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INTERACTIONS BETWEEN SYMPATHETIC BAROREFLEX SENSITIVITY AND VASCULAR TRANSDUCTION IN MALES AND FEMALES

Sarah Hissen BHSc (Hons II-I)

School of Science and Health

Western Sydney University

Supervisor:

Doctor Chloe Taylor

Co-supervisor:

Professor Vaughan Macefield

Co-supervisor:

Doctor Rachael Brown

A thesis submitted to Western Sydney University in fulfilment of the requirements of Doctor of Philosophy 2019

ACKNOWLEDGEMENTS

My research would not have been possible without the aid and support of my three supervisors, Dr Chloe Taylor, Professor Vaughan Macefield and Dr Rachael Brown. I have learnt so much from each of you over the last four years and being under your supervision has been a pleasure. Thank you for providing me the opportunity to conduct this research and opening many doors which have and will advance my career in research.

I am profoundly grateful to Chloe who first introduced me to the world of microneurography. From day one you showed me constant support, encouragement and the potential I never thought I had. I really do appreciate the time, effort and patience you've put in throughout my PhD and your assistance in putting my thesis together, especially in the last few months.

I would like to thank the Integrative Physiology team for their assistance in the lab and advice whenever I needed it. I am lucky to have shared all the highs and lows of my PhD journey with other postgraduate students in both Sport Science and School of Medicine at WSU.

To my mum, dad and brothers and sisters - Thank you for always being there when I needed to talk and supporting me throughout this journey even though you didn't always understand what I was doing but knew how important it was to me. Love you all.

A special thanks to all the people I've met since moving to Perth a year ago. Thanks for putting up with my crazy moments while writing this thesis. You have made Perth my home away from home.

STATEMENT OF ORIGINAL AUTHORSHIP

The work presented in this thesis is based entirely on my own independent work, except where research has been done in collaboration with other colleagues and is stated in the studies listed below. This thesis has not been submitted for a degree or diploma at any institution of higher education.

[redacted]

Sarah Hissen

Publications arising from the work conducted in this thesis

Hissen, S. L., El Sayed, K., Macefield, V. G., Brown, R., & Taylor, C. E. (2017). Muscle sympathetic nerve activity peaks in the first trimester in healthy pregnancy: a longitudinal case study. *Clin Auton Res*. 27(6): 401-406. doi:10.1007/s10286-017-0439-1

Hissen, S. L., Sayed, K. E., Macefield, V. G., Brown, R., & Taylor, C. E. (2018). The stability and repeatability of spontaneous sympathetic baroreflex sensitivity in healthy young individuals. *Front Neurosci, 12*, 403. doi:10.3389/fnins.2018.00403

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EXPERIMENTAL STUDIES

LIST OF ABBREVIATIONS

µm	Micrometre
bpm	Beats per minute
BHS(Hons)	Bachelor of Health Science (Honours)
α	Alpha
α′	Intercept
ATP	Adenosine triphosphate
AU	Arbitrary units
b′	Slope
β	Beta
B2	Beta2
BRS	Baroreflex sensitivity
BRS_{inc}	Baroreflex sensitivity characterised using MSNA burst incidence
BRS_{total}	Baroreflex sensitivity characterised using total MSNA
cm	Centimetre
CI	Confidence interval
CO	Cardiac output
DBP	Diastolic blood pressure
ECG	Electrocardiogram

HB	Heartbeat
HR	Heart rate
Hz	Hertz
ICC	Intra-class correlation
kHz	Kilohertz
m	Metre
mA	Milliamps
Min	Minute
mm	Millimetre
mmHg	Millimetres of mercury
MAP	Mean arterial pressure
MHz	Megahertz
ms	Millisecond
MSNA	Muscle sympathetic nerve activity
MVC	Maximum voluntary contraction
NPY	Neuropeptide-Y
NTS	Nucleus tractus solitarus
OLP	Ordinary least products
OLS	Ordinary least squares

RAAS	Renin-angiotensin-aldosterone system
RMS	Root mean square
ROI	Region of interest
RVLM	Rostral ventrolateral medulla
s	Second
SBP	Systolic blood pressure
SD	Standard deviation
SFA	Superficial femoral artery
T50	Diastolic pressure at which 50% of cardiac cycles are associated with a burst of MSNA
TPR	Total peripheral resistance
VI	Virtual instrument
VT_{CON}	Vascular transduction characterised using leg vascular conductance
VT_{DBP}	Vascular transduction characterised using diastolic blood pressure
VT_{MAP}	Vascular transduction characterised using mean arterial pressure
V_{mean}	Mean blood velocity
Vs.	Versus

ABSTRACT

The control of muscle sympathetic nerve activity (MSNA) via the baroreflex is an important mechanism of blood pressure control. Spontaneous sympathetic baroreflex sensitivity (BRS) is a tool used to examine how well the baroreflex buffers beat-to-beat changes in arterial pressure. Due to the lack of research around the baroreflex control of MSNA, it is unknown if an individual's sympathetic BRS reflects the end organ response and thus is indicative of how effective they are at regulating their blood pressure. It was hypothesised that poor baroreflex sensitivity was compensated for by enhanced vascular transduction, and vice versa. Given that sex differences are known to exist in regulatory mechanisms involved in cardiovascular control, these interactions were explored and contrasted in young males and females. In order to further our understanding of the regulatory mechanism of the sympathetic baroreflex, MSNA, blood pressure and superficial femoral artery (SFA) blood flow were measured to i) examine the stability and repeatability of measures of spontaneous sympathetic BRS, ii) examine whether vascular transduction, quantified on a beat-to-beat basis using two different approaches, were different between males and females, iii) examine the relationship between sympathetic BRS and vascular transduction, and iv) examine sympathetic BRS and vascular transduction during physiological stressors that drive increases in MSNA.

Here I present evidence of sex differences in sympathetic baroreflex function in healthy young adults. Spontaneous sympathetic BRS was moderately stable in the same recording period and also when examined on different days. Recording periods of at least 5 min should be used when quantifying BRS as shorter durations can overestimate BRS values. Using the Fairfax method, sympathetic vascular transduction was significantly lower in males when compared with females. In contrast, the Briant method did not reveal sex differences in vascular transduction between males and females. Sympathetic BRS and vascular transduction was negatively correlated under resting conditions. This means that individuals with high sympathetic BRS have less effective vascular transduction during spontaneous changes in blood pressure. However, this was only apparent in young males; there was no relationship observed in females. Furthermore, resting MSNA did not predict sympathetic BRS or vascular transduction in either males or females. Finally, vascular transduction was significantly greater in males when quantified as the relationship between MSNA and leg vascular conductance during isometric handgrip and the cold pressor test. Sympathetic BRS

was not different between males and females during the cold pressor test but was reset to a higher blood pressure range.

Collectively, the studies conducted in this thesis provide insight into the dynamic nature of the baroreflex control of arterial pressure at rest, and during increases in muscle vasoconstrictor drive. Whilst this thesis provides evidence of sex differences in sympathetic BRS and vascular transduction, it also highlights the differences between the various approaches available for quantifying vascular transduction. The method chosen can have a profound effect on the findings regarding sex differences and the interaction vascular transduction has with sympathetic BRS.

Chapter 1: INTRODUCTION

The autonomic nervous system plays an important role in cardiovascular control, and the way in which arterial pressure is regulated differs between males and females. Blood pressure is determined by both cardiac output (CO) and the total peripheral resistance (TPR) and is the radial distension pressure exerted on the systemic arteries (Magder, 2018). Adequate blood pressure is required for the perfusion of blood to all tissues to effectively deliver oxygen and nutrients and to remove carbon dioxide and metabolic waste. Cardiac output is determined by cardiac contractility and end diastolic volume (i.e., stroke volume) and heart rate. End diastolic volume is determined by venous blood volume and venous smooth muscle tone (i.e., venous pressure) (Guyenet, 2006). Highly vascularized skeletal muscle plays a primary role in determining TPR and blood flow through the muscle vascular bed and is controlled by muscle sympathetic nerve activity (MSNA), which is vasoconstrictor in function. Total peripheral resistance is determined exclusively by increases or decreases in sympathetic vasoconstrictor drive, whereas CO is governed by the sympathetic and parasympathetic branches (Guyenet, 2006), as described below. The beat-to-beat maintenance of blood pressure is regulated by the baroreflex. The baroreflex performs this task by modulating heart rate and MSNA at rest and during physiological challenges, such as exercise. Resting levels of MSNA have been shown to be repeatable over time (Fagius and Wallin, 1993), are similar to levels in other vascular beds (heart and kidney), and have also shown to be correlated with whole body noradrenaline spillover (Wallin et al., 1992, Wallin et al., 1996). Although sympathetic outflow can be measured across individuals, there are significant interindividual differences in resting MSNA in young normotensive individuals (Fagius and Wallin, 1993, Sundlof and Wallin, 1977). Evidence suggests that significant interindividual variability also exists in vascular transduction, i.e., the end organ response of the peripheral vasculature to MSNA (Fairfax et al., 2013b). However, it is not yet known how this interindividual variability influences blood pressure control and if this variability is similar between males and females. To date, sympathetic baroreflex sensitivity (BRS) has not been examined in conjunction with sympathetic vascular transduction. Therefore, current methods of examining sympathetic BRS may not encompass the whole baroreflex response. The aim of this chapter was to review the literature on how the autonomic nervous system is involved in cardiovascular control, in particular, the baroreflex modulation of MSNA to efficiently buffer beat-to-beat changes in blood pressure. A major theme throughout this literature review was how sex influences cardiovascular control.

1.1. Autonomic nervous system

The peripheral nervous system is composed of a sensory and motor division directed at both somatic and visceral pathways. The autonomic nervous system is a visceral division of the peripheral nervous system and is responsible for a number of involuntary motor functions such as breathing, sweating, heart rate, and vascular tone to ensure survival and to maintain homeostasis during physical and emotional stress (Levick, 2010, Amerman, 2016). The autonomic nervous system has three divisions, the parasympathetic (rest and digest), sympathetic (fight or flight) and the enteric division. For this review, the focus was on the role of the sympathetic nervous system on the peripheral vasculature with some reference to the parasympathetic division on the baroreflex control of heart rate (cardiac baroreflex sensitivity). Table 1.1 summarises the primary functions of the parasympathetic and sympathetic divisions of the autonomic nervous system on the body.

Parasympathetic preganglionic neurones are known as the craniosacral division of the autonomic nervous system as they are located in the cranial nerves (III, VII, IX and X) and sacral spinal cord (S2-S4) (Wehrwein et al., 2016). The axons of preganglionic neurons are relatively long as they synapse on postganglionic neurones (which have short axons) located near or within the wall of the target organ (Wehrwein et al., 2016). Acetylcholine is the primary neurotransmitter for parasympathetic postganglionic neurones (Amerman, 2016). The cell bodies of sympathetic preganglionic neurones are thinly myelinated and reside in the intermediolateral column of the thoracolumbar spinal cord (T1-L2/L4) (Charkoudian and Wallin, 2014, Wehrwein et al., 2016). Preganglionic neurones receive synaptic input from premotor neurones in the brainstem via the dorsolateral funiculus as well as input from spinal and propriospinal interneurones (Charkoudian and Wallin, 2014). They leave the spinal cord in the ventral roots and the white rami and their short axons synapse to unmyelinated postganglionic neurones in the paravertebral ganglia of the sympathetic chain and prevertebral (visceral) ganglia (Wallin and Charkoudian, 2007). After synapsing, unmyelinated postganglionic axons project via the grey rami and peripheral nerves to their target organs. Most vascular sympathetic nerves in humans release noradrenaline and cause vasoconstriction as well as co-transmitters, neuropeptide-Y (NPY) and adenosine triphosphate (ATP) (Wallin and Charkoudian, 2007).

An example of the role of the autonomic nervous system is the detection and maintenance of blood pressure. Visceral baroreceptors detect the stretch of the carotid sinus and aortic arch during increases in pressure and cause a motor effector response at both the heart and peripheral vasculature to return blood pressure to its homeostatic set point. Both parasympathetic and sympathetic arms of the autonomic nervous system are essential in the regulation of CO (heart rate and stroke volume) and TPR (MSNA) to ensure arterial pressure is maintained via the homeostatic mechanism, the baroreflex.

Table 1.1. Main effects of parasympathetic and sympathetic nervous system on target cells (modified from Amerman, 2016)

Target organ or system effects	**Sympathetic**	**Parasympathetic**
Eye	Dilation of pupil	Constriction of pupil, adjustment of lens for near vision
Salivary glands	Increase in secretion in certain cells	Increase salivary secretions
Heart	Increase in heart rate and force of contraction	Decrease in heart rate
Lung, bronchus	Dilation of bronchioles (bronchodilation)	Bronchoconstriction
Sweat glands	Increase in sweat secretion	
Blood vessels supplying skeletal muscle	Vasoconstriction of blood vessels supplying skin, vasodilation of blood vessels supplying muscle	
Liver and Pancreas	Increase in release of glucose	
Adrenal gland	Release of epinephrine and norepinephrine of cells from the adrenal medulla	
Digestive and urinary organs	Vasoconstriction of blood vessels supplying digestive and urinary organs. Relaxation of digestive and urinary tracts, constriction of sphincters. Decrease in secretion of digestive glands	Contraction of digestive tract smooth muscle, relaxation of sphincters. Increase in secretions of digestive glands

1.2. The baroreflex

The baroreflex is a negative feedback system that regulates beat-to-beat changes in arterial pressure and is initiated when the stretch sensitive mechanoreceptors (baroreceptors) detect distension of the arterial walls in the carotid sinus and aortic arch during increases in blood pressure. This excites afferent projections in the Glossopharyngeal and Vagus nerves (cranial nerves IX and X) to the cardiovascular control centre, the nucleus tractus solitarus (NTS) of the medulla oblongata (Benarroch, 2008). Excitatory projections to the nucleus ambiguus and the dorsal motor nucleus of the Vagus increase cardiac vagal outflow and decrease heart rate (Benarroch, 2008). Baroreceptor signals from the NTS also project directly to and excite inhibitory GABAergic neurones in the caudoventrolateral medulla, which then in turn project to inhibit preganglionic neurones within the rostral ventrolateral medulla (RVLM) (Dampney et al., 2003b). The RVLM is the primary output nucleus for vasoconstrictor drive to the muscle, splanchnic and renal vascular beds (Guyenet, 2006). Inhibition of the RVLM results in a reduction of sympathetic outflow to the vasculature, and therefore vascular resistance and a decrease in blood pressure back to baroreflex set point (Leowry, 1990, Macefield et al., 2006, Sander et al., 2010). These pathways have been identified through previous work performed using animal models (Dampney et al., 2003a, Dampney et al., 2003b) and, more recently, in humans using direct recordings of MSNA concurrently with functional magnetic resonance imaging of the medulla (Macefield and Henderson, 2010). When arterial pressure is low, cardiac vagal outflow is withdrawn, and sympathetic terminals on the outer layer of vasculature walls are excited, releasing noradrenaline and causing constriction of blood vessels (Levick, 2010). This process leads to an increase in blood pressure (via an increase in TPR) back to its operational set point (Dampney et al., 2003a, Leowry, 1990, Levick, 2010) (Fig 1.1).

Sympathetic outflow increases with age in both males and females, although age explains 53% of MSNA variance in females, but only 8% in males (Narkiewicz et al., 2005). In young normotensive adults, there is no significant relationship between MSNA and blood pressure (Narkiewicz et al., 2005). However, as humans age the link between resting MSNA and blood pressure becomes more apparent; i.e., a higher resting MSNA is associated with higher resting blood pressure (Narkiewicz et al., 2005). Since some forms of hypertension are sympathetically driven, it is essential to understand how blood pressure is controlled acutely

via the sympathetic nervous system. The ability of the baroreflex to efficiently buffer changes in blood pressure is known as baroreflex sensitivity. Sympathetic BRS can be quantified by relating MSNA to changes in diastolic pressure. Muscle sympathetic nerve activity is measured directly using a technique known as microneurography.

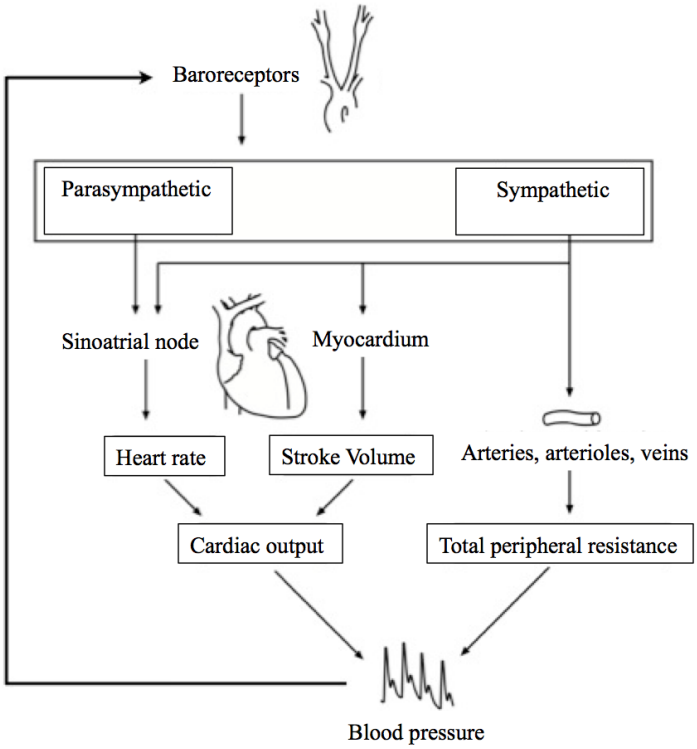

Figure 1.1. Schematic showing the major mechanistic pathways involved with short-term systemic blood pressure control (Taylor et al., 2014)

1.3. Sympathetic outflow – how is it measured?

Measurement of sympathetic outflow in humans is critical for understanding cardiovascular control with respect to how vasoconstrictor drive influences the homeostatic maintenance of blood perfusion to tissues. Two of the primary methods developed to assess sympathetic outflow are regional noradrenaline spillover and microneurography as described below.

1.3.1 Regional noradrenaline spillover

Noradrenaline spillover was developed by Murray Esler and colleagues in 1979 to measure the noradrenaline released from sympathetic nerves of the whole body (Esler et al., 1979), and also regionally in specific organs such as the heart (Esler et al., 1982). Regional

noradrenaline spillover enables the investigator to measure the amount of noradrenaline released from sympathetic nerves of individual organs. Other techniques such as sampling noradrenaline from urine and plasma noradrenaline only allow investigators to record whole body noradrenaline (Esler, 2011). This is a limitation as there is no differentiation between the clearance rate of noradrenaline and the amount that is released (Esler, 2011). The noradrenaline spillover technique uses radiotracer-derived measurements of the appearance rate of noradrenaline in plasma from individual organs (Esler et al., 1982). The regional rate of noradrenaline spillover is measured during a constant rate infusion of radiolabelled noradrenaline and the spillover rate to plasma is determined by isotope dilution (Esler et al., 1982, Esler, 1993). Regional noradrenaline spillover allows for the recording of sympathetic outflow from regions of the body that is not possible with more direct measures such as microneurography.

