0.0005) and mean burst area/s (*P*<0.0005), with smaller burst area/s observed during inspiration/post-inspiration versus mid-late expiration (5752 ± 2248 versus 6560 ± 2700 % and 11.7 ± 2.3 versus 13.3 ± 2.3 %, for total burst area/s and mean burst area/s respectively, across both groups). The absolute reduction in burst area with inspiration did not differ between groups (*P*=0.097 and *P*=0.082 for total burst area/s and mean burst area/s respectively, Table 6.19; Figure 6.9). However, the percentage reduction in burst area/s was greater in hypertensive versus normotensive postmenopausal females (total burst area/s -18 ± 7 versus -6 ± 7 %, *P*=0.006; mean burst area/s -17 ± 7 versus -8 ± 7 %, *P*=0.040; Table 6.19). There was no main effect of group on either total burst area/s (*P*=0.421) or mean burst area/s (*P*=0.156), indicating that overall burst area was similar between the groups. Overall, these data show that respiratory modulation of burst area occurs in both hypertensive and normotensive postmenopausal females, but that hypertensive females may show greater inspiratory inhibition of burst area compared to normotensive females.

6.4.2.5 Respiratory sympathetic coupling across 10 respiratory phases in postmenopausal females

To further investigate the respiratory modulation of MSNA, data were considered in terms of 10 respiratory phases (Figure 6.12). In hypertensive and normotensive postmenopausal females, there was no significant interaction between group and respiratory phase for burst incidence (*P*=0.167), burst frequency (*P*=0.756), total burst area/s (*P*=0.363), or mean burst area/s (P=0.201; statistical test data in Table 6.20). There was a significant main effect of respiratory phase for burst incidence (*P*=0.029) and mean burst area/s (*P*=0.022). However, there was no significant main effect of respiratory phase for burst frequency (*P*=0.257) or total burst area/s (*P*=0.071). Therefore, the respiratory modulation of burst frequency and total burst area/s that was

observed across two respiratory phases was not observed when data were expressed at 10 respiratory phases. There was no significant main effect of group for any measure of MSNA (Table 6.20), suggesting that MSNA did not differ between the hypertensive and normotensive groups of postmenopausal females.

6.4.2.6 Traube-Hering waves and respiratory modulation of heart rate in postmenopausal females

Traube-Hering wave amplitude, respiratory-modulated heart rate, respiratory-modulated MSNA and respiratory cycle duration did not differ between hypertensive and normotensive postmenopausal females (Figure 6.14, statistical test data in Table 6.21).

6.4.2.7 Correlations between respiratory-modulated haemodynamic variables in postmenopausal females

Spearman's rho correlation coefficient for correlations between respiratory and haemodynamic variables did not differ between hypertensive and normotensive females (Figure 6.15, statistical test data in Table 6.22).

6.4.2.8 Participant characteristics in older males

Hypertensive older males were older than the normotensive older males (*P*=0.015, mean ranks 12.12 versus 6.22 for hypertensive versus normotensive), but did not differ in height, weight, or BMI (Table 6.14). Clinic systolic blood pressure was higher in the hypertensive versus normotensive males (156 ± 20 versus 120 ± 8 mmHg, *P*=0.003) but there was no group difference in clinic diastolic pressure (87 ± 12 versus 75 ± 6 mmHg, *P*=0.084) (clinic blood pressure data were available for 5/9 normotensive males). Heart rate during clinic and ambulatory blood pressure measurements were not different between hypertensive and normotensive older males (Table 6.14). Daytime ambulatory blood pressure for hypertensive older males (N=5/8) are shown in Table 6.14. Ambulatory blood pressure data were available for only two of nine normotensive older males, so a statistical comparison to hypertensive males was not done. Of the eight hypertensive older males, three were untreated, two were treated with controlled blood pressure, and three were treated with uncontrolled blood pressure (Table 6.15). Among the five treated older males, one reported taking one anti-hypertensive medication, three reported taking two anti-hypertensive

medications, and one reported taking three anti-hypertensive medications. The anti-hypertensive drug classes taken by participants are listed in Table 6.15.

6.4.2.9 Resting sympathetic nerve activity and haemodynamic variables in older males

In the older males, MSNA was higher in the hypertensive versus normotensive group (burst incidence mean ranks 12.0 versus 6.33 (hypertensive versus normotensive), *P*=0.021; burst frequency mean ranks 12.12 versus 6.22, P=0.015; Table 6.16). Mean MSNA burst latency was lower in the hypertensive versus normotensive group (1.24 ± 0.03 versus 1.32 ± 0.07 s, *P*=0.007). Among the male participants in which continuous blood pressure was measured by photoplethysmography (Finometer; N=8 hypertensive and N=5 normotensive males), systolic and diastolic blood pressure were higher in hypertensive versus normotensive males (systolic mean ranks 9.0 versus 3.8, *P*=0.019; diastolic 65 ± 11 versus 51 ± 4 mmHg, *P*=0.024). Pulse pressure and mean arterial pressure were also greater in hypertensive versus normotensive males (pulse pressure mean ranks 8.88 versus 4.00, *P*=0.030; mean arterial pressure, mean ranks 9.5 versus 3.0, *P*=0.002). Among the participants in which continuous blood pressure was measured by brachial artery pressure transducer (N=4 normotensive males), systolic, diastolic, pulse pressure and mean arterial blood pressure were 140 [35], 72 [14], 68 [22] and 95 [21] mmHg respectively. Heart rate and overall baroreflex sensitivity slope did not differ between hypertensive and normotensive older males (Table 6.16). Frequency domain measures of heart rate variability were not different between hypertensive and normotensive older males (Table 6.17). For time domain measures, normotensive older males had more RR intervals >50 ms versus hypertensive males (8.0 [27.0] versus 1.5 [5.0] %, *P*=0.015). However, there was no group difference in SDRR or RMSSD (Table 6.17).

6.4.2.10 Respiratory sympathetic modulation of burst firing in older males

Respiratory sympathetic modulation of burst firing appeared to be similar between hypertensive and normotensive older males (Figure 6.10). There was no significant respiratory phase x group interaction for burst incidence (*P*=0.319) or burst frequency (*P*=0.375; statistical test data in Table 6.18). There was a significant main effect of respiratory phase on burst incidence (*P*=0.011) and burst frequency (*P*=0.023), with MSNA reduced during inspiration/post-inspiration

versus mid-late expiration (75 ± 15 versus 84 ± 10 bursts/100 heartbeats; 44 ± 10 versus 49 ± 8 bursts/min, across both groups). Furthermore, absolute and percentage change in burst incidence and burst frequency did not differ between groups (Figure 6.11; burst incidence -6 ± 9 versus -12 ± 15 bursts/100 heartbeats, *P*=0.319, and -6.3 ± 11.5 versus -15.3 ± 17.7 %, *P*=0.375; burst frequency -4 ± 8 versus -8 ± 10 bursts/min, *P*=0.375, and mean ranks for percentage change 10.5 versus 7.67, *P*=0.277; for hypertensive versus normotensive). There was a significant main effect of group on burst frequency (*P*=0.006) but not burst incidence (*P*=0.197), indicating that, across the respiratory cycle, burst frequency was greater in hypertensive versus normotensive older males (52 ± 8 versus 40 ± 8 bursts/min). Overall, these data show that respiratory modulation of burst firing occurs similarly in hypertensive and normotensive older males, despite an overall greater burst frequency in hypertensive males.

Given that the hypertensive older males were significantly older than the normotensive older males, age was included in a further model analysing group differences in absolute and percentage changes in MSNA between mid-late expiration and inspiration/post-inspiration. Absolute change in burst incidence did not differ between hypertensive and normotensive males when age was accounted for (univariate ANCOVA F(1, 13) = 0.837, *P*=0.377, partial η2=0.060). However, the data violated the assumptions of normality (standardised residual for normotensive group deviated from normality (Shapiro-Wilk)) and linearity of relationship between dependent variable and covariate but met the other assumptions of univariate ANCOVA. Transforming the data did not produce a linear relationship between dependent variable and covariate, therefore the data are not suitable for analysis by ANCOVA.

6.4.2.11 Respiratory sympathetic modulation of burst area in older males

Respiratory modulation of burst area was also similar in hypertensive and normotensive older males (Figure 6.10). There was no significant respiratory phase x group interaction for either total burst area/s (*P*=0.331) or mean burst area/s (*P*=0.441; with statistical test data in Table 6.18). There was a significant main effect of respiratory phase for total burst area/s (*P*=0.019) and mean burst area/s (*P*=0.008), with smaller burst area seen during inspiration/post-inspiration versus mid-late expiration (4983 ± 2332 versus 5738 ± 2631 % and 10.8 ± 2.4 versus 12.7 ± 3.2 %, for total burst area/s and mean burst area/s respectively,

across both groups). However, absolute and percentage change in both total burst area/s and mean burst area/s did not differ between the groups (Figure 6.11; absolute change in total burst area/s -467 ± 663 versus -1042 ± 1486 %, P=0.316; percentage change in total burst area/s mean ranks 10.5 versus 7.67, P=0.277; absolute change in mean burst area/s mean ranks 9.88 versus 8.22. P=0.541; percentage change in mean burst area/s mean ranks 10.38 versus 7.78, P=0.321). There was a significant main effect of group for mean burst area/s (*P*=0.046) but not for total burst area/s (*P*=0.211), with a higher mean burst area/s seen in hypertensive versus normotensive males (13.0 ± 2.5 versus 10.4 ± 2.5 %). Overall, these data show that MSNA burst area is modulated by respiration similarly in hypertensive and normotensive older males, despite a larger overall burst area observed in hypertensive males.

6.4.2.12 Respiratory sympathetic modulation across 10 respiratory phases in older males

Respiratory modulation of MSNA was further investigated using 10 respiratory phases (Figure 6.13). In older males, there was no significant respiratory phase x group interaction for burst incidence (*P*=0.676), burst frequency (*P*=0.776), total burst area/s (*P*=0.776), or mean burst area/s (*P*=0.686; statistical test data in Table 6.20). There was a significant main effect of respiratory phase for all measures of MSNA (Table 6.20), thus the respiratory modulation of burst firing and burst area observed during two respiratory phases was still observed when the data were displayed as 10 respiratory phases. In agreement with the two-respiratory phase data, there was a significant main effect of group for burst frequency (*P*=0.006) and mean burst area/s (*P*=0.049), with larger burst frequency and mean burst area/s seen in hypertensive versus normotensive males (52 ± 8 versus 40 ± 8 bursts/min and 12.9 ± 2.4 versus 10.4 ± 2.4 %). However, there was no main effect of group for burst incidence (*P*=0.183) or total burst area/s (*P*=0.211).

6.4.2.13 Traube-Hering waves and respiratory modulation of heart rate in older males

Traube-Hering wave amplitude, respiratory modulation of heart rate, respiratory-modulated MSNA, and respiratory cycle duration did not differ between hypertensive and normotensive older males (Figure 6.16; statistical test data in Table 6.21).

6.4.2.14 Correlations between respiratory-modulated variables in older males

Spearman's rho correlation coefficient for correlations between respiratory and haemodynamic variables did not differ between hypertensive and normotensive older males (Figure 6.17; statistical test data in Table 6.22).

6.4.2.15 Summary of data for aim 2

Study 2 aimed to compare respiratory sympathetic coupling in hypertensive versus normotensive postmenopausal females, and separately in hypertensive versus normotensive older males. Overall, the data suggest, firstly, that hypertensive and normotensive postmenopausal females and older males all demonstrate respiratory sympathetic coupling, in terms of both burst firing and burst area. Secondly, it appears that respiratory sympathetic coupling is generally not affected by hypertension in either postmenopausal females, or older males. However, the percentage reduction in burst area between mid-late expiration and inspiration/post-inspiration appeared to be greater in hypertensive versus normotensive postmenopausal females. Thirdly, the data suggest that there is no effect of hypertension on respiratory modulation of either heart rate or mean arterial pressure (Traube-Hering waves).

6.4.3 The relationship between resting burst incidence, sex, age, hypertension, and respiratory sympathetic modulation

A final analysis of this chapter assessed whether respiratory modulation of burst incidence was related to resting burst incidence, when sex, age and hypertension were also included as covariates. With datasets 1 and 2 combined, respiratory modulation of burst incidence (percentage change between mid-late expiration and inspiration/post-inspiration) was predicted by the model (Figure 6.18; $F(4, 58)=12.374$, $P<0.0005$, $R=0.679$, $R^2=0.460$, adjusted $R^2=0.423$). Age was the only variable with a significant contribution to the model (B=0.427, 95 % confidence intervals 0.047 to 0.806, $P=0.028$). This indicates that every additional year was associated with a 0.429 % increase in percentage change in burst incidence between the two respiratory phases (as percentage change was negative for the vast majority of participants, an increase represents a smaller reduction in percentage burst incidence from mid-late expiration to inspiration/post-inspiration). The contribution of burst incidence to the model approached but did not reach significance (B=0.361, 95 % confidence intervals -

0.005 to 0.728, *P*=0.053). Sex and hypertension did not contribute significantly to the model (Table 6.23).

6.5 Discussion

6.5.1 Summary of results

This chapter aimed to investigate the roles of sex, age, and hypertension on respiratory modulation of sympathetic nerve activity. The main findings were 1) that respiratory sympathetic coupling was reduced in healthy postmenopausal females versus premenopausal females and younger males; 2) that respiratory sympathetic coupling did not differ between healthy premenopausal females and younger males; 3) that there was no effect of hypertension on respiratory sympathetic coupling in postmenopausal females and older males; and 4) that age was the strongest predictor of respiratory modulation of MSNA burst incidence among variables of age, sex, hypertension, and resting burst incidence.

6.5.2 Respiratory sympathetic coupling in healthy ageing

Given that sympathetic control of blood pressure is altered in healthy ageing (Narkiewicz et al., 2005), there may be age-related changes to respiratory sympathetic coupling. Shantsila et al. investigated this in healthy males and concluded that respiratory sympathetic coupling is similar in older and younger males, despite older males having an increased resting level of sympathetic activation (Shantsila et al., 2015). In contrast, the data presented in this chapter indicate that respiratory sympathetic coupling is reduced in healthy postmenopausal females versus premenopausal females. In agreement, postmenopausal females demonstrated a smaller average Traube-Hering wave amplitude compared to premenopausal females and younger males. There are several possible explanations for this observation. Firstly, there may be sex differences in age-related changes in lung function that could affect pulmonary afferent activity. Lung function decreases with age in older males and females (Thomas et al., 2019). Some longitudinal studies have demonstrated that ageing females show a greater rate of lung function decline versus ageing males, when normalised to initial lung function (Luoto et al., 2019). Similarly, postmenopausal females have a greater rate of decline in lung function versus premenopausal females (Triebner et al., 2017) and lung function in postmenopausal females is positively related to the length of time in which they were fertile (Lim et al., 2020). In contrast, Thomas et al., reported that across several studies, there was no overall difference in the rate of lung function decline between males and females

(Thomas et al., 2019), however a variety of methods were used across the studies evaluated. Overall, it appears that there is an effect of sex hormones on lung function. As such, the loss of female sex hormones in the postmenopausal females studied here may have affected lung function and reduced respiratory sympathetic coupling versus premenopausal females. This could explain why older males show similar levels of respiratory-sympathetic coupling to younger males (Shantsila et al., 2015), but that older females show less inhibition of SNA versus premenopausal females (as seen in the current data). Lim et al. found that early-onset menopause is associated with greater risk of restrictive but not obstructive lung disease (Lim et al., 2020). In restrictive lung disease, lung and chest wall compliance is decreased and a smaller lung volume is achieved for a given (negative) intrapleural pressure (Lutfi, 2017). Smaller lung volumes would be associated with reduced contribution of pulmonary stretch receptor activity to respiratory sympathetic coupling (Seals et al., 1990). Therefore, it is possible that menopause is associated with reduced pulmonary stretch receptor activity, and, through this mechanism, respiratory sympathetic coupling is reduced. Along these lines, the current data showed that MSNA burst incidence was similar in postmenopausal females and younger adults around end-expiration, but different around peak inspiration, when the pulmonary stretch receptors would be activated (10 respiratory phase data, Figure 6.5). However, there is no direct evidence to support this suggestion yet.

Alternatively, it is possible that there is a sex difference in the central mechanisms driving respiratory sympathetic coupling. Work in animal models has shown that chronic intermittent hypoxia has a sex-specific effect on respiration in rats. Females exhibit lengthening of early expiration following chronic intermittent hypoxia, whereas males show longer periods of late expiration (Souza et al., 2016). Souza et al. suggest that this could be due to sex differences in the neural networks regulating respiration, given that the behaviour of ex vivo respiratory neural networks (pre-Bötzinger complex) following a hypoxic stimulus also differs between male and female animals (Garcia et al., 2013). The data in this chapter show no sex difference in respiratory sympathetic coupling in younger adults, suggesting that any potential sex differences in neural networks may only become important in later life.

Finally, it is possible that the reduction in respiratory sympathetic coupling seen in postmenopausal females is related to changes in sympathetic nerve activity.

Resting levels of sympathetic nerve activity increase with healthy ageing in males and females (Keir et al., 2020) and Shantsila et al. hypothesised that the increased MSNA in older males would be associated with reduced respiratory coupling (Shantsila et al., 2015). In contrast, they found that respiratory coupling in older males was similar to younger males despite the greater resting MSNA in the older group. The average resting MSNA of the postmenopausal females in the current study was greater than that reported in the older males of Shantsila et al. (median 79 bursts/100 heartbeats in the current postmenopausal females, versus mean 42 bursts/100 heartbeats in the older males of Shanstila et al.'s study). Therefore, it is possible that the reduced respiratory coupling in postmenopausal females shown here is linked to their relatively high resting level of MSNA. This is unlikely to be characteristic of the wider population however, as resting MSNA is thought to be similar in males and females older than about 50 years (Keir et al., 2020). The differences in resting burst incidence may be a consequence of the subjective nature of marking bursts of MSNA. However, the current analysis was conducted blind to participant group, reducing the likelihood that the current results were subject to bias of this nature.

6.5.3 Respiratory sympathetic modulation of burst firing versus burst area

The current data showed that respiratory modulation of burst occurrence (i.e. whether a cardiac cycle is associated with a burst of MSNA or not) was reduced in postmenopausal females compared to younger adults, but burst area (i.e. how many sympathetic action potentials make up a burst and how large those action potentials are (Shoemaker, 2017)) was not. This is similar to Shantsila et al. who reported that MSNA total activity was not modulated by respiration differently in older versus younger males (Shantsila et al., 2015), although this method of quantifying burst area was not exactly the same as the method used in the current study. If valid, these data may raise questions about whether respiration modulates different populations of sympathetic action potentials differently. It has already been demonstrated that firing of larger amplitude sympathetic action potentials is controlled differently to the firing of smaller amplitude action potentials (Shoemaker, 2017). For example, larger amplitude action potentials which are normally silent at rest are active during sympathoexcitatory stimuli, such as apnoea (Badrov et al., 2016a). Furthermore, small-midsized action potentials appear to be more tightly controlled by the baroreflex than larger action potentials (Salmanpour and Shoemaker, 2012). Therefore, there is evidence that different action potential populations are controlled differently, and this control

may extend to respiratory modulation of sympathetic action potentials, although more work is needed to confirm this.

6.5.4 Respiratory sympathetic coupling in hypertension

The data presented in this chapter showed no difference in respiratory sympathetic modulation between hypertensive and normotensive postmenopausal females, or between hypertensive and normotensive older males. This agrees with previous studies who used a different method to quantify respiratory sympathetic modulation (waveform correlation), but similarly showed little difference in modulation between hypertensive patients and healthy older male and female adults (Fatouleh and Macefield, 2011). Fatouleh and Macefield included few female hypertensive participants and did not report menopausal status, so the influence of hypertension on respiratory sympathetic modulation in postmenopausal females could not be determined. However, the results of this chapter suggest that hypertension does not appear to influence respiratory modulation in postmenopausal females. Furthermore, multiple linear regression analysis demonstrated that age was the most important predictor of respiratory modulation of MSNA burst incidence when sex, hypertension status and resting burst incidence were also included as potential predictors.

The current data did show that hypertensive postmenopausal females showed a significantly larger percentage reduction in burst area with inspiration, compared to normotensive controls (Figure 6.9), although this was not shown in the raw data (either absolute change (Figure 6.9) or mixed-model ANOVA (Figure 6.8)).

6.5.5 Relationship between respiratory sympathetic modulation and age, sex, hypertension, and resting level of sympathetic activation

Goso et al. showed that in participants with heart failure, lower individual respiratory sympathetic modulation was associated with enhanced resting sympathetic activation (Goso et al., 2001). The current work aimed to extend this analysis to hypertensive patients, testing whether resting burst incidence was predictive of the degree of respiratory modulation of burst incidence when age, sex and hypertension status were also included as predictors. Age was the only significant predictor of respiratory modulation of burst incidence (P=0.028), with

resting burst incidence approaching but not reaching significance (*P*=0.053) as a predictor of respiratory modulation of burst incidence. Given that resting sympathetic activity increases with age (Keir et al., 2020), the near significance of resting burst incidence as a predictor in this model may be a function of the age/burst incidence relationship. Indeed, in the current data, resting burst incidence and age were highly correlated (R=0.82). However, they were not considered multicollinear, as variance inflation tolerance was >0.1 (Field, 2018). Goso et al.'s high and low respiratory sympathetic modulation groups were matched for age (Goso et al., 2001), therefore there may be some role of resting sympathetic activity in regulating the degree of respiratory sympathetic modulation in heart failure that is not present in hypertension. This may not be unexpected, given that heart failure is a more severe pathological state than hypertension, with additional factors that could influence respiratory sympathetic coupling like breathlessness (Schultz et al., 2015) and high peripheral chemoreflex activation (Ponikowski et al., 2001). Sex and hypertension status did not contribute to the prediction of respiratory modulation of burst incidence (Table 6.22). As such, it appears that age is the most important factor regulating respiratory sympathetic modulation among those tested. The current regression model included younger and older males, and therefore the effect of age on respiratory sympathetic modulation is in contrast to previous findings in healthy males (Shantsila et al., 2015). However, the current analysis did not compare these groups directly.

6.5.6 Limitations of this study

The studies presented here have several limitations. Firstly, no participants were characterised in terms of lung function. Whilst none reported current respiratory disease, no lung function tests were performed, so the presence of undiagnosed disease cannot be ruled out. Similarly, lung function testing may have provided insight into whether reduced respiratory sympathetic modulation in postmenopausal versus premenopausal females was related to poorer (non-pathological) lung function. Secondly, respiration was recorded by a pressure transducer belt placed around the abdomen, which cannot provide the information on lung volumes that other methods, e.g., spirometry, can. No measure of vital capacity was available, so it was not possible to normalise the ventilation during the analysis window to individual vital capacity. Furthermore, spirometry would have provided more detailed insight into the quality of individual

ventilation (e.g. faster, shallower breathing versus slower, deeper breathing) and allowed this to be controlled for between groups. Thirdly, all measurements were conducted during normal, quiet breathing, and as such no information on respiratory sympathetic modulation during increased pulmonary stretch (deliberate deeper breathing) was available.

The main limitation of the study addressing aim 2 however, is the heterogeneity of the hypertensive groups of participants. Given that some participants were untreated, some treated with controlled blood pressure, and some treated with uncontrolled blood pressure. Additionally, participants were not screened for sleep apnoea, which is a known predictor of hypertension and increased sympathetic nerve activity (Narkiewicz and Somers, 2003) (although none reported diagnosis).

6.5.7 Future work

Initial work would directly compare respiratory sympathetic modulation in the healthy younger and older males of the current cohort to address whether reduced respiratory modulation is indeed specific to postmenopausal females. Further work could then focus on understanding the mechanisms by which respiratory coupling is reduced in older females (and possibly males). Studies could compare the effect of controlled deep breathing on respiratory sympathetic modulation in older males and females to determine whether pulmonary stretch receptor activation influences respiratory sympathetic modulation differently by sex.

6.6 Tables and Figures

6.6.1 Tables and figures for Aim 1: respiratory sympathetic coupling in healthy postmenopausal females, premenopausal females, and younger males

Table 6.1 Studies contributing data to chapter 6.

Study short name	Ethical approval board	Number of participants
Hypertension, Brain Blood Flow and Nerve Activity.	NHS REC 11/SW/0207	25
Carotid Body Removal for the Treatment of Resistant Hypertension	NHS REC 12/SW/0277	1
Mayo Clinic study	Mayo Clinic Institutional Review Board	41

NHS REC; National Health Service Research Ethics Committee.

Table 6.2 Participant characteristics in healthy postmenopausal females, premenopausal females, and younger males.

	YF	YM	PMF	*P* value	Pairwise comparisons	
N	12	12	13			
Age (years)	25 [5]	26 [7]	55 [9]	***<0.0005***	PMF vs. YF	***<0.0005***
					PMF vs. YM	***<0.0005***
					YF vs. YM	1.0
Height (m)	1.67	1.78	1.67	***<0.0005***	PMF vs. YF	1.0
	[0.09]	[0.07]	[0.10]		PMF vs. YM	***0.001***
	(12.82)	(24.79)	(9.94)		YF vs. YM	***0.007***
Weight (kg)	65 [11]	78 [12]	67 [11]	***0.006***	PMF vs. YF	1.0
	(13.00)	(23.33)	(11.67)		PMF vs. YM	***0.014***
					YF vs. YM	***0.025***
BMI (kg/m^2)	23.7 ±	24.7 ±	24.2 ±	0.317	PMF vs. YF	-
	1.0	2.2	2.5		PMF vs. YM	-
					YF vs. YM	-
Plasma	152	167	278	***0.008***	PMF vs. YF	***0.016***
noradrenaline	[115]	[141]	[145]		PMF vs. YM	***0.029***
(units)	(14.50)	(15.33)	(26.54)		YF vs. YM	1.0

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, N; sample size, BMI; body mass index. Group differences in means (BMI) were tested for by one-way ANOVA, whilst group differences in medians (age) and mean ranks (height, weight, plasma noradrenaline) were tested for by Kruskal-Wallis test. Where significant group differences were found, *P* values for Bonferroni-corrected pairwise comparisons are shown. Data are presented as either mean ± SD or median [interquartile range], with (mean rank) where appropriate.

Table 6.3 Resting sympathetic nerve activity and haemodynamic data in healthy postmenopausal females, premenopausal females, and younger males.

	YF	YM	PMF	*P* value	Pairwise comparisons	
Burst incidence (bursts/100 HB)	53 [13] (13.33)	51 [11] (12.92)	79 [17] (29.85)	***<0.0005***	PMF vs. YF PMF vs. YM YF vs. YM	***<0.0005*** ***<0.0005*** 1.0
Burst frequency (bursts/min)	33 [10] (14.42)	29 [6] (11.67)	52 [13] (30.00)	***<0.0005***	PMF vs. YF PMF vs. YM YF vs. YM	***0.001*** ***<0.0005*** 1.0
Heart rate (beats/min)	62 ± 10	58 ± 9	64 ± 7	0.247	PMF vs. YF PMF vs. YM YF vs. YM	- - -
Systolic BP (mmHg)	136 [17] (20.17)	125 [8] (11.08)	146 [21] (25.23)	***0.004***	PMF vs. YF PMF vs. YM YF vs. YM	0.728 ***0.003*** 0.119
Diastolic BP (mmHg)	78 [13] (21.75)	69 [9] (15.67)	72 [13] (19.54)	0.378	PMF vs. YF PMF vs. YM YF vs. YM	- - -
Pulse pressure (mmHg)	59 ± 7	52 ± 5	71 ± 16	***0.001***	PMF vs. YF PMF vs. YM YF vs. YM	0.050 ***0.002*** ***0.023***
Mean arterial pressure (mmHg)	98 [12] (21.58)	88 [8] (13.00)	97 [14] (22.15)	0.065	PMF vs. YF PMF vs. YM YF vs. YM	- - -
Breathing rate (breaths/min)	14 [2] (20.58)	13 [6] (17.92)	13 [5] (18.54)	0.818	PMF vs. YF PMF vs. YM YF vs. YM	- - -
Overall sBRS (%/mmHg)	-5.3 [1.9] (18.67)	-6.6 [4.7] (13.83)	-3.4 [3.8] (24.08)	0.061	PMF vs. YF PMF vs. YM YF vs. YM	- - -

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, N; sample size, BP; blood pressure, HB; heartbeats, sBRS; sympathetic baroreflex sensitivity. Data are presented as either mean ± SD or median [interquartile range]. Group differences in means (heart rate, pulse pressure) were tested for by one-way ANOVA, whilst group differences in mean ranks (burst incidence, burst frequency, systolic BP, diastolic BP, mean arterial

pressure, overall sympathetic baroreflex sensitivity) were tested for by Kruskal-Wallis test. Where significant group differences were found, *P* values for Bonferroni-corrected pairwise comparisons are shown.

Table 6.4 Frequency and time domain analyses of heart rate variability in healthy postmenopausal females, premenopausal females, and younger males.

	YF	YM	PMF	*P* value	Pairwise comparisons	
LF/HF ratio	0.57 [1.31] (16.42)	0.68 [0.98] (17.08)	1.19 [1.47] (23.15)	0.226	PMF vs. YF PMF vs. YM YF vs. YM	- - -
LF (nu)	42.3 ± 18.9	41.7 ± 20.8	52.6 ± 17.4	0.284	PMF vs. YF PMF vs. YM YF vs. YM	- - -
HF (nu)	55.1 ± 17.4	53.4 ± 18.9	42.7 ± 14.5	0.150	PMF vs. YF PMF vs. YM YF vs. YM	- - -
LF (ms^2)	1183 [1971] (22.25)	1771 [2492] (26.67)	219 [230] (8.92)	***<0.0005***	PMF vs. YF PMF vs. YM YF vs. YM	***0.006*** ***<0.0005*** 0.953
HF (ms^2)	1671 [3906] (22.33)	2191 [10175] (26.17)	132 [208] (9.31)	***<0.0005***	PMF vs. YF PMF vs. YM YF vs. YM	***0.008*** ***<0.0005*** 1.0
LF (%)	28.8 ± 12.1	30.3 ± 13.8	25.7 ± 9.2	0.613	PMF vs. YF PMF vs. YM YF vs. YM	- - -
HF (%)	38.9 ± 16.5	41.9 ± 18.8	25.3 ± 17.0	0.052	PMF vs. YF PMF vs. YM YF vs. YM	- - -
SDRR (ms)	67.3 [40.7] (21.92)	80.2 [71.7] (25.50)	35.2 [20.7] (10.31)	***0.001***	PMF vs. YF PMF vs. YM YF vs. YM	***0.022*** ***0.001*** 1.0
RMSSD (ms)	66.5 [67.7] (22.00)	70.1 [129.5] (25.67)	20.9 [23.3] (10.08)	***0.001***	PMF vs. YF PMF vs. YM YF vs. YM	***0.018*** ***0.001*** 1.0
pRR50 (%)	44.3 [55.0] (22.08)	56.2 [54.0] (26.50)	2.2 [5.0] (9.23)	***<0.0005***	PMF vs. YF PMF vs. YM YF vs. YM	***0.009*** ***<0.0005*** 0.953

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, LF/HF ratio; ratio of high to low frequency, LF; low frequency domain, HF; high frequency domain, SDRR; standard deviation of RR intervals, RMSSD;

root mean square of RR intervals, pRR50; RR intervals longer than 50 ms as a percentage of all RR intervals. Data are presented as either mean ± SD or median [interquartile range]. Group differences in means (LF and HF (nu), LF and HF (%)) were tested for by one-way ANOVA, whilst group differences in mean ranks (LF/HR ratio, LF and HF (ms^2), SDRR, RMSSD, pRR50) were tested for by Kruskal-Wallis test. Where significant group differences were found, *P* values for Bonferroni-corrected pairwise comparisons are shown.

Table 6.5 Statistical test data for group differences in participant characteristics, resting haemodynamic variables, and heart rate variability.

Variable	Test statistic	*P* value	Effect size
Age	$\chi^2(2) = 18.959$	***<0.0005***	-
YF vs. YM	z=-0.201	1.0	0.042
YF vs. PMF	z=-3.902	***<0.0005***	0.873
YM vs. PMF	z=-3.787	***<0.0005***	0.826
Height	$\chi^2(2) = 15.522$	***<0.0005***	-
YF vs. YM	-3.063	***0.007***	0.639
YF vs. PMF	0.683	1.0	0.153
YM vs. PMF	3.596	***0.001***	0.785
Weight	$\chi^2(2) = 10.297$	***0.006***	-
YF vs. YM	z=-2.640	***0.025***	0.550
YF vs. PMF	z=0.316	1.0	0.071
YM vs. PMF	z=2.822	***0.014***	0.616
BMI	F(2, 15.582) = 1.239	0.317 (Welch)	0.058
Plasma noradrenaline	$\chi^2(2) = 9.757$	***0.008***	-
YF vs. YM	z=-0.189	1.0	0.039
YF vs. PMF	z=-2.778	***0.016***	0.556
YM vs. PMF	z=-2.586	***0.029***	0.517
Burst incidence	$\chi^2(2) = 20.131$	***<0.0005***	-
YF vs. YM	z=0.094	1.0	0.019
YF vs. PMF	z=-3.811	***<0.0005***	0.762
YM vs. PMF	z=-3.907	***<0.0005***	0.781
Burst frequency	$\chi^2(2) = 21.09$	***<0.0005***	-
YF vs. YM	z=0.622	1.0	0.127
YF vs. PMF	z=-3.597	***<0.0005***	0.719
YM vs. PMF	z=-4.231	***0.001***	0.846
Heart rate	F(2, 34) = 1.458	0.247	0.079
Systolic BP	$\chi^2(2) = 10.866$	***0.004***	-
YF vs. YM	z=2.056	0.119	0.420
YF vs. PMF	z=-1.169	***0.003***	0.234
YM vs. PMF	z=-3.265	0.728	0.653
Diastolic BP	$\chi^2(2) = 1.945$	0.378	-
Pulse pressure	F(2, 21.118) = 10.545	***0.001 (Welch)***	0.389
YF vs. YM		***0.023***	
YF vs. PMF		0.050	
YM vs. PMF		***0.002***	
Mean arterial pressure	$\chi^2(2) = 5.474$	0.065	-

Breathing rate	$\chi^2(2) = 0.401$	0.818	-
Overall sBRS	$\chi^2(2) = 5.605$	0.061	-
LF/HF ratio	$\chi^2(2) = 2.974$	0.226	-
LF (nu)	$F(2, 34) = 1.306$	0.284	0.071
HF (nu)	$F(2, 34) = 2.010$	0.150	0.106
LF (ms^2)	$\chi^2(2) = 18.368$	***<0.0005***	-
YF vs. YM	z=-0.999	0.953	0.204
YF vs. PMF	z=3.076	***0.006***	0.615
YM vs. PMF	z=4.095	***<0.0005***	0.819
HF (ms^2)	$\chi^2(2) = 16.821$	***<0.0005***	-
YF vs. YM	z=-0.867	1.0	0.177
YF vs. PMF	z=3.006	***0.008***	0.601
YM vs. PMF	z=3.891	***<0.0005***	0.778
LF (%)	$F(2, 34) = 0.497$	0.613	0.028
HF (%)	$F(2, 34) = 3.239$	0.052	0.160
SDRR	$\chi^2(2) = 13.582$	***0.001***	-
YF vs. YM	z=-0.811	1.0	0.166
YF vs. PMF	z=2.679	***0.022***	0.536
YM vs. PMF	z=3.506	***0.001***	0.701
RMSSD	$\chi^2(2) = 14.308$	***0.001***	-
YF vs. YM	z=-0.830	1.0	0.169
YF vs. PMF	z=2.752	***0.018***	0.550
YM vs. PMF	z=3.598	***0.001***	0.720
pRR50	$\chi^2(2) = 17.324$	***<0.0005***	-
YF vs. YM	z=-0.999	0.953	0.204
YF vs. PMF	z=2.966	***0.009***	0.593
YM vs. PMF	z=3.985	***<0.0005***	0.797

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, BMI; body mass index, BP; blood pressure, sBRS; spontaneous baroreflex sensitivity, LF; low frequency, HF; high frequency, SDRR; standard deviation of RR intervals, RMSSD; root mean square of RR intervals, pRR50; RR intervals longer than 50 ms as a percentage of all RR intervals, F; ANOVA test statistic, χ^2; Kruskal-Wallis test statistic, z; standardised Kruskal-Wallis test statistic. Group differences were tested by one-way ANOVA or Kruskal-Wallis test. ANOVA effect size is partial η^2. Effect size for Kruskal-Wallis was determined for pairwise comparisons only following a significant effect of group (calculated as z/(square root of N)). N=12 YF, N=12 YM, N=13 PMF.

Table 6.6 Statistical test data for respiratory sympathetic coupling of burst firing during mid-late expiration and inspiration/post-inspiration.

Variable	Test statistic	*P* value	Partial η^2
Burst incidence			
Interaction	F(2, 34)=6.38	***0.004***	0.273
Simple main effect phase:			
Expiration vs. inspiration	YF F(1, 11)=36.25	***<0.0005***	0.767
	YM F(1, 11)=64.84	***<0.0005***	0.855
	PMF F(1, 12)=17.75	***0.001***	0.597
Simple main effect group:			
Expiration	Overall F(2, 34)=8.11	***0.001***	0.323
	YF vs. YM	0.841	
	YF vs. PMF	***0.002***	
	YM vs. PMF	***0.009***	
Inspiration	Overall F(2, 34)=38.62	***<0.0005***	0.694
	YF vs. YM	0.938	
	YF vs. PMF	***<0.0005***	
	YM vs. PMF	***<0.0005***	
Burst frequency			
Interaction	F(2, 34)=3.93	***0.029***	0.188
Simple main effect phase:			
Expiration vs. inspiration	YF F(1, 11)=34.65	***<0.0005***	0.759
	YM F(1, 11)=48.03	***<0.0005***	0.814
	PMF F(1, 12)=11.49	***0.005***	0.489
Simple main effect group:			
Expiration	Overall F(2, 34)=11.87	***<0.0005***	0.680
	YF vs. YM	1.0	
	YF vs. PMF	***0.001***	
	YM vs. PMF	***0.001***	
Inspiration	Overall F(2, 34)=36.12	***<0.0005***	0.411
	YF vs. YM	0.983	
	YF vs. PMF	***<0.0005***	
	YM vs. PMF	***<0.0005***	

YF; premenopausal females, YF; younger males, PMF; postmenopausal females, F; ANOVA test statistic. Two-way mixed model ANOVA. Simple main effect of phase tested by univariate repeated measures ANOVA in each group separately. Simple main effect of group tested by univariate ANOVA during each phase separately, with Tukey pairwise comparisons.

Table 6.7 Statistical test data for respiratory sympathetic coupling of burst area during mid-late expiration and inspiration/post-inspiration.

Variable	Test statistic	*P* value	Partial η^2
Total burst area			
Interaction	F(2, 34) = 0.465	0.632	0.027
Phase	F(1, 34) = 39.959	***<0.0005***	0.540
Group	F(2, 34) = 0.465	***0.009***	0.240
	YF vs. YM	1.0	
	YF vs. PMF	***0.013***	
	YM vs. PMF	0.050	
Mean burst area			
Interaction	F(2, 34) = 0.700	0.503	0.040
Phase	F(1, 34) = 36.109	***<0.0005***	0.535
Group	F(2, 34) = 11.259	***<0.0005***	0.398
	YF vs. YM	0.748	
	YF vs. PMF	***<0.0005***	
	YM vs. PMF	***0.006***	

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, F; ANOVA test statistic. Two-way mixed model ANOVA.

Table 6.8 Statistical test data for absolute and percentage change in MSNA from mid-late expiration to inspiration/post-inspiration.

Variable	Test statistic	*P* value	Effect size
Δ Burst incidence	$\chi^2(2) = 12.285$	***0.002***	-
YF vs. YM	z=0.509	1.0	0.104
YF vs. PMF	z=-2.723	***0.019***	0.545
YM vs. PMF	z=-3.243	***0.004***	0.649
%Δ Burst incidence	$\chi^2(2) = 15.875$	***<0.0005***	-
YF vs. YM	z=0.245	1.0	0.050
YF vs. PMF	z=-3.296	***0.003***	0.659
YM vs. PMF	z=-3.546	***0.001***	0.709
Δ Burst frequency	$\chi^2(2) = 7.161$	***0.028***	-
YF vs. YM	z=0.434	1.0	0.089
YF vs. PMF	z=-2.050	0.121	0.410
YM vs. PMF	z=-2.493	***0.038***	0.499
%Δ Burst frequency	F(2, 34) = 10.893	**<0.0005**	0.391
YF vs. YM		0.951	
YF vs. PMF		***0.001***	
YM vs. PMF		***0.001***	
Δ Total burst area/s	$\chi^2(2) = 1.484$	0.476	
%Δ Total burst area/s	$\chi^2(2) = 4.731$	0.094	
Δ Mean burst area/s	$\chi^2(2) = 1.395$	0.498	
%Δ Mean burst area/s	$\chi^2(2) = 4.604$	0.100	

Δ; absolute change, %Δ; percentage change, YF; premenopausal females, YM; younger males, PMF; postmenopausal females, F; ANOVA test statistic, χ2; Kruskal-Wallis test statistic, z; Kruskal-Wallis standardised test statistic. Group differences tested by one-way ANOVA or Kruskal-Wallis test. ANOVA effect size is partial η^2. Effect size for Kruskal-Wallis was done for pairwise comparisons only, following a significant effect of group, calculated as z/(square root N).

Table 6.9 Statistical test data for respiratory sympathetic coupling across 10 respiratory phases.

Variable	Test statistic	*P* value	Partial η^2
Burst incidence			
Interaction	F(10.42, 117.141) = 2.153	***0.021*** (GG)	0.430
Burst frequency			
Interaction	F(7.562, 128.551) = 1.746	0.098 (GG)	0.093
Phase	F(3.781, 128.551) = 24.819	***<0.0005*** (GG)	0.422
Group	F(2, 34) = 24.840	***<0.0005***	0.594
Total burst area/s			
Interaction	F(7.964, 135.387) = 1.157	0.330 (GG)	0.064
Phase	F(3.982, 135.387) = 18.509	***<0.0005*** (GG)	0.352
Group	F(2, 34) = 5.372	***0.009***	0.240
Mean burst area/s			
Interaction	F(8.462, 143.856) = 1.351	0.220 (GG)	0.074
Phase	F(4.231, 143.856) = 21.312	***<0.0005*** (GG)	0.385
Group	F(2, 34) = 11.366	***<0.0005***	0.400

F; ANOVA test statistic, GG; Greenhouse-Geisser correction.

Table 6.10 Statistical test data for group differences in respiratory-modulated haemodynamic variables.

Variable	Test statistic	*P* value
Traube-Hering wave amplitude	$\chi^2(2) = 15.8$	***<0.0005***
Respiratory modulation of heart rate	$\chi^2(2) = 21.449$	***<0.0005***
Respiratory modulated MSNA amplitude	$\chi^2(2) = 1.221$	0.543
Respiratory cycle duration	$\chi^2(2) = 0.707$	0.702

X^2; Kruskal-Wallis test statistic.

Table 6.11 Group differences in correlation coefficient.

Variable	Test statistic	*P* value
Respiratory trace amplitude vs. Traube-Hering wave amplitude	$\chi^2(2) = 5.623$	0.060
Respiratory trace amplitude vs. respiratory-modulated heart rate	$F(2, 34) = 5.847$	***0.007***
Respiratory trace amplitude vs. respiratory-modulated MSNA	$F(2, 34) = 2.925$	0.067
Respiratory-modulated MSNA vs. Traube-Hering wave amplitude	$\chi^2(2) = 2.169$	0.338
Respiratory-modulated MSNA vs. Traube-Hering wave +1	$\chi^2(2) = 3.234$	0.198
Traube-Hering wave -1 vs. respiratory-modulated MSNA	$\chi^2(2) = 1.265$	0.531

F; ANOVA test statistic, χ^2; Kruskal-Wallis test statistic, MSNA; muscle sympathetic nerve activity.

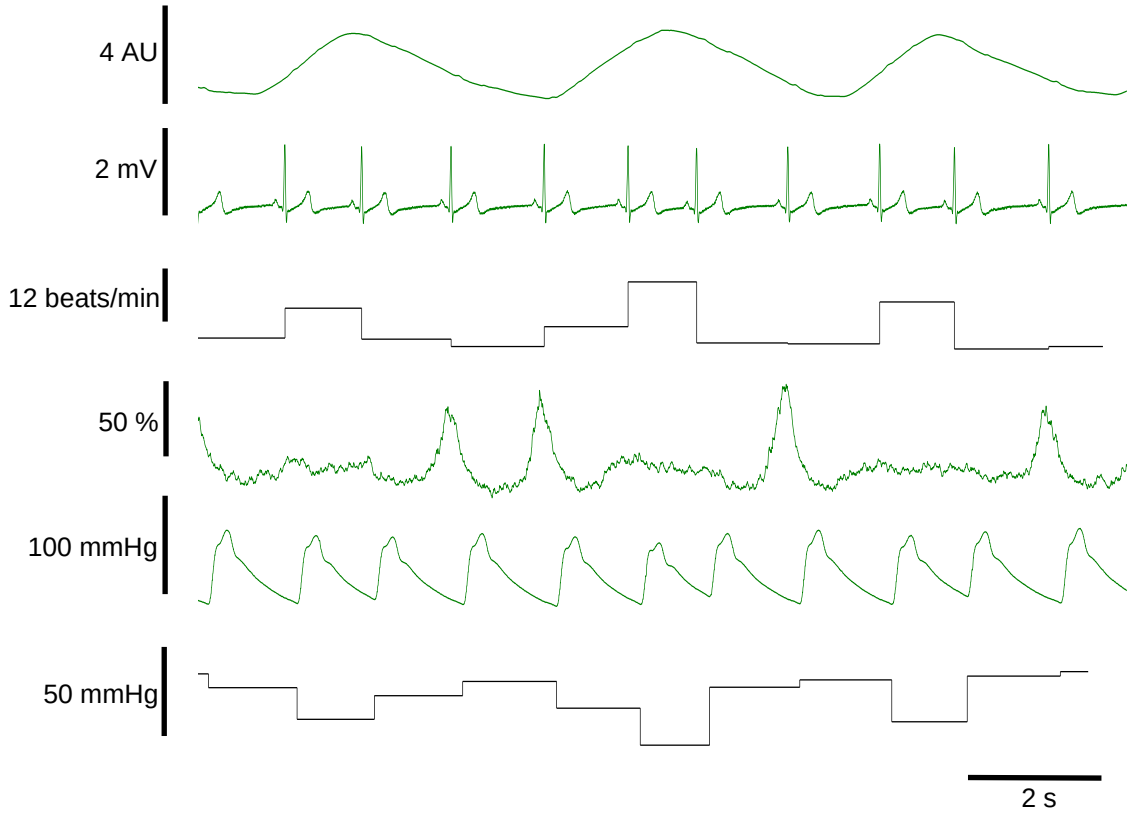

Figure 6.1 Example recording from one healthy premenopausal female participant.

From top: respiratory trace, ECG, heart rate, integrated MSNA neurogram normalised to tallest burst and shifted by mean latency, arterial blood pressure, beat to beat mean arterial pressure (integrated blood pressure waveform between consecutive diastolic blood pressures).