However, there are limitations in measuring sympathetic activity using regional noradrenaline spillover, specifically the complexity of the method of assessment and the invasiveness of the procedure (Charkoudian and Rabbitts, 2009). When noradrenaline is released from sympathetic nerve endings, the concentration of noradrenaline circulating in the blood is influenced by being reabsorbed into nearby nerve endings and smooth muscle; and may also be subjected to enzymatic degradation (Watson et al., 1979, Iversen, 1973). Sympathetic nervous system responses may also differ between vascular beds, with increased sympathetic outflow to some organs whilst others are unaffected or inhibited (Esler, 1993). For instance, measuring blood from the forearm may not be related to changes in the lower limb when performing exercise such as dorsiflexion of the ankle. Unlike noradrenaline spillover, microneurography is a direct measure of sympathetic outflow and provides beat-to-beat changes in sympathetic vasoconstrictor activity in response to changes in arterial pressure. Microneurography has a much finer time resolution that cannot be achieved with noradrenaline spillover.

1.3.2 Muscle sympathetic nerve activity

Microneurography is a technique that allows for the direct measurement of vasoconstrictor neural activity in skeletal muscle (Vallbo et al., 2004). Prior to the mid-1960s, sympathetic

outflow was only measured indirectly in humans until Karl-Erik Hagbarth and Ake Valbo developed the microneurography technique (Vallbo and Hagbarth, 1967). Microneurography allows researchers to directly record action potentials from groups of axons (multi-unit recording) or individual axons (single-unit recording) (Macefield, 2013). The microneurography technique involves inserting a microelectrode percutaneously into a muscle or skin fascicle of a peripheral nerve where both single and multi-unit sympathetic activity can be recorded (Hagbarth and Vallbo, 1968a). Measurements are made using electrolytically sharpened and insulated tungsten microelectrodes with a shaft diameter of 200 µm and a rounded uninsulated tip with a diameter of approximately 5µm that provides a focal recording tip (Macefield, 2013). In this review, the focus was on sympathetic nerve activity recorded from fascicles innervating muscle, i.e., MSNA. Muscle sympathetic nerve activity is typically quantified by the number of sympathetic bursts per minute (burst frequency); the number of bursts per 100 heartbeats (burst incidence), burst amplitude/area (size of the sympathetic bursts, sometimes referred to as burst strength) and the integration of both burst incidence and burst amplitude/area (total MSNA). The absolute strength of MSNA cannot be obtained with microneurography as it is dependent on how close the microelectrode tip is to the active nerve fibres (Macefield, 2013). However, MSNA burst strength can still be utilised and compared between individuals by normalising the MSNA bursts to the strongest burst in the recording (Halliwill, 2000, Sverrisdottir et al., 1998).

The penetration of a muscle fascicle (as opposed to a fascicle innervating the skin) is confirmed by the following criteria: i) a cramp, dull ache in the muscle innervated by the nerve; ii) muscle twitches without skin parathesia during electrical stimulation; iii) spontaneous bursts of muscle sympathetic activity that are locked to the cardiac cycle and respiratory rhythms; iv) muscle spindle afferent discharges when the muscle is mechanically affected by contracting, stretching or tapping the muscle belly without an increase in activity when stroking the skin (Fagius et al., 1989, Hagbarth and Vallbo, 1968a, Hagbarth and Vallbo, 1968b). Figure 1.2 displays the cardiac locked synchronicity of MSNA at rest.

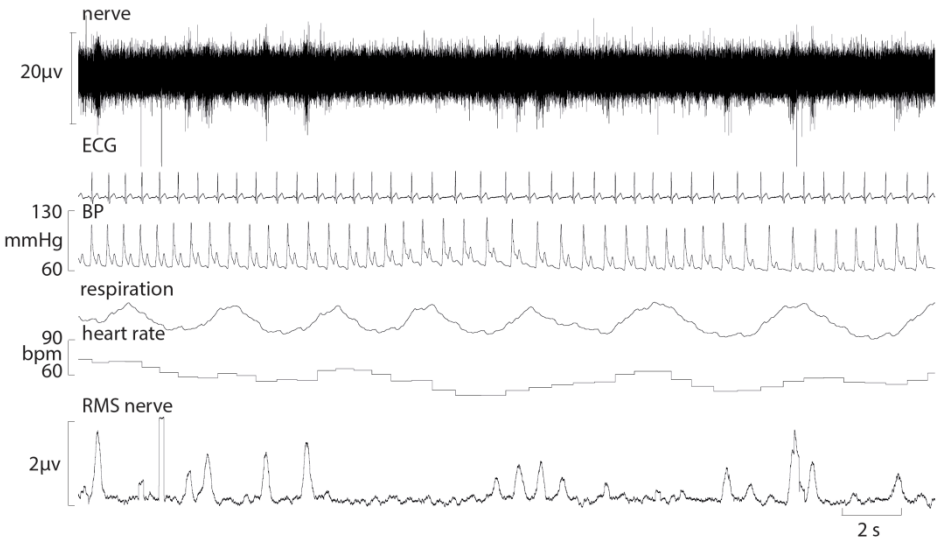

Figure 1.2. Experimental recording of blood pressure, respiration, heart rate and MSNA at rest in a 22-year old male (collected during Honours)

1.4. Baroreflex sensitivity

The cardiac and sympathetic arms of the baroreflex are critical in regulating blood pressure at rest and during physiological stress. Baroreflex sensitivity is quantified by plotting the beat-to-beat changes in blood pressure against RR-interval (cardiac BRS) or MSNA (sympathetic BRS) via linear regression analysis. The slope of the linear portion of this relationship is used as an index of BRS (Parati et al., 1988, Sundlof and Wallin, 1978). Since the vasculature is innervated by the sympathetic nervous system, the baroreflex control of MSNA is most commonly referred to as sympathetic baroreflex sensitivity. It should be noted, however, that the use of this term has the potential to be misleading as the sympathetic nervous system also influences the heart. However, for consistency with the current literature, the term sympathetic baroreflex was used throughout this thesis. While the cardiac and sympathetic arms of the baroreflex share the same afferent pathway, the effectiveness of the efferent pathways may differ within individuals. It should not be assumed that an individual with low cardiac BRS also possesses poor sympathetic baroreflex function. In support of this, evidence suggests no direct correlations between cardiac and sympathetic BRS (Dutoit et al., 2010, Rudas et al., 1999). However, there does seem to be an interaction with regards to sex. When assessed separately according to sex, there is a modest correlation between cardiac and sympathetic BRS in females that is not apparent in males (Dutoit et al., 2010). This correlation has been found using two different methods of quantifying sympathetic BRS,

active perturbation via the modified Oxford method (Dutoit et al., 2010) and through spontaneous changes in arterial pressure, RR-interval and MSNA (Taylor et al., 2015). The evaluation of BRS is similar in both arms of the baroreflex, but certain methods are primarily used for cardiac and others for sympathetic BRS.

1.4.1 Assessment of cardiac baroreflex sensitivity

Cardiac baroreflex sensitivity can be evaluated through spontaneous fluctuations in blood pressure and also by actively perturbing blood pressure. Examples of techniques that are commonly used to estimate cardiac BRS include the sequence method, spectral analysis, the Valsalva manoeuvre, neck suction and pressure, and the modified Oxford method (Ebert and Cowley, 1992, Parati et al., 1988, Robbe et al., 1987, Leon et al., 1970, Eckberg, 1976, Elisberg, 1963). Cardiac BRS is expressed as the change in RR-interval in millisecond per mmHg change in systolic pressure (ms/mmHg). The slope of the linear portion of this relation is used as an index of BRS (Parati et al., 1988). Cardiac BRS can also be expressed relative to changes in heart rate. This is important, particularly when comparing conditions in which heart rate differs, because of the non-linear relationship between heart rate and RR-interval (Ludbrook, 1983).

Spontaneous baroreflex sensitivity

Assessment of cardiac BRS can be performed using techniques from both the time and frequency domains. The most commonly used technique in the time domain is the sequence method. This involves the identification of baroreflex sequences of three or more cardiac cycles where corresponding systolic blood pressure and RR-intervals change in the same direction (increase or decrease) (Hughson et al., 1993). The slope is quantified for each sequence and the average value of the slopes is then taken as an index of the sensitivity of the cardiac baroreflex (Parati et al., 1988). Non-baroreflex effects on RR-interval, such as respiratory sinus arrhythmia, may distort cardiac BRS values (Hughson et al., 1993). Previous reports indicate that the sequence method correlates strongly with estimations of BRS in the frequency domain, such as spectral analysis (Hughson et al., 1993). Transfer function analysis, a form of spectral analysis, can be used to examine the relationship between systolic

blood pressure and RR-interval as a power density spectrum (Robbe et al., 1987). Transfer function analysis involves determining the gain, phase, and coherence of systolic blood pressure to RR-interval relationship. Gain measures the amplitude of the output response relative to the input, i.e., the ratio between changes in RR-interval time and changes in systolic blood pressure in a specified frequency band (Robbe et al., 1987). Phase provides a measure of delay between input and output response and coherence indicates how closely the two variables are correlated. Cardiac BRS is found within the low frequency range (0.04-0.15 Hz) or more specifically 0.1 Hz when using transfer function analysis (1996, Linden and Diehl, 1996, Robbe et al., 1987). Spontaneous methods, including spectral analysis, require at least a 5 minute recording to provide reliable results (Robbe et al., 1987). Spectral analysis only uses a steady-state measurement so this is not appropriate when assessing baroreflex function during stressors (Wirch et al., 2006). Another limiting factor of spontaneous methods of BRS is that they only provide an assessment of BRS around the operating point. This can involve a fairly small blood pressure range, thus limiting the assessment of the full baroreflex curve. In order to examine the full baroreflex curve, methods involving active perturbation of arterial pressure are commonly used.

Active perturbation

An alternate approach to the spontaneous methods is to actively perturb blood pressure. An acute fall and rise in blood pressure can be achieved with vasoactive drugs or the Valsalva manoeuvre. A pharmacological method of assessing cardiac BRS is through bolus injections of a vasoactive drug until systolic blood pressure increases between 15-40 mmHg. This is known as the Oxford method. This method was initially carried out using injections of angiotensin or phenylephrine to examine the cardiac baroreflex responses to an increase in arterial pressure (Smyth et al., 1969). The Oxford method was then adapted to include the use of a vasodilator, such as sodium nitroprusside, to observe changes in the rise as well as fall in blood pressure (Ebert and Cowley, 1992). This is advantageous as it partially opens the closed-loop system of the baroreflex, allowing estimates of the ratio of the inputs and outputs of the baroreflex to be observed, and directly measures the linearity of the relationship between blood pressure and RR-interval (Lipman et al., 2003, Diaz and Taylor, 2006). It has been argued that the use of vasoactive drugs affects properties of the baroreflex. The use of nitric oxide may cause central neural effects that may lead to an inhibition of nuclei

responsible for sympathetic outflow, resulting in an underestimated BRS value (Hogan et al., 1999, Kienbaum and Peters, 2004). Also, bolus infusions of sodium nitroprusside and phenylephrine can alter central venous pressure and therefore activate cardiopulmonary baroreceptors (Martin and Charkoudian, 2005). The use of the modified Oxford method is also limited in some research laboratories because it is an invasive technique and may cause side effects including headaches and facial parathesia in myocardial infarction patients (Farrell et al., 1992).

The Valsalva manoeuvre provides a non-pharmacological way of actively assessing BRS. The technique is performed by blowing into a tube connected to an aneroid manometer and maintaining a pressure of 40 mmHg for 15 seconds (s) (Elisberg, 1963). There are four phases of the Valsalva manoeuvre: I) an increase in blood pressure and decrease in heart rate immediately after onset of straining; II) a decrease in blood pressure and an increase in heart rate during straining; III) a sudden brief further reduction of blood pressure and an increase of heart rate immediately following the release of straining; IV) an increase in blood pressure above control levels and slowing of heart rate (Elisberg, 1963). Phase IV, in particular, has been used to quantify BRS and can be simplified further into a ratio between RR-interval and systolic blood pressure. This ratio has been reported to produce reliable and reproducible BRS values (Kautzner et al., 1996). The increase in heart rate present in late phase II has an influence on blood pressure elevation in phase IV, and may also alter the heart rate response (Zollei et al., 2003). Using the Valsalva manoeuvre to assess cardiac BRS is limited in that it only involves a few cardiac cycles with which to plot the relationship between systolic blood pressure and RR-interval. It is especially limited when trying to assess sympathetic BRS as there is often no muscle sympathetic bursts during phase IV, meaning that the sympathetic baroreflex response to rising pressures cannot be evaluated. Late phase II, during which blood pressure is falling, is also associated with difficulties since almost all cardiac cycles tend to have a MSNA burst, often with little difference in burst amplitude. An example of this from our work is displayed in figure 1.3. At least two Valsalva manoeuvres are needed to ensure reliable results, whereas it has been demonstrated that the modified Oxford method can supply reliable cardiac BRS measurements in one recording (Airaksinen et al., 1993).

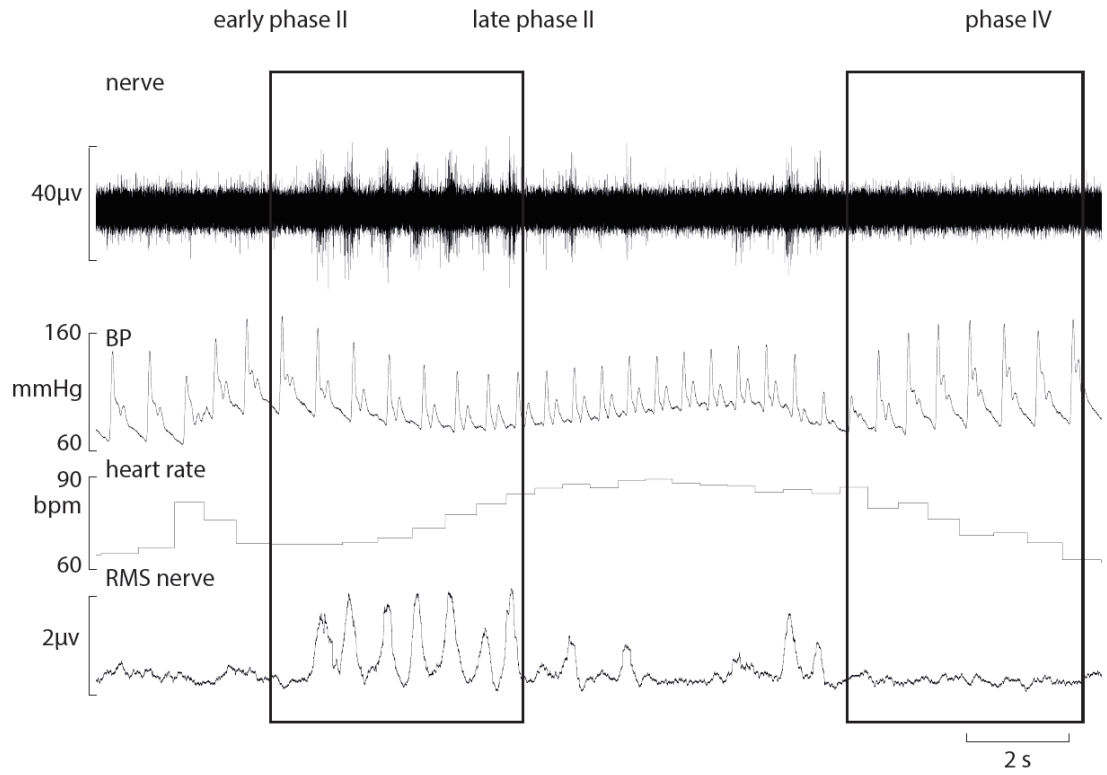

Figure 1.3. Trace of raw muscle sympathetic nerve activity, blood pressure, heart rate and RMS muscle sympathetic nerve activity when performing the Valsalva manoeuvre (pilot work). Phase II and IV are highlighted to indicate the main areas of BRS analysis

Application of neck suction and pressure using a neck chamber can also be used to directly load and unload the carotid baroreceptors, allowing the corresponding changes in heart rate or RR-interval to be examined (Zollei et al., 2003). Responsiveness to the neck chamber/suction method of baroreceptor stimuli depends on the timing of the method within the cardiac cycle (Eckberg, 1976). Goldstein and colleagues (1982) conducted a study that compared the above techniques for estimating cardiac BRS. It was found that there was quite a large variability between techniques and the authors concluded that the different techniques might measure different characteristics of the baroreflex (Goldstein et al., 1982).

The techniques above have been discussed predominantly in relation to cardiac BRS. Whilst similar methods can be applied to the sympathetic baroreflex, there has been considerably less research conducted on sympathetic BRS due to the invasive nature of microneurography.

These experiments are more technically demanding and require significant expertise. In the following section, the most frequently used methods for calculating sympathetic BRS were discussed.

1.4.2 Assessment of sympathetic baroreflex sensitivity

Sympathetic baroreflex sensitivity is the ability of the baroreflex to efficiently regulate changes in blood pressure through the modulation of MSNA (Kienbaum et al., 2001, Sundlof and Wallin, 1978). This is determined by quantifying the slope of the relationship between diastolic blood pressure and MSNA. The steeper the slope, the more effective the baroreflex is at buffering beat-to-beat changes in blood pressure (Taylor et al., 2014, Charkoudian and Wallin, 2014). Diastolic pressure is used in the analysis of sympathetic BRS as it has a stronger correlation to MSNA than systolic pressure (Vallbo et al., 1979). It is recommended that a minimum of 5 minutes of data in sympathetic baroreflex analysis be used, as shorter periods may not provide sufficient diastolic pressure ranges to provide a valid baroreflex slope (Holwerda et al., 2012). To date, the quantification of sympathetic BRS has been performed predominantly through spontaneous techniques. Although, methods involving active perturbation of blood pressure have been applied, such as the modified Oxford method. Whilst the modified Oxford method is regarded as the 'gold standard' for quantifying cardiac BRS, there are limitations when determining sympathetic BRS (Dutoit et al., 2010, Taylor et al., 2014). In cardiac baroreflex assessment, there is always an RR-interval to slope changes in systolic blood pressure. However, with regards to sympathetic baroreflex assessment, not every cardiac cycle is associated with a MSNA burst, so the number of data points included to determine sympathetic BRS is reduced (Taylor et al., 2014), particularly when blood pressure is driven up during phenylephrine administration. Spontaneous methods are therefore often chosen for sympathetic baroreflex assessment. Spontaneous techniques that estimate sympathetic BRS include MSNA burst strength (amplitude or area), MSNA burst incidence or a combination of MSNA burst strength and burst incidence (total MSNA).

Sympathetic BRS quantified using MSNA burst strength has been reported to be less successful and less reliable than when using MSNA burst incidence or total MSNA (Keller et al., 2006, Kienbaum et al., 2001). Therefore MSNA burst strength alone is no longer

commonly used in sympathetic baroreflex assessment (Kienbaum et al., 2001). This is because the burst strength method involves removal of cardiac cycles that are not associated with a burst which poses some problems, particularly during high pressures, where sympathetic outflow is significantly decreased (Taylor et al., 2014). The elimination of cardiac cycles removes potentially important data, and therefore may not allow for a complete representation of muscle sympathetic outflow to changes in blood pressure. Also, the baroreceptor influence on MSNA burst amplitude may not be as dominant as other inputs that adjust muscle sympathetic outflow (Kienbaum et al., 2001). Muscle sympathetic burst amplitude may have a closer relationship with the transduction of MSNA to the vasculature rather than the baroreflex control of blood pressure (Fairfax et al., 2013b). However, investigations into this are needed to confirm the role of MSNA burst amplitude in regulating blood pressure.