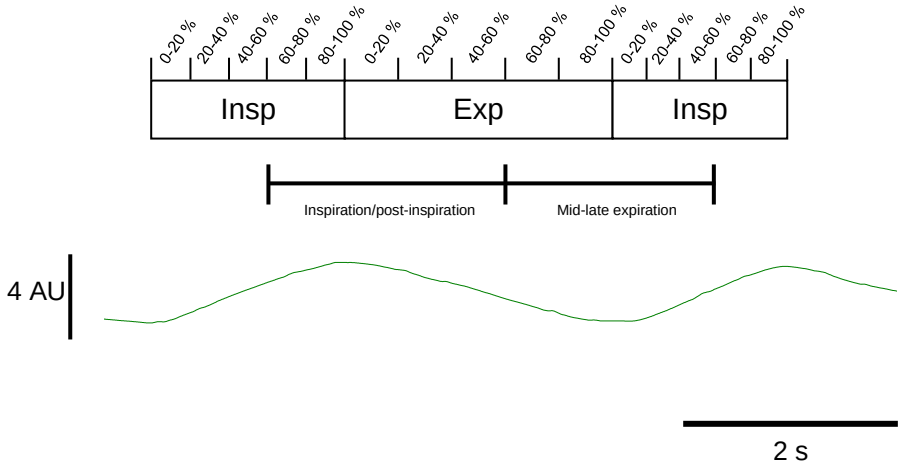

Figure 6.2 Example respiratory trace in one premenopausal female participant, with 20 % phases and mid-late expiration and inspiration/post-inspiration regions identified. Insp; inspiration, exp; expiration.

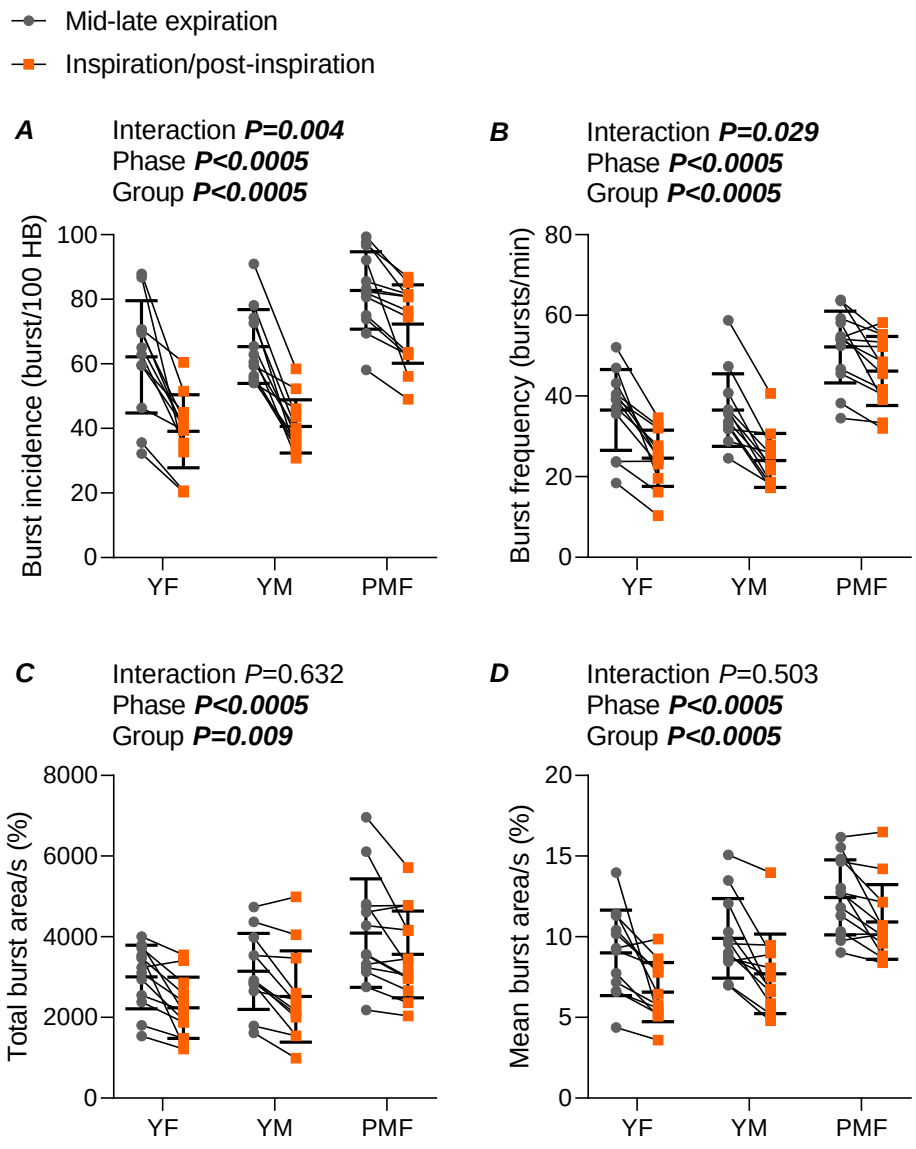

Figure 6.3 Respiratory modulation of burst firing and burst area in healthy premenopausal females, younger males and postmenopausal females. (A) burst incidence, (B) burst frequency, (C) total burst area/s, and (D) mean burst area/s across two respiratory phases in healthy postmenopausal females (N=13), premenopausal females (N=12), and younger males (N=12). YF; premenopausal females, YM; younger males, PMF; postmenopausal females, HB; heartbeats. Data are expressed as mean ± SD. A phase x group interaction was tested by two-way mixed model ANOVA.

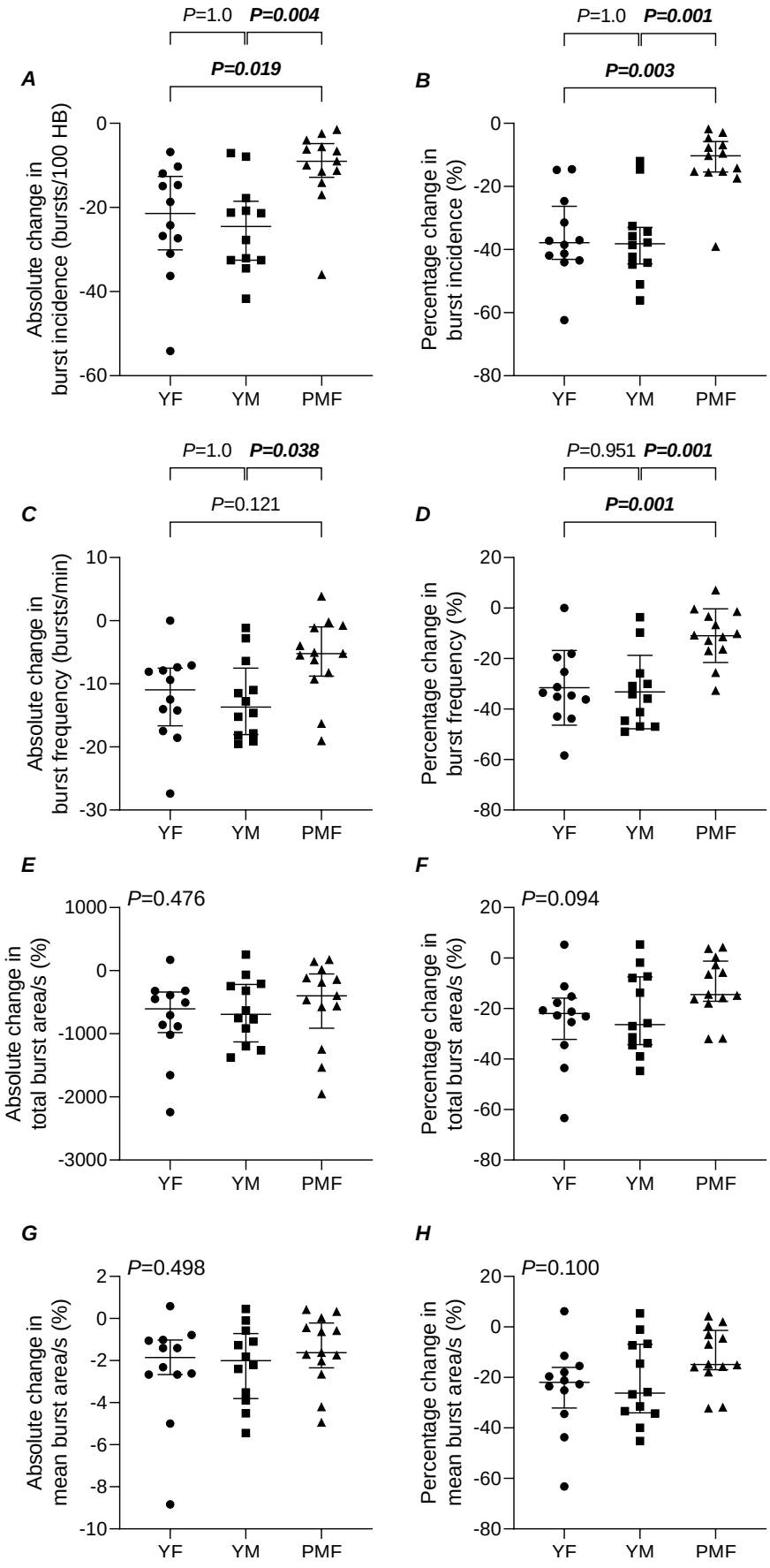

Figure 6.4 Absolute and percentage change in MSNA (A-B) burst incidence,

(C-D) burst frequency, (E-F) total burst area/s, and (G-H) mean burst area/s between mid-late expiration and inspiration/post-inspiration in healthy postmenopausal females, premenopausal females and younger males. PMF; postmenopausal females, YF; premenopausal females, YM; younger males; HB; heartbeats. Data are median ± interquartile range (A-C and E-H) or mean ± SD (D). Group differences in absolute and percentage change were tested by Kruskal-Wallis test (A-C and E-H) or one-way ANOVA (D).

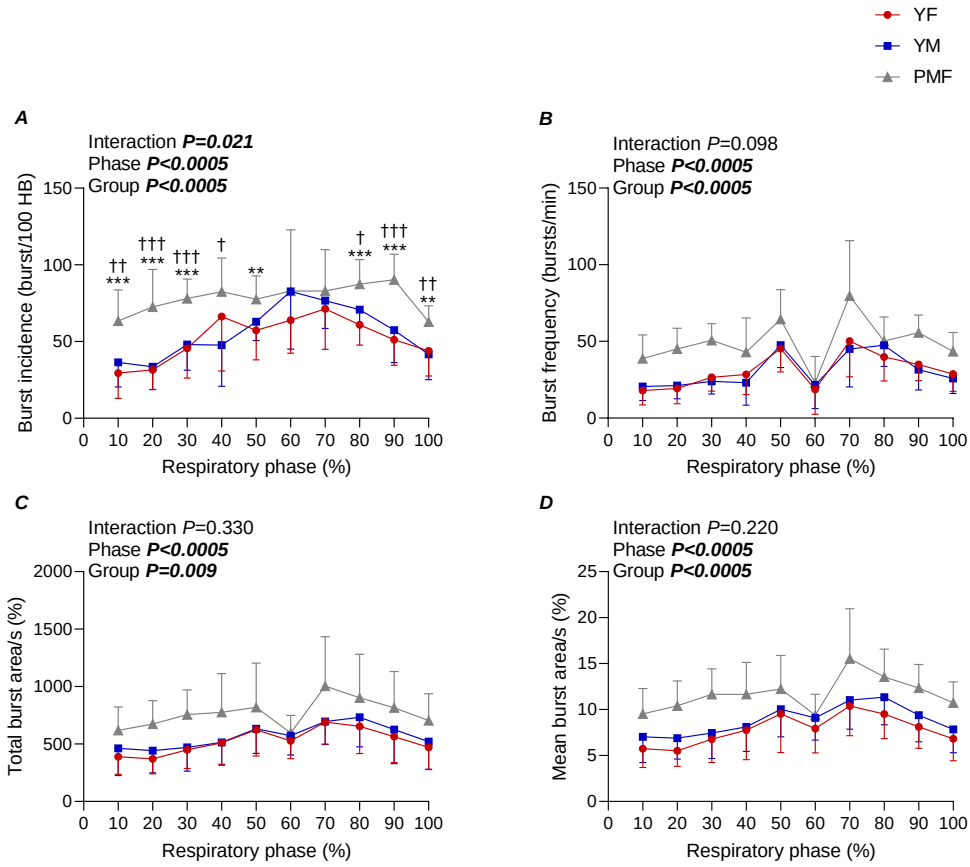

Figure 6.5 Respiratory modulation of (A) burst incidence, (B) burst frequency, (C) total burst area/s and (D) mean burst area/s across 10 percentage phases of the respiratory cycle in healthy postmenopausal females (N=13), premenopausal females (N=12) and younger males (N=12). YF; premenopausal females, YM; younger males, PMF; postmenopausal females, HB; heartbeats. Data are expressed as mean ± SD. A phase x group interaction was tested by two-way mixed model ANOVA. *P<0.05, **P<0.01, ***P<0.001 premenopausal females versus postmenopausal females; †P<0.05,

††P<0.01, †††P<0.001 younger males versus postmenopausal females (Tukey post-hoc test for pairwise comparison of groups at each respiratory phase following a significant simple main effect of group).

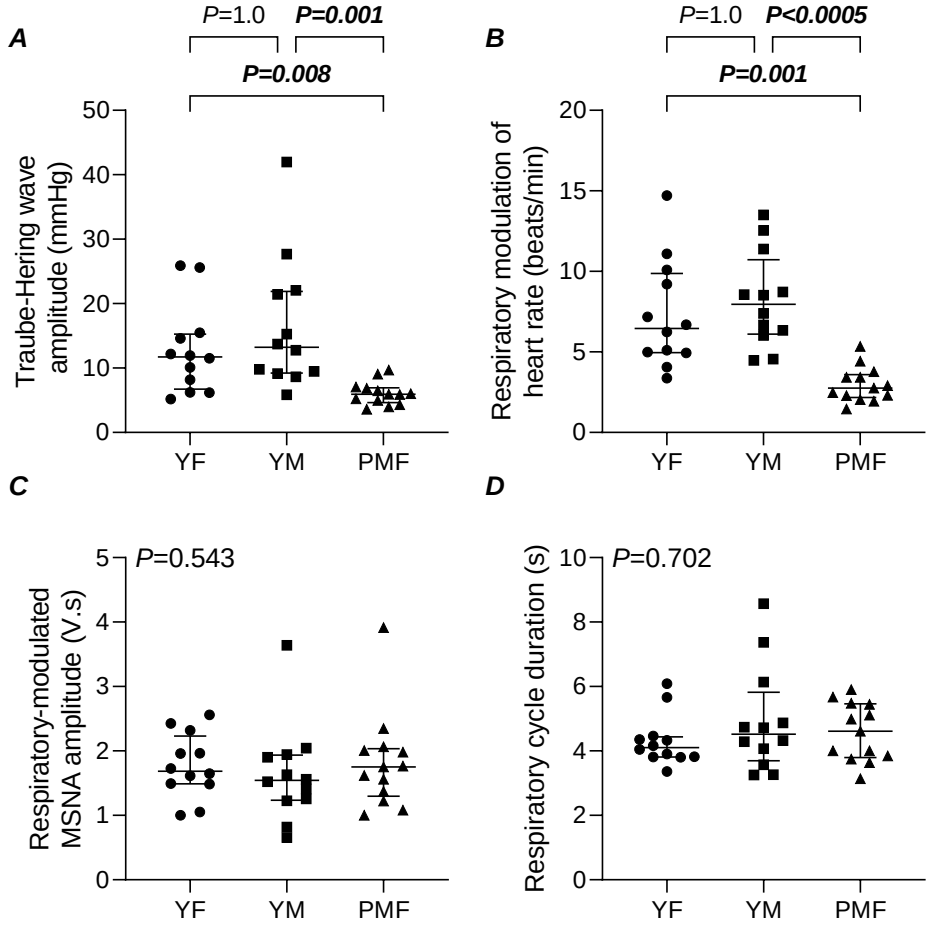

Figure 6.6 Group differences in (A) Traube-Hering wave amplitude, (B) respiratory-modulated heart rate, (C) respiratory-modulated MSNA, and (D) respiratory cycle duration in healthy postmenopausal females (N=13) and premenopausal females (N=12) and males (N=12). YF; premenopausal females, YM; younger males, PMF; postmenopausal females, MSNA; muscle sympathetic nerve activity. Data are median ± interquartile range. Group differences tested by Kruskal-Wallis test and Bonferroni-corrected pairwise comparisons.

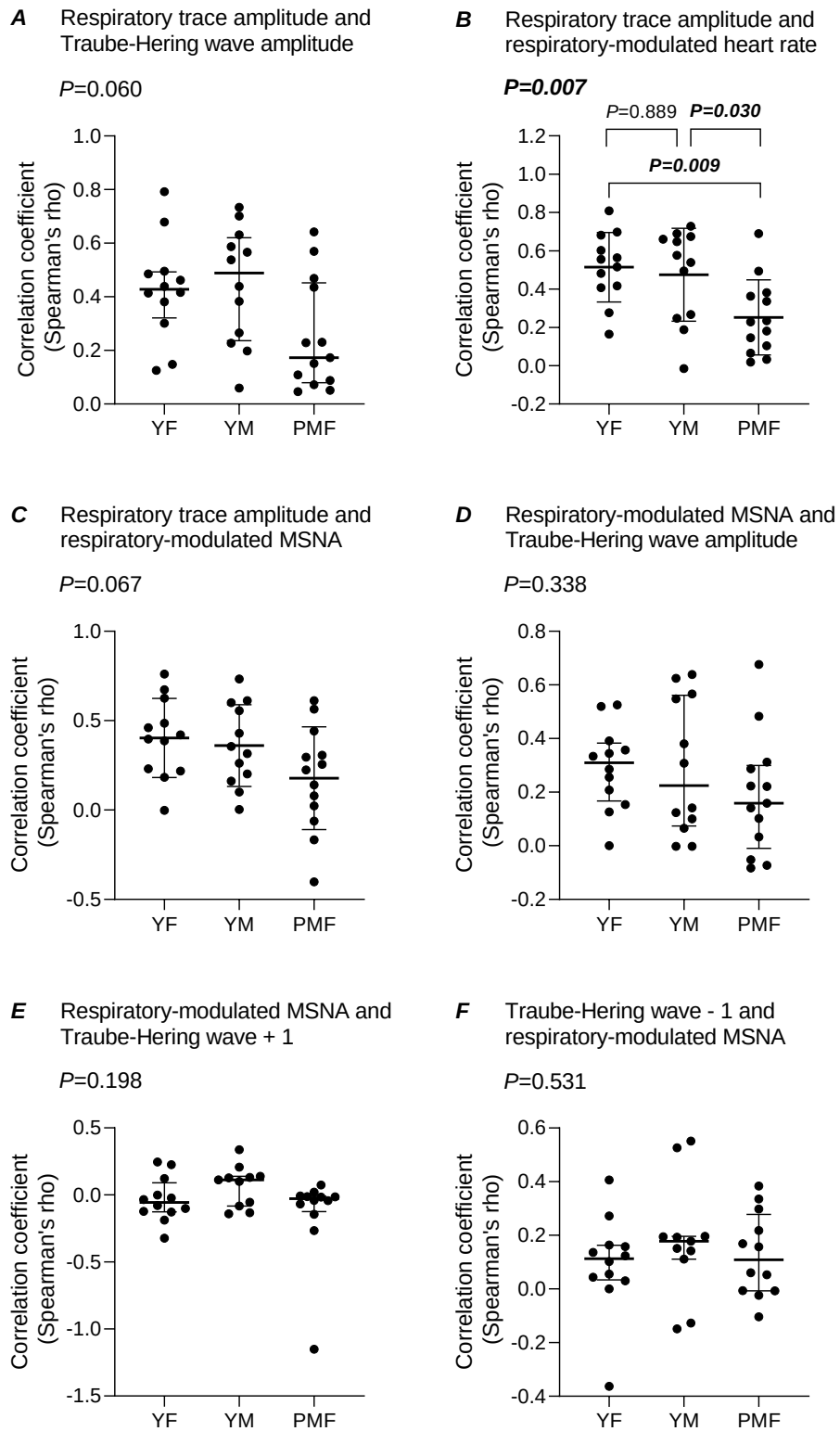

Figure 6.7 Spearman's rank correlation coefficients for associations between respiratory sympathetic coupling variables in healthy postmenopausal females, premenopausal females and younger males. YF; premenopausal females, YM; younger males, PMF; postmenopausal females,

MSNA; muscle sympathetic nerve activity. Data are median ± interquartile range (A, D-F) or mean ± SD (B-C). Group differences in correlation coefficient were tested by Kruskal-Wallis test (A, D-F) or one-way ANOVA (B-C). For B, Tukey post-hoc test was used to determine the significance of pairwise comparisons. Correlation coefficients were included in the analysis regardless of the significance of the correlation. For E and F, there was no correlation coefficient for one younger male participant and one postmenopausal female participant.

6.6.2 Tables and figures for Aim 2: respiratory sympathetic coupling in hypertensive and normotensive postmenopausal females and older males

Table 6.12 Participant characteristics for hypertensive and normotensive postmenopausal females.

	HTN PMF	NTN PMF	Test statistic	*P* value	Effect size
N	7	7			
Age (years)	60 ± 6	59 ± 3	T(12)=0.234	0.819	0.125
Height (m)	1.56 [0.10]	1.63 [0.08]	z=0.705	0.535	0.188
Weight (kg)	75.2 [24.5]	67.3 [23.2]	z=-0.256	0.805	0.068
BMI (kg/m^2)	29.1 [7.3] (9.0)	28.9 [7.7] (6.0)	z=-1.342	0.209	0.359
Clinic SBP (mmHg)	149 [34] (9.14)	130 [8] (5.86)	z=-1.471	0.165	0.393
Clinic DBP (mmHg)	90 ± 9	78 ± 6	T(12)=2.928	***0.013***	1.565
Clinic heart rate (beats/min)	64 [11] (8.36)	63 [7] (6.64)	z=-0.767	0.456	0.205
Daytime ambulatory SBP (mmHg)	136 [10] (11.0)	125 [10] (4.0)	z=-3.137	***0.001***	0.838
Daytime ambulatory DBP (mmHg)	86 [7] (10.29)	73 [11] (4.71)	z=-2.50	***0.011***	0.668
Daytime ambulatory heart rate (beats/min)	73 [17] (8.5)	68 [3] (5.71)	z=-1.287	0.234	0.357

HTN; hypertensive, NTN; normotensive, PMF; postmenopausal females, N; sample size, BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, z; standardised test statistic for Mann-Whitney U. Data are mean ± SD or median [interquartile range], with (mean ranks) where appropriate. Effect size is Cohen's D. Group differences tested by independent samples T test, or

Mann-Whitney U test of medians or mean ranks. Ambulatory heart rate data available for N=6 hypertensive females.

Table 6.13 Hypertensive sub-group and anti-hypertensive medication information for hypertensive postmenopausal females.

Hypertension sub-group	N / 7 postmenopausal females
Untreated	1
Treated controlled	2
Treated uncontrolled	4
Anti-hypertensive medication data among treated participants	
Drug class	N of 5 treated HTN participants for which data are available
Angiotensin converting enzyme inhibitor	3
Angiotensin receptor blocker	1
Calcium channel blocker	2
Beta-blocker	1
Thiazide or Thiazide-like diuretic	1

N; sample size, HTN; hypertension. Drug classes not listed were not taken by any participant.

Table 6.14 Participant characteristics for hypertensive and normotensive older males.