A method that has proven to be more successful is the use of MSNA burst incidence, also referred to as the blood pressure threshold diagram, in which diastolic pressure is related to the incidence of MSNA bursts (Kienbaum et al., 2001, Sundlof and Wallin, 1978). Diastolic pressure values are grouped into 1 or 3 mmHg bins, and MSNA burst incidence is plotted against the mean diastolic pressure for each bin (Hart et al., 2010, Kienbaum et al., 2001). The slope of this relationship represents the variability of the blood pressure threshold required to initiate a burst of MSNA and reflects the baroreflex control of MSNA (Kienbaum et al., 2001, Sundlof and Wallin, 1978). However, this threshold is not constant due to the varying degree of inputs to the cardiovascular control centre as cardiac cycles with the same diastolic pressure are not always associated with a burst of MSNA (Charkoudian et al., 2005). The T50 value is defined as the diastolic pressure at which 50% of cardiac cycles are associated with a MSNA burst and provides information on the average setting of the baroreflex over the range of blood pressures in a recording (Wallin et al., 1974). Sundlof and Wallin (1978) reported a systematic relationship between MSNA burst incidence, mean diastolic pressure and the T50 value. When an individual's MSNA burst incidence is 50 bursts/100HB, their mean diastolic pressure equals their T50 value. Furthermore, when mean diastolic pressure is less than T50, MSNA burst incidence is greater than 50 bursts/100HB, and less than 50 bursts/100HB when mean diastolic pressure is greater than T50 (Sundlof and Wallin, 1978). A way in which burst strength can be used as a valid measurement of

sympathetic BRS is by using total MSNA in analysis. This allows both MSNA burst incidence and burst amplitude to be taken into account, providing an overall representation of the sympathetic baroreflex in regulating blood pressure (Hissen et al., 2015, Taylor et al., 2015, Keller et al., 2006). Figure 1.4 provides examples of sympathetic BRS slopes using the burst incidence and total MSNA method in the morning and afternoon. There is an inverse relationship between MSNA and diastolic pressure, whereby MSNA bursts are more likely to occur when diastolic pressures are low compared to when pressures are high. These data were obtained and analysed as part of my Honours project, and the study has since been published in Frontiers in Neuroscience and is presented in Appendix A (Hissen et al., 2015). Burst amplitude/area can also be utilised using the segregated signalling average approach where total MSNA is quantified across all cardiac cycles and is then averaged for each diastolic pressure bin (Halliwill, 2000).

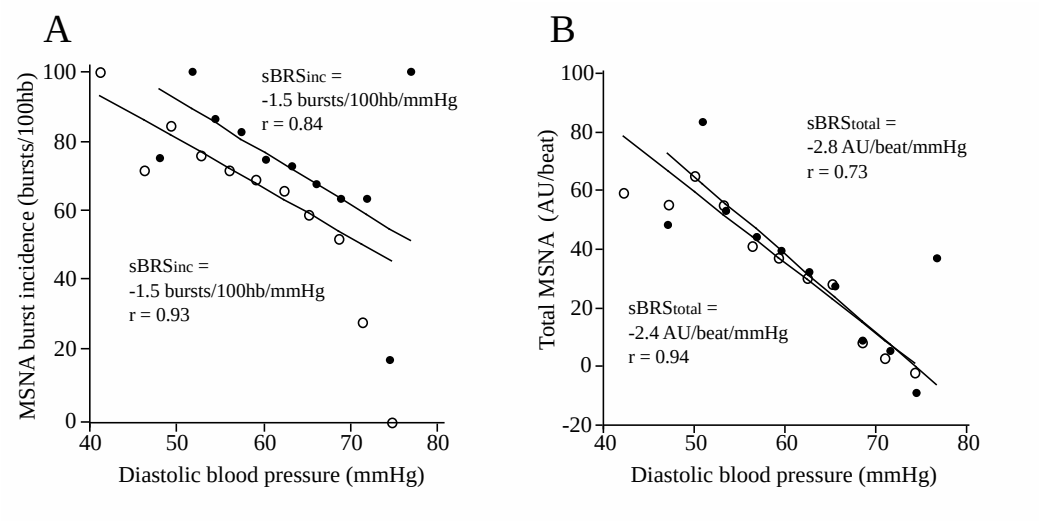

Figure 1.4. Sympathetic baroreflex slopes for a 21-year old male in the morning (closed circles) and afternoon (open circles) using A) the sympathetic BRS incidence method, and B) the sympathetic BRS total MSNA method (Hissen et al, 2015)

Baroreflex sensitivity can be further divided into both its mechanical and neural components through diameter changes in the carotid artery measured using ultrasound. Hunt and colleagues (2001a) used ultrasound images of the carotid artery to separate BRS into its mechanical (transfer of changes in blood pressure to changes in carotid diameter) and neural (transduction of carotid diameter to changes in RR-interval/MSNA) components. Dividing

the baroreflex into its mechanical and neural components provides insight into the mechanisms that underlie changes in baroreflex function and possible causes of cardiovascular pathologies that may not be answered with BRS alone. Figure 1.5 provides an example of the edge tracking software used to detect the changes in carotid artery diameter. This method of baroreflex analysis has been used to examine the effects of time of day (Taylor et al., 2011), aerobic training (Deley et al., 2009), changes in posture (Taylor et al., 2013) and ageing (Studinger et al., 2009).

1.5. The application of baroreflex sensitivity in understanding blood pressure control

Baroreflex sensitivity is a tool that has been utilised to examine how ageing, disease, intervention and lifestyle factors influence cardiovascular control. Literature on how cardiac and sympathetic BRS are applied is discussed below.

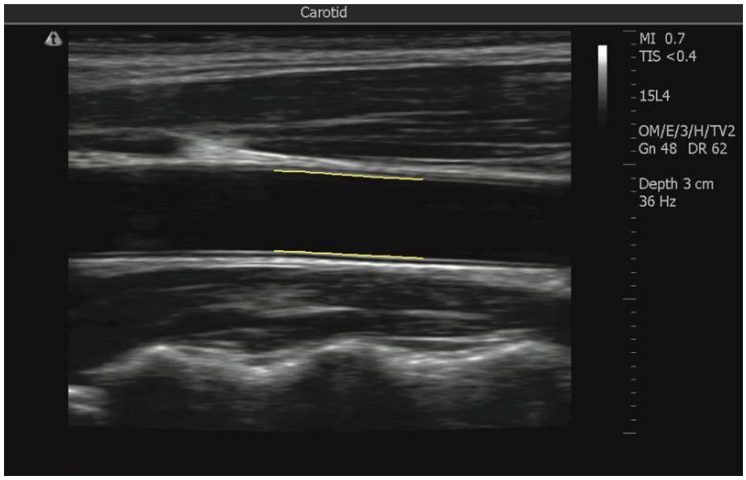

Figure 1.5. Sample screen shot showing edge tracking of the carotid diameter (Taylor et al, 2014)

1.5.1 Baroreflex sensitivity in clinical and ageing populations

Several aspects of cardiovascular control are altered as humans age (Dinenno et al., 1999, Dinenno et al., 2001), and these effects differ between males and females (Hart et al., 2009a, Schmitt et al., 2010). Cardiac BRS decreases with healthy ageing, and across all age ranges, males have greater cardiac BRS than females (Laitinen et al., 1998, Monahan et al., 2001, Ebert et al., 1992). Age and sex have previously been shown to account for 52% of the

interindividual variation in cardiac BRS (Laitinen et al., 1998). Carotid artery compliance is significantly correlated with cardiac BRS and reductions in compliance may play a role in the age-associated decrease in cardiac BRS in healthy sedentary humans (Monahan et al., 2001). Age-related increases in carotid and aortic arterial stiffness have previously been reported to be responsible for the decrease in cardiac BRS (Lenard et al., 2001). Okada et al. (2012) reported that sympathetic BRS is inversely correlated with carotid and aortic arterial stiffness. Barosensory vessel stiffness associated with age may be one independent determinant of sympathetic BRS in elderly males and females.

Although low BRS has been associated with elevated cardiovascular risk, the evidence has come predominantly from studies of cardiac BRS (Johansson et al., 2007). This includes the establishment of clinical thresholds for cardiac BRS, particularly after myocardial infarction. A cardiac BRS of 3 ms/mmHg or less has been associated with increased risk of death following myocardial infarction (La Rovere et al., 1988). Cardiac BRS has been shown to decrease with age in normotensive and hypertensive individuals, and the effect of hypertension cannot be distinguished from the effect of ageing when over the age of 50 (Kornet et al., 2005). This suggests that the neural component of the baroreflex decreases with age irrespective of changes occurring in arterial wall structure and dynamics (Kornet et al., 2005). Evidence indicates that cardiac BRS is reduced after acute stroke when compared to aged hypertensive controls (Robinson et al., 1997). However, regular aerobic training in middle-aged and older sedentary individuals has previously been shown to increase cardiac BRS (Monahan et al., 2001). Rehabilitative exercise improved cardiac BRS from 3.06 ± 0.3 ms/mmHg to 5.36 ± 0.7 ms/mmHg following two weeks of stationary cycling in patients with coronary artery disease (Iellamo et al., 2000). Although this is essential information, very little research has been performed on the sympathetic baroreflex, which leaves a significant gap in the literature. While resting levels of MSNA increase with age, Ebert et al. (1992) reported that sympathetic BRS is not significantly different between young (ages 18-34), middle-aged (ages 35-50) or older (ages 51-71) individuals. In addition to this, Okada et al. (2012) recently found that elderly females have a lower sympathetic BRS than elderly males and may predispose them to an increased risk of hypertension.

The age-related increase in the stiffening of the carotid artery may have implications relating to the mechanical component of the baroreflex. Hunt and colleagues (2001b) examined the effects of ageing and fitness on both the mechanical and neural components of the cardiac baroreflex and found both to be lower in older than in untrained younger men. However, when the comparisons were made between fit older men and untrained younger men, cardiac BRS was not significantly different, due to maintenance of the neural component. Since both arms of the baroreflex share the same afferent pathway, it makes sense to assume that sympathetic BRS is also influenced by the mechanical component of the baroreflex. Studinger et al. (2009) investigated this question by examining sympathetic BRS using the modified Oxford method. Ageing is associated with reductions in the mechanical component of the baroreflex during both increases and decreases in blood pressure (Studinger et al., 2009). However, this seems to be counteracted by a larger neural component when compared with younger individuals.

1.5.2 Factors influencing sympathetic BRS

There are contradictory findings in the literature with regards to sex differences in baroreflex modulation of heart rate and MSNA, with some studies indicating similar BRS values between males and females (Tank et al., 2005), and others greater BRS in females (Hogarth et al., 2007), or males (Christou et al., 2005). The discrepancies between these studies may be due to the choice of analytical technique used to quantify sympathetic BRS. In females, the set point of the sympathetic baroreflex operates at a lower blood pressure range so, at a given blood pressure, MSNA tends to be lower in females than males (Tank et al., 2005). Many studies have utilised BRS to evaluate how arterial blood pressure regulation is altered or enhanced. Examples include the cardiovascular benefits of lifestyle interventions, such as exercise training (Deley et al., 2009, Laterza et al., 2007) and diet (Lambert et al., 2011), which have been associated with improvements in both cardiac and sympathetic BRS. Baroreflex sensitivity has been examined in studies of diurnal variation in baroreflex function, which show that cardiac BRS is attenuated in the morning compared with the afternoon, whilst sympathetic BRS is unaffected by time of day (Taylor et al., 2011, Hissen et al., 2015). During changes in posture from supine to standing, cardiac BRS is attenuated during rising pressures in the standing position due to a decrease in the neural component of the cardiac baroreflex while standing (Taylor et al., 2013). Heat stress has also been shown to

alter sympathetic BRS. Keller et al. (2006) reported that an increase in body temperature augments sympathetic BRS by approximately 2-fold.

1.5.3 Repeatability of BRS

Whilst there is considerable variability in resting MSNA from person to person, interindividual differences in MSNA have been shown to be reproducible on the same day (Notay et al., 2016), to months and even years between trials (Sundlof and Wallin, 1977, Fagius and Wallin, 1993, Kimmerly et al., 2004). The interindividual variability in resting MSNA is likely to be of genetic origin as it is similar in identical twins, but not in fraternal twins (Wallin et al., 1993, Lundblad et al., 2017). What is not certain is whether sympathetic BRS is reproducible over time. Baroreflex modulation of heart rate appears to be repeatable. Dawson and colleagues (1997) previously reported that spontaneous cardiac BRS, assessed via the sequence method, is repeatable between recording sessions. Sympathetic BRS has been shown to be consistent during the same recording period (Kienbaum et al., 2001), but more research is required to confirm whether this holds true over time and also within different population groups. The use of MSNA burst incidence when quantifying sympathetic BRS has previously been shown to be repeatable, whereas methods involving MSNA burst strength have not (Kienbaum et al., 2001). However, these conclusions were based on minimal statistical analysis (paired t-tests), and so the variability between sympathetic BRS values within individuals has yet to be revealed. By assessing segments of data from the same recording period we can assess the stability of sympathetic BRS within subjects.

It is understood that MSNA burst incidence is correlated to changes in diastolic pressure and that baroreflex driven changes in MSNA have a direct effect on vascular resistance. However, it is not clear how this response varies between individuals at the level of the vasculature. Enhanced sympathetic BRS is only of importance if this leads to more effective vasoconstriction, which is dependent upon the response of the blood vessels to MSNA (referred to as ‘vascular transduction’).

1.6. Vascular transduction

Sympathetic nerves innervate peripheral vessels and vascular tone is regulated by noradrenaline released from nerve terminals. Noradrenaline release is also accompanied by co-transmitters, ATP and NPY (Burnstock, 2009). Noradrenaline binds to post-synaptic *a*-1 and *a*-2 adrenergic receptors, NPY binds to NPY-Y1 and ATP binds to purinergic (P2X) receptors. The degree of vasoconstriction varies according to the amount of sympathetic stimulation. During low discharge frequencies, the contribution of ATP is greater whereas NPY is co-released at high firing rates (Burnstock, 2009, Haniuda et al., 1997). However, it has been demonstrated that NPY acts as more of a neuromodulator rather than a co-transmitter, increasing the effect of other co-transmitters, with a greater contribution to vasoconstriction at lower firing frequencies (Bradley et al., 2003). The vasoconstrictor effects of noradrenaline and its co-transmitters on vascular tone contend with vasodilatory effects of ß-adrenergic activity and circulating levels of nitric oxide. For example, it is proposed that young females have lower transduction of MSNA than young males due to competing vasodilatory factors such as greater ß-adrenergic sensitivity (Hart et al., 2011a). As females reach menopause, ß-adrenergic sensitivity is thought to be diminished or abolished, so it is no longer able to offset the vasoconstrictor effects of MSNA (Hart et al., 2011a). The response of a MSNA burst occurs with a short latency period of 1.1-1.5 s and a transduction time of approximately 5.5 s, increasing vascular resistance and arterial pressure (Fagius and Wallin, 1980, Sundlof and Wallin, 1978, Wallin and Nerhed, 1982). The way in which vascular transduction can be measured directly is by looking at the response of the vasculature to sympathetic outflow through the use of vascular ultrasound.

Vascular ultrasound is not only used to quantify the mechanical and neural components of BRS, it can also be used to examine the end organ response of the arteries (supplying blood to the skeletal musculature) to changes in MSNA. The advantage of using ultrasound in baroreflex analysis is that it allows for beat-to-beat measurements in both arterial diameter and blood velocity, and when coupled with recordings of MSNA and blood pressure, allows the quantification of sympathetic vascular transduction. Other methods of recording blood flow are also available but are limited in that they do not allow beat-to-beat measurements (i.e., venous occlusion plethysmography) (Charkoudian et al., 2006) or involve invasive procedures to record blood flow (thermodilution) (Jorfeldt et al., 1978). For the scope of this

review, only Doppler ultrasound was discussed. For information on other techniques for quantifying blood flow, refer to review (Casey et al., 2008).

1.6.1 Physics of Ultrasound

Doppler ultrasound measures blood flow through simultaneous measurements of arterial diameter (in cm) and mean blood velocity (V_{mean}). The transducer used in ultrasound assessments generates and receives ultrasound waves. Ultrasound emits sound waves that have a frequency too high for the human ear to hear (over 20 kHz) (Martin et al., 2015). Ultrasound systems range in frequencies that are much higher, from 2-4 MHz used in recording deeper structures such as abdominal organs and cerebral vessels (transcranial Doppler), to 3-15 MHz to image the peripheral vasculature (Aaslid et al., 1982). The piezoelectric crystals within the transducer vibrate when electricity is applied sending a short burst of sound into tissue. Repeated pulses made by the ultrasound transducer create an ultrasound beam. The transducer becomes a receiver and waits for the reflected sound waves to return (i.e., an echo). Higher frequency ultrasound probes have shorter wavelengths and provide a higher resolution than lower frequency ultrasound probes (which have longer wavelengths). A limiting factor of transducers with higher frequencies is they are less able to penetrate tissue. However, imaging the SFA is possible, as it is within the range that the ultrasound waves can penetrate and acquire an image. The time between transmitting a pulse and receiving an echo is used to determine the depth of the ultrasound beam (Pellerito and Pollack, 2012). The speed of the returning echo depends on properties of the transmitting medium and not on frequency and wavelength (Pellerito and Pollack, 2012). For soft tissue, the average speed of sound is 1540 m/sec.

When recording the velocity of blood flowing through a vessel, the pulse waved Doppler function in the ultrasound system is used. The Doppler function utilises the Doppler effect where the frequency of the echo changes when it is reflected off a moving interface (Martin et al., 2015). The Doppler shift is the difference between the transmitted and observed frequency. When an ultrasound beam is aimed at an artery, red blood cells moving towards the ultrasound beam cause the reflected waves to be compressed and return to the transducer more rapidly, shortening the wavelength, and increasing the frequency (Martin et al., 2015).

The change in frequency is proportional to the velocity of the red blood cells and also the angle between the probe and vessel being imaged. The position of the probe should be angled so that it is directed towards the vessel. An angle $<60^0$ can be used in conjunction with an angle correction function on the ultrasound machine.

1.6.2 Calculating sympathetic vascular transduction

The quantification of vascular transduction provides insight into the end organ response of vasoconstrictor drive to the peripheral vasculature and is a component of the baroreflex response that is often neglected when examining sympathetic BRS. With the use of Doppler ultrasound to record beat-to-beat artery diameter and velocity, blood flow is calculated from the following equation:

V_{mean} (in cm/s)$\cdot\pi\cdot$[mean artery diameter (in cm) $\div 2]^2\cdot 60$ s/min

Blood flow is then divided by mean arterial pressure to calculate vascular conductance. Vascular conductance, which represents the ease of blood flow through a vessel, is dependent on the width of the vessel. The larger the width of a vessel the larger the conductance, and the larger the flow with any given pressure gradient (Levick, 2010). Vascular resistance is the inverse of vascular conductance and is used to describe the difficulty that blood experiences when passing through a vessel (Tan et al., 2013a). According to Darcy's law of flow, resistance is the difference in mean pressure needed to drive blood through a vessel at steady state (Levick, 2010). The relationship between MSNA and both vascular conductance and vascular resistance has been used to quantify sympathetic vascular transduction (Best et al., 2014, Fairfax et al., 2013b). According to O'Leary (1991), changes in conductance reflect the response in pressure regulation better than changes in regional resistance. When CO is kept constant, changes in vascular conductance result in the same change in mean arterial pressure regardless of the baseline conductance. In contrast, a change in vascular resistance is dependent on baseline level (O'Leary, 1991). When baseline resistance is low, changes in resistance lead to greater changes in arterial pressure than when baseline resistance is high.

The transduction of MSNA to vasoconstrictor drive has been measured using many techniques. This includes simply dividing mean peripheral resistance or conductance by MSNA at rest (Jarvis et al., 2012), and also during sympathetic activation such as isometric handgrip and post-exercise ischaemia (Minson et al., 2000a). A number of methods have been used to quantify vascular transduction during sympathetic activation such as dividing the percent change in resistance by the percent change in MSNA (Ray and Monahan, 2002), plotting MSNA against corresponding peripheral resistance in 30 s bins at rest during sympathetic activation (Minson et al., 2000a), and also using an adapted Poiseuille relation between arterial pressure, MSNA and blood flow (Tan et al., 2013a). These methods have provided insight into sympathetic vascular transduction, but they do not provide information on a beat-by-beat basis, particularly at rest (except for (Tan et al., 2013a)). Wallin and Nerhed (1982) studied blood pressure and heart rate responses for 15 cardiac cycles after a MSNA burst and found blood pressure to increase following each burst with a peak response after approximately 7 cardiac cycles (or 5.5 s). Later findings by Vianna and colleagues (2012) using a similar approach demonstrated that changes in blood pressure were positively related to the size of a MSNA burst, i.e., bursts with higher amplitudes caused a greater increase in blood pressure when compared to smaller bursts. These studies involve the effects of MSNA on blood pressure but, with the use of Doppler ultrasound, the beat-to-beat vascular responses to MSNA can be quantified.

1.6.3 Quantifying vascular transduction using ultrasound

Fairfax and colleagues (2013b) extended the analysis of Vianna et al. (2012) by investigating the effects of MSNA burst size and burst pattern (single vs. multiple bursts) on leg vascular conductance. Each MSNA burst was normalized to the three largest bursts and ranked into four quartiles according to MSNA burst amplitude. The bursts were then arranged into four burst clusters (Fairfax et al., 2013b). A burst cluster was defined as either a single burst or a number of serial bursts separated by more than one cardiac cycle (Fairfax et al., 2013b). As expected, higher levels of MSNA (as indicated by larger bursts or a greater number of consecutive bursts) were associated with more significant reductions in vascular conductance and larger increases in blood pressure (Fairfax et al., 2013b). When clusters of bursts were made up of similar total MSNA (amplitude multiplied by burst frequency) but different numbers of bursts, the clusters with fewer (but larger) bursts were the most effective in

decreasing leg vascular conductance (Fairfax et al., 2013b). It was also confirmed that after each burst, the percentage decrease in leg vascular conductance peaked after approximately 6 cardiac cycles. This approach enables the transduction of MSNA to be assessed with regard to the time course and magnitude of the response at the level of the vasculature. This method has previously been validated as a way of quantifying sympathetic vascular transduction (Fairfax et al., 2013a, Fairfax et al., 2013b, Fairfax et al., 2013c). The analytical approach established by Fairfax and colleagues (2013a) has been implemented to demonstrate that α-adrenergic receptor function is exclusively responsible for the vasoconstrictor effect of MSNA on the peripheral vasculature. The bursting pattern observed in these studies illustrates how the central nervous system can modulate the rate and amount of noradrenaline release to provide a variety of durations and strengths of vascular responses (Fairfax et al., 2013a, Fairfax et al., 2013b). This approach has also been used to examine ethnic differences in resting sympathetic vascular transduction (Vranish et al., 2018). Despite similar resting levels of MSNA, sympathetic vascular transduction was reported to be greater in young African American males when compared with young Caucasian males and may provide insight into why African American are at greater risk of developing cardiovascular disease (Vranish et al., 2018). However, this method may be limited in individuals with high levels of resting MSNA. This is because it depends on locating single or clusters of bursts that are followed by cardiac cycles not associated with a burst. For individuals with high MSNA, there are few periods of no MSNA and so the time course of the effects of sympathetic outflow to the vasculature may be difficult to track. It is also not known whether the same trend occurs in females. Using longer recording periods or focusing on the strength of the bursts could be a way around this limiting factor.

1.6.4 Quantifying vascular transduction using diastolic pressure

Briant and colleagues (2016) developed an approach for quantifying vascular transduction using the relationship between MSNA burst area and diastolic pressure. This is particularly useful in instances where measurements of blood flow cannot be acquired. This method of vascular transduction uses diastolic pressure as a proxy for the vascular response to sympathetic outflow. With this approach, the total burst area across two cardiac cycles is identified at a fixed lag of 8-6 cardiac cycles prior to each diastolic pressure (Fig 1.6A). Normalised MSNA burst area is binned into 1% s bins and plotted against their

corresponding diastolic pressure and put through linear regression analysis (Fig 1.6B) (Briant et al., 2016). The slope of the regression line represents an individual's vascular transduction. This method is similar to that of spontaneous sympathetic BRS, but has distinct differences. The threshold method looks for changes in MSNA that result from changes in arterial pressure, whereas transduction analysis looks for changes in blood pressure that result from the effect of MSNA on the circulation (Briant et al., 2016). However, this method of vascular transduction completely bypasses the end organ response of MSNA and therefore may not provide as in depth information on the vasoconstrictor drive of MSNA than through direct measures such as blood flow using Doppler ultrasound. It is not yet known whether these two methods of vascular transduction can be used interchangeably. Therefore, in this thesis, both measures of vascular transduction were implemented.

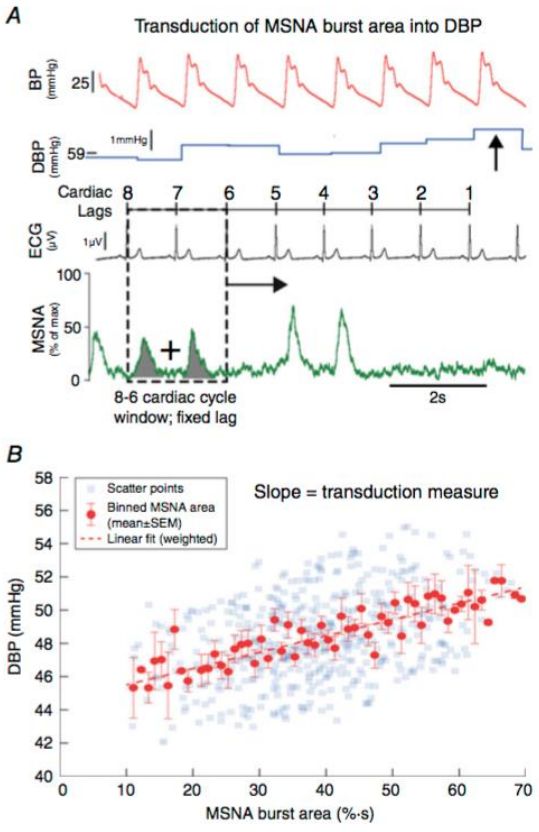

Figure 1.6 Method of quantifying vascular transduction into diastolic pressure in humans A) For each diastolic blood pressure (arrowed), MSNA burst area (shaded) in a 2-cardiac cycle window was summed at a fixed lag of 8-6 cardiac cycles preceding the diastolic pressure (dashed window). B) MSNA burst area is binned into 1% s bins, and the corresponding diastolic pressure is plotted. A weighted linear regression was then fitted to these data, the slope of which provided the measurement of vascular transduction (units of mmHg (% s)$^{-1}$) (Briant et al., 2016)

1.6.5 Sex differences in vascular transduction

Evidence suggests that vascular transduction, which is key to an effective baroreflex response to changes in blood pressure, is influenced by age and sex (Briant et al., 2016, Vianna et al., 2012). Although it is known that the autonomic control of MSNA is vital for ensuring that adequate blood pressure and perfusion is maintained, the influence of sex and ageing on the end organ response of this mechanism warrant investigation. Lower vascular transduction in premenopausal females, when compared with young males, may be attributed to women having lower levels of arterial pressure, MSNA and CO (Joyner et al., 2015). Also, women are lighter, have more fat mass, lower lean mass and higher levels of oestrogen (Joyner et al., 2015). Vianna et al. (2012) found that both males and females have age-related declines in sympathetic vascular transduction. Conversely, Briant et al. (2016) reported that females have an age-related increase in vascular transduction, whereas males have an age-related decline in vascular transduction. Following ß-blockade, premenopausal females had a significant increase in transduction while postmenopausal women and young men had no systematic changes. This supports a greater role of ß2-mediated sympathetic vasodilation at rest in young women (Hart et al., 2011a). The different findings by Vianna et al. (2012) and Briant et al. (2016) may be explained in part by different analytical techniques used. Vianna et al. (2012) quantified vascular transduction as the mean peak increase in mean arterial pressure following spontaneous bursts of MSNA whereas Briant et al. (2016) used the relationship between diastolic pressure and MSNA burst area that occurred at a fixed lag. The techniques described by Fairfax et al. (2013a, 2013b, 2013c) have only been examined in young normotensive males. It is unknown if this method (i.e., Fairfax method) of characterising sympathetic vascular transduction displays the same results as that of Briant et al. (2016) with regards to differences between males and females. This is important because if diastolic pressure is used as a proxy for the end organ response, it is vital that they are actually providing the same information.

1.7. Compensatory interactions in cardiovascular control

There is evidence to suggest that the body provides compensatory interactions in an attempt to maintain blood pressure homeostasis. The effects of resting MSNA and CO are inversely related (Charkoudian et al., 2005). Individuals with high MSNA have low CO, more

specifically stroke volume, and those with low levels of MSNA have high levels of CO and stroke volume (Charkoudian et al., 2005). However, these relationships have only been established in young normotensive males; the relationship between CO and MSNA does not exist in females (Hart et al., 2009a). Previous evidence suggests this may be due to enhanced ß-adrenergic sensitivity in females, which balances the vasoconstrictor effects of sympathetic outflow (Hart et al., 2011a, Kneale et al., 2000). This highlights the fact that there are variables other than MSNA that control TPR, and that these variables may be more dominant in premenopausal females.

Levels of MSNA may also be counterbalanced by the degree of sympathetic vascular transduction. Figure 1.7 illustrates the individual variability in the dynamic changes in leg vascular conductance following each MSNA burst (Fairfax, 2013). The change in leg vascular conductance following MSNA bursts is also inversely related to MSNA burst frequency, which accounts for 35% of the variability in vascular transduction. Those with low resting MSNA have greater vascular transduction, and those with high resting MSNA have lower vascular transduction. The authors also speculate that impairments in sympathetic vascular transduction may be compensated by higher sympathetic BRS (Fairfax et al., 2013b). However, the relationship between resting MSNA and vascular transduction has only been examined in young and older males. It is not known if this compensatory interaction exists in females. The control of cerebral blood flow is, in part, reliant on the baroreflex to buffer the changes in blood pressure (Tzeng et al., 2010). Interestingly, it has been demonstrated that individuals with poor cerebral autoregulation possess elevated cardiac BRS, and vice versa (Tzeng et al., 2010), again highlighting the compensatory ability between two mechanisms. Recent evidence also suggests that an inverse relationship exists between cerebral autoregulation and sympathetic BRS (Witter et al., 2017). These findings reflect the integrated response to rapid changes in pressure evoked pharmacologically by the modified Oxford method. Witter et al. (2017) also observed this inverse relationship when sympathetic BRS was quantified during spontaneous MSNA and diastolic blood pressure.

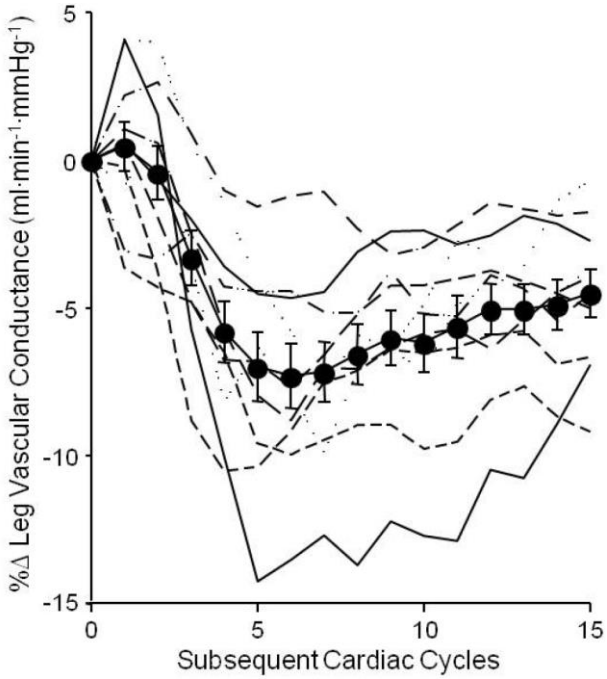

Figure 1.7. Peak changes of beat-to-beat responses of leg vascular conductance following MSNA bursts in 10 individuals (lines) and group average (circles) (Fairfax, 2013)

Baroreflex sensitivity is essential in determining an individual's blood pressure buffering capability, but current methods of assessment do not take into account sympathetic BRS in conjunction with the direct effects of MSNA on the vasculature. Establishing whether there is a relationship between sympathetic BRS and vascular transduction is essential, as it will confirm whether or not an enhanced sympathetic BRS does lead to more effective control of blood pressure. If these cardiovascular variables do share a relationship, they may potentially be used as a tool for assessing cardiovascular risk. These investigations will provide information on inter-individual differences in blood pressure control at rest.

1.8. Effects of the menstrual cycle on autonomic control

As demonstrated in the sections above, sex has major implications for cardiovascular control when maintaining homeostasis. The disparity between males and females may, in part, be due to the fluctuating levels of sex hormones in females across the menstrual cycle. The female sex hormones, oestradiol, and progesterone are not only crucial for reproductive purposes but are also important in other physiological functions such as cardiovascular control. This is particularly apparent across the menstrual cycle in premenopausal women, and reductions in these hormones following menopause may be a core reason as to why post-menopausal

women have a higher risk for hypertension and cardiovascular disease. Minson et al. (2000a) reported that MSNA is greater during the mid-luteal (high hormone) phase of the menstrual cycle when compared with the early follicular (low hormone) phase of the menstrual cycle. The elevated sympathetic outflow during the mid-luteal phase compensates for the greater vasodilation that occurs due to increased oestrogen-mediated nitric oxide release (Minson et al., 2000a).

Sympathetic BRS is greater during the mid-luteal (high hormone) phase of the menstrual cycle when compared with the early follicular (low hormone) phase in females with naturally occurring menstrual cycles (Minson et al., 2000a). Moreover, Minson et al. (2000a) had one participant that did not ovulate (did not produce progesterone) and their sympathetic BRS was greater where oestrogen was highest which was in the low hormone phase. This suggests that oestrogen enhances sympathetic BRS whereas progesterone antagonises this effect in menstruating females. Conversely, Fu and colleagues (2009) found no differences in sympathetic BRS between the two menstrual phases. MSNA burst frequency responses during head-up tilt were similar between males and females and between low and high hormone phases (Fu et al., 2009). However, total MSNA during the upright position tended to be lower in women during the early follicular phase when compared with men (Fu et al., 2009).

Previous reports suggest there are no differences in vascular transduction between the low and high hormone phases in females with natural menstrual cycles (Minson et al., 2000a). Oestrogen supplementations have been shown to lower MSNA in premenopausal women by enhancing the availability of nitric oxide (Best et al., 1998), which may counteract the vasoconstrictor effects of MSNA. Charkoudian and colleagues (2006) examined the relationship between MSNA and vascular response to brachial infusions of neurotransmitters, noradrenaline and tyramine in young males and females. Those with low MSNA displayed a stronger noradrenaline-induced vasoconstriction than those with high MSNA. The vasoconstrictor effects of sympathetic activity are blunted in individuals with high levels of MSNA and may be why individuals with similar levels of blood pressure have varying levels of MSNA (Charkoudian et al., 2006). In this study sex differences in the pressor response to

these infusions were not examined and thus it is not clear how consistent these effects are between males and females. Ettinger et al. (1998) studied the sympathetic nervous system response to isometric handgrip across the menstrual cycle when circulating levels of oestrogen were low (early follicular) versus when they were high (late follicular). The increase of sympathetic outflow during isometric handgrip was greater during the early follicular phase of the menstrual cycle when compared with the late follicular phase. Despite this, a cycle related effect on blood pressure was not observed. The blunted increase of sympathetic outflow in response to isometric handgrip during the high hormone phase may be due to a dampened sympathetic vascular transduction during sympathetic activation (Ettinger et al., 1998). This has also been observed during chemoreflex stimulation where despite a greater increase in MSNA during the early follicular phase of the cycle than the mid-luteal, the increase in cardiovascular haemodynamics such as TPR and mean arterial pressure was not different between the two phases of the menstrual cycle (Usselman et al., 2015a).

At rest, CO and TPR are not different in females with natural menstrual cycles and those who take oral contraceptives (Minson et al., 2000b). However, the use of oral contraceptives further influences the MSNA and BRS responses across the menstrual cycle (Minson et al., 2000b). In normal menstruating females, MSNA and sympathetic BRS are greater during the high hormone phase of the menstrual cycle (Minson et al., 2000b). In females taking oral contraceptives, sympathetic BRS is elevated in the low hormone phase, but MSNA is not different between the two phases of the menstrual cycle (Minson et al., 2000b). Based on this evidence, it is important when designing a research study on the sympathetic baroreflex to control for the menstrual cycle and confirm that each female participant is tested in the same phase to ensure that the changes that are observed are due to the intervention or stressor (Minson et al., 2000a), and not because of the different phase of their cycle. Whether or not the individuals take oral contraception should also be considered. Therefore, the data for this thesis was only collected when female participants were in the early follicular phase of their menstrual cycle. The studies in this thesis did not control for oral contraceptive use, but was recorded for potential comparisons between those who do and do not take oral contraceptives.

1.9. Cardiovascular control during pregnancy

A number of the cardiovascular control mechanisms discussed so far are influenced by pregnancy. During pregnancy, physiological changes occur in the body to support the foetus, protect both mother and foetus and to prepare the mother for delivery. Such physiological changes include an increase in CO (via increases in heart rate and stroke volume), blood volume, renal and systemic vasodilation and a decrease in mean arterial pressure. Elevated blood volume during pregnancy is associated with the activation of the renin-angiotensin-aldosterone system (RAAS) and results in increased sodium and water reabsorption from the kidney (Fu and Levine, 2009). Hormonal changes associated with pregnancy result in renal vasodilation, overriding vasoconstriction elicited by the RAAS system (Chapman et al., 1998). A simultaneous increase in systemic vasodilation occurs, possibly due to increased endothelial nitric oxide release stimulated by elevated circulating oestradiol (Fu and Levine, 2009). Despite the increase in CO and blood volume, mean arterial pressure is reduced early in pregnancy (Chapman et al., 1998). Elevated levels of MSNA may represent a compensatory mechanism to balance these vasodilator effects and prevent blood pressure from dropping to dangerous levels (Fu and Levine, 2009). During pregnancy, resting levels of MSNA have shown to be linked with the release of arginine vasopressin and may contribute to arterial pressure regulation during pregnancy (Charkoudian et al., 2017, Reyes et al., 2018). Charkoudian et al. (2017) reported resting levels of MSNA to be strongly correlated with the amount of vasopressin released in normotensive pregnant women in the third trimester but not in non-pregnant controls, demonstrating that vasopressin is a significant contributor to the variability of resting MSNA in normotensive pregnant females.