	HTN OM	NTN OM	Test statistic	*P* value	Effect size
N	8	9			
Age (years)	63 [7] (12.12)	54 [11] (6.22)	z=-2.410	***0.015***	0.585
Height (m)	1.75 ± 0.06	1.80 ± 0.05	T(15)=-1.766	0.094	0.868
Weight (kg)	84.0 ± 20.0	81.8 ± 9.9	T(15)=0.291	0.775	0.141
BMI (kg/m^2)	27.2 ± 4.9	25.2 ± 2.8	T(15)=1.033	0.318	0.502
Clinic SBP (mmHg)	156 ± 20	120 ± 8	T(11)=3.859	***0.003***	2.200
Clinic DBP (mmHg)	87 ± 12	75 ± 6	T(11)=1.898	0.084	1.082
Clinic heart rate (beats/min)	65 ± 7	60 ± 6	T(11)=1.408	0.187	0.803
Daytime ambulatory SBP (mmHg)	140 [16]				
Daytime ambulatory DBP (mmHg)	82 [14]				
Daytime ambulatory heart rate (beats/min)	70 [16]				

HTN; hypertensive, NTN; normotensive, OM; older males, N; sample size, BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure. Data are mean ± SD or median [interquartile range] with (mean ranks) where appropriate. Group differences tested by independent samples T test or Mann-Whitney U test. For clinic BP and heart rate, data were available for N=5 normotensive older males. For daytime ambulatory BP and heart rate, data were only available for N=2 normotensive older males, so are not included.

Table 6.15 Hypertensive sub-group and anti-hypertensive medication information for hypertensive older males.

Hypertension sub-group	N / 8 older males
Untreated	3
Treated controlled	2
Treated uncontrolled	3
Anti-hypertensive medication data among treated participants	
Drug class	N of 5 treated HTN participants
Angiotensin converting enzyme inhibitor	4
Angiotensin receptor blocker	1
Calcium channel blocker	3
Beta-blocker	0
Thiazide or Thiazide-like diuretic	2

N; sample size, HTN; hypertension. Drug classes not listed were not taken by any participant.

Table 6.16 Resting sympathetic nerve activity and haemodynamic data for hypertensive versus normotensive postmenopausal females and older males.

Postmenopausal females					
	HTN	NTN	Test statistic	*P* value	Effect size
Burst incidence (bursts/100 HB)	80 ± 13	75 ± 11	T(12)=0.754	0.465	0.403
Burst frequency (bursts/min)	48 ± 8	43 ± 5	T(12)=1.390	0.190	0.743
Mean burst latency (s)	1.23 [0.07] (6.14)	1.29 [0.09] (8.86)	z=-1.214	0.259	0.324
Heart rate (beats/min)	63 [15] (8.43)	55 [8] (6.57)	z=-0.831	0.456	0.222
Systolic BP (mmHg)	125 ± 23	125 ± 8	T(12)=0.024	0.981	0.013
Diastolic BP (mmHg)	64 [18] (9.71)	53 [10] (5.29)	z=-1.981	0.053	0.529
Pulse pressure (mmHg)	60 ± 13	72 ± 10	T(12)=-1.936	0.077	1.035
Mean arterial pressure (mmHg)	80 [20] (9.29)	77 [5] (5.71)	z=-1.597	0.128	0.427
Overall sBRS (%/mmHg)	-1.6 ± 1.6	-2.7 ± 2.7	T(12)=0.937	0.367	0.501
Older males					
Burst incidence (bursts/100 HB)	84 [12] (12.00)	68 [12] (6.33)	z=-2.309	***0.021***	0.560
Burst frequency (bursts/min)	50 [15] (12.12)	39 [11] (6.22)	z=-2.406	***0.015***	0.584
Mean burst latency (s)	1.24 ± 0.03	1.32 ± 0.07	T(12.039)=-3.208	***0.007 (GG)***	1.503
Heart rate (beats/min)	63 ± 10	56 ± 4	T(9.432)=1.909	0.087 (GG)	0.968
Finometer blood pressure	*N=8*	*N=5*			

Systolic BP (mmHg)	153 [21] (9.00)	114 [12] (3.80)	z=-2.342	***0.019***	0.650
Diastolic BP (mmHg)	65 ± 11	51 ± 4	T(11)=2.608	***0.024***	1.487
Pulse pressure (mmHg)	87 [20] (8.88)	61 [9] (4.00)	z=-2.196	***0.030***	0.609
Mean arterial pressure (mmHg)	93 [21] (9.50)	73 [7] (3.00)	z=-2.928	***0.002***	0.812
Brachial artery catheter pressure		*N=4*			
Systolic BP (mmHg)		140 [35]			
Diastolic BP (mmHg)		72 [14]			
Pulse Pressure (mmHg)		68 [22]			
Mean arterial pressure (mmHg)		95 [21]			
Overall sBRS (%/mmHg)	-2.8 ± 1.6	-3.3 ± 1.5	T(14)=1.418	0.178	0.709

HTN; hypertensive, NTN; normotensive, HB; heartbeats, BP; blood pressure. N=7 HTN postmenopausal females, N=7 NTN postmenopausal females, N=8 HTN older males, N=9 NTN older males. Data are mean ± SD or median [interquartile range]. Group differences tested by independent samples T-test (burst incidence, burst frequency, systolic BP and pulse pressure for postmenopausal females; mean burst latency, heart rate, diastolic BP and mean arterial pressure for older males) or Mann-Whitney U test (mean burst latency, heart rate, diastolic BP and mean arterial pressure for postmenopausal females; burst incidence, burst frequency, systolic BP and pulse pressure for older males).

Table 6.17 Frequency and time domain measures of heart rate variability in hypertensive and normotensive postmenopausal females (N=7/7 for HTN/NTN) and older males (N=8/9 for HTN/NTN).

Postmenopausal females

	HTN PMF	NTN PMF	Test statistic	*P* value	Effect size
LF/HF ratio	0.85 [0.92]	0.73 [1.54]	z=0.064	1.0	0.017
LF (nu)	43.7 ± 15.1	44.1 ± 20.5	T(12)=-0.050	0.961	0.027
HF (nu)	53.5 ± 16.4	49.4 ± 16.7	T(12)=0.463	0.651	0.248
LF (ms^2)	238.2 [201.2] (6.57)	489.7 [979.7] (8.43)	z=0.831	0.456	0.222
HF (ms^2)	299.4 [275.4]	257.5 [1327.6]	z=0.192	0.902	0.051
LF (%)	20.4 [14.5] (6.86)	21.9 [18.8] (8.14)	z=0.575	0.620	0.154
HF (%)	25.9 [11.1] (7.14)	30.8 [28.6] (7.86)	z=0.319	0.805	0.085
SDRR (ms)	38.1 [21.1] (7.00)	21.1 [57.3] (8.00)	z=0.447	0.710	0.119
RMSSD (ms)	26.4 ± 9.6	40.2 ± 27.6	T(7.431)=-1.249	0.250 (GG)	0.667
pRR50 (%)	6.8 [11]	4.7 [52]	z=0.449	0.710	0.120

Older males

	HTN OM	NTN OM	Test statistic	P value	Effect size
LF/HF ratio	3.34 [4.25] (9.62)	0.93 [7.79] (8.44)	z=-0.481	0.673	0.117

LF (nu)	73.7 ± 13.5	56.9 ± 31.8	T(11.055)=1.441	0.177	0.670
HF (nu)	23.0 [22.8] (8.38)	50.0 [55.2] (9.56)	z=0.481	0.673	0.117
LF (ms^2)	550.7 ± 527.0	750.1 ± 538.4	T(15)=-0.770	0.453	0.374
HF (ms^2)	139.5 [181.0] (6.88)	264.0 [1011.8] (10.89)	z=1.636	0.114	0.397
LF (%)	37.4 ± 14.2	30.0 ± 17.3	T(15)=0.950	0.357	0.462
HF (%)	12.1 ± 5.1	24.5 ± 22.4	T(8.928)=-1.608	0.143	0.738
SDRR (ms)	37.0 ± 10.8	50.1 ± 15.4	T(15)=-2.033	0.064	0.973
RMSSD (ms)	22.0 [17.9] (6.88)	30.5 [25.4] (10.89)	z=1.636	0.114	0.397
pRR50 (%)	1.5 [5.0] (5.88)	8.0 [27.0] (11.78)	z=2.406	***0.015***	0.584

HTN; hypertensive, NTN; normotensive; PMF, postmenopausal females, OM; older males, LF/HF ratio; ratio of high to low frequency, LF; low frequency domain, HF; high frequency domain, SDRR; standard deviation of RR intervals, RMSSD; root mean square of RR intervals, pRR50; RR intervals longer than 50 ms as a percentage of all RR intervals. Data are mean ± SD or median [interquartile range] with (mean ranks) where appropriate. Group differences were tested by independent samples T-test or Mann-Whitney U test.

Table 6.18 Statistical test data for respiratory modulation of MSNA between mid-late expiration and inspiration/post-inspiration in postmenopausal females and older males.

Variable	Test statistic	*P* value	Partial η^2
	Postmenopausal females		
Burst incidence			
Interaction	F(1, 12)=1.318	0.273	0.099
Phase	F(1, 12)=12.264	***0.004***	0.505
Group	F(1, 12)=0.707	0.417	0.056
Burst frequency			
Interaction	F(1, 12)=1.04	0.328	0.080
Phase	F(1, 12)=13.162	***0.003***	0.523
Group	F(1, 12)=1.929	0.190	0.138
Total burst area/s			
Interaction	F(1, 12)=2.496	0.140	0.172
Phase	F(1, 12)=27.905	***<0.0005***	0.699
Group	F(1, 12)=0.695	0.421	0.055
Mean burst area/s			
Interaction	F(1, 12)=3.601	0.082	0.231
Phase	F(1, 12)=38.529	***<0.0005***	0.763
Group	F(1, 12)=2.285	0.156	0.160
	Older males		
Burst incidence			
Interaction	F(1, 15)=1.061	0.319	0.066
Phase	F(1, 15)=8.401	***0.011***	0.359
Group	F(1, 15)= 1.824	0.197	0.108
Burst frequency			
Interaction	F(1, 15)=0.834	0.375	0.053
Phase	F(1, 15)=6.409	***0.023***	0.299
Group	F(1, 15)=1.153	***0.006***	0.404
Total burst area/s			
Interaction	F(1, 15)=1.011	0.331	0.063
Phase	F(1, 15)=6.968	***0.019***	0.317
Group	F(1, 15)=1.706	0.211	0.102
Mean burst area/s			
Interaction	F(1, 15)=0.628	0.441	0.040
Phase	F(1, 15)=9.293	***0.008***	0.383
Group	F(1, 15)=4.715	***0.046***	0.239

F; ANOVA test statistic.

Table 6.19 Statistical test data for group difference in absolute and percentage change from mid-late expiration to inspiration/post-inspiration in postmenopausal females and older males

Variable	Test statistic	*P* value	Effect size
Postmenopausal females			
Δ Burst incidence	T(12)=-1.148	0.273	0.612
%Δ Burst incidence	T(12)=-1.011	0.332	0.540
Δ Burst frequency	T(12)=-1.019	0.328	0.544
%Δ Burst frequency	T(12)=-1.014	0.331	0.542
Δ Total burst area/s	z=1.752	0.097	0.468
%Δ Total burst area/s	T(12)=-3.325	***0.006***	1.792
Δ Mean burst area/s	T(12)=-1.90	0.082	1.016
%Δ Mean burst area/s	T(12)=-2.296	***0.040***	1.227
Older males			
Δ Burst incidence	T(15)=1.030	0.319	0.501
%Δ Burst incidence	T(15)=1.224	0.240	0.595
Δ Burst frequency	T(15)=0.914	0.375	0.444
%Δ Burst frequency	z=-1.155	0.277	0.280
Δ Total burst area/s	T(11.329)=1.048	0.316 (GG)	0.488
%Δ Total burst area/s	z=-1.155	0.277	0.280
Δ Mean burst area/s	z=-0.674	0.541	0.163
%Δ Mean burst area/s	z=-1.058	0.321	0.257

Δ; absolute change, %Δ; percentage change, z; standardised test statistic for Mann Whitney U test. Group difference tested by independent samples T-test or Mann-Whitney U test. Effect size is Cohen's D.

Table 6.20 Statistical test data for respiratory modulation of MSNA across 10 respiratory phases in postmenopausal females and older males.

Variable	Test statistic	*P* value	Partial η^2
	Postmenopausal females		
Burst incidence			
Interaction	F(9, 108)=1.473	0.167	0.106
Phase	F(9, 108)=2.172	***0.029***	0.153
Group	F(1, 12)=0.682	0.425	0.054
Burst frequency			
Interaction	F(3.276, 39.309)=0.420	0.756 (GG)	0.034
Phase	F(3.276, 39.309)=1.397	0.257 (GG)	0.104
Group	F(1, 12)=2.206	0.163	0.155
Total burst area/s			
Interaction	F(2.286, 27.432)=1.075	0.363 (GG)	0.082
Phase	F(2.286, 27.432)=2.821	0.071 (GG)	0.190
Group	F(1, 12)=0.695	0.421	0.055
Mean burst area/s			
Interaction	F(3.793, 45.521)=1.5667	0.201 (GG)	0.116
Phase	F(3.793, 45.521)=3.234	***0.022*** (GG)	0.212
Group	F(1, 12)=1.551	0.237	0.114
	Older males		
Burst incidence			
Interaction	F(4.085, 61.282)=0.588	0.676 (GG)	0.038
Phase	F(4.085, 61.282)=4.590	***0.002***	0.234
Group	F(1, 15)=1.945	0.183	0.115
Burst frequency			
Interaction	F(9, 135)=0.622	0.776	0.040
Phase	F(9, 135)=9.237	***<0.0005***	0.305
Group	F(1, 15)=10.116	***0.006***	0.403
Total burst area/s			
Interaction	F(3.764, 56.46)=0.426	0.776	0.028
Phase	F(3.764, 56.46)=6.571	***<0.0005***	0.381
Group	F(1, 15)=1.706	0.211	0.102
Mean burst area/s			
Interaction	F(3.187, 47.8)=0.513	0.686	0.033
Phase	F(3.187, 47.8)=7.398	***<0.0005***	0.330
Group	F(1, 15)=4.567	***0.049***	0.233

F; ANOVA test statistic.

Table 6.21 Statistical test data for group differences in respiratory-modulated haemodynamic variables in postmenopausal females and older males.

Variable	Test statistic	*P* value	Effect size
Postmenopausal females			
Traube-Hering wave amplitude	T(12)=0.626	0.543	0.334
Respiratory modulation of heart rate	z=0.447	0.710	0.119
Respiratory modulated MSNA amplitude	T(12)=0.550	0.592	0.294
Respiratory cycle duration	T(12)=-0.242	0.813	0.130
Older males			
Traube-Hering wave amplitude	T(14)=-0.707	0.491	0.356
Respiratory modulation of heart rate	T(15)=-1.348	0.198	0.655
Respiratory modulated MSNA amplitude	z=-0.840	0.442	0.210
Respiratory cycle duration	z=0.096	1.0	0.023

z; Mann-Whitney U standardised test statistic. Effect size is Cohen's D.

Table 6.22 Statistical test data for group differences in coefficients for correlations between respiratory-modulated haemodynamic variables.

Correlation	Test statistic	*P* value
Postmenopausal females		
Respiratory trace amplitude vs. Traube-Hering wave amplitude	T(12)=-0.559	0.587
Respiratory trace amplitude vs. respiratory-modulated heart rate	T(12)=-0.258	0.801
Respiratory trace amplitude vs. respiratory-modulated MSNA	T(12)=-0.237	0.817
Respiratory-modulated MSNA vs. Traube-Hering wave amplitude	z=-0.958	0.383
Respiratory-modulated MSNA vs. Traube-Hering wave +1	T(11)=1.749	0.108
Traube-Hering wave -1 vs. respiratory-modulated MSNA	z=-0.857	0.445
Older males		
Respiratory trace amplitude vs. Traube-Hering wave amplitude	T(14)=-0.606	0.554
Respiratory trace amplitude vs. respiratory-modulated heart rate	z=1.636	0.114
Respiratory trace amplitude vs. respiratory-modulated MSNA	T(15)=-0.189	0.852
Respiratory-modulated MSNA vs. Traube-Hering wave amplitude	z=0.370	0.758
Respiratory-modulated MSNA vs. Traube-Hering wave +1	z=0.424	0.681
Traube-Hering wave -1 vs. respiratory-modulated MSNA	z=0.634	0.681

MSNA; muscle sympathetic nerve activity, z; Mann-Whitney U standardised test statistic. Effect size is Cohen's D.

Table 6.23 Multiple linear regression for percentage change in burst incidence between inspiration and expiration.

	B	β	95 % CI		*P* value
			Lower	Upper	
Constant	-64.972		-80.871	-48.948	***<0.0005***
Age	0.429	0.399	0.047	0.806	***0.028***
Burst incidence	0.361	0.338	-0.005	0.728	0.053
Sex	1.118	0.031	-6.08	8.067	0.780
Hypertension	-2.543	-0.060	-11.841	6.982	0.607

B; unstandardised coefficient, β; standardised coefficient, CI; confidence intervals. Reference category for sex was female and for hypertension was normotensive. R=0.679, R^2=0.460, R^2 adjusted=0.423. N=63.

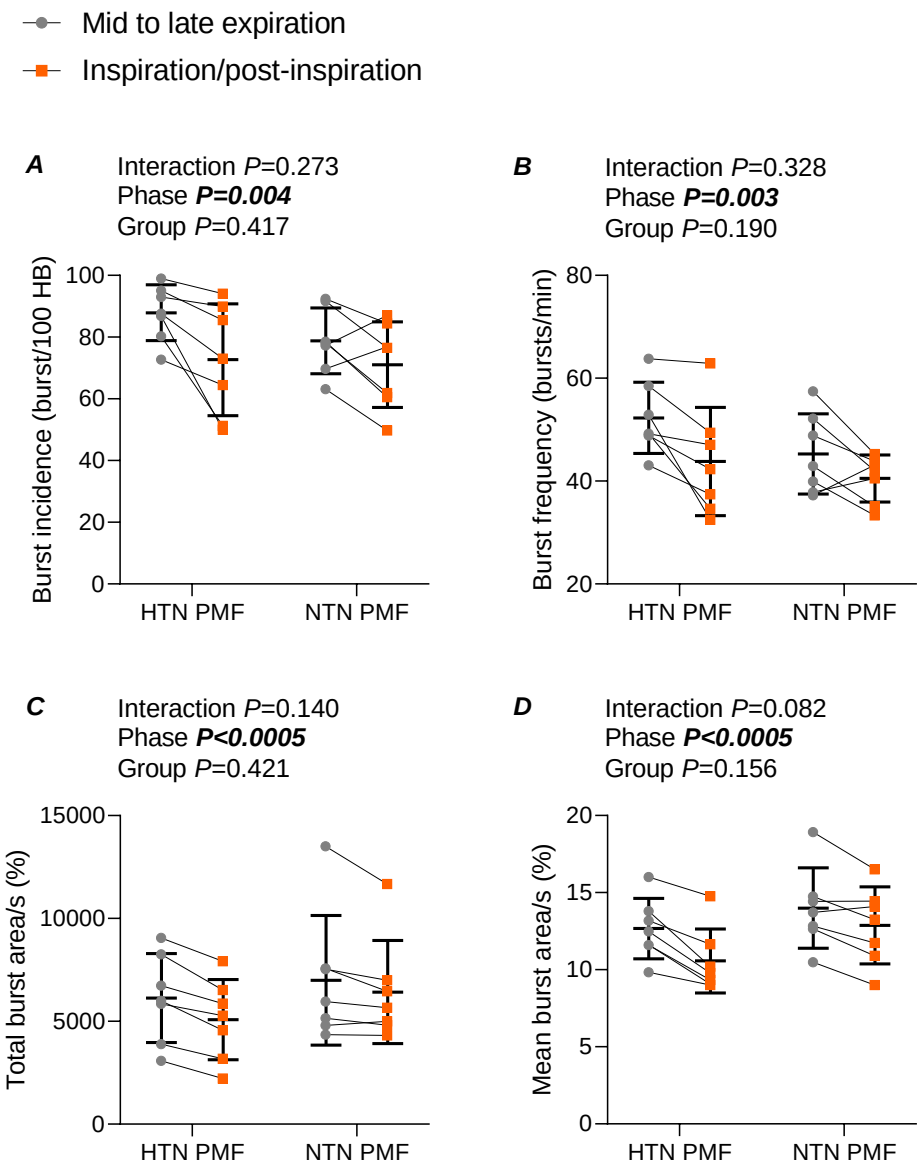

Figure 6.8 Respiratory modulation of (A) burst incidence, (B) burst frequency, (C) total burst area/s, and (D) mean burst area/s across two respiratory phases in hypertensive (N=7) and normotensive (N=7) postmenopausal females. HTN; hypertensive, NTN; normotensive, PMF; postmenopausal female, HB; heartbeats. Data are expressed as mean ± SD. A respiratory phase x group interaction was tested by two-way mixed model ANOVA.

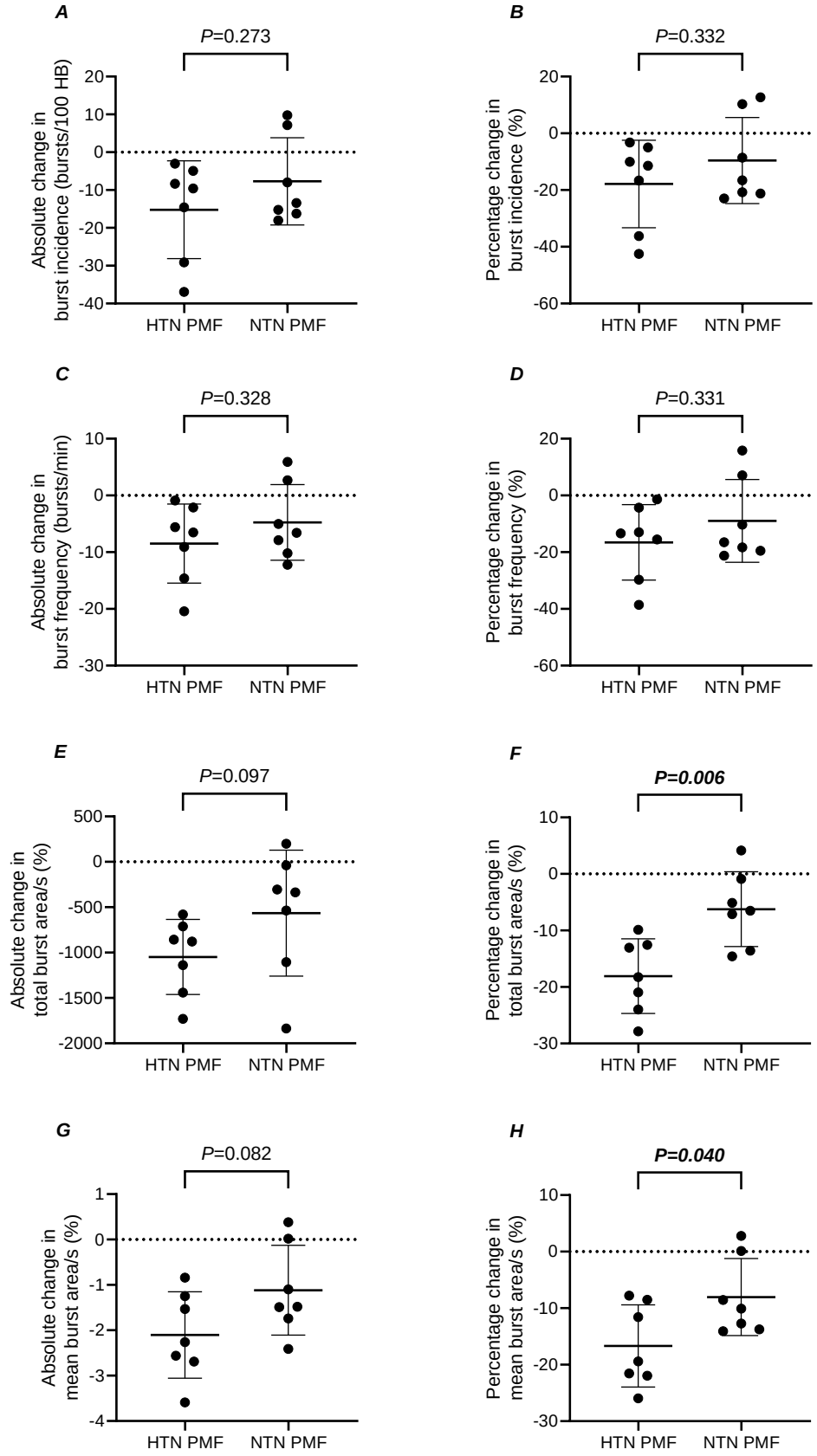

Figure 6.9 Absolute and percentage change in MSNA burst incidence (A-B), burst frequency (C-D), total burst area/s (E-F), and mean burst area/s (G-H) between mid-late expiration and inspiration/post-inspiration in hypertensive

and normotensive postmenopausal females. HTN; hypertensive, NTN; normotensive, PMF; postmenopausal female, HB; heartbeats. Data are mean ± SD (A-D and F-H) or median ± interquartile range (E). Group differences were tested by independent samples T-test (A-D and F-H) or Mann-Whitney U test (E).