Literature on the role of the sympathetic nervous system during pregnancy is limited. In some studies, MSNA has been reported to increase as early as 6 weeks into the gestation period (Jarvis et al., 2012, Okada et al., 2015), and in others towards the later stages (Fischer et al., 2004, Greenwood et al., 2001, Greenwood et al., 1998, Okada et al., 2015). However, these studies have been performed longitudinally either only in the first half or second half of pregnancy or have compared pregnant women with non-pregnant controls (Fischer et al., 2004, Greenwood et al., 2001, Greenwood et al., 1998, Jarvis et al., 2012, Okada et al., 2015). Okada and colleagues (2015) conducted the first longitudinal examination of MSNA before, during and after pregnancy. It was reported that MSNA increased early in the first

trimester of pregnancy and increased further into the third trimester (Okada et al., 2015). Reyes and colleagues (2018) conducted a longitudinal case series on two females. The increase in sympathetic outflow during pregnancy was strongly correlated with elevated levels of progesterone and oestrogen (Reyes et al., 2018). Furthermore, augmented sympathetic outflow during pregnancy was offset by a reduction in vascular transduction (Reyes et al., 2018). Despite the elevated levels of sympathetic outflow during pregnancy, vascular transduction decreased by up to 53%. Previous studies have also reported vascular transduction to decrease during early (Jarvis et al., 2012) and late pregnancy (Usselman et al., 2015c). Schmidt et al. (2018) reported that the elevated sympathetic outflow in the third trimester of normotensive pregnancy is not due to the number of action potential or action potential clusters within an individual MSNA burst but rather an increased incidence of multi-unit bursts when compared with non-pregnant controls. This suggests that the attenuated vascular transduction during pregnancy previously reported (Jarvis et al., 2012, Reyes et al., 2018, Usselman et al., 2015c) occurs downstream of neural signalling such as neurotransmitter release and/or receptor sensitivity. For example, nitric oxide-mediated vasodilation is enhanced during pregnancy due to increased circulating oestradiol (Williams et al., 1997). In previous reports, vascular transduction was not quantified on a beat-to-beat basis, so the magnitude of change in vascular conductance or arterial pressure following MSNA bursts is not known.

In order to increase our understanding of sympathetic activation throughout a healthy pregnancy, it is important to define the levels of MSNA before, during and after pregnancy using longitudinal design. Although we have some insight into the effect pregnancy has on sympathetic outflow, it is unclear how the baroreflex modulation of MSNA is affected during pregnancy. In previous studies, both cardiac and sympathetic BRS are attenuated during the third trimester of pregnancy (Moertl et al., 2009, Usselman et al., 2015b), with further reductions in cardiac BRS associated with gestational hypertension and preeclampsia (Silver et al., 2001). Even though cardiac BRS has been assessed longitudinally from early pregnancy to the post-partum period (Moertl et al., 2009), studies of sympathetic BRS have been limited to a single assessment in the third trimester of pregnancy in a cross-sectional design with non-pregnant controls (Usselman et al., 2015b). This does not tell us how MSNA or sympathetic BRS is affected throughout pregnancy in the same individual.

Until this point, the focus of this review has been on cardiovascular control at rest. However, humans are exposed to a range of physical and mental stressors on a daily basis, posing physiological challenges to which the autonomic nervous system must respond. And yet, our understanding of blood pressure regulation and MSNA responses to physiological stress and how they are influenced by sex is limited. In the following section, the response of the cardiovascular system during both mental and physical stressors and how this response differs between males and females was discussed.

1.10. Sex differences in cardiovascular responses to physiological stress

The control of blood pressure is not only essential during rest but also during everyday tasks. Given that there are known sex differences in cardiovascular control, it is easy to assume that the response to physiological stress may be different between males and females. Any increases in MSNA during stressors will be reliant upon effective vascular transduction in order to have an effect on blood pressure. Sex differences in MSNA, vascular transduction, and sympathetic BRS may influence the blood pressure reactivity to stressors in males and females. Physiological stressors that drive increases in MSNA, such as isometric handgrip and cold pressor test, can be used to examine sympathetic BRS and vascular transduction over larger blood pressure and sympathetic activity ranges. Since physiological stress is associated with activation of the sympathetic nervous system, evaluating the mechanisms that regulate MSNA during mental and physical stress may provide insight into how stress influences cardiovascular regulation during everyday tasks.

1.10.1 Mental stress

Cardiovascular responses to mental stress are commonly examined using standard laboratory tasks such as mental arithmetic and the Stroop colour word test. Although it is debatable whether these tasks imitate the pressor response to everyday stress, the utilization of these tasks in previous studies suggests that marked increases in blood pressure can occur (Carter et al., 2005, Durocher et al., 2009, Kuipers et al., 2008, Rusch et al., 1981). It has been reported that an exaggerated response during mental stress is a predictor of future hypertension and elevated cardiovascular risk (Carroll et al., 2012, Carroll et al., 2011, Chida and Steptoe,

2010). Males experience a greater increase in diastolic blood pressure during mental stress when compared with females (Carter and Ray, 2009, Traustadottir et al., 2003, Satish et al., 2015). It is believed that the greater increase in arterial pressure in males during mental stress is due to males having greater sympathetic arousal or parasympathetic withdrawal due to the influence of circulating male hormones that affect vagal activity (Montano et al., 2009). However, other reports have shown that despite the greater increase in blood pressure observed in males during mental stress, the increase in heart rate and MSNA seem to be similar between the sexes (Durocher et al., 2009). The changes in blood pressure in response to mental stress may be due to MSNA acting as a driving force for this pressor response, although the evidence from previous studies is inconsistent.

The increases in blood pressure in response to mental stress have previously been associated with increases (Durocher et al., 2011, Durocher et al., 2009, Klein et al., 2010, Yang et al., 2013), decreases (Durocher et al., 2009) and no changes (Kuipers et al., 2008) in MSNA. Increases in sympathetic outflow have also been demonstrated following the cessation of the mental task and may be mediated by the baroreflex (Anderson et al., 1987, Carter et al., 2005). This is because the drop in pressure during recovery may have led to an increase in MSNA as the baroreflex was reset to a higher pressure during the mental stressor task (Anderson et al., 1987, Carter et al., 2005). The inconsistent results between studies may be due to inter-individual variability in response to mental stress. In response to this variation, Carter and Ray (Carter and Ray, 2009) divided individuals into groups based on whether MSNA increased (positive responders), decreased (negative responders) or did not change (non-responders) in response to mental stress. Even though MSNA changed in the responders, mean arterial pressure and heart rate increased similarly across all groups (Carter and Ray, 2009). This suggests that the changes in MSNA may not be correlated with changes in blood pressure during mental stress and the response to this stressor is not as straightforward as once thought (Carter and Ray, 2009). El Sayed et al. (2016) recently explored the rate of rise in diastolic blood pressure during mental stress in young male responders and non-responders and how it influenced the direction of change in MSNA. Individuals who had a greater rate of rise in diastolic pressure at the onset of mental stress had a suppressed MSNA response, which may reflect activation of the baroreflex. In those individuals with a slower rate of rise in arterial pressure the baroreflex was reset to higher

pressures, allowing MSNA to increase and contribute to the elevation in blood pressure during mental stress (El Sayed et al., 2016). This approach was also performed in females and showed, that regardless of sex, reactivity of blood pressure early in the task determines whether MSNA has a role in the pressor response (El Sayed et al., 2018).

The vascular responses to mental stress have been examined in both upper and lower limbs. It has been reported that, during mental stress, vasodilation occurs in the forearm leading to increases in vascular conductance, whereas it remains unaltered in the calf (Carter et al., 2005, Durocher et al., 2009). Nitric oxide, a major vasodilator, plays a role in this increase in vascular conductance during mental stress (Cardillo et al., 1997, Dietz et al., 1994), which may explain the varied response of the vasculature between the upper and lower limbs. However, Kuipers and colleagues (2008) reported that during mental stress, vascular conductance increased in both the forearm and leg. The inconsistent results between these two studies may be due to the site at which blood flow was measured in the leg. In one study, blood flow was measured at the popliteal artery (Carter et al., 2005), whilst in the other, it was measured at the common femoral artery (Kuipers et al., 2008). It is suggested that the variation between the common femoral and popliteal artery may be due to the length and size of the vascular bed measured (Kuipers et al., 2008). Nevertheless, calf blood flow has been shown to increase (Blair et al., 1959, Hjemdahl et al., 1989, Hjemdahl et al., 1984, Kamiya et al., 2000) and not change (Carter et al., 2005, Rusch et al., 1981) during mental stress.

The dissociation between the levels of vasodilation shown in the upper and lower limbs may also be influenced by sex. Butt et al. (1999) reported that during mental stress, females experience significantly greater calf vasodilation than males, and males demonstrate greater calf vasoconstriction than females. The authors speculate that there is a greater ß2-adrenoreceptor density in the calf vascular bed in females and greater α-adrenoreceptor density in the calf vascular beds of males (Butt et al., 1999). This may be the source of inconsistent findings in the calf vascular response to mental stress. Yang et al. (2013) previously reported significantly greater increases in calf blood flow and vascular conductance during mental stress, which was positively correlated with increases in forearm vascular conductance in females only. In contrast, there was an inverse relationship between

calf vascular conductance and MSNA responses in males only (Yang et al., 2013). This suggests a decoupling of limb vascular responses to mental stress in men but not females. The transduction of MSNA to vasoconstrictor drive may be different between males and females; i.e., males are more sensitive to the vasoconstrictor action of MSNA during mental stress than females (Yang et al., 2013). Differences in sympathetic BRS, and hence the beat-to-beat control of MSNA, between participant groups, may also cause this variation in changes in leg vascular conductance.

Durocher and colleagues (2011) investigated the response of sympathetic BRS to 5 minutes of mental arithmetic. Sympathetic BRS was reduced when performing mental arithmetic, but further analysis revealed it was only during the first 2 minutes of the task. Sympathetic BRS during the subsequent three minutes was not different from baseline (Durocher et al., 2011). Assessing baroreflex slopes with only two minute and three minute segments may not provide enough data to accurately quantify an individual's sympathetic BRS (Holwerda et al., 2012). Evaluating sympathetic BRS in conjunction with vascular transduction may help to unravel the effects of mental stress on the cardiovascular system.

1.10.2 Physical Stress

During exercise, neural mechanisms are involved to accommodate the increased demand of oxygen to tissues for energy production, removal of metabolic waste, and to ensure arterial pressure is maintained. At the onset of exercise, the cardiovascular pressor response is initiated via central command, which involves higher brain regions that project to the cardiovascular control centre to increase CO, heart rate and blood pressure via parasympathetic vagal withdrawal and sympathetic activation (Rowell, 1974, Rowell, 1997). This is maintained by the exercise pressor reflex where type III and IV muscle afferents respond to muscle contraction (mechanoreflex) and metabolic by-products caused by muscle work (metaboreflex) resulting in a reflex increase in MSNA (Rowell, 1992). In addition, the baroreflex is reset around a higher blood pressure range during exercise. The magnitude of cardiac baroreflex resetting is dependent on the intensity of the exercise performed, with no change in the sensitivity of the baroreflex (Papelier et al., 1994, Potts, 2006). Sympathetic BRS has also been shown to reset during physical stressors such as isometric handgrip

(Ichinose et al., 2006) and the cold pressor test (Cui et al., 2001). During isometric handgrip, sympathetic BRS was augmented, and the slope of the relationship between diastolic pressure and MSNA was shifted upwards and rightwards and is maintained during post-exercise ischaemia (Ichinose et al., 2006, Kamiya et al., 2001). Also, sympathetic BRS using total MSNA, quantified using the modified Oxford method, improves in response to the cold pressor test (Cui et al., 2002). Blood pressure increases consistently in individuals in response to a number of standard laboratory physical stressor tasks. The increase in MSNA during the cold pressor test (Fagius et al., 1989, Victor et al., 1987) and isometric handgrip (Mark et al., 1985, Minson et al., 2000a, Seals, 1989b) correlates strongly with an increase in blood pressure and thus both make a suitable manoeuvre to stimulate the sympathetic nervous system. These responses are in contrast to those during mental stress, for which there is considerable inter-individual variation in MSNA responses regardless of the similar increases in arterial pressure (Durocher et al., 2009).

Experimental work in rats has shown that the central neural pathways involved in autonomic activation during the cold pressor test include the RVLM and nucleus ambiguous (Nakamura et al., 2008). These cardiovascular brain regions drive increases in both muscle sympathetic activity and heart rate when exposed to a cold stimulus (Nakamura et al., 2008). The sympathetic response to isometric handgrip involves a number of mechanisms. Reflex activation of central command increases heart rate within the first 30 s of isometric handgrip (Mark et al., 1985). The rate of the increase in MSNA is dependent on the size of the muscle performing the exercise (i.e., single vs. double isometric handgrip) (Seals, 1989a, Vianna et al., 2010). Furthermore, there is an intensity-dependent increase in muscle vasoconstrictor drive in both active and non-active muscle (Boulton et al., 2014, Saito et al., 1986). Sympathetic outflow is elevated during isometric handgrip to counteract the locally induced vasodilation of the forearm muscles (Seals et al., 1988). Both mechano- and chemo-sensitive receptors activate group III and IV muscle afferents that project to the NTS, evoking reflex increases in MSNA (Rowell, 1992). Previous evidence suggests that central command has little involvement in the elevated sympathetic outflow to the non-active muscle during isometric exercise and is mediated by the metaboreflex (Vissing et al., 1991). The increase in MSNA and blood pressure elicited by isometric handgrip remain elevated during post exercise ischaemia whereas heart rate returns to baseline levels (Macefield and Henderson,

2015, Ray et al., 1992). However this is only evident in the non-active muscle. Recent reports have demonstrated central command to be the primary neural mechanism for sympathetic vasoconstrictor drive in the active muscle during isometric exercise (Boulton et al., 2014, Boulton et al., 2016). At the onset of exercise, Boulton and colleagues (2014, 2016) reported a rapid increase in MSNA that maintained throughout the task and a decrease in MSNA following cessation of the task during post exercise ischaemia.

Previous research demonstrates that cardiovascular responses to isometric handgrip and the cold pressor test follow a similar pattern in males and females and these responses are not significantly different (Hogarth et al., 2007, Jones et al., 1996, Shoemaker et al., 2001, Dishman et al., 2003). However, when expressed in absolute changes, MSNA during isometric handgrip has been shown to be greater in males than females (Jones et al., 1996). Hogarth et al. (2007) reported smaller increases in arterial pressure in response to isometric handgrip in females, while absolute increases in MSNA were not different when compared to males. As young females tend to have lower resting levels of MSNA, the percent increase in MSNA was greater in females (Hogarth et al., 2007). However, the changes in calf vascular resistance during isometric handgrip were blunted in females when compared with males. This suggests that the transduction of MSNA to vasoconstrictor drive is attenuated in females during sympathetic activation (Hogarth et al., 2007).

Use of sympathetic activation to quantify sympathetic vascular transduction

Previous studies have involved the use of stressors, such as lower body negative pressure (Ray and Monahan, 2002), isometric handgrip (Tan et al., 2013a) and post-exercise ischaemia (Minson et al., 2000a), to compare the change in vascular resistance for a given change in MSNA and thus quantify sympathetic vascular transduction. Isometric handgrip stimulates a reproducible pressor response where MSNA and vascular resistance increase in a progressive, parallel fashion (Minson et al., 2000a, Seals, 1989b). This fundamental relation between MSNA and vascular resistance during static handgrip provides a non-pharmacological means for quantifying sympathetic vascular transduction over a wider sympathetic range (Minson et al., 2000a). The gradual increase in MSNA during static handgrip coincides with the gradual decrease in calf blood flow. However, Saito and colleagues (1990) found the increase in calf

vascular resistance during static handgrip plateaued within the first minute despite the continued increase in MSNA. The dissociation between MSNA and calf vascular resistance during handgrip may be that when the arterioles reach a certain threshold, they start to relax and dilate back to baseline due to the increase in local metabolites (Marshall, 1982).

Although both the cold pressor test and isometric handgrip induce significant increases in heart rate, blood pressure, and MSNA, it is still unclear as to how inter-individual differences in the vascular transduction of MSNA may influence the magnitude of the blood pressure response and whether these inter-individual differences are due to sex differences in cardiovascular control. Furthermore, the use of physical stressors to drive increases in MSNA and blood pressure may allow for sex differences in vascular transduction to be examined over larger MSNA and pressure ranges.

1.11. Conclusion

Cardiovascular control is dependent on a number of variables including sex, age, menstrual cycle, and pregnancy. Having high sympathetic BRS does not necessarily mean that the transduction of MSNA to the peripheral vessels is also efficient. Evidence suggests compensatory interactions occur in the body and whether these interactions exist between sympathetic BRS and vascular transduction is yet to be investigated. Due to the lack of research around the baroreflex control of MSNA, it is unknown if an individual's sympathetic BRS reflects the end organ response and thus is indicative of how effective they are at regulating their blood pressure and whether this relationship exists in both males and females. Determining whether there is a relationship between sympathetic BRS and vascular transduction will enable us to better understand the regulatory mechanism of the sympathetic arm of the baroreflex. It is important that this is assessed at rest, but also to address how these mechanisms may be impacted by physiological stress associated with tasks of everyday living.

1.12. Study hypotheses and aims

Study 1

Hypotheses:

i) Spontaneous sympathetic BRS was stable within the same recording period and repeatable when recorded on a separate day in both males and females.

ii) Recording periods of longer durations were associated with greater diastolic pressure ranges at rest and that poor correlations exist between sympathetic BRS values derived from recording periods of different durations.

Aims:

i) To determine whether the current methods employed to quantify sympathetic BRS were stable within the same recording period and repeatable for separate experimental sessions.

ii) To determine whether the duration of the recording period influenced values of sympathetic BRS. These analyses were performed separately for males and females.

The primary variables were diastolic blood pressure and MSNA enabling the assessment of sympathetic BRS.

Study 2

Hypotheses:

i) Sympathetic vascular transduction was lower in young premenopausal females than when compared with young males.

ii) Sex differences were apparent using both the Fairfax and Briant methods of vascular transduction analysis.

Aims:

i) To examine whether vascular transduction, quantified using Doppler ultrasound and spontaneously occurring MSNA bursts differs between healthy young males and females.

ii) To compare the Fairfax method with an alternative method that does not involve ultrasound in which the transduction of MSNA to changes in blood pressure is quantified

Alongside diastolic blood pressure and MSNA, the primary variables were Doppler ultrasound imaging of the SFA to calculate beat-to-beat changes in leg vascular conductance in response to MSNA (vascular transduction).

Study 3

Hypothesis: Sympathetic BRS and sympathetic vascular transduction, acquired using Doppler ultrasound were negatively correlated under resting conditions in young healthy individuals. This would mean that individuals with a high sympathetic BRS had less effective vascular transduction during spontaneous changes in blood pressure.

Aim: To examine the relationship between sympathetic BRS and sympathetic vascular transduction. This was assessed at rest during spontaneous changes in MSNA, leg vascular conductance and blood pressure.

Study 4

Hypothesis: Vascular transduction is greater in males when compared with females during physiological stress and sympathetic BRS is reset to higher blood pressures during the cold pressor test.

Aim: To examine sympathetic BRS and vascular transduction during physiological stressors that drive increases in MSNA.

1.13. Outcomes

The studies that were performed in this thesis have furthered our understanding of baroreflex control of blood pressure via MSNA in males and females, taking into account both sympathetic BRS and the transduction of MSNA at the level of the vasculature. It was postulated that having a high sympathetic BRS does not necessarily mean that the transduction of MSNA to the peripheral vessels is also efficient at regulating acute changes in arterial pressure. Determining this relationship has provided insight into the regulatory mechanism of the baroreflex, from initial changes in blood pressure to the end organ response. Identifying the extent of inter-individual variability in response to stress in healthy young individuals will allow potentially abnormal responses to be identified in diseased states.

Chapter 2: GENERAL METHODS

2.1. Project overview

This thesis was designed to contribute to the body of knowledge about the influence of the sympathetic arm of the autonomic nervous system in baroreflex function in young, healthy males and females. In each study, muscle sympathetic nerve activity, heart rate and blood pressure were measured and superficial femoral artery blood flow was measured in Studies 2, 3 and 4. Respiration was also measured to monitor breathing but the data was not used in analysis. Study 1 was conducted to confirm the stability and repeatability of the analytical technique used to quantify spontaneous sympathetic BRS. Study 2 was conducted to examine whether sympathetic vascular transduction, quantified using Doppler ultrasound and spontaneously occurring MSNA bursts, differs between males and females. This method was compared with a method that does not involve ultrasound, in which transduction of MSNA to changes in blood pressure is quantified. Study 3 was performed to determine whether a compensatory interaction exists between spontaneous sympathetic BRS and sympathetic vascular transduction in the regulation of arterial pressure at rest. Lastly, Study 4 was conducted to compare vascular transduction between males and females during a number of standard laboratory stressors (isometric handgrip and cold pressor test) that drive MSNA and therefore produce a greater cardiovascular range compared with rest. Data for each of the studies above were collected within the same experimental session. However, Study 1 was retrospective in design as it also included data that were collected in previous studies in the same laboratory.

Participants were informed of what was involved in the experiment both in writing and verbally before signing a consent form (Appendix C). The studies that are presented in this thesis were conducted with the approval of the Human Research Ethics Committee, Western Sydney University, and satisfied the Declaration of Helsinki (approval number H11138). All studies were completed in a laboratory in the School of Medicine, Western Sydney University, Campbelltown campus.

2.2. Participants

Study 1 was a retrospective analysis of data recorded from 84 participants, consisting of 40 recordings made during this candidature, combined with data from 44 participants presented in previous publications (Hissen et al., 2015, Taylor et al., 2015, El Sayed et al., 2016). Studies 2, 3 and 4 were not retrospective in design as the data for each were collected during the same experimental session and comprised of participants who were recruited throughout this candidature. Exclusion criteria include those who smoked or took regular medication or had a known history of cardiovascular, respiratory, or endocrine disease. Fifty-two young healthy individuals were recruited for Studies 2, 3 and 4. Of these, 13 participants were excluded, as we were unable to gain spontaneous MSNA (7 participants) or did not acquire high quality microneurography or Doppler ultrasound recordings (6 participants). Therefore, 39 (19 males) participants were used in Studies 2 and 3. For Study 4, 25 (12 male) participants successfully performed isometric handgrip and 22 (13 males) participants successfully completed the cold pressor test. Not every participant was able to complete all of the stressor tasks. This was because at some point throughout the experimental session, the microelectrode had dislodged from the recording site and regaining a recording of spontaneous MSNA was not achieved. Participants were instructed to abstain from alcohol and vigorous activity for 24 hours before the experiment, and not to consume any caffeine on the day. Females were tested during the early follicular, low hormone phase (days 1-7) of their cycle to control for the variation of MSNA and BRS that occurs from fluctuations of sex hormones during the menstrual cycle (Minson et al., 2000a).

2.3. Experimental setup

In each experimental session, participants sat in a semi-recumbent (45°) posture with the left leg supported in an extended position in a temperature-controlled laboratory (22-23^{o}C). Beat-to-beat measurements of ECG, blood pressure, respiration, MSNA and SFA blood velocity and diameter were recorded. After a 10-min baseline period, cardiovascular stressors were performed. A schematic of the experimental protocol is shown in figure 2.1.

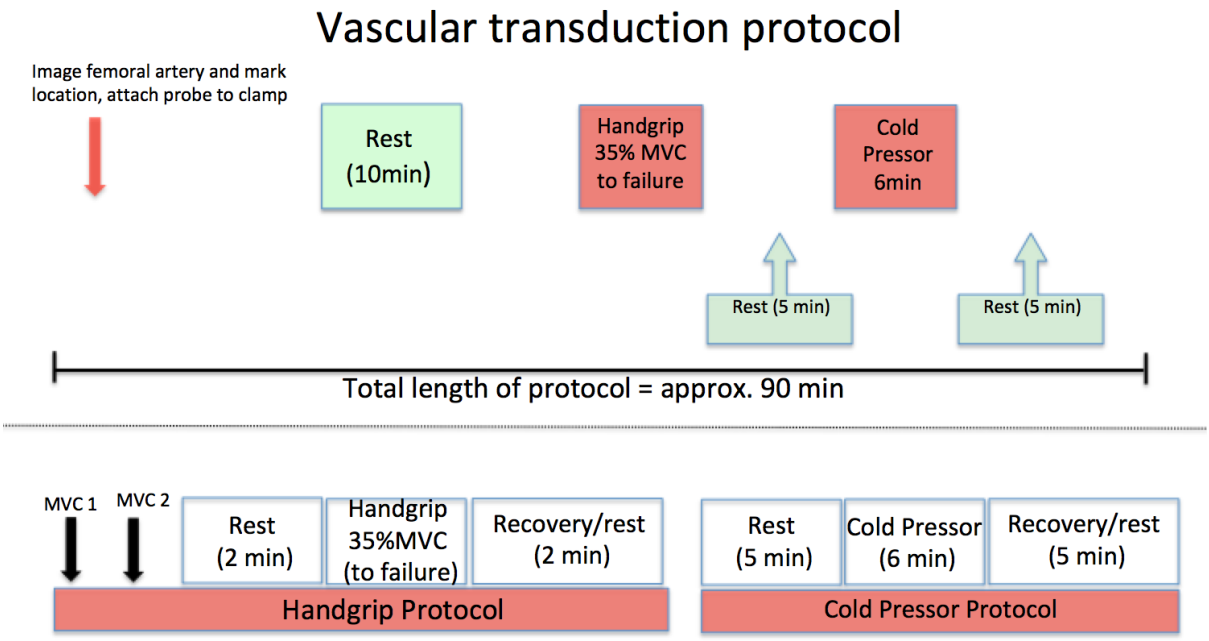

Figure 2.1. Schematic of experimental protocol of data collected in studies 2-4

2.4. Measurements

2.4.1 Cardiorespiratory measurements

Heart rate was recorded via a single lead (II) electrocardiogram (0.3-1 kHz) using 3 Ag-AgCl surface electrodes, ground on the left clavicle, negative electrode under the right clavicle and positive electrode on the left fifth intercostal space (BioAmplifier, ADInstruments, Dunedin, New Zealand). Respiration was measured via a strain-gauge transducer (DC-100Hz; Pneumotrace II, UFI, Morro Bay, CA, USA) wrapped around the chest. This was used to ensure participants were breathing at a normal rate throughout the experiment and were not holding their breath at any point.

2.4.2 Blood Pressure

Beat-to-beat measurement of blood pressure was continuously recorded throughout the experiments using a non-invasive, finger cuff on the second or third digit of the left hand (NOVA, Finapres Medical Systems, The Netherlands), height corrected for the distance between the heart and finger and sampled at 400 Hz. The inflatable bladder and infrared

plethysmograph within the finger cuff enables beat-to-beat blood pressure to be measured. The Finapres device achieves this by first determining the unloaded diameter of the arteries in the finger by inflating the bladder to a pressure that is equal to the intra-arterial pressure and where the transmural pressure across the arterial walls is zero. The unloaded diameter does not remain constant during a measurement so a physiological recalibration (Physiocal) must be performed periodically. The Physiocal algorithm uses arterial diameter and the shape of the plethysmograph signal during periods of constant pressure to track the unloaded diameter of a finger artery. Physiocals were performed to ensure the finger cuff air pressure was equal to the finger arterial blood pressure. In order to prevent drifting of the blood pressure signal, physiocals were turned on during the stabilisation period and between stressor tasks, but turned off during the baseline recording and laboratory stressors. Physiocals occur over 2-4 cardiac cycles and, if included in the analysis, will provide a misrepresentation of blood pressure during those cardiac cycles. Continuous beat-to-beat measurements of blood pressure are integral to the analytical techniques described below for sympathetic BRS and vascular transduction. Brachial blood pressure measurements (Omron, Kyoto, Japan) were also made during the initial baseline and between stressor tasks to ensure the finger cuff was accurately measuring blood pressure.

2.4.3 Muscle sympathetic nerve activity

In each study, microneurography was performed to directly record sympathetic outflow from a muscle fascicle. The common peroneal nerve was mapped out on the leg at the level of the fibular head using external stimulation (constant-current stimuli, 0.2 ms pulses, 2-10 mA) on the surface of the skin at 1 Hz (Stimulus Isolator, ADInstruments, Dunedin, New Zealand). Once the best site for inserting the microelectrode was located, a tungsten microelectrode (Frederick Haer and Co, Bowdoin, ME, USA) was inserted into the skin and guided to the nerve through weak electrical stimuli (0.02-1 mA) to induce muscle twitches. A reference electrode with 1 mm insulation removed was inserted just under the skin about 1-2 cm from the recording site. Following internal stimulation, small adjustments of the microelectrode tip were made until it penetrated a muscle fascicle and spontaneous bursts of MSNA were apparent. Listening to the neural recording speakers aided in identifying MSNA. The sound of bursts of sympathetic outflow is described as *"waves approaching the distant shore"* (Macefield, 2013). Penetration of a muscle fascicle was also confirmed by tapping and

stretching the muscle belly to evoke muscle spindle afferent activity and ensuring there was no increase in cutaneous afferent activity when stroking the skin (Sundlof and Wallin, 1977). Each participant was also asked to perform an inspiratory apnoea, which loads the low-pressure baroreceptors, causing an increase in MSNA, which does not occur when the electrode is recording neural activity from a skin fascicle. Neural activity was amplified (gain 20,000) and filtered (bandpass 0.3-5.0 kHz) using an isolated headstage (NeuroAmpEx, ADInstruments, Dunedin, New Zealand), and stored on a computer (10 kHz sampling) using a computer-based data acquisition and analysis system (PowerLab 16SP hardware and LabChart 8 software; ADInstruments, Dunedin, New Zealand). A root-mean-square-processed version of the signal was computed with a moving average of 200 ms. Recording of data did not begin until spontaneous MSNA was achieved and stabilized for at least 10 minutes. Experimental setup is shown in figure 2.2.

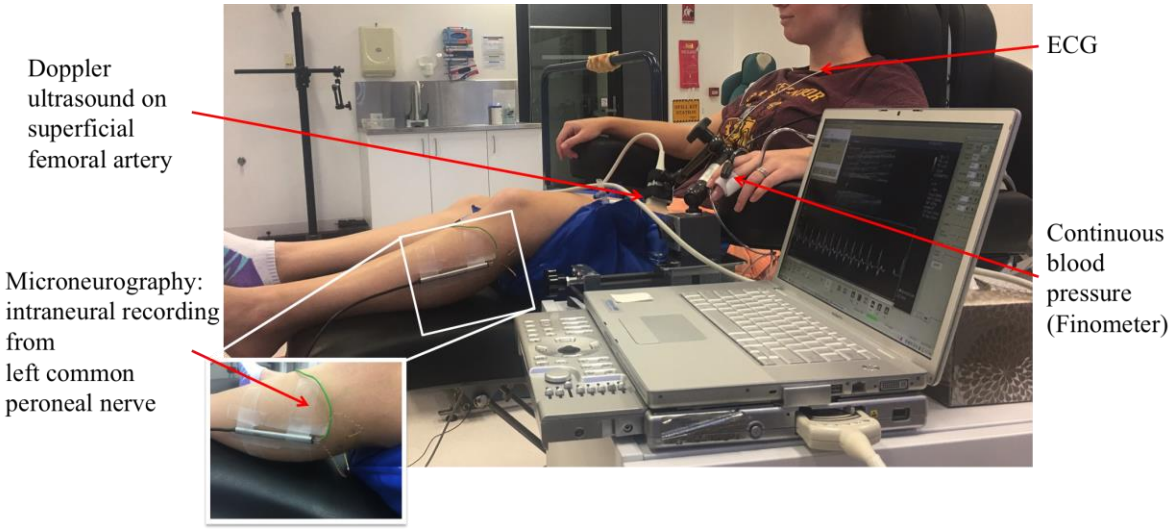

Figure 2.2. Experimental setup for microneurography and continuous measurements of ECG, blood pressure, respiration and blood flow using Duplex Doppler ultrasound

2.4.4 Superficial femoral artery diameter and blood flow assessment

Diameter and blood flow of the left SFA was measured with a Duplex Doppler ultrasound (T3000, Terason, Burlington, MA) using a linear array transducer operating at a frequency of 12 MHz. The SFA was imaged 2 to 3 cm distal to the bifurcation of the common femoral artery. Due to the semi-recumbent posture the participants were in, it was too difficult to

image the common femoral artery. Therefore, the SFA was chosen as it was more easily accessible and supplies blood to the lower leg (where the common femoral artery supplies blood to both the thigh and lower leg). The SFA was imaged in B-mode simultaneously with pulse-wave at an insonation angle of 60^{o} and operating at a linear frequency of 5 MHz. The Duplex mode of the ultrasound system was used to simultaneously record SFA diameter and velocity. Once an image was acquired and optimised, the location was marked before the probe was held in place by a clamp. A video of the ultrasound image was recorded and saved as an AVI file using Camtasia (TechSmith, Michigan, USA) (figure 2.3).

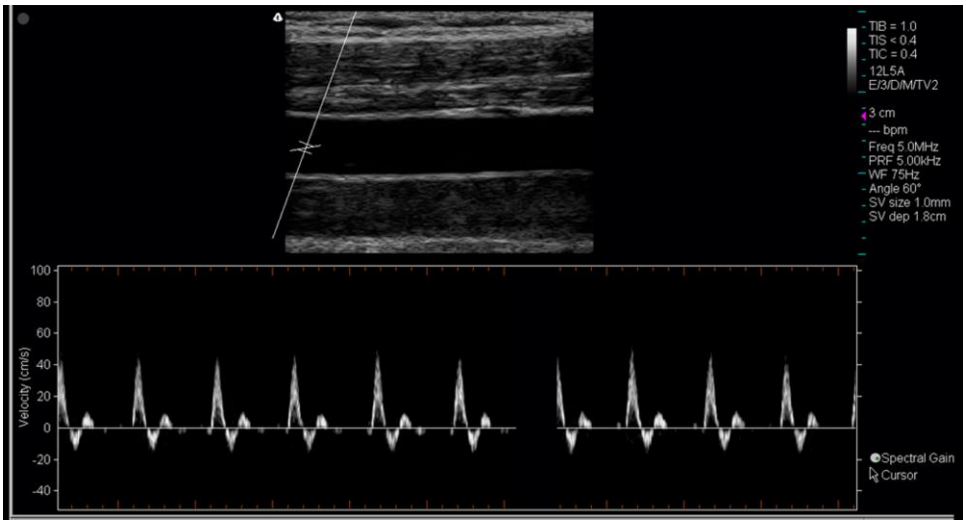

Figure 2.3. Ultrasound image of superficial femoral artery during pulse wave mode

The video recording of the ultrasound was used to extract beat-to-beat diameter and velocity of the SFA at 30 frames per second using custom-designed, edge-detection, and wall tracking software (BloodFlow Analysis, version 4.0). This approach is independent of investigator bias and has previously been validated with an average intra-observer coefficient of variation of 0.36% (Woodman et al., 2001, Black et al., 2008). The edge detection and wall tracking software was created using LabView (National Instrumetns, Austin, Texas) and IMAQ (National Instruments), which are used to build software programs called a virtual instrument (VI). Edge detection analysis has previously been described in detail (Black et al., 2008, Woodman et al., 2001, Green et al., 2002). Four regions of interest (ROI) were used to measure the beat-to-beat diameter and velocity for each recording:

- A “calibrate diameter” ROI converted the image size on the computer, measured in pixels, to the actual diameter of the artery (in cm). A box was drawn on the screen between two marks on the ultrasound image that were known to be 1 cm apart.

- The “calibrate Doppler” ROI calibrates the Doppler velocity by drawing a region between two points of the y-axis of the Doppler strip and inputting the appropriate scaling factor (~ 80-100 cm/s depending on the scale of the Doppler trace).

- The “diameter” ROI was used to select the most stable part of the artery on the B-mode image. An IMAQ pixel-density VI algorithm automatically identified the angle-corrected near and far wall of the artery and measured the diameter (in cm). The parallel lines on the near and far wall were then plotted to confirm that the system was accurately tracking the arterial walls.

- The “Doppler” ROI was drawn around the Doppler waveform strip. Within this region, an IMAQ automatic threshold VI was used to filter the grey-scale image. Each column of pixels within the ROI was analysed to detect the first white pixel in the vertical array to track the waveform envelope. The detected points were then plotted to provide visual feedback to ensure it was accurately tracking the Doppler trace.

The synchronized diameter and velocity data was used to calculate blood flow. Figure 2.4 displays an example of the edge detection analysis detecting each ROI. Since SFA diameter and velocity data were measured on a separate device to ECG, blood pressure and MSNA, a 2 1 countdown was made prior to each recording. This was done to ensure that the data from the ultrasound was time aligned to the data recorded in LabChart. For each participant, ECG, blood pressure and MSNA were extracted from LabChart as a text file. A text file was also extracted for each participant containing the SFA diameter and velocity output from the edge tracking software. Both files were put through a custom designed LabView program that aligned the two files for the transduction analysis.