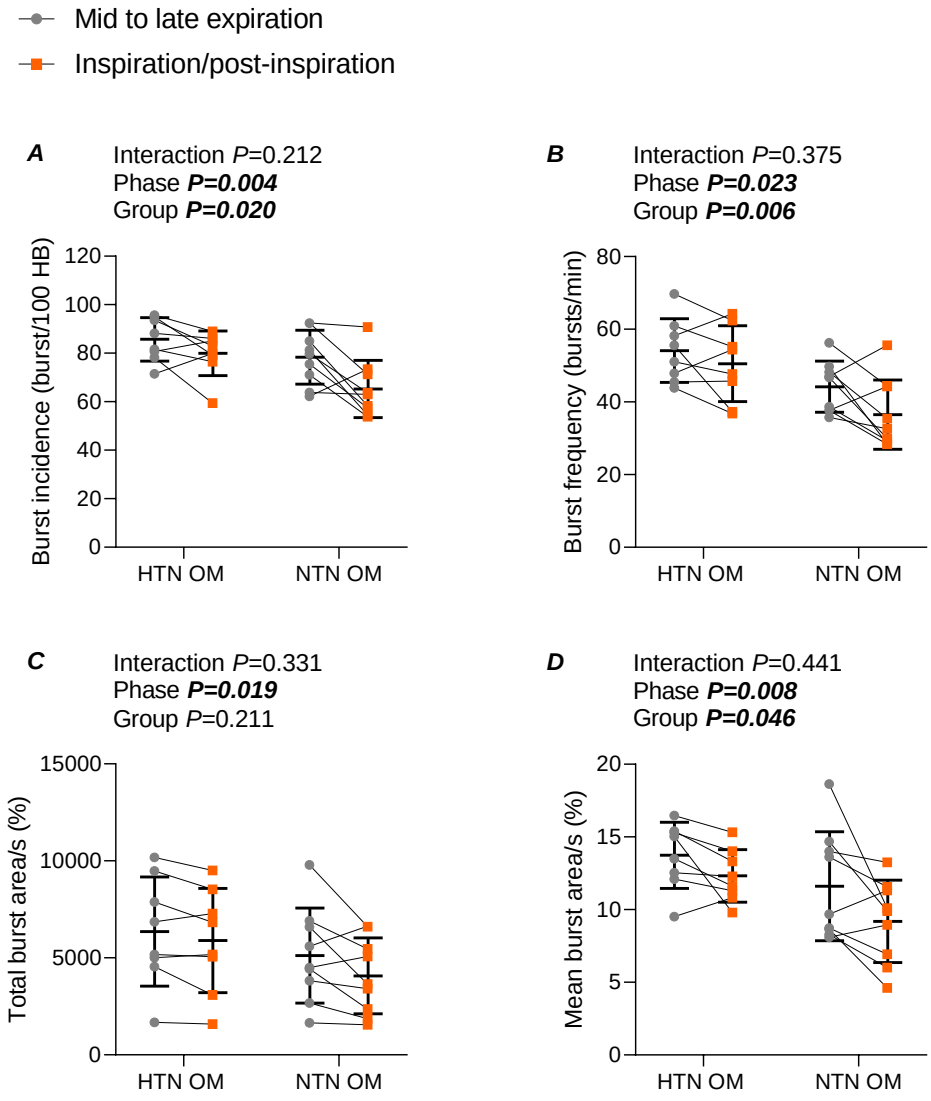

Figure 6.10 Respiratory modulation of (A) burst incidence, (B) burst frequency, (C) total burst area/s, and (D) mean burst area/s across two respiratory phases in hypertensive (N=8) and normotensive (N=9) older males. HTN; hypertensive, NTN; normotensive, OM; older males, HB; heartbeats. Data are expressed as mean ± SD. A respiratory phase x group interaction was tested by two-way mixed model ANOVA.

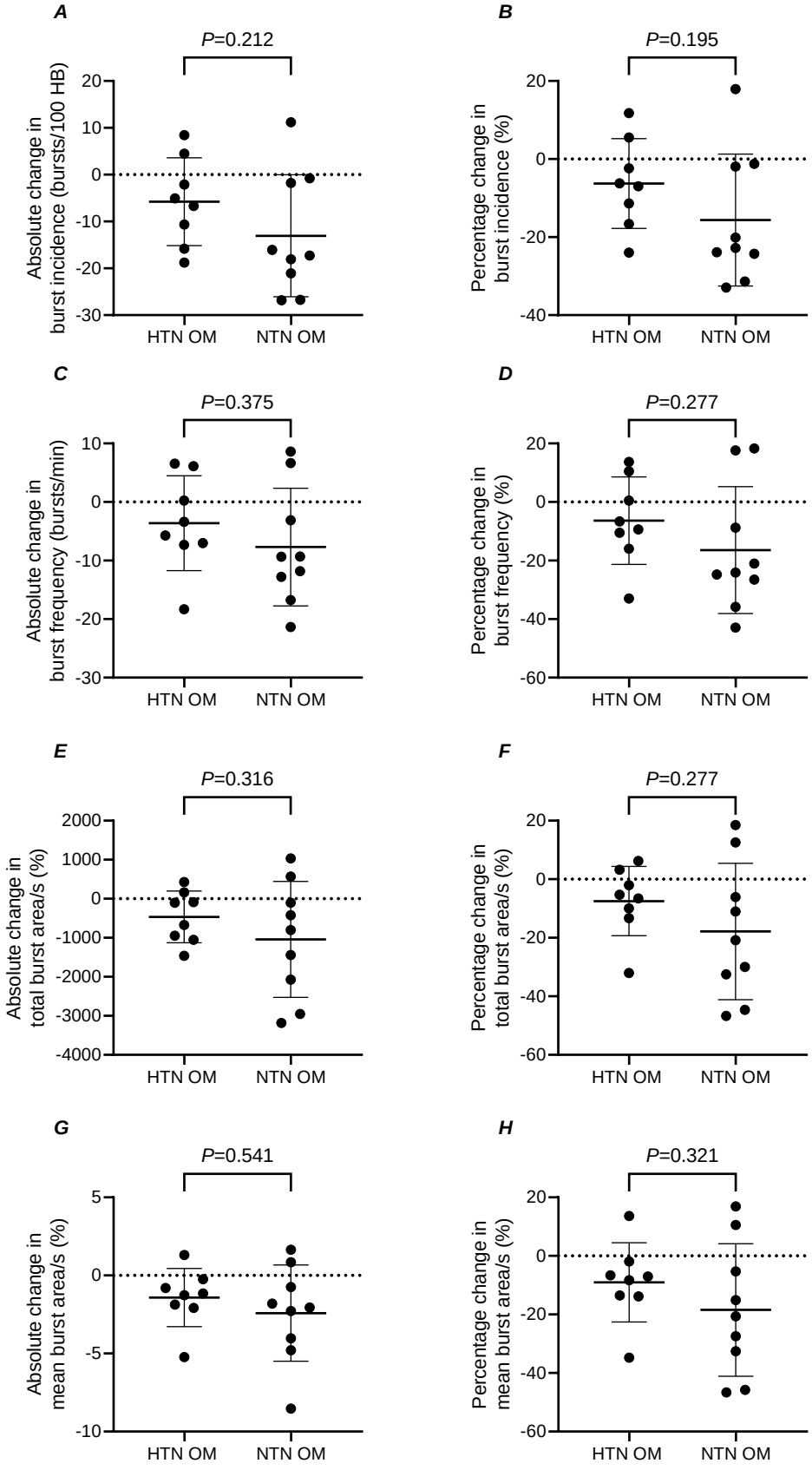

Figure 6.11 Absolute and percentage change in MSNA burst incidence (A-B), burst frequency (C-D), total burst area/s (E-F), and mean burst area/s (G-H) between mid-late expiration and inspiration/post-inspiration in

hypertensive and normotensive older males. HTN; hypertensive, NTN; normotensive, OM; older males, HB; heartbeats. Data are mean ± SD (A-C and E) or median ± interquartile range (D and F-H). Group differences were tested by independent samples T-test (A-C and E) or Mann-Whitney U test (D and F-H).

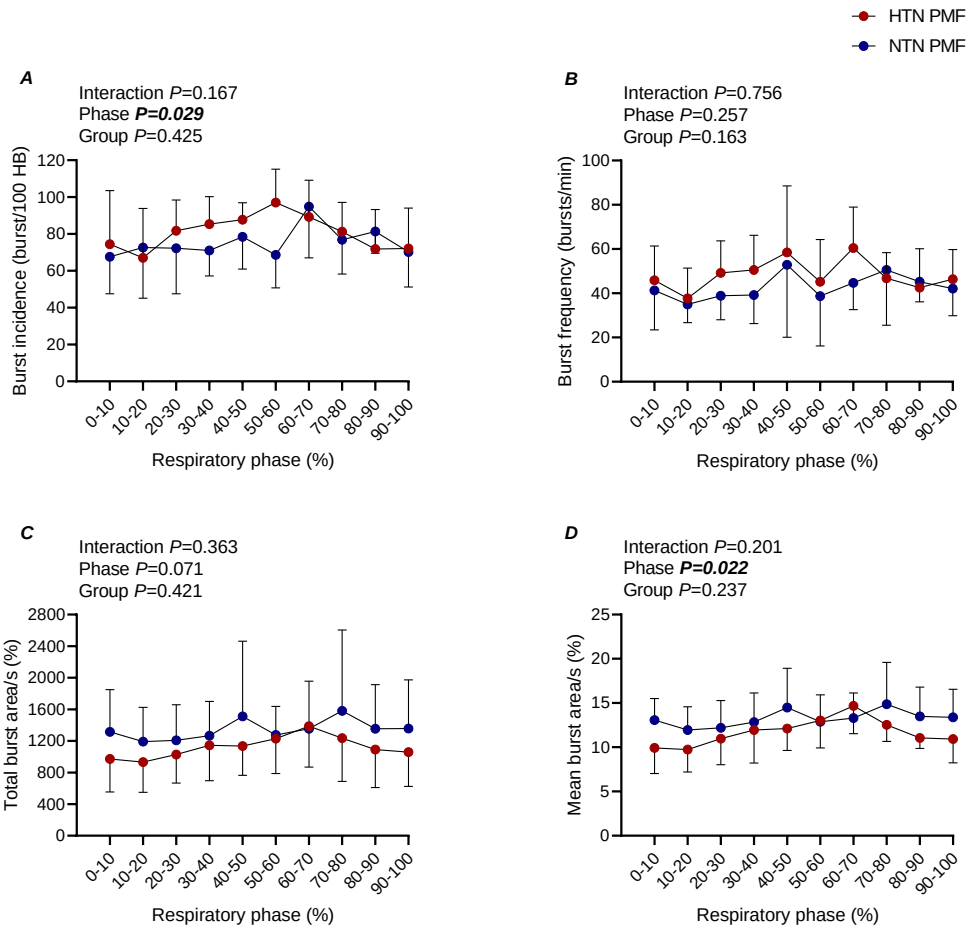

Figure 6.12 Respiratory modulation of (A) burst incidence, (B) burst frequency, (C) total burst area/s and (D) mean burst area/s across 10-percentage phases of the respiratory cycle in hypertensive (N=7) and normotensive (N=7) postmenopausal females. HTN; hypertensive, NTN; normotensive, PMF; postmenopausal females, HB; heartbeats. Data are mean ± SD. Two-way mixed model ANOVA.

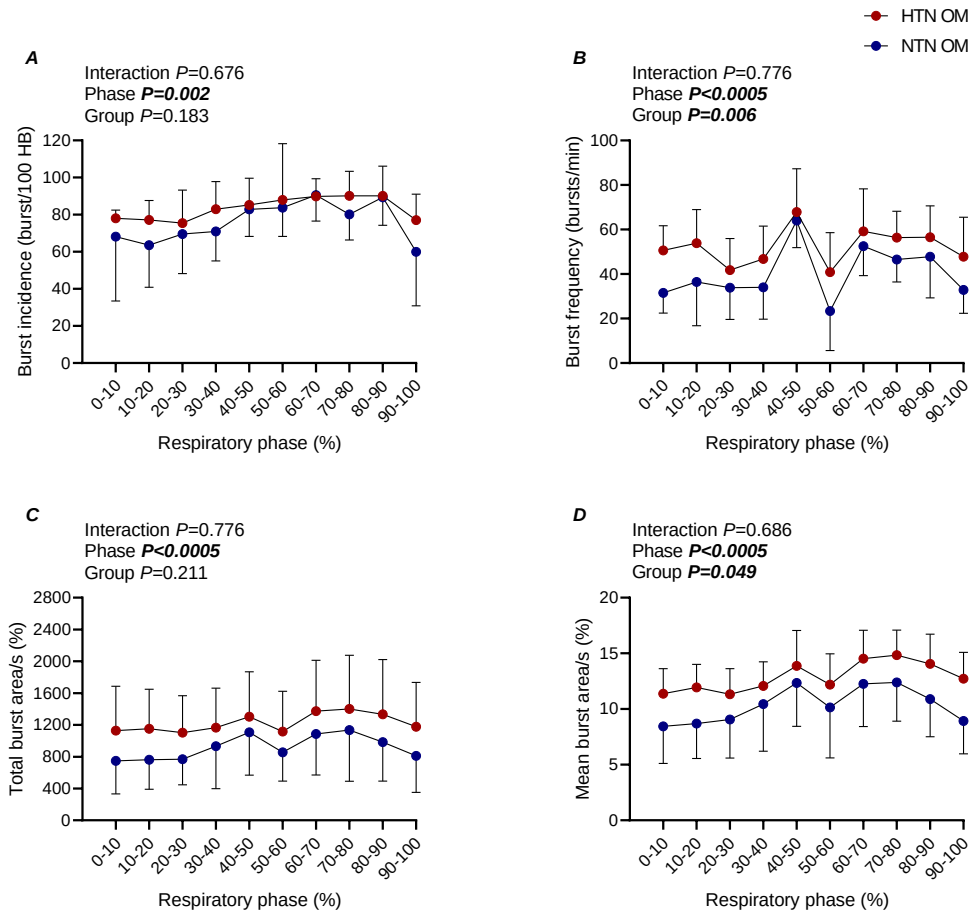

Figure 6.13 Respiratory modulation of (A) burst incidence, (B) burst frequency, (C) total burst area/s and (D) mean burst area/s across 10-percentage phases of the respiratory cycle in hypertensive (N=8) and normotensive (N=9) older males. HTN; hypertensive, NTN; normotensive, OM; older males, HB; heartbeats. Data are mean ± SD. Two-way mixed model ANOVA.

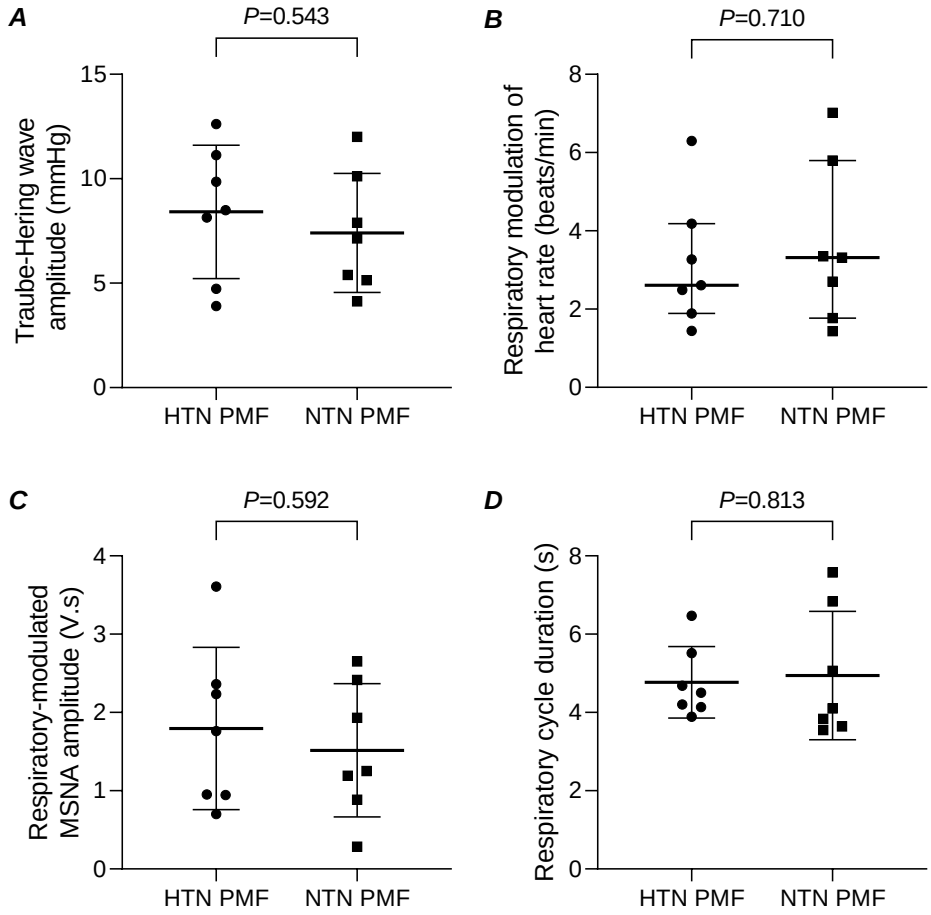

Figure 6.14 Average (A) Traube-Hering wave amplitude, (B) respiratory modulation of heart rate over one respiratory cycle, (C) respiratory-modulated MSNA over one respiratory cycle, and (D) respiratory cycle duration in hypertensive (N=7) and normotensive (N=7) postmenopausal females. HTN; hypertensive, NTN; normotensive, PMF; postmenopausal females; MSNA; muscle sympathetic nerve activity. Data are mean ± SD (A, C and D) or median ± interquartile range (B). Group differences were tested by independent samples T-test (A, C and D) or Mann-Whitney U test (B).

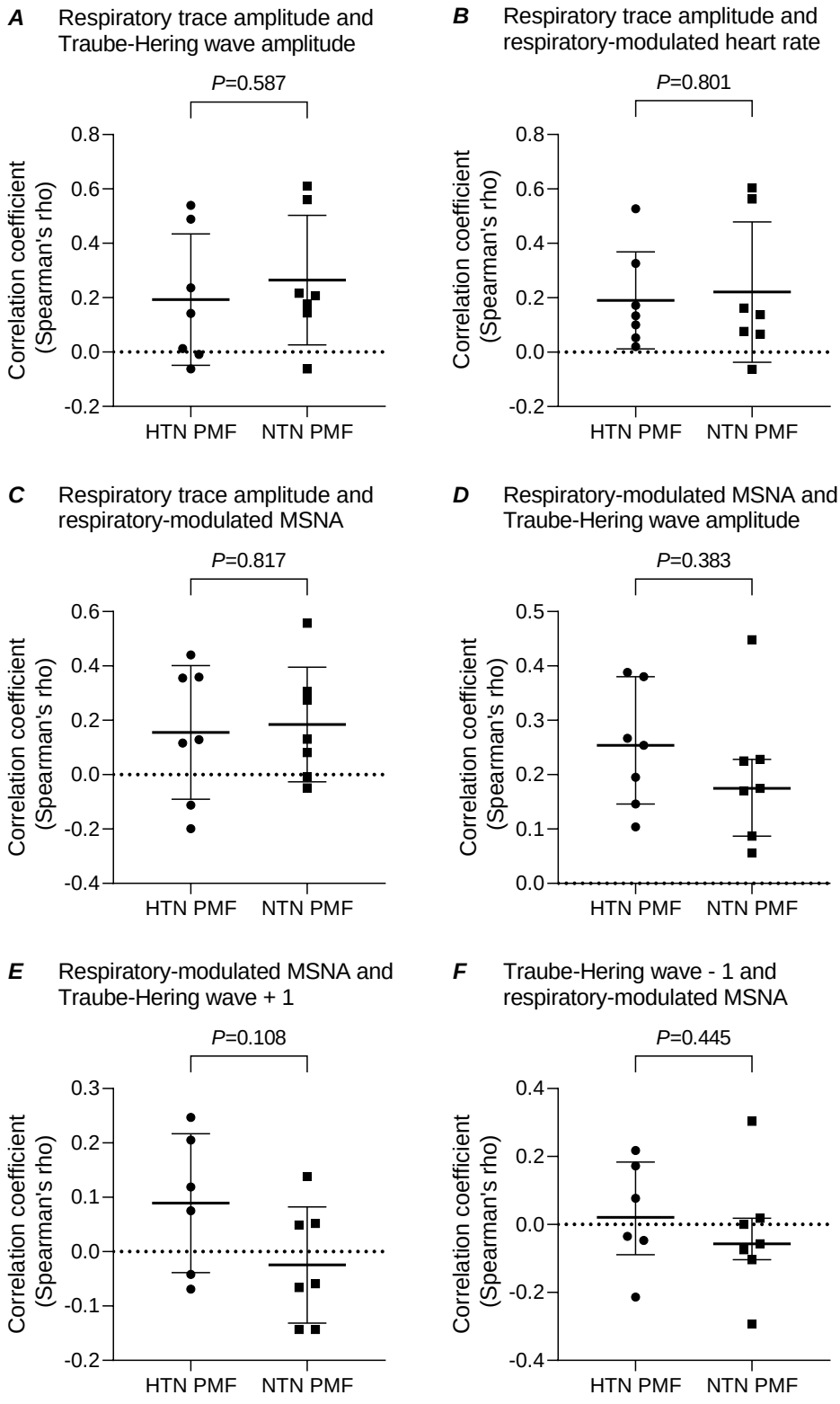

Figure 6.15 Spearman's rho correlation coefficient for associations between respiratory and haemodynamic variables in hypertensive (N=7) and normotensive (N=7) postmenopausal females. HTN; hypertensive, NTN; normotensive, PMF; postmenopausal females; MSNA; muscle sympathetic nerve

activity. Data are mean ± SD (A-C, E) or median ± interquartile range (D). Group differences were tested by independent samples T-test (A-C, E) or Mann-Whitney U test (D).

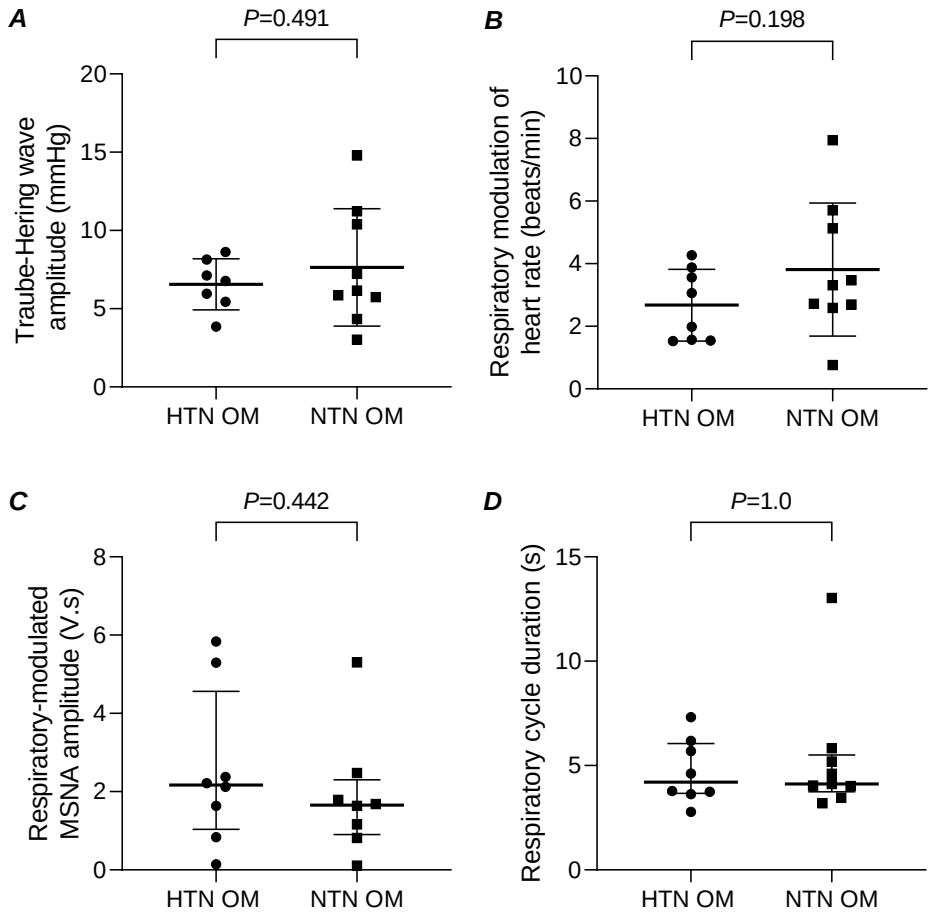

Figure 6.16 Average (A) Traube-Hering wave amplitude, (B) respiratory modulation of heart rate over one respiratory cycle, (C) respiratory-modulated MSNA over one respiratory cycle, and (D) respiratory cycle duration in hypertensive (N=8) and normotensive (N=9) older males. HTN; hypertensive, NTN; normotensive, OM; older males, MSNA: muscle sympathetic nerve activity. Data are mean ± SD (A and B) or median ± interquartile range (C and D). Group differences were tested by independent samples T-test (A and B) or Mann-Whitney U test (C and D).

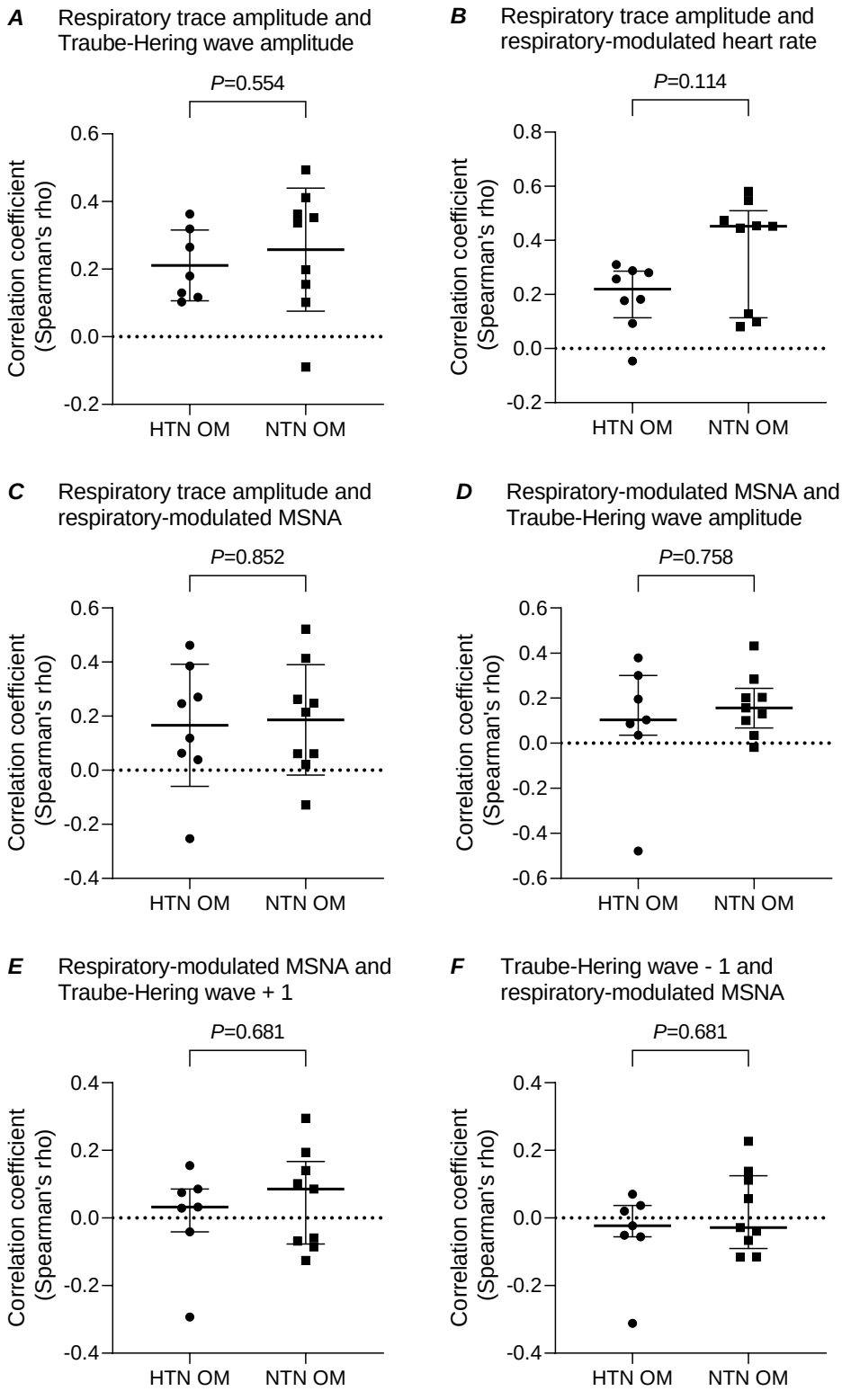

Figure 6.17 Spearman's rho correlation coefficient for associations between respiratory and haemodynamic variables in hypertensive (N=8) and normotensive (N=9) older males. HTN; hypertensive, NTN; normotensive, OM; older males, MSNA; muscle sympathetic nerve activity. Data are mean ± SD (B

and C) or median ± interquartile range (A, D-F). Group differences were tested by independent samples T-test (B and C) or Mann-Whitney U test (A, D-F).

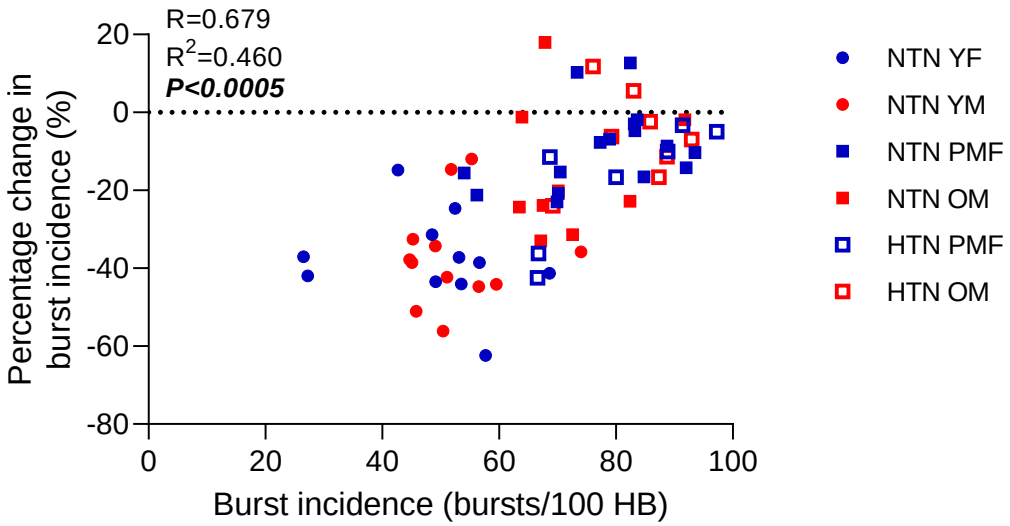

Figure 6.18 Multiple linear between resting burst incidence and percentage change in burst incidence from mid-late expiration to inspiration/post-inspiration. NTN; normotensive, HTN; hypertensive, YF; premenopausal females, YM; younger males; PMF; postmenopausal females, OM; older males; HB, heartbeats. N=63, NTN PMF grouped from aims 1 and 2.

Chapter 7 General discussion

7.1 Overview

The mechanisms driving hypertension are not yet fully understood. Given the sex differences in sympathetic regulation of blood pressure in healthy adults, this thesis aimed to contribute to the understanding of the role of the sympathetic nervous system in the development of hypertension in females. Two aspects of sympathetic blood pressure regulation were the focus: firstly, the ability of the sympathetic nerves to promote vasoconstriction (sympathetic transduction) and secondly, the modulation of sympathetic outflow by the respiratory system.

There is some evidence to suggest that premenopausal females show lower sympathetic transduction compared to healthy young males (Hart et al., 2009, Briant et al., 2016, Hogarth et al., 2007a) although this finding has not been replicated universally (Jarvis et al., 2011, Vianna et al., 2012, Robinson et al., 2019, Hissen et al., 2019). If sympathetic transduction is lower in young females, this may in part explain the reduced risk of hypertension in young females (Hart et al., 2011a). However, the level of transduction in *hypertensive* young females had not been studied. Additionally, sympathetic transduction in healthy females increases after the menopause, which may contribute to the increased hypertension risk in older females (Hart et al., 2011a, Briant et al., 2016, Hogarth et al., 2008). However, it was unclear whether sympathetic transduction differed between hypertensive and normotensive postmenopausal females. Therefore, the first study of this thesis aimed to measure sympathetic transduction in hypertensive versus normotensive premenopausal females, and hypertensive versus normotensive postmenopausal females.

One of the key mechanisms underlying the sex difference in sympathetic transduction among healthy premenopausal females versus younger males and postmenopausal females involves the vascular beta-adrenergic receptors (Hart et al., 2011a), which appear to have a greater vasodilatory effect in premenopausal females (Kneale et al., 2000, Hart et al., 2011a), perhaps secondary to oestradiol upregulation of nitric oxide production (Miller and Duckles, 2008). If sympathetic transduction was shown to be enhanced in hypertensive versus normotensive

premenopausal females, dysfunction of this beta-adrenergic vasodilatory mechanism could be responsible. Therefore, the second study of this thesis aimed to assess beta-adrenergic contribution to sympathetic transduction in hypertensive versus normotensive premenopausal females, using systemic beta-adrenergic receptor blockade as a stimulus.

Additionally, the contribution of sympathetic transduction to the pressor response to exercise was considered in healthy males and females. If lower transduction in young females versus males is maintained during exercise, this may contribute to the smaller exercise pressor response in young females (Smith et al., 2019). As such, sympathetic transduction was quantified before and during isometric handgrip exercise. Whilst the study also aimed to measure sympathetic transduction during exercise in hypertensive participants, data collection was limited and insufficient numbers within each participant group were recruited.

Finally, respiratory modulation of sympathetic outflow was assessed in premenopausal and older females, and in hypertension. Sympathetic activity is subject to respiratory modulation such that a quietening of activity is associated with inspiration (Seals et al., 1990). Loss or weakening of this modulation may increase the overall level of sympathetic activity, which is a characteristic of hypertension (Grassi et al., 2018). Previous work reported no change to respiratory sympathetic modulation in older versus younger healthy males (Shantsila et al., 2015). However, ageing females are subject to additional factors that may influence respiratory sympathetic modulation, for example the decline in oestrogen, which is sympathoinhibitory (Saleh et al., 2000). Therefore, this thesis aimed to quantify respiratory sympathetic modulation in healthy postmenopausal versus premenopausal females. Furthermore, given the role of increased sympathetic nerve activity in hypertension (Grassi et al., 2018), a final aim was to assess respiratory sympathetic modulation in hypertensive versus normotensive postmenopausal females.

7.2 Summary of findings

7.2.1 Chapter 3: Sympathetic transduction in hypertensive females

Analysis in chapter 3 demonstrated that sympathetic transduction into diastolic blood pressure was increased in hypertensive versus normotensive

premenopausal females. As such, the data suggest that exaggerated conversion of sympathetic nerve activity into blood pressure is a mechanism contributing to hypertension in premenopausal females, that before this research was only speculative. Therefore, whilst the hypertensive premenopausal females of the current study had a similar level of sympathetic activity directed to their vasculature as normotensive controls, the sympathetic vasoconstrictor mechanisms were more effective in the hypertensive females. Sympathetic activity is associated with changes to vascular structure (Grassi, 2006). As such, vascular changes may accompany increased sympathetic transduction in hypertensive premenopausal females (before and/or after the development of hypertension).

The underlying cause of enhanced sympathetic transduction in the current hypertensive premenopausal females could not be fully identified. Chapter 4 aimed to determine the contribution of one of the potential mechanisms underlying increased sympathetic transduction, the function of the beta-adrenergic receptors (discussed more below). However, a number of other factors could increase sympathetic transduction. Alpha-adrenergic receptor sensitivity may have been increased in the current hypertensive premenopausal females. Alpha-adrenergic sensitivity is not thought to explain the reduced sympathetic transduction in *normotensive* premenopausal females versus young males (given that males and females exhibited similar alpha-adrenergic sensitivity under ganglionic blockade (Christou et al., 2005)). However, others have shown enhanced alpha-adrenergic sensitivity in females versus males among a mixed hypertensive/normotensive cohort, although there was no separate effect of hypertension (Sherwood et al., 2017). As such, the role of alpha-adrenergic receptors in premenopausal hypertension is unclear. Alternatively, poorer endothelial function or nitric oxide production may underlie the enhanced sympathetic transduction in hypertensive premenopausal females, given that existing evidence points to worse endothelial function in hypertension versus normotension (Gokaslan et al., 2020, Taddei et al., 1997) and in hypotensive females versus hypertensive males (Routledge et al., 2012). However, this was not assessed as part of the current study. Oestrogen is known to increase nitric oxide production via upregulation of eNOS (Miller and Duckles, 2008), therefore any hormonal dysfunction could have affected nitric oxide production in the current cohort. However, participants all reported regular menstrual cycles (such that the study could be arranged during the early follicular

phase), and a similar number of hypertensive and normotensive premenopausal females used hormonal contraception (three hypertensive versus two normotensive).

Hypertensive postmenopausal females did not demonstrate increased sympathetic transduction compared to normotensive controls, indicating that sympathetic transduction may be less important in driving hypertension in postmenopausal females. In agreement, sympathetic transduction was negatively correlated with age in hypertensive females, indicating that sympathetic transduction was generally greater in premenopausal hypertensive females and as such may be a more important mechanism in premenopausal hypertension compared to postmenopausal hypertension. The postmenopausal groups of the current study showed similar resting levels of sympathetic activity, in contrast to previous reports where MSNA was greater in hypertensive versus normotensive postmenopausal females (Hogarth et al., 2011). Therefore, the mechanism underlying the hypertension in the current group of postmenopausal females is unclear. BMI was greater in the hypertensive versus normotensive group, which may offer one explanation (Janghorbani et al., 2017). However, a number of other factors with the potential to drive hypertension were not assessed in the current study, for example arterial stiffness (Vaitkevicius et al., 1993) or blood pressure during exercise (Berger et al., 2015).

Recent developments in the field support the idea that sympathetic transduction is an important measure to assess in various participant groups. For example, sympathetic transduction was shown to be negatively correlated with sympathetic baroreflex sensitivity in healthy males, whereas female participants demonstrated no relationship between those variables (Hissen et al., 2019). Given that the current data show increased sympathetic transduction in hypertensive versus normotensive premenopausal females, it may also be important to assess the relationship between transduction and baroreflex sensitivity in these groups. Additionally, it was recently shown that sympathetic transduction is elevated in normotensive female participants using oral contraception compared controls (Takeda et al., 2021). As the current data have shown that elevated transduction is an important mechanism in hypertension particularly in premenopausal females, it may be that elevated sympathetic transduction contributes to the increased risk of hypertension associated with oral contraception (Perol et al., 2019).

Overall, the current findings contribute to the understanding of sex differences in blood pressure regulation and the sex-specific development of hypertension. There is argument by some for sex-specific blood pressure targets (Gerdts and de Simone, 2021) and understanding sex-specific mechanisms behind hypertension development is an important contribution to this debate.

7.2.2 Chapter 4: Effect of beta-adrenergic receptor blockade on sympathetic transduction in hypertensive females

Chapter 4 aimed to investigate one of the potential mechanisms underlying increased sympathetic transduction in hypertensive premenopausal females. The study was unable to determine whether increased sympathetic transduction in hypertensive premenopausal females was associated with reduced contribution of the vascular beta-adrenergic receptors to overall vasoconstrictor tone (data collection was limited by the SARS-CoV-2 pandemic). Therefore, this question remains unanswered. However, there is existing evidence that supports the role of beta-adrenergic receptors in premenopausal hypertension. Hypertension is associated with beta-adrenergic receptor downregulation (Peng et al., 2000, Sherwood et al., 2017), which would reduce the beta-adrenergic vasodilator potential of the vasculature. Nitric oxide is thought to reduce beta-adrenergic downregulation by GPRK (via s-nitrosylation) (Whalen et al., 2000). Therefore, if endothelial production of nitric oxide is poorer in hypertensive premenopausal females, this may further contribute to beta-adrenergic receptor downregulation. Furthermore, epidemiology studies have identified SNPs in the beta-adrenergic receptor gene which could promote poorer vasodilation (Brodde, 2008). As such, several mechanisms could reduce beta-adrenergic expression and function in hypertensive premenopausal females. However, current evidence for these mechanisms in hypertensive premenopausal females specifically is limited. Instead, chapter 4 shows pilot data assessing the effect of systemic beta-adrenergic receptor blockade on sympathetic blood pressure regulation at rest and during isometric handgrip exercise in healthy participants. Whilst resting MSNA and blood pressure were unaffected by beta-blockade, sympathetic burst latency was reduced under beta-blockade despite no change in the level of MSNA. Given that bursts latency correlates with burst amplitude (taller bursts have shorter latencies) (Wallin et al., 1994), the decrease in burst latency could be associated with taller bursts, which would indicate recruitment of more

sympathetic action potentials, larger sympathetic action potentials, or both (Shoemaker, 2017) during beta-blockade. However, the current data showed that median normalised burst amplitude was not significantly greater during beta-blockade compared to baseline (*P*=0.08). Furthermore, when sympathetic action potentials were assessed during handgrip exercise in the current study, there was no significant beta-blockade x time interaction for the number of action potentials per burst, indicating that MSNA bursts during beta-blockade did not contain more sympathetic action potentials than bursts during baseline. The sample size of those data were small however (N=3) and average action potential amplitude was not assessed. Alternatively, given that propranolol may be able to cross the blood brain barrier (Laurens et al., 2019), there may have been a central effect of propranolol that altered sympathetic firing. Along these lines, beta-adrenergic receptors have been identified in the RVLM of Wistar rats (Oshima et al., 2014). However, this remains a purely speculative suggestion.

7.2.3 Chapter 5: Sympathetic transduction during isometric handgrip exercise

Data in chapter 5 demonstrate that sympathetic transduction appears to be unaltered during isometric handgrip exercise in healthy young adults and postmenopausal females. As such, low transduction in premenopausal females may persist during exercise and therefore contribute to the smaller pressor response to exercise seen in premenopausal females (Smith et al., 2019). However, individual response of transduction slope to handgrip exercise was variable and the sample size is relatively small. Given the variable responses, an alternative method of assessing sympathetic transduction may provide more information. Methods assessing the change in vascular resistance or blood pressure over the cardiac cycles following a burst of MSNA (Vianna et al., 2012, Robinson et al., 2019) would be informative, but may be difficult when burst incidence becomes very high. Determining the ratio of change in blood pressure to change in MSNA over short intervals (e.g., 30 s, as was used by Minson et al.) may be more effective (Minson et al., 2000). Indeed, recent work assessing sympathetic transduction during handgrip exercise as the ratio of change in peripheral resistance to change in MSNA was able to demonstrate an effect of oral contraception use on transduction in premenopausal female participants (Takeda et al., 2021).

Although the current data in this chapter were variable, the work still contributes to understanding the effect of age on sympathetic and blood pressure responses to static exercise in females. This is an active area of research with recent work by others demonstrating, for example, that hormone replacement in postmenopausal female participants was associated with smaller pressor and sympathetic responses to handgrip exercise (Wenner et al., 2022). Meanwhile, others have shown that age appears to affect the vasodilatory response to acute static contractions differently in male and female participants (Hanson et al., 2021). Overall, work in this field can contribute to understanding the mechanisms underlying pressor responses to exercise, and how this may differ with age and sex.

7.2.4 Chapter 6: Respiratory sympathetic modulation in premenopausal and postmenopausal females

Chapter 6 focused on the role of respiration in modulating sympathetic outflow. In healthy adults, postmenopausal females were found to have smaller respiratory modulation of sympathetic nerve activity compared to premenopausal females (and younger males). Given that respiratory sympathetic modulation was similar in young females and males, there did not appear to be an effect of sex hormones on this regulatory mechanism. However, the age-related change in respiratory modulation seen in postmenopausal females differs from previous studies in healthy males, where age did not affect respiratory sympathetic modulation (Shantsila et al., 2015). Whether females exhibit a particular age-related mechanism that reduces respiratory sympathetic modulation is unclear, although there are some potential such mechanisms for example the loss of oestrogen, which inhibits sympathetic activity in the brainstem (Saleh et al., 2000). When respiratory sympathetic modulation was assessed in hypertensive versus normotensive older adults, there was no effect of hypertension in either males or females, suggesting that hypertension has little influence on respiratory sympathetic modulation. To confirm this, age, sex, hypertension, and resting level of sympathetic activation (burst incidence) were entered into a linear regression model with percentage respiratory sympathetic modulation. Age was the only significant predictor of respiratory modulation, with modulation decreasing with increasing age. Given that the model included younger and older males, the data further contradict the results of Shantsila et al., however younger and older males were not directly compared in the current study.

These data have implications for understanding the mechanisms driving increased sympathetic activation with ageing. Given that MSNA was similar in postmenopausal females and the younger groups during expiration but higher around peak inspiration, it appears that the inspiratory-related inhibition of sympathetic activity is reduced in older females compared to younger adults (rather than postmenopausal females showing increased sympathetic activation at expiration). As such, inspiration may be less able to inhibit sympathetic activity and therefore, older adults likely experience a greater overall level of sympathetic activity.

This chapter contributes to the body of work investigating sex differences in respiratory sympathetic modulation. Recent advances in this field are strengthening the argument that sex differences in respiratory sympathetic modulation exist, particularly in relation to the peripheral chemoreflex. For example, one group has shown that female participants had a greater sympathetic response to hypoxia versus male participants (Sayegh et al., 2022), whilst others demonstrated that respiratory sympathetic coupling is altered following an intermittent hypoxia stimulus differently in male and female participants (Edmunds et al., 2021). The current data showed no sex difference among younger adults under normoxic conditions, suggesting that sex differences in young adults may only become apparent under hypoxic stress. However, the current data do show reduced respiratory sympathetic modulation in postmenopausal versus premenopausal females during normoxia. As such, the effect of hypoxia on respiratory sympathetic modulation in postmenopausal females may be worthy of investigation. Overall, the study of sex differences in respiratory sympathetic modulation may help to understand whether there may be sex-specific mechanisms underlying conditions such as obstructive sleep apnoea. The current work contributes to this field and to the understanding of the link between respiration and the sympathetic nervous system in females.