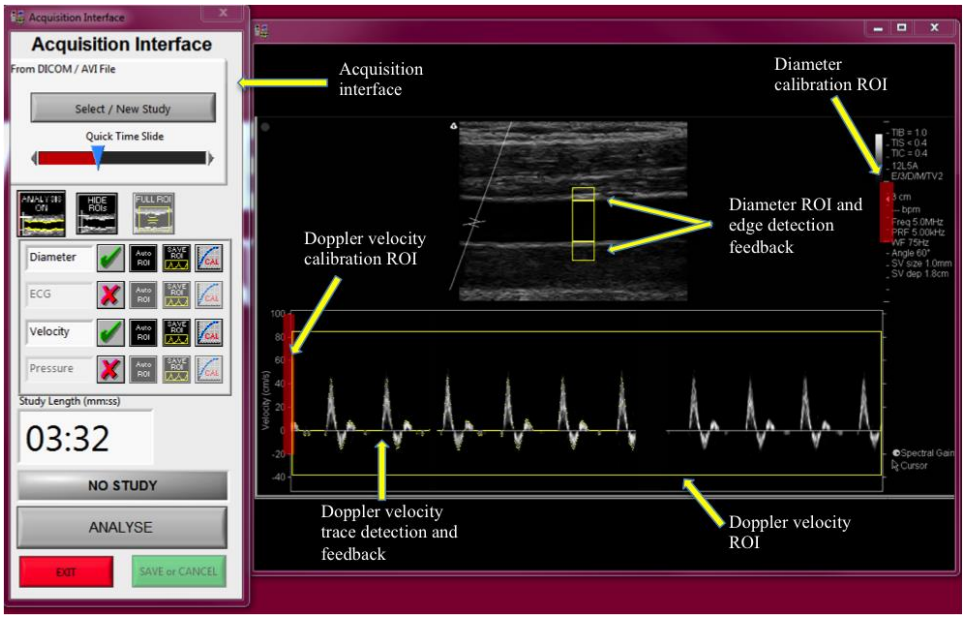

Figure 2.4. Example of the edge tracking software which provides beat-to-beat measurements of changes in SFA diameter and blood velocity displaying calibration ROI of the vessel and Doppler trace, diameter ROI, and the Doppler velocity ROI

2.5. Data analysis

Beat-to-beat values were extracted from LabChart (ADInstruments, Dunedin, New Zealand) for systolic blood pressure, diastolic blood pressure, mean arterial pressure, R-R interval and MSNA. The detection and area of each MSNA burst and the quantification of sympathetic BRS was performed using Ensemble (Elucimed Ltd, Wellington, New Zealand). The number of bursts per minute (MSNA burst frequency) and bursts per 100 heartbeats (MSNA burst incidence) was determined for each individual. Two approaches were used for the quantification of sympathetic BRS. These will be referred to as the 'burst incidence method' and 'total MSNA method', and both are described below.

2.5.1 Sympathetic baroreflex sensitivity: burst incidence method

Sympathetic BRS was quantified using methods originally described by Kienbaum et al. (2001). The nerve trace was optimally shifted for each participant by ~1.2-1.4 s for both methods of sympathetic BRS to account for the conduction delay of sympathetic outflow to the common peroneal nerve. Diastolic blood pressures were assigned to 3 mmHg bins for

each participant to remove potential non-baroreflex stimuli, such as respiration (Ebert and Cowley, 1992, Tzeng et al., 2009). For each bin, the corresponding MSNA burst incidence was determined. Sympathetic BRS was quantified by plotting MSNA burst incidence against the mean diastolic pressure for each bin. Each data point was weighted according to the number of cardiac cycles, as the bins at the highest and lowest diastolic pressures contain fewer cardiac cycles (Kienbaum et al., 2001). The value of the slope ($r \geq 0.5$ acceptance level) (Hart et al., 2011b), determined via linear regression analysis, provided the sympathetic BRS for the individual, which will be referred to as 'sympathetic BRS_{inc}'. An example of the slope for BRS_{inc} is displayed in figure 2.5. It is important that the BRS value represents the linear portion of the baroreflex slope. However, in cases where a sigmoidal relationship was present the weighting procedure limits the influence of saturation and threshold regions, for which there are very few cardiac cycles.

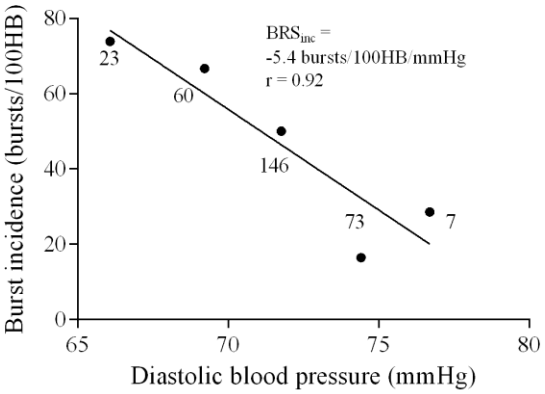

Figure 2.5. Sympathetic baroreflex assessment in a 24-year-old female using the burst incidence method. MSNA burst incidence is determined for each 3 mmHg diastolic pressure bin and then plotted against diastolic pressure. Number of cardiac cycles per bin indicated next to each point

2.5.2 Sympathetic baroreflex sensitivity: total MSNA method

The total MSNA method is based on a segregated signal averaging approach introduced by Halliwill (Halliwill, 2000). The largest MSNA burst for each recording period was assigned a value of 1000 and the remaining MSNA bursts during each period were normalized against

this (Halliwill, 2000). The relationship between diastolic pressure and total MSNA was assessed using 3 mmHg bins. Total integrated MSNA was determined for each bin using segregated signal averaging and expressed as arbitrary units (AU) per beat. Figure 2.6A shows the mean MSNA burst amplitude for each bin. The lower diastolic pressure bins have higher mean amplitude, and the higher diastolic pressure bins have lower mean amplitude. Linear regression was used to determine the relationship between total MSNA and diastolic pressure as shown in figure 2.6B, with the application of the weighting procedure described above to account for the number of cardiac cycles per bin. These baroreflex values will be referred to as 'sympathetic BRS_{total}' to differentiate them from the MSNA burst incidence method for assessing sympathetic BRS.

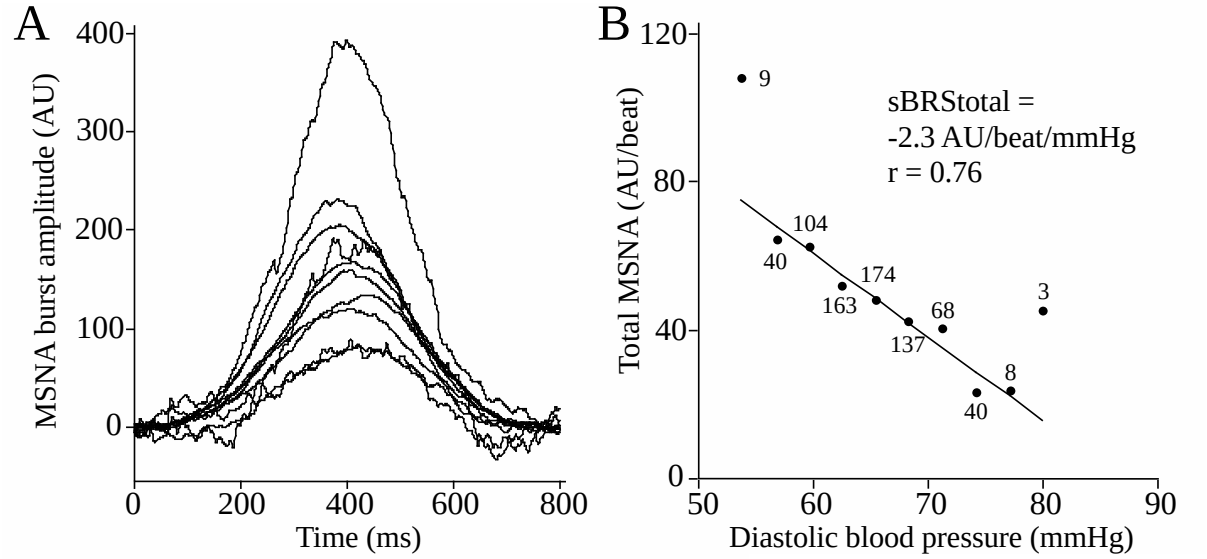

Figure 2.6. Sympathetic baroreflex assessment in a 21-year-old male using the segregated signal averaging approach. (A) MSNA bursts are normalized to the burst with the largest amplitude and entered into diastolic pressure bins of 3 mmHg. Each line represents the mean MSNA burst amplitude for a different bin, with the largest representing the lowest diastolic pressure bin. (B) Total MSNA per beat is determined for each bin and plotted against diastolic pressure (Taylor et al., 2015). Number of cardiac cycles per bin indicated next to each data point.

2.6. Statistical analysis

Statistical analyses were performed using Prism v6.00 for Windows (GraphPad Software, San Diego, California, USA). Acceptance levels for BRS slopes were $r \geq 0.5$. For all statistical tests, a probability of $p \leq 0.05$ was regarded as significant. All values are expressed as mean ± standard deviation (SD). Statistical analyses will be described in each corresponding chapter.

Chapter 3: Study 1

THE STABILITY AND REPEATABILITY OF SPONTANEOUS SYMPATHETIC BAROREFLEX SENSITIVITY IN YOUNG HEALTHY INDIVIDUALS

This chapter was published in the Journal *Frontiers in Neuroscience* on the 14th of June 2018. The authors of this article were Sarah L Hissen, Khadigeh El Sayed, Vaughan G Macefield, Rachael Brown and Chloe E Taylor. *Front. Neurosci.* 12, 403. doi: 10.3389/fnins.2018.00403

3.1. Abstract

Spontaneous sympathetic BRS is a valuable tool for assessing how well the baroreflex buffers beat-to-beat changes in blood pressure. However, there has yet to be a study involving appropriate statistical tests to examine the stability of sympathetic BRS within an experimental session and the repeatability between separate sessions. The aim of this study was to use intra-class correlations, ordinary least products regression, and Bland-Altman analyses to examine the stability and repeatability of spontaneous sympathetic BRS assessment. In addition, the influence of recording duration on values of BRS was assessed. In eighty-four healthy young individuals (49 males, 35 females), continuous measurements of blood pressure, heart rate and MSNA were recorded for 10 min. In a subgroup of 13 participants (11 male, 2 female) the measurements were repeated on a separate day. Sympathetic BRS was quantified using MSNA burst incidence (BRS_{inc}) and total MSNA (BRS_{total}) for the first 5-min period, the second 5-min period, and a 2-min segment taken from the second 5-min period. Intra-class correlation coefficients indicated moderate stability in sympathetic BRS_{inc} and BRS_{total} between the first and second 5-min periods in males (BRS_{inc} r = 0.63, BRS_{total} r = 0.78) and females (BRS_{inc} r = 0.61, BRS_{total} r = 0.47) with no proportional bias, but with fixed bias for BRS_{inc} in females. When comparing the first 5-min with the 2-min period (n = 76), the intra-class correlation coefficient indicated poor to moderate repeatability in sympathetic BRS_{inc} and BRS_{total} for males (BRS_{inc} r = -0.01, BRS_{total} r = 0.70) and females (BRS_{inc} r = 0.46, BRS_{total} r = 0.39). However, Bland-Altman analysis revealed a fixed bias for BRS_{total} in males and proportional bias for BRS_{total} in females, with lower BRS values for 5-min recordings. In the subgroup, intra-class correlations indicated moderate repeatability for measures of BRS_{inc} (9 male, 2 female, r = 0.63) and BRS_{total} (6 male, 2 female, r = 0.68) assessed using 5-min periods recorded on separate days. However, Bland-Altman analysis indicated proportional bias for BRS_{inc} and fixed bias for BRS_{total}. In conclusion, measures of spontaneous sympathetic BRS are moderately stable and repeatable within and between testing sessions in healthy young adults, provided that the same length of recording is used when making comparisons.

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3.2. Introduction

Blood pressure is homeostatically maintained at an optimal level to suit a given task or situation, such as changes in posture, exercise or mental stress (Benarroch, 2008), through baroreflex-mediated changes in TPR, as determined by the degree of vasoconstriction in systemic arterioles, and CO. The ability of the baroreflex to efficiently buffer beat-to-beat changes in blood pressure is known as BRS; it does this by modulating both heart rate (cardiac BRS) and MSNA (sympathetic BRS) (Benarroch, 2008, Wehrwein and Joyner, 2013). Spontaneous sympathetic BRS is typically quantified by binning the diastolic pressures into either 1, 2 or 3 mmHg bins and plotting the mean for each bin against either MSNA burst incidence, burst strength (amplitude/area) or integrated activity (Kienbaum et al., 2001, Halliwill, 2000). The slope of this relationship provides an individual's baroreflex sensitivity (Sundlof and Wallin, 1978, Kienbaum et al., 2001). The steeper the slope, the more efficient the baroreflex is in correcting for changes in blood pressure (Charkoudian and Wallin, 2014, Taylor et al., 2014). Hart and colleagues (2010) have previously found that the sympathetic BRS values determined by the spontaneous burst incidence technique (also known as the threshold method) correlate well with those defined by the "gold-standard" modified Oxford method. Thus, the diastolic pressure-MSNA burst incidence slope produced via the spontaneous threshold method has been accepted as a robust non-pharmacological alternative to the modified Oxford method (Hart et al., 2010).

Kienbaum and colleagues (2001) have theorized that the modulation of muscle sympathetic outflow via the baroreflex, has two central nervous system pathways; one modulating the gating (i.e. incidence) of bursts, and the other modulating the strength (i.e. amplitude) of a burst. It has been reported that computation of sympathetic BRS values is more successful when quantified using the gating (incidence) of sympathetic bursts rather than the strength of the sympathetic outflow (amplitude/area of MSNA burst) (Kienbaum et al., 2001). Keller et al. (2006) later went on to combine the incidence and strength of sympathetic bursts in the quantification of sympathetic BRS. Another method of analysis is the segregated signal averaging approach, developed by Halliwill (2000). This technique involves the quantification of total integrated MSNA across all cardiac cycles, which is then averaged for each diastolic pressure bin. These methods of assessing spontaneous sympathetic BRS have been used to evaluate the cardiovascular benefits of lifestyle interventions, such as exercise

training (Laterza et al., 2007) and diet (Lambert et al., 2011). They have also been applied to studies of diurnal variation in baroreflex function (Hissen et al., 2015) and the effects of heat stress (Keller et al., 2006). To confidently report enhancements in baroreflex function following an intervention, it is essential to assess spontaneous sympathetic BRS with a technique that is both accurate and repeatable. However, while the accuracy of the spontaneous baroreflex technique is accepted (Hart et al., 2010), a comprehensive assessment of the repeatability of this method has yet to be conducted. Dawson and colleagues (1997) previously reported that the analytical techniques commonly used to measure spontaneous cardiac BRS, such as the sequence method, are repeatable between recording sessions. Whilst there is considerable variability in resting MSNA from person to person, inter-individual differences in MSNA have been shown to be reproducible on the same day (Notay et al., 2016), to months and even years between trials (Sundlof and Wallin, 1977, Fagius and Wallin, 1993, Kimmerly et al., 2004). The use of MSNA burst incidence when quantifying sympathetic BRS has previously been shown to be repeatable, whereas methods involving MSNA burst strength have not (Kienbaum et al., 2001). However, these conclusions were made based on minimal statistical analysis (paired t-tests), and so the variability between sympathetic BRS values within each participant has yet to be revealed. By assessing segments of data from the same recording period we can assess the stability of sympathetic BRS within subjects.

Therefore, the aim of this study is to employ appropriate statistical tests to determine whether sympathetic BRS, quantified using spontaneous techniques, is stable during the same recording session in healthy young adults. It is hypothesized that sympathetic BRS is highly stable during a single recording session. It is also hypothesized that recording periods of longer durations are associated with greater diastolic pressure ranges at rest and that poor correlations exist between sympathetic BRS values derived from recording periods of different durations. Therefore, a secondary aim is to determine whether the duration of the recording period influences values of sympathetic BRS. Finally, it is hypothesized that measures of sympathetic BRS are repeatable between experimental sessions on different days. Therefore, the final aim is to assess test-retest repeatability in a subgroup of participants in whom measurements are made on two separate days.

3.3. Methods and materials

3.3.1 Participants

This study was a retrospective analysis of unpublished data combined with data presented in previous publications (Hissen et al., 2015, Taylor et al., 2015, El Sayed et al., 2016). The participants were 49 male (aged 21 ± 2 yrs, height 177 ± 8 cm, weight 77 ± 12 kg, BMI 24 ± 3 kg/m) and 35 female (aged 23 ± 4 yrs, height 164 ± 6 cm, weight 65 ± 16 kg, BMI 24 ± 6 kg/m) healthy young individuals. From this cohort, 13 participants (2 females) returned to the lab on a separate day (ranging from 3 to 43 months) where the between day test-retest repeatability of sympathetic BRS was examined. Each experiment was conducted with the approval of the Human Research Ethics committee, Western Sydney University, and satisfied the Declaration of Helsinki.

3.3.2 Measurements and experimental protocol

Beat-to-beat changes in ECG, blood pressure and MSNA were measured during a 10-min recording. Experiments were set up according to the description in Chapter 2, section 2.3 and 2.4.

3.4. Data analysis

After a stable period of baseline activity (> 10 min) had been obtained, a 10-min recording of beat-to-beat values of systolic blood pressure, diastolic blood pressure, mean arterial pressure, R-R interval and MSNA was extracted from LabChart (ADInstruments, Dunedin, New Zealand). The 10-min recording period was then split into two 5-min periods to determine the stability of spontaneous sympathetic BRS within the same session. A 2-min recording was taken from the second 5-min recording to compare BRS values obtained from recording periods of different duration. 5- and 2-min recordings periods were chosen as this study included data that had already been collected previously. Baseline recordings in some experimental sessions were not long enough to compare 5-min with longer recording periods (i.e., 10-min) without overlapping recording periods. Although these comparisons were made at rest, previous studies have used shorter recording periods to examine sympathetic BRS.

Examples include before and during isometric handgrip (Ichinose et al., 2006), post exercise ischaemia (Ichinose et al., 2006), and mental stress (Durocher et al., 2011). The detection and area of each MSNA burst and the quantification of sympathetic BRS was performed using Ensemble (Elucimed Ltd, Wellington, New Zealand). The number of bursts per minute (MSNA burst frequency) and per 100 heartbeats (MSNA burst incidence) was determined for each individual. The following analyses were performed for both of the 5-min periods, and the 2-min recording. In a sub group of 13 participants (11 male, 2 female), a second 5-min recording period was recorded on a separate day to determine test-retest repeatability of spontaneous sympathetic BRS. Two approaches were used for the assessment of sympathetic BRS: the burst incidence method, and a segregated signal averaging approach that incorporates total MSNA. Both of these approaches were quantified according to the description in Chapter 2, section 2.5.1 and 2.5.2.

3.4.1 Diastolic pressure range

The diastolic pressure range was determined for the first 5-min and the 2-min recordings for each participant by calculating the difference between the highest and lowest diastolic pressure bins from the BRS analysis. Diastolic pressure ranges in males and females were compared between 5 and 2-min recording periods to determine whether the duration of the recording period affects the range of diastolic pressures involved in the baroreflex assessment.

3.5. Statistical analysis

The following statistical analysis was performed separately for males and females. Comparisons were made between the two 5-min periods to examine stability of BRS within a single recording session. Intra-class correlations, ordinary least products regression (OLP) and Bland-Altman plots were employed to compare the two 5-min periods to determine if sympathetic BRS is stable; to compare the first 5-min period with the 2-min recording to examine the effect of recording duration; and finally, in a subgroup of participants, the first 5-min recording was compared with another 5-min recording measured on a separate day to examine between day repeatability.

3.5.1 Intra-class correlations

These were performed for each comparison to test for repeatability in BRS values. Intra-class correlations of <0.5, 0.5-0.74, 0.75-0.89, and >0.90 are indicative of poor, moderate, good, and excellent repeatability, respectively (Koo and Li, 2016).

3.5.2 Ordinary least products regression

The goal of repeatability studies is not to find similarities between two recording periods but to determine whether any systematic differences or bias exists (Ludbrook, 1997). Therefore, OLP regression analyses were performed to determine whether there is any error or bias between the two measurement values. Ordinary least products regression is recommended over the more common ordinary least squares (OLS) regression as the recordings being compared both have some form of error (Ludbrook, 1997). All calculations were performed using techniques described by Ludbrook (1997). The 95% confidence intervals (CI) of the intercept (α') and slope (b') of the relationship will allow the presence of any fixed (where the intercept differs from zero) or proportional (where the slope of the regression differs from unity, i.e., 1) bias to be determined (Ludbrook, 1997). If the 95% CI for the intercept contains '0' and the 95% CI for the slope contains '1' then no proportional or fixed bias exists.

3.5.3 Bland-Altman plots

This analysis allows us to plot the difference between the two measurement values against the mean value of the measurement values (Altman and Bland, 1983, Bland and Altman, 1986, Ludbrook, 1997). Bland-Altman plots also provided a visual representation of how the data is spread (Altman and Bland, 1983, Bland and Altman, 1986, Ludbrook, 1997). A secondary examination of bias/error was performed on the Bland-Altman test. Proportional bias was determined using OLS regression of the mean against the difference of the two measurement values. If the slope of the OLS regression was significantly different from '0', then proportional bias exists. A one-sample t-test of the mean difference was tested against a value of '0' to determine whether fixed bias exists. Fixed bias was evident if the one-sample t-test was significantly different from 0.

3.5.4 Comparing diastolic pressure ranges

Students paired t-tests were performed to compare diastolic blood pressure ranges between the first 5-min recording and the 2-min recording (taken from the second 5-min period). Linear regression analysis was performed between diastolic pressure ranges in the 5 and 2-min recordings with the corresponding BRS values to examine the influence of diastolic pressure ranges on BRS values. Statistical analyses were performed using Prism v6.00 for Windows (GraphPad Software, San Diego, California, USA). Acceptance levels for BRS slopes were $r \geq 0.5$. For all statistical tests, a probability level of $p \leq 0.05$ was regarded as significant. All values are expressed as mean ± SD.

3.6. Results

3.6.1 Sex differences in cardiovascular variables

When comparing males and females using the first 5-min rest period, males had higher MSNA burst incidence and MSNA burst frequency. There was no significant difference in systolic pressure, diastolic pressure, mean arterial pressure or heart rate between males and females (Table 3.1). However, females had significantly greater sympathetic BRS when expressed using both MSNA burst incidence ($p = 0.001$) and MSNA total activity ($p = 0.03$). There was no significant difference in the number of cardiac cycles between the first and second 5-min periods in males (331 ± 33 vs. 331 ± 33 cardiac cycles; $p = 0.67$) and females (344 ± 38 vs. 342 ± 39 cardiac cycles; $p = 0.24$).

Table 3.1. Cardiovascular variables during 5 and 2-min recording periods in 47 males and 35 females (mean ± SD).

Cardiovascular variable	Males 5-min	Males 2-min	Females 5-min	Females 2-min
Systolic blood pressure (mmHg)	128 ± 18	128 ± 19	125 ± 19	124 ± 19
Diastolic blood pressure (mmHg)	65 ± 12	65 ± 11	64 ± 12	63 ± 12
Mean arterial pressure (mmHg)	83 ± 12	83 ± 12	83 ± 13	82 ± 13
Heart rate (bpm)	66 ± 8	66 ± 8	69 ± 7	69 ± 8

Burst incidence (bursts/100heartbeats)	45 ± 13	52 ± 14	38 ± 11	37 ± 11*
Burst frequency (bursts/minute)	29 ± 7	33 ± 8	25 ± 6	24 ± 6*

* Indicates a significant difference ($p < 0.05$) between males and females.

3.6.2 Intra-class correlations

Acceptable sympathetic BRS_{inc} values ($r \geq 0.5$) were obtained for both 5-min periods in 82 participants (47 males and 35 females). The intra-class correlation coefficient suggests moderate stability in sympathetic BRS_{inc} between the first and second 5-min periods in males (-3.2 ± 1.4 vs. -3.2 ± 1.5 bursts/100HB/mmHg; r = 0.63) and females (-4.4 ± 1.8 vs. 3.8 ± 1.6 bursts/100HB/mmHg; r = 0.61). Acceptable sympathetic BRS_{total} values were obtained for both 5-min periods in 42 participants (26 males and 16 females). There was a strong correlation in sympathetic BRS_{total} between the first and second 5-min periods in males (-6.9 ± 3.6 vs. -6.5 ± 3.8 AU/beat/mmHg; r = 0.78) but a poor repeatability in females (-9.4 ± 3.8 vs. -9.1 ± 5.2 AU/beat/mmHg; r = 0.47).

Acceptable sympathetic BRS_{inc} values were obtained in 76 participants (45 males, 31 females) when comparing the 5 and 2-min recordings. The intra-class correlation coefficient indicated poor repeatability in BRS_{inc} between the 5-min and 2-min recording periods in males (-3.2 ± 1.3 vs. -3.3 ± 1.4 bursts/100HB/mmHg; r = -0.01) and females (-4.4 ± 1.9 vs. -4.5 ± 2.3 bursts/100HB/mmHg; r = 0.46). Acceptable BRS_{total} values were acquired in 44 participants (30 males, 14 females) when comparing 5 and 2-min recording periods. There was a moderate intra-class correlation for sympathetic BRS_{total} between the first 5-min and 2-min recording periods in males (-6.6 ± 3.4 vs. -8.8 ± 4.2 AU/beat/mmHg; r = 0.70) but a poor correlation in females (-9.2 ± 4.0 vs. -13.6 ± 8.4 AU/beat/mmHg; r = 0.39).

Of the 13 participants who returned on a separate day for a second experimental session, acceptable sympathetic BRS_{inc} values were obtained for 11 participants (2 female). The intra-class correlation coefficient suggests a moderate correlation between the two 5-min periods (-

3.28 ± 1.0 bursts/100HB/mmHg vs. -2.79 ± 1.84 bursts/100HB/mmHg; r = 0.63). Acceptable BRS_{total} values were acquired in 8 participants with a moderate intra-class correlation (-4.60 ± 3.2 AU/beat/mmHg vs. -8.28 ± 5.7 AU/beat/mmHg; r = 0.68).

3.6.3 Ordinary least products regression analysis

A summary of OLP regression analysis for BRS_{inc} and BRS_{total} is provided in table 3.2. The results of the OLP analysis indicate that there was no evidence of fixed or proportional bias between the two baroreflex slopes for both BRS_{inc} and BRS_{total} when comparing the two 5-min recording periods, between the first 5-min and 2-min periods in both males and females, and also in the subgroup of participants who returned on a separate day. This means that i) one recording period did not have values that were different from the second by a constant amount over the total range of BRS values (fixed bias); and ii) one recording did not give values that were different from those from the second by an amount that was proportional to the level of the BRS variable (proportional bias) (Kimmerly et al., 2004, Ludbrook, 1997). Figures 3.1a and 3.1b illustrate the comparisons between the first and second 5-min recording periods for BRS_{inc} and BRS_{total} in males, and figures 3.2a and 3.2b illustrates these comparisons in females. The comparison between the 5-min and 2-min recording periods in males is illustrated in figures 3.3a and 3.3b, and in females in figures 3.4a and 3.4b. The between-session comparisons are illustrated in figures 3.5a and 3.5b.

3.6.4 Bland-Altman analysis

Results of the Bland-Altman plots are detailed in table 3.3. In males, the mean difference in BRS_{inc} between the two 5-min periods was 0.07 ± 1.5 bursts/100HB/mmHg, and for BRS_{total} was -0.40 ± 3.1 AU/beat/mmHg. For females, the mean difference in BRS_{inc} between the two 5-min periods was 0.65 ± 1.8 bursts/100HB/mmHg, and for BRS_{total} was 0.35 ± 5.4 AU/beat/mmHg. In males, the mean difference in BRS_{inc} between the 5 and 2-min periods was -0.01 ± 1.9 bursts/100HB/mmHg, and for BRS_{total} was -2.13 ± 3.3 AU/mmHg. For females, the mean difference in BRS_{inc} between the 5 and 2-min periods was -0.05 ± 2.5 bursts/100HB/mmHg, and for BRS_{total} was -4.38 ± 7.7 AU/beat/mmHg. When comparing the BRS values between the two 5-min recording periods, there was no evidence of proportional

bias in BRS_{inc} or BRS_{total}, as illustrated in figures 3.1c and 3.1d (males) and figures 3.2c and 3.2d (females). However, there was evidence of fixed bias for BRS_{inc} in females with higher BRS values for the first 5-min recording. When comparing the BRS values from the first 5-min with the 2-min recording period, there was no proportional or fixed bias when BRS was quantified using MSNA burst incidence. However, there was evidence of fixed bias for BRS_{total} in males, with higher BRS values acquired from the 2-min recording, and proportional bias for BRS_{total} in females. When comparing the BRS values that were acquired from two experimental sessions, there was evidence of proportional bias in BRS_{inc} and fixed bias in BRS_{total}. These comparisons are illustrated in figures 3.3c and 3.3d for males, figures 3.4c and 3.4d for females, and figures 3.5c and 3.5d in the subgroup of participants.

Table 3.2. Ordinary least products regression of sympathetic baroreflex sensitivity

Variable	ICC	α'	95%CI for α'	b'	95% CI for b'	Fixed bias	Proportional bias
MALES							
BRS_{inc} 5min1 vs. 5min2 (n=47)	0.63	0.24	-1.93, 2.42	1.08	0.44, 1.71	NO	NO
BRS_{total} 5min1 vs. 5min2 (n=26)	0.78	0.78	-3.42, 4.98	1.06	0.51, 1.6	NO	NO
BRS_{inc} 5min1 vs. 2min (n=45)	-0.01	-4.9	-85.53, 75.73	-0.54	-23.51, 22.49	NO	NO
BRS_{total} 5min1 vs. 2min (n=30)	0.70	-0.71	-5.02, 3.6	1.21	-0.63, 1.79	NO	NO
FEMALES							
BRS_{inc} 5min1 vs. 5min2 (n=35)	0.61	-0.14	-2.66, 2.91	0.88	0.30, 1.47	NO	NO
BRS_{total} 5min1 vs. 5min2 (n=16)	0.47	3.8	-20.97, 28.56	1.37	-1.08, 3.81	NO	NO
BRS_{inc} 5min1 vs. 2min (n=31)	0.46	0.98	-6.2, 8.2	1.23	-0.26, 2.7	NO	NO
BRS_{total} 5min1 vs. 2min (n=14)	0.39	5.78	-24.68, 36.24	2.11	-0.95, 5.17	NO	NO

ICC, intra-class correlation coefficient; α', b' coefficients of ordinary least products regression model (Y) = $\alpha' + b'$(X); α', y-intercept; b', slope, fixed bias, if 95%CI for α' does not include '0', proportional bias, if 95% CI for b' does not include '1'.

Table 3.3. Proportional and fixed bias outcomes for sympathetic baroreflex sensitivity from Bland-Altman

Variable	R	*b′*	p (OLS)	Proportional bias	Mean difference ±SD	Mean difference 95%CI	p (t-test)	Fixed bias
MALES								
BRS_{inc} 5min1 vs. 5min2	0.08	0.09	0.61	NO	0.07 ±1.5	-0.43, 0.44	0.98	NO
BRS_{total} 5min1 vs. 5min2	0.07	0.07	0.73	NO	-0.40 ±3.1	-0.87, 1.66	0.52	NO
BRS_{inc} 5min1 vs. 2min	0.04	0.08	0.80	NO	-0.01 ±1.9	-0.59, 0.58	0.98	NO
BRS_{total} 5min1 vs. 2min	0.24	0.24	0.19	NO	-2.13 ±3.3	-3.37, -0.88	0.002	YES
FEMALES								
BRS_{inc} 5min1 vs. 5min2	0.14	0.17	0.42	NO	0.65 ±1.8	0.04, 1.25	0.04	YES
BRS_{total} 5min1 vs. 5min2	0.32	0.47	0.23	NO	0.35 ±5.4	-2.5, 3.2	0.80	NO
BRS_{inc} 5min1 vs. 2min	0.22	0.33	0.23	NO	-0.05 ±2.5	-0.98, 0.87	0.90	NO
BRS_{total} 5min1 vs. 2min	0.66	0.97	0.009	YES	-4.38 ±7.7	-8.85, 0.09	0.05	NO

r, product-moment correlation for Bland-Altman method of differences plots; *b′*, ordinary least squares slope of Bland-Altman method of differences, P(OLS), the p-value for the ordinary least square slope (versus 0); CI, confidence interval; p (t-test), the p-values for the one sample t-test on the mean differences (versus 0).

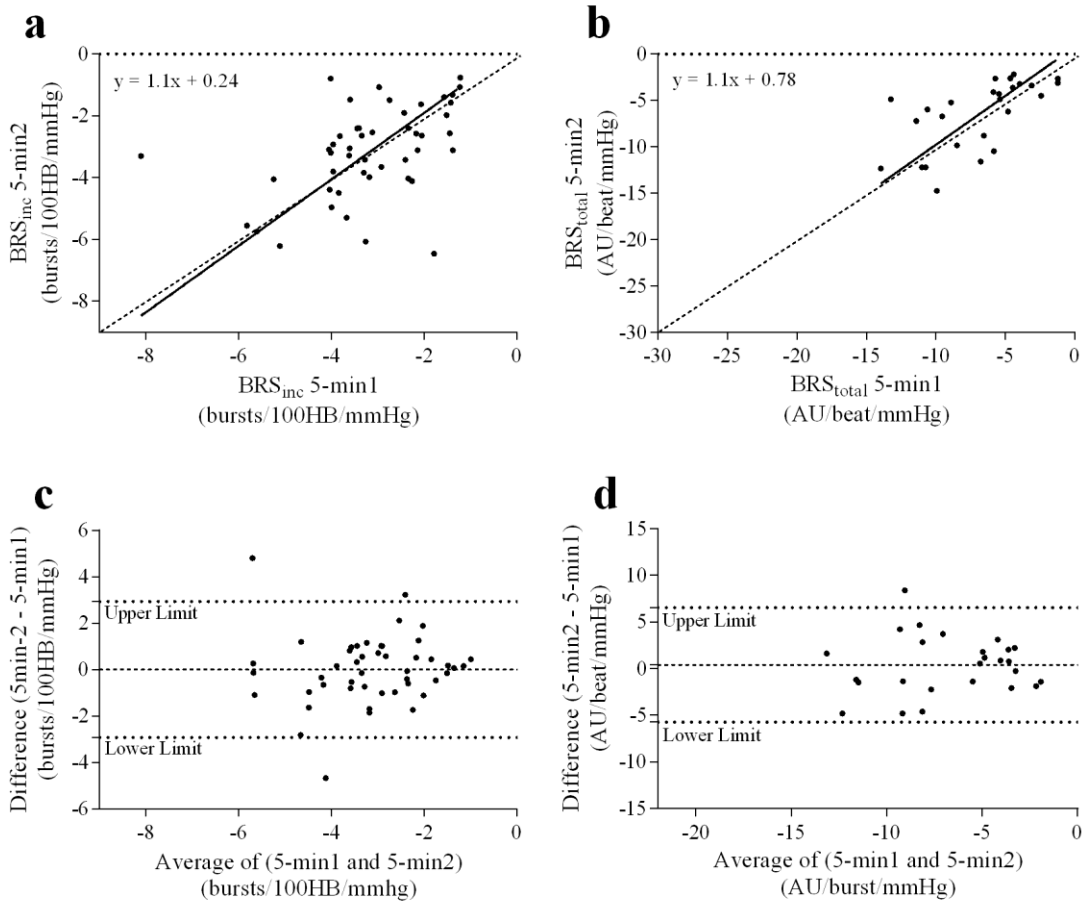

Figure 3.1. Sympathetic BRS quantified using the first 5-min period (5-min1) and second 5-min period (5-min2) in males. Ordinary least products (OLP) regression analysis using a) BRS_{inc} and b) BRS_{total} and Bland-Altman plots c) BRS_{inc} and d) BRS_{total}. The solid and dashed lines in the OLP plots represent the line of regression and line of unity, respectively. The dashed and dotted lines in the Bland-Altman plots represent mean difference and 95% limits of agreement, respectively

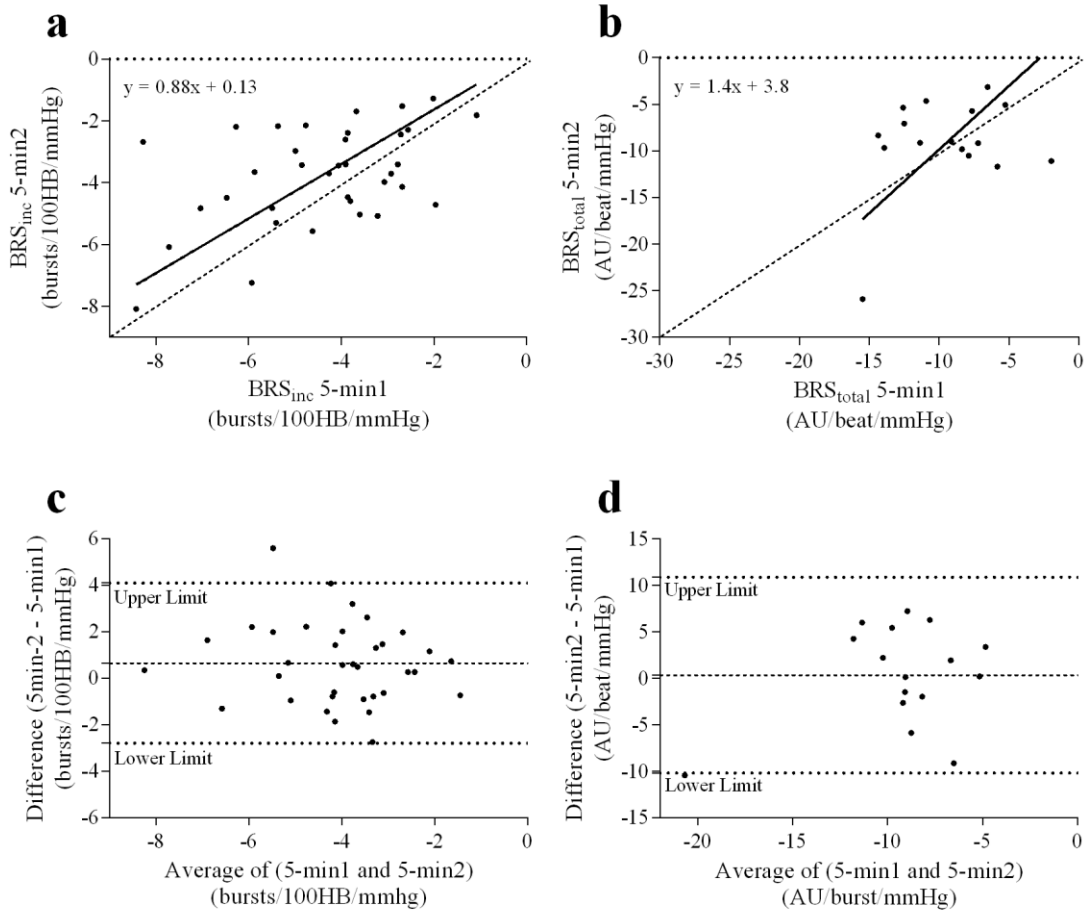

Figure 3.2. Sympathetic BRS quantified using the first 5-min period (5-min1) and second 5-min period (5-min2) in females. Ordinary least products (OLP) regression analysis using a) BRS_{inc} and b) BRS_{total} and Bland-Altman plots c) BRS_{inc} and d) BRS_{total}. The solid and dashed lines in the OLP plots represent the line of regression and line of unity, respectively. The dashed and dotted lines in the Bland-Altman