7.3 Summary and implications

Overall, this thesis has contributed new data to the understanding of sympathetic regulation of blood pressure in females. The data have shown that increased sympathetic transduction may be key in driving high blood pressure in hypertensive premenopausal females, instead of high MSNA. Further study of the

mechanisms driving increased sympathetic transduction could help to identify the most effective targets for anti-hypertensive treatment in premenopausal females. For example, anti-hypertensive medications that reduce sympathetic transduction (e.g., calcium channel blockers) may be more effective than others, however this is purely speculative and would need further investigation. On the other hand, sympathetic transduction is a less important mechanism in hypertension development in postmenopausal females. Therefore, treatments that reduce sympathetic outflow may be more important after the menopause, particularly in postmenopausal females with high levels of sympathetic nerve activity. Alternatively, it could be more effective to target other risk factors for hypertension in postmenopausal females, such as reducing BMI (Rappelli, 2002).

Finally, the data have shown that poorer respiratory modulation of sympathetic nerve activity may be an important driver of increased symapthoexcitation in postmenopausal females. The data suggest that females likely have fewer non-bursting periods after the menopause, due to reduced inspiratory inhibition of SNA. Therefore, over the longer term, postmenopausal females would experience more MSNA bursts compared to premenopausal females. Given that age was identified as the only significant contributor to respiratory sympathetic modulation in a model of age, sex, hypertension, and resting burst incidence, the data have implications for ageing females and males. As such, the data support the notion that poorer respiratory sympathetic modulation contributes to the age-related increase in resenting level of sympathetic activation.

Whilst there was a clear respiratory phase x group interaction for burst incidence and frequency, the current data showed that respiratory modulation of burst area is similar in postmenopausal females and younger adults. This lends some support to the idea that burst firing (i.e. burst or no burst for a given blood pressure) is controlled differently from burst area (i.e. how many sympathetic action potentials occur and which size axons are recruited) (Shoemaker, 2017). However, further work would be required to confirm this.

7.4 Future directions

The current data suggest several directions for future work. Given that the involvement of beta-adrenergic receptors in enhancing sympathetic transduction in hypertensive premenopausal females remains unclear, attempting to complete

these experiments would be useful. However, the beta-blockade study was logistically challenging, and it may be beneficial to approach the question from an epidemiological stance. For example, the role of beta-adrenergic receptor SNPs specifically in hypertensive premenopausal females could be studied, using longitudinal data sets with genomic and health data. An association between SNP/s and hypertension could indicate a role for the beta-adrenergic receptors in enhancing sympathetic transduction in hypertensive premenopausal females. However, these studies would not reveal whether poor beta-adrenergic receptor function independent of genetic variation is present in this cohort.

The mechanism promoting poorer respiratory sympathetic modulation in older adults remains unclear. Given that several physiological mechanisms contribute to respiratory modulation (central processing of SNA outflow, lung-stretch receptors, the peripheral chemoreflex), the contribution of each of these to poorer age-related respiratory sympathetic modulation could be determined. For example, the role of lung-stretch receptors could be quantified using a protocol that measures sympathetic modulation under controlled deep and shallow breathing. Furthermore, the respiratory modulation of burst firing versus burst area could be investigated by interrogating the respiratory modulation of sympathetic action potentials. It could be that sympathetic action potentials of all amplitudes are similarly regulated by respiration, or it could be that only certain action potential subpopulations are modulated by respiration. In support of this, when sympathetic action potentials are grouped by amplitude, some populations are under tighter baroreflex control than others (small/mid-sized groups exhibited greater baroreflex sensitivity) (Salmanpour and Shoemaker, 2012). Whether respiration regulates sympathetic action potential firing in a similar way is unknown, in both health and disease. Such a study would contribute further to the understanding of sympathetic regulatory mechanisms.

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Appendices

Appendix 1: Study literature

Participant Information Sheet

University of BRISTOL | University Hospitals Bristol NHS Foundation Trust | CRiCBristol Clinical Research & Imaging Centre

Sex Differences in the Role of Sympathetic Nerve Activity in the Development of Hypertension in Humans

PARTICIPANT INFORMATION SHEET

Chief Investigator: Dr. Emma Hart, School of Physiology, Pharmacology & Neuroscience, Faculty of Biomedical Sciences, University of Bristol, Bristol, BS8 1TD. Email: emma.hart@bristol.ac.uk

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Please contact Zoe Adams if you have any questions about this study.

You are being invited to take part in a research study at the University of Bristol, which is looking at how high blood pressure develops differently in men and women. This information sheet explains why the study is being done and what happens during the study. If you are interested in taking part in this study, it is important that you read this sheet so that you fully understand what taking part involves. Feel free to discuss this information with your family, friends and GP. You can, of course, also contact us if you have any questions about the study. The study will form part of the PhD thesis for Zoe Adams.

1. Why is this study being done?
High blood pressure is a common condition in the UK, affecting 24% of men and 20% of women in England (in 2015). Having high blood pressure increases the risk of stroke, heart attack and heart failure. However, medications to treat high blood pressure do not work well in everyone. To improve the treatment of high blood pressure, we are trying to better understand how high blood pressure develops.

An individual's chance of developing high blood pressure depends on their age and whether they are male or female. In general, younger women are less likely to get high blood pressure than younger men. This is thought to be because female sex hormones, such as oestrogen, affect the way blood pressure is controlled by the

nervous system. These hormones make it harder for the nerves that control blood pressure (the sympathetic nerves) to raise blood pressure in younger women. This means that most young women are protected against high blood pressure by their hormones. However, despite this protection, a number of young women do still get high blood pressure. Why this happens is not known. Possibly, female sex hormones may affect the sympathetic nerves differently in these women.

Furthermore, women are more likely to get high blood pressure after the menopause, when their levels of female sex hormones have dropped. This might be because the sympathetic nerves find it easier to raise blood pressure when levels of hormones are lower. However, this still needs further investigation.

This study aims to investigate why some young women develop high blood pressure and why the risk of high blood pressure increases after the menopause. To do this we will measure how active the sympathetic nerves are in an individual, using a technique called microneurography. We aim to measure nerve activity in premenopausal and postmenopausal women, as well as younger and older men, with both normal and high blood pressure.

2. What will taking part involve?

Taking part involves one visit to the Clinical Research and Imaging Centre (CRIC), Bristol. For premenopausal women and younger men (younger than approximately 40 years), the visit will last up to **approximately 4 hours 45 min**. For postmenopausal women and older men (older than approximately 40 years), the visit will last up to **approximately 3 hours**. The first part of the visit involves screening. This is where we go through your medical history and complete some screening tests to make sure you are safely able to take part in the study. All participants will complete the screening part of the visit. The second part of the visit is the experimental part. The procedures you complete in this part of the study help us to answer the research questions we are interested in. Which experimental procedures you complete depends on your age (details given below). The flow chart on page 6 shows what happens during the study visit.

<u>Before consent</u>

- After reading this information sheet, if you are interested in taking part please contact Zoe Adams (details at end of sheet).
- We will then contact you to check your eligibility to take part. This will either be by email or over the phone. A member of the Research Team will call you and complete a brief pre-screening questionnaire with you. We will also be able to answer any questions you have about the study.
- If you are eligible to take part according to the pre-screening questionnaire, and you would like to take part, we will then arrange a time for you to come in for the study visit.

- For premenopausal women, we will arrange for the study to take place at the end of a period or in the first few days after a period. Therefore, we will ask about your cycle when arranging the appointment.
- When you arrive for the study visit, we will first explain the study to you and answer any questions you have.
- If then you decide you would like to take part in the study, we will ask you to sign a consent form.
- **Taking part in the study is completely voluntary and you can withdraw at any point, without giving a reason.**
- If you do decide to take part, you will be given a study ID number, which will be used to anonymously label your information.
- During consent, we will also ask for your permission to contact your GP to inform them about any abnormal results we find during the screening tests. This is optional and you are welcome to decline this. This will not affect your participation in the study.

Screening

After consent, we will then complete the following screening tests to ensure you are able to participate in the study. The screening tests may show that you are not eligible to participate in the study, in which case we will not be able to include you in this study and your involvement will end.

- Medical history questionnaire
- Height and weight measurements
- Blood pressure and heart rate measurements
- *Electrocardiography (ECG):* **We will make a recording of your heart's electrical** activity to check for any abnormalities. This involves attaching stickers to your chest. The ECG will be checked by a Doctor.
- *Urine sample:* We will test your urine to check for kidney damage or signs of diabetes. For premenopausal women, we will also complete a urine pregnancy test. Your urine sample is discarded immediately after testing.
- *24-hour blood pressure monitor:* At the end of your visit, you will be given a blood pressure monitor to wear at home for 24 hours. This measures your blood pressure every 30 minutes during the day and every hour at night. The 24-hour monitor provides a more accurate measurement of blood pressure than the measurements we take during screening. You will also be given a blood pressure diary to fill in with details of when you went to sleep, when you ate etc. over the 24 hours. We will provide stamped addressed packaging for you to return the blood pressure monitor to us after you have worn it.

Note: **These screening tests and the Doctor's analysis of the ECG are for the** purposes of the study only and should not be relied upon for the identification of undiagnosed medical conditions.

Experimental procedures

After the screening tests, we will then complete the experimental procedures. The procedures you complete depend on your age; premenopausal women and men younger than 40 years old have more procedures to complete.

For everyone:

- Blood sample

We will take a blood sample to test for levels of sex and catecholamine hormones. This will confirm premenopausal status in women. Your blood sample will be tested at the Department for Clinical Biochemistry at the Bristol Royal Infirmary. After testing, the sample will be disposed of in accordance with the Human Tissue Authorities Code of Practice.

- Microneurography

This is a technique used to measure the activity of the nerves that control blood pressure (the sympathetic nerves). We use two small electrodes, similar to acupuncture needles. One is inserted into a nerve in the lower leg called the peroneal nerve. The other is placed into the surface of the skin nearby the nerve and acts as a reference. It may take up to an hour to position the electrodes correctly. We will record your sympathetic nerve activity at rest and whilst you perform some handgrip exercise.

- Handgrip exercise

Handgrip exercise involves squeezing a hand-held device. You will squeeze the device at 40% of your maximal voluntary contraction (the contraction generated with maximum effort). To work out 40%, we will ask you to first squeeze the device with maximum effort three times. The handgrip procedure will tell us how the changes in nerve activity and blood flow to the arm seen during exercise differ between men and women, younger and older people, and people with high or normal blood pressure. We will measure the blood flowing to your arm using an ultrasound probe (see below).

- Vascular ultrasound

This technique uses ultrasound to measure the blood flow to the arm before, during and after handgrip exercise. To do this we place an ultrasound probe (similar to those used in a pregnancy ultrasound scan) on your upper arm. We will also use ultrasound to scan the carotid arteries by placing the probe on your neck. This is to measure blood flow in the carotid artery.

- Echocardiography

This is another ultrasound technique used to measure the volume of blood your heart pumps during each heartbeat and other measurements, such as the wall thickness of the main artery taking blood away from your heart (aorta). To do this we place an ultrasound probe to your chest.

- Physiological monitoring

Throughout the study we will record your heart rate using three ECG stickers. Blood pressure will be measured by a small cuff that inflates around the end of a finger. Breathing will be monitored by a belt placed around the chest (at the diaphragm).

For premenopausal women and men younger than approx. 40 years old:

- Infusion of propranolol

In younger men and women, we will also measure the activity of your sympathetic nerves whilst you receive a beta-blocker drug (propranolol). Propranolol is a drug that is routinely used as a treatment for different cardiovascular conditions. Propranolol blocks certain receptors in the blood vessels called beta-adrenergic receptors. These receptors are important in explaining how female sex hormones keep blood pressure low in healthy young women. Using propranolol in this study will help us to understand the role of these receptors in young women with *high* blood pressure.

After 10 minutes of recording resting nerve activity, younger men and premenopausal women will receive a propranolol infusion. During the infusion, we will make a further 10-minute recording of your nerve activity, and then ask you to perform handgrip exercise again. We will also re-measure the blood pumped by your heart each heartbeat (echocardiography). Propranolol will be given to you via a cannula, which is a small, flexible tube inserted into a vein in your arm. The cannula will be inserted by either a Research Nurse or Doctor with a needle. The needle is removed but the cannula remains in place for the duration of the study. At the end of the study, the Nurse will remove the cannula and put a small dressing over the site. At the end of the study, we will ask you to wait at CRIC for one hour so that we can check for side effects of propranolol.

Note on incidental findings
Some of the study procedures (ECG, blood pressure monitoring, urine sample testing and blood sample testing for sex and catecholamine hormones) could identify abnormal results. If abnormal results are found in ECG or urine you may be unable to take part in the rest of the study. We will inform you of any abnormalities in urine or ECG during the study visit. 24-hour blood pressure monitoring and testing of your blood sample takes place after the study visit. If abnormal results are found in your blood pressure or blood sample, we will contact you to inform you. However, please note that the testing of blood samples for catecholamine hormones will take place at the end of the study. Therefore, we may not know the results of this test for 2-3 years after you took part in the study. We will also inform your GP of any abnormal results, if you have given permission for this.

3. Where will the study take place?

This study will take place at the Clinical Imaging and Research Centre, Bristol. The full address is:

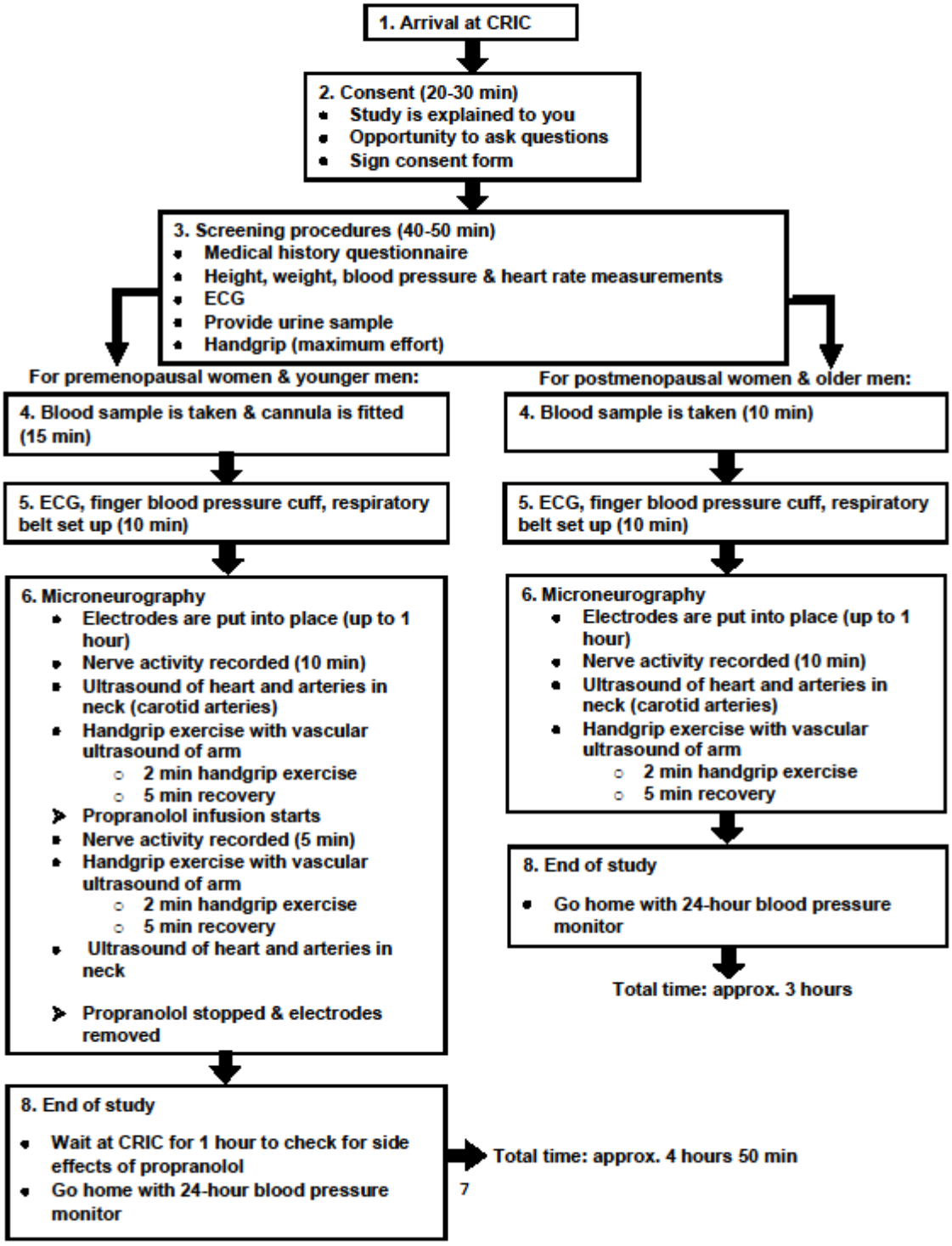
Clinical Research and Imaging Centre (CRIC Bristol)
60 St Michael's Hill,
Bristol,
BS2 8DX
Tel (reception): 0117 342 1500

4. What do I need to do before the study visit?

There are several things we will ask you to do before you attend the appointment:

- Please wear or bring comfortable clothing. To make recordings from the nerve in your leg we will need one of your legs to be bare from mid-thigh down during the experiment. Therefore, you may wish to bring your own shorts or trousers that roll up comfortably.
- Please do not take painkillers such as aspirin, paracetamol or ibuprofen for 24 hours before the study.
- However, you should take any medications that have been prescribed by your Doctor. Please let us know which medications you have taken on the day of the study.
- Please do not drink alcohol on the day of the study or the evening before the study.
- Please do not drink caffeine on the morning of the study.
- Please do not exercise on the day of the study or after 7pm the evening before the study.

Flow chart showing the order of the study visit



5. Must I take part?

No. It is entirely your decision. If you are interested, please let us know by contacting us (details at the top and bottom of this sheet). Signing a consent form only happens when you are completely happy that all your questions have been answered. You are also free to withdraw from the study at any time point, without having to give a reason.

6. What are the possible side effects of taking part?

For everyone:

- Microneurography

Microneurography sometimes causes numbness or tingling (parathesia) in the lower leg for 3-7 days after the procedure is completed. However, this is reported to occur in less than 10% of cases. To minimise the risk of infection, we use sterile, single-use electrodes and a 'no-touch' technique, after we have sterilised your skin.

- Physiological monitoring

Repeated blood pressure measurements may lead to mild discomfort and numbness in the arm or finger. There are no known risks associated with monitoring of heart rate or breathing.

- Handgrip exercise and vascular ultrasound/echocardiography

There are no known risks associated with handgrip exercise, or use of ultrasound to measure either blood flow in the arm or neck (vascular ultrasound) or blood pumped by the heart (echocardiography).

- Venepuncture for blood sample

During the study a Research Nurse or Doctor will take a blood sample, which will be tested for levels of sex steroid hormones. You may experience mild discomfort during venepuncture and/or mild swelling at the site.

For premenopausal women and men younger than 40 years old:

- Propranolol infusion

Propranolol has side effects including:

Uncommon (1 to 10 patients in 1000):

- Especially at start of treatment: Tiredness, vertigo, dazedness, confusion, nervousness, sweating, headache, sleep disorders, depressive mood, nightmares, hallucinations, false sensations (paraesthesia), feeling of cold in the limbs.
- Transient gastrointestinal symptoms (diarrhoea, constipation, nausea, vomiting).
- Allergic skin reactions (redness, itching, exanthema) and hair loss
- Increased fall in blood pressure, severe slowing of heart rate (bradycardia), convulsive, short-term loss of consciousness (syncope), heart pounding (palpitations), atrio-ventricular conduction disorders or increase in heart muscle weakness (heart failure).

Rare (1 to 10 patients in 10,000):

- A clinical picture similar to that of myasthenia gravis (pathological muscle weakness or tiredness)
- Dry mouth

- Inflammation of connective eye tissue (conjunctivitis), reduced tear flow (look for this in wearers of contact lenses)
- Small areas of bleeding in the skin and muccus membranes (purpura) or reduction in the thrombocyte count (thrombocytopenia)
- Occurrence of previously undetectable sugar disease (latent diabetes mellitus) or worsening of already existing sugar disease.

Very rare (fewer than 1 patient in 10,000):

- Increase in existing myasthenia gravis.
- Outbreak of psoriasis vulgaris, increase in symptoms of the disease, scaly skin-like (psoriasiform) skin rashes.
- Increase in seizures in pain occurring convulsively in the region of the heart (angina pectoris), increase in the symptoms of peripheral blood flow disorders, including intermittent limping (intermittent claudication) and spasms of the arteries in the fingers **(Raynaud's syndrome).**
- In long-term treatment, joint diseases (arthropathy), in which one joint (monoarthritis) or several (polyarthritis) may be affected.
- Libido and potency disorders.
- Elevation of liver enzymes (GOT, GPT) in the blood.
- In severe kidney function disorders: worsening of kidney function. Kidney function should be appropriately monitored during treatment with Dociton.

Frequency unknown:

- Hypoglycaemia including hypoglycaemic seizures.

Additionally, there is a risk of allergic reaction to the propranolol.

Propranolol is used in this study to completely block the beta-adrenergic receptors. For this reason, the dose used in the study is above the maximum dose used clinically. This dose and greater doses have been used in previous research studies, including studies conducted by Dr Hart (Chief Investigator of this study), to completely block the beta-adrenergic receptors. There were no adverse events associated with using this/greater doses of propranolol in the study previously conducted by Dr Hart. Due to the short duration of propranolol infusion, we expect the risk of side effects to be low.

The propranolol will be given to you by a Research Nurse, who will also monitor you throughout. A Doctor will also be available. At the end of the study, we will ask you to wait at CRIC for one hour to confirm that you have not had any side effects. To reduce the risk of you having an allergic reaction to these drugs, we will ask about your allergies during screening.

- <u>Cannulation</u>

Propranolol will be given to you by a cannula. This is a small, flexible tube inserted with a needle into a vein in your arm, by a Research Nurse or Doctor. You may experience mild discomfort during fitting of the cannula.

7. Who is able to take part in the study?

Men and women aged 18-75 years old with either high blood pressure or normal blood pressure are able to take part in this study. Both premenopausal and postmenopausal women can take part. Women are classed as postmenopausal if they have not had a period for at least one year.

8. Who is not able to take part in the study?

For safety reasons, people with the following conditions/criteria are unable to take part in the study:

For everyone:

- Major illness e.g. cancer, inflammatory disease (including vasculitis) or receiving palliative care
- Diagnosed cardiovascular (including arrhythmia), respiratory (including asthma), psychiatric, renal or ophthalmic disease
- Congenital or acquired neurological conditions (including dementia), language disorders, repeated or chronic pain conditions (excluding menstrual pain and minor sporadic headaches)
- Diabetes
- **A body mass index ≥30 kg**/m^2
- Pregnancy
- Ovaries removed before natural menopause
- Taking hormone replacement therapy
- Taking nitrate, steroid, anti-coagulant or immunosuppressant medication or medication as part of a clinical trial
- Flu-like symptoms or fever less than a week before the study
- Regularly drink more than 28 units of alcohol per week or use recreational drugs
- Needle phobia
- Unable to understand instructions given in English

In addition, for people with high blood pressure:

- Secondary causes of high blood pressure

In addition, for premenopausal women and men younger than 40 years old:

- Known hypersensitivity to propranolol, beta-blockers or any other ingredients in the propranolol preparation; history of severe hypersensitivity (allergic reaction) or receiving treatment to weaken allergic reactions.
- Low heart rate (<50 beats/min at rest) or low blood pressure
- Current or history of asthma
- Late-stage peripheral blood flow disorders
- Poor liver or kidney function

- Recent lengthy strict fasting or severe physical stress
- History (or family history) of psoriasis
- Taking monoamine-oxidase inhibitors, medication to lower blood pressure or any other medications that are contraindicated or known to interact with propranolol

9. What are the possible benefits of taking part?

This study will help us to better understand how high blood pressure develops differently in men and women. We will investigate why some young women get high blood pressure, even though most women this age are protected against high blood pressure by female sex hormones. Furthermore, the study will help us to understand why the risk of getting high blood pressure increases after the menopause.

10. Will I receive any reimbursement for taking part in the study?

We will reimburse you for your time and travel to the Clinical Research and Imaging Centre, Bristol. Younger participants taking part in the study (which is a longer duration) will receive a total reimbursement of £50. Older participants, taking part in the shorter study, will receive a total reimbursement of £25.

11. What happens if I don't want to carry on with the study?

You are free to withdraw from the study at any time, without giving a reason. Identifiable data already collected, with consent, will be retained and used in the study. A decision to withdraw at any time, or a decision not to take part, will not affect your ongoing medical care in any way.

12. What if something goes wrong?

In the unlikely event that you are harmed in this study, there are no specific **compensation arrangements. If you are harmed due to someone's negligence, t**hen you may have grounds for a legal action but you may have to pay for it. This study will be sponsored by the University of Bristol. The University has Public Liability insurance to cover the liability of the University to research participants. If you have any general complaints regarding the study please contact Dr. Emma Hart. Contact details are listed at the start of this information sheet.

13. Will my identity be protected/what happens to my personal data?

The University of Bristol is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. The University of Bristol will keep identifiable information about you for 15 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum

personally-identifiable information possible. You can find out more about how we use your information by contacting a member of the study team (PhD student: Zoe Adams, zoe.adams@bristol.ac.uk; Chief Investigator: Dr Emma Hart, emma.hart@bristol.ac.uk).

This study is a collaboration between the University of Bristol and University Hospitals Bristol NHS Foundation Trust. The staff working on this study may be employed by either organisation or have dual contracts with both. Study staff will collect information from you and your medical records for this research study in accordance with our instructions.

The University of Bristol and University Hospitals Bristol will use your name, date of birth and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from University Hospitals Bristol and regulatory organisations may look at your medical and research records to check the accuracy of the research study. Study staff will pass these details to University Hospitals Bristol along with the information collected from you and your medical records. The only people in University Hospitals Bristol who will have access to information that identifies you will be people who need to contact you, such as study staff, or those auditing the data collection process. The people who analyse the information collected in the study will be members of the study team who will have access to your name, date of birth and contact details.

The University of Bristol and University Hospitals Bristol will keep identifiable information about you from this study for 15 years after the study has finished.

Anonymised data collected in this study may be used in future research. Should this occur, the data will be shared anonymously with other researchers. If in the course of the research, results are found that could be important for your personal health, study staff will contact you and, with your permission, your GP who may subsequently require further information. Your blood sample will be analysed by both study staff and University Hospitals Bristol staff in the Department of Clinical Biochemistry. Staff in Clinical Biochemistry will only have access to your study ID, age and sex.

14. What happens to my blood sample?

Whole blood is not stored but serum/plasma, which contains hormones, is stored. We will only analyse the sample for different levels of hormones. We do not look at DNA. Samples will be stored in a locked freezer at a University of Bristol or a NHS UH Bristol site, with an ID number, your sex and your age on the sample. Samples will be discarded after testing.

15. What happens at the end of the study?

The full results of the study will not be known until the last participant has been tested. If you are interested in receiving a summary of the results, please contact Zoe Adams (contact details are given at the head and base of this sheet).

16. Who is funding the study?

The study is funded by the British Heart Foundation, as part of the PhD Studentship Grant for Zoe Adams.

17. Who has reviewed the study?

The study has been reviewed by South West – Frenchay Research Ethics Committee and adheres to the approved safety and ethical procedures of the Clinical Research and Imaging Centre (CRIC), Bristol.

18. Insurance

Insurance/Indemnity will be provided by the Sponsor, University of Bristol.

19. What do I do now?

If you are interested in taking part in the study, or have any questions, please contact Zoe Adams by email or phone (details below).

Thank you for reading this information sheet and for considering whether you would like to take part in the study.

Contact information

If you are interested in taking part in the study, or have any questions, please contact:

Zoe Adams

Email: zoe.adams@bristol.ac.uk

Phone: 0117 342 1513

University of BRISTOL | University Hospitals Bristol NHS Foundation Trust | CRiCBristol Clinical Research & Imaging Centre

Telephone Screening Questionnaire

Sex Differences in the Role of Sympathetic Nerve Activity in the Development of Hypertension in Humans

Name			Screen ID			
Age (years)		Sex			Screen date	
Height (specify units)		**Hypertension status**	**YES**		**NO**	**NOTES**
Weight (specify units)		Diagnosed hypertension?				
BMI (kg/m^2) If >30, excluded.		Taking anti-hypertensive medication?				

Younger potential participants must provide an accurate weight, as this will be used to order the propranolol.

	YES		NO	NOTES
Answering yes to the following excludes the individual from taking part.				
Major illness e.g. cancer, inflammatory disease; receiving palliative care.				
Diagnosed cardiovascular, respiratory, psychiatric, renal, ophthalmic disease.				
Neurological conditions, language disorders, chronic pain.				
Diabetes.				
Taking nitrate, steroid, anti-coagulant or immunosuppressant medication.				
Taking medication as part of clinical trial.				
Consume >28 units alcohol per week or take recreational drugs.				
Phobia of needles.				
Recent febrile illness (within 2 weeks of study visit).				
In addition, for women only. Only certain criteria exclude the individual from taking part.				
Postmenopausal?				
If postmenopausal, has it been >1 year since last period? *(If no, excluded).*				
If postmenopausal, taking HRT *(If yes, excluded).*				
Ovaries removed before natural menopause *(If yes, excluded).*				

Pregnant or breastfeeding *(If yes, excluded).*				
Taking oral contraceptives *(If yes, study visit will be arranged during placebo phase).*				
In addition, for premenopausal women and men <40 years only. Answering yes to the following excludes the individual from taking part.				
Current/history of asthma.				
Known hypotension or bradycardia (<50 beats/min).				
Known allergy to propranolol/beta-blockers.				
History of severe allergic reaction/receiving treatment to weaken allergic reactivity.				
Fasting or severe physical stress.				
Late stage peripheral blood flow disorders.				
Known impaired liver or kidney function.				
History/family history of psoriasis.				
Take any medications? Taking the following medications excludes the individual from taking part: - Insulin/oral antidiabetics. - Anti-hypertensive medications (nitroglycerin, diuretics, vasodilators, calcium antagonists of nifedipine/verapamil/diltiazem types). - Tricyclic anti-depressants - Phenothiazines - Barbiturates - Antiarrhythmics e.g. disopyramide or others - Cardiac glycosides - Reserpine - Alpha-methyldopa - Guanfacine, clonidine - Adrenaline, noradrenaline - Monoamine oxidase inhibitors - Indometacin - Narcotics - Peripheral muscle relaxants e.g. suxamethonium, tubocurarine - Cimetidine - Quinidine, propafenone, rifamycin, theophylline, warfarin, thioridazine, nifedipine, nisoldipine, nicardipine, isradipine, lacidipine				

Is there any other information that the individual feels is relevant?
Convenient times/dates:

Screening Questionnaire

Sex Differences in the Role of Sympathetic Nerve Activity in the Development of Hypertension in Humans
Screening Questionnaire v1 24/09/2018

University of BRISTOL | University Hospitals Bristol NHS NHS Foundation Trust | CRiCBristol

Screening Questionnaire

Date:		**Study ID:**
Patient initials:		
Date of Birth (dd/mm/yyyy): **Age:**		**Gender (tick box)** **Male** ☐ **Female** ☐
Height:	**Weight:**	**BMI:**
GP contact details:		

Patient Questions (*tick relevant box*)

Questions	Yes/No	Comments
1. Do you have any allergies? ***Please comment on which allergies if yes***	**Yes** ☐ **No** ☐	
2. Do you have asthma? **2A. If YES do you use an inhaler?** **2B. If YES what type of inhaler?**	**Yes** ☐ **No** ☐ **Yes** ☐ **No** ☐	**Inhaler type:** _____ **Used times/month?** _____
3. Have you ever been diagnosed with hypertension?	**Yes** ☐ **No** ☐	**If YES: for how many years?** **Comment on family history:** **MAT:** **PAT:** **SIB:**

Please turn over STUDY ID: ☐☐☐☐☐☐

4. Have you ever been diagnosed with heart disease? **e.g. Heart attack** **Chronic heart failure** **Chronic arrhythmia** **4A. If YES what was diagnosed?** **4B. Have any of your immediate family members had a heart attack when they were <60 yrs old?**	**Yes** ☐ **No** ☐ **Yes** ☐ **No** ☐	**What was diagnosed?** **Date of diagnosis?** **Comment on family history:** **MAT:** **PAT:** **SIB:**
5. Have you ever had a stroke or a trans-ischemic attack? **5A. If YES what was diagnosed?**	**Yes** ☐ **No** ☐	**What was diagnosed?** **Date of diagnosis?** **Comment on family history:** **MAT:** **PAT:** **SIB:**
6. Do you have diabetes? **6B. If YES, which type?**	**Yes** ☐ **No** ☐ **Type I** ☐ **Type II** ☐	**Comment on family history:** **MAT:** **PAT:** **SIB:**
7. Do you have sleep apnoea?	**Yes** ☐ **No** ☐	**Date of diagnosis?**
8. Do you have any respiratory disorders? **e.g. COPD**	**Yes** ☐ **No** ☐	**What was diagnosed?** **Date of diagnosis?** **Comment on family history:**

Please turn over

STUDY ID: ☐☐☐☐☐☐

8A. If YES what was diagnosed?		**MAT:** **PAT:** **SIB:**
9. Do you have a nerve or neurological disorder? **e.g. Parkinsons disease, peripheral neuropathy** **9A. If YES what was diagnosed?**	**Yes** ☐ **No** ☐	**What was diagnosed?** **Date of diagnosis?** **Comment on family history:** **MAT:** **PAT:** **SIB:**
10. Do you have kidney disease or renal failure? **10A. If YES what was diagnosed?**	**Yes** ☐ **No** ☐	**What was diagnosed?** **Date of diagnosis?** **Comment on family history:** **MAT:** **PAT:** **SIB:**
11. Are you being treated for cancer? **11A. Have you ever had cancer?**	**Yes** ☐ **No** ☐ **Yes** ☐ **No** ☐	**Date of remission?**
12. Are you receiving palliative care? **e.g. Receiving care for a terminal medical condition**	**Yes** ☐ **No** ☐	

13. Have you ever had significant or major surgery? ***If yes please state where (e.g. left knee/stomach/head)***	**Yes** ☐ **No** ☐	**Which procedures/surgeries?** **Date of surgery or procedure:**
14. Have you currently got any lower leg injuries?	**Yes** ☐ **No** ☐	**What injuries and where?**
15. Have you ever smoked/used tobacco? If yes, please state how long you smoked for (in years) or when you gave up.	**Yes** ☐ **No** ☐	**Years smoked:** _____ **Date you gave up if applicable (mm/yyyy):** _____
16. How many units of alcohol do you drink per week?	**< 10 units** ☐ **< 14 units** ☐ **< 28 units** ☐	
17. Do you use reactional drugs?	**Yes** ☐ **No** ☐	**Type of drug** **Date last used (dd/mm/yy)** **Frequency of use**
18. Do you take any medications? ***If yes please list types of medication in the comments box*** **18A. If yes, are you currently taking any steroids, immunosupressants, anti-coagulants or nitrates?**	**Yes** ☐ **No** ☐ **Yes** ☐ **No** ☐	**Please state which medications**

Please turn over

STUDY ID: ☐☐☐☐☐☐

18B. Are you taking medication as part of a clinical trial?	**Yes** ☐ **No** ☐	
19. Do you exercise regularly?	**Yes** ☐ **No** ☐	**Type of activity** **Times per week**
20. Have you had a viral illness in the last two weeks (e.g. Flu)	**Yes** ☐ **No** ☐	
21. Do you have a phobia of needles that would prevent your participation in this study?	**Yes** ☐ **No** ☐	
FOR WOMEN ONLY		
22. Are you postmenopausal?	**Yes** ☐ **No** ☐	**What type of HRT?**
22A. If YES, are you taking HRT?	**Yes** ☐ **No** ☐	
22B. If NO, could you be pregnant?	**Yes** ☐ **No** ☐	
23. Are you using oral / injectable / implantable contraceptives? **23A. If YES, what type?**	**Yes** ☐ **No** ☐	**What type?** **e.g. POP, COCP, IUD, Depot injection, implant**

Please turn over

STUDY ID: ☐☐☐☐☐☐

24. Are you currently menstruating? **24A. If NO, when was your last menstrual period?**	**Yes** ☐ **No** ☐	
25. Have you ever been diagnosed with pre-eclampsia?	**Yes** ☐ **No** ☐	
26. Do you have any other medical condition that you feel is relevant to this research study?	**Yes** ☐ **No** ☐	

Participant
Print Name: _____ Sign: _____ Date: _____

Investigator
Print Name: _____ Sign _____ Date: _____

Please turn over STUDY ID: ☐☐☐☐☐☐

Consent Form

Sex Differences in the Role of Sympathetic Nerve Activity in the Development of Hypertension in Humans
IRAS project ID: 243054
Consent Form V3 04/10/2019

University of BRISTOL

University Hospitals Bristol NHS Foundation Trust

CRICBristol Clinical Research & Imaging Centre

Sex Differences in the Role of Sympathetic Nerve Activity in the Development of Hypertension in Humans

Chief investigator: Dr. Emma C. Hart, School of Physiology and Pharmacology, University of Bristol, Biomedical Sciences Building, University Walk, Bristol, BS8 1TD. Email: emma.hart@bristol.ac.uk. Tel: 0117 331 1472

Research Staff: Zoe Adams, PhD Student, School of Pharmacology and Physiology, Faculty of Biomedical Sciences, University of Bristol, BS8 1TD. Email: zoe.adams@bristol.ac.uk Tel: 0117 342 1513

CONSENT FORM

Participant Identification Number.....

Please initial boxes

1. I confirm that I have read and understand the participant information sheet (version 3 dated: 19/07/2019) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, and that I can decline any part of this study at any time without my medical care or legal rights being affected. ☐
3. I agree for a venous blood sample to be taken and tested for levels of sex steroid and catecholamine hormones. I understand that the sample will be discarded after testing. ☐
4. I understand that sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
5. I understand that anonymised information collected about me may be used in future research and may be shared anonymously with other researchers. ☐
6. I agree to my General Practitioner (GP) being informed that I am taking part in this study and/or if any abnormality is found in ECG, blood pressure, blood sample or urine tests. Yes ☐ No ☐
7. If any situation arose in which I became unable to continue giving informed consent, I would be excluded from further participation, however I agree that data already collected may be included in the study. ☐
8. I agree to take part in the above study ☐
9. Please send me the lay summary of results via (delete as appropriate) email/post. Y/N

Name of Participant	Date	Signature

Name of person taking consent	Date	Signature

Laboratory protocol for measurement of plasma oestradiol

Redacted due to permissions issue.

Transcript of instruction for use for intravenous propranolol

Redacted due to permission issue.

Propranolol prescription and dilution calculation sheet

PROPRANOLOL INFUSION FOR SEX DIFFERENCES STUDY

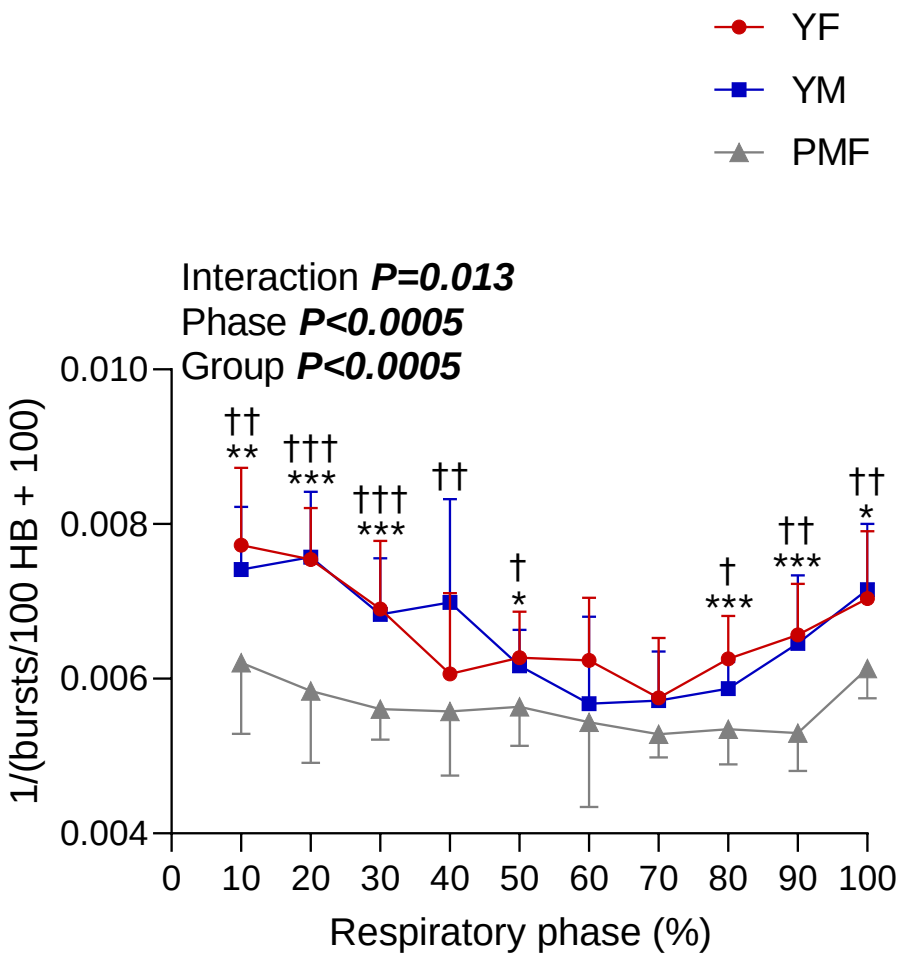
BHI CardioNomics

INSTRUCTIONS FOR USE
1. Enter participant's weight (kg) into the green box.
2. Follow the dilution procedures for the loading and maintenance doses to make up the infusions.

GENERAL POINTS
Propranolol is supplied in 1ml vials at a concentration of 1mg/ml. Propranolol will be diluted with 0.9 % saline and administered at a concentration of 0.5 mg/ml.
Propranolol is administered in a loading dose (0.15 mg/kg) over 10 minutes, followed immediately by a maintenance infusion (dose of 0.004 mg/kg/min) for 30 min.
Both the loading and maintenance infusions are made up directly into 60 ml or smaller syringes, although the syringes may not be full. The concentration in the syringes is 0.5 mg/ml for both infusions.
The loading infusion is administered manually by giving one-tenth of the volume in the loading syringe per minute. The cannula should be flushed with saline after the loading dose has been administered. The maintenance infusion is administered at the rate specified in this sheet using the infusion pump.

PROPRANOLOL INFUSION PRESCRIPTION				
Study visit date:			Total number of propranolol vials required:	
Study ID:	Participant age:	years	18	vials
Participant's weight	65	kg	Propranolol concentration: 0.5 mg/ml	
Loading dose dilution performed by:			Checked by:	
Maintenance dose dilution perfomed by:			Checked by:	
Maintenance dose infusion rate:	31.2	ml/hour	Maintenance dose duration:	30 min
Prescriber name:	Sign:			
Administration and infusion rate check:				
Time loading dose given:		Given & cannula flushed by:	Sign:	
Time maintenance infusion started:		Given by:	Sign:	
Time maintenance infusion finished:		Removed by:	Second check:	

Appendix 2: Supplementary data for chapter 6



Supplementary Figure 1 Respiratory modulation of transformed burst incidence over 10-percentage phases of the respiratory cycle in healthy premenopausal females (N=11), postmenopausal females (N=12) and younger males (N=11). A reciprocal transform was applied to the data after the addition of a constant of 100. One premenopausal female participant and one postmenopausal female participant with residuals >3 SD were removed from the analysis. After transformation the data met the assumption of equal variance of residuals. Two-way mixed model ANOVA on the transformed data produced a significant phase x group interaction, similar to the result on the untransformed data, therefore the untransformed data is included in the main results section of the chapter. Greenhouse-Geisser correction was applied to the interaction P value to correct for violation of the assumption of sphericity. F(10.068, 161.096) = 2.347, *P*=0.013 (Greenhouse-Geisser corrected), partial η^2=0.128). Data are mean ± SD. P values denote the significance of pairwise comparisons (Tukey post-hoc test following separate univariate analysis of group differences at each

respiratory phase); *P<0.05, **P<0.01, ***P<0.001 postmenopausal versus premenopausal females; †P<0.05, ††P<0.01, ††P<0.001 postmenopausal females versus younger males. There were no significant pairwise comparisons between premenopausal women and younger males at any respiratory phase.

Supplementary Table 1 Statistical test data for 10-respiratory phase comparison of burst incidence.

Respiratory phase (%)	Burst incidence (bursts/100 HB)			Test statistic	*P* value	Partial η^2	Pairwise comparison	*P* value
	YF	YM	PMF					
0-10	29 ± 16	36 ± 16	63 ± 20	F(2, 34) = 12.813	***<0.0005***	0.430	PMF vs. YF	***<0.0005***
							PMF vs. YM	***0.002***
							YF vs. YM	0.601
10-20	32 ± 13	34 ± 15	73 ± 25	F(2, 34) = 19.964	***<0.0005***	0.540	PMF vs. YF	***<0.0005***
							PMF vs. YM	***<0.0005***
							YF vs. YM	0.960
20-30	46 ± 19	48 ± 17	78 ± 13	F(2, 34) = 15.685	***<0.0005***	0.480	PMF vs. YF	***<0.0005***
							PMF vs. YM	***<0.0005***
							YF vs. YM	0.924
30-40	66 ± 36	48 ± 27	83 ± 22	F(2, 34) = 4.675	***0.016***	0.216	PMF vs. YF	0.338
							PMF vs. YM	***0.012***
							YF vs. YM	0.261
40-50	57 ± 19	63 ± 12	78 ± 15	F(2, 34) = 5.525	***0.008***	0.245	PMF vs. YF	0.068
							PMF vs. YM	***0.008***
							YF vs. YM	0.654
50-60	64 ± 22	83 ± 38	83 ± 40	F(2, 34) = 1.234	0.304	0.068	PMF vs. YF	0.359
							PMF vs. YM	1.0
							YF vs. YM	0.382

60-70	71 ± 26	77 ± 18	83 ± 27	F(2, 34) =0.718	0.495	0.041	PMF vs. YF	0.463
							PMF vs. YM	0.800
							YF vs. YM	0.848
70-80	61 ± 13	71 ± 10	87 ± 16	F(2, 34) = 12.306	***<0.0005***	0.420	PMF vs. YF	***<0.0005***
							PMF vs. YM	***0.011***
							YF vs. YM	0.189
80-90	51 ± 17	57 ± 21	90 ± 17	F(2, 34) = 16.754	***<0.0005***	0.496	PMF vs. YF	***<0.0005***
							PMF vs. YM	***<0.0005***
							YF vs. YM	0.680
90-100	44 ± 16	42 ± 16	63 ± 10	F(2, 34) = 8.042	***0.001***	0.321	PMF vs. YF	***0.003***
							PMF vs. YM	***0.001***
							YF vs. YM	0.705

YF; premenopausal females, YM; younger males, PMF; postmenopausal females, HB; heartbeats F; ANOVA test statistic. Simple main effects analysis of group was tested by one-way ANOVA at each respiratory phase, following a significant phase x group interaction (two-way mixed model ANOVA). Pairwise comparisons are Tukey, data are mean ± SD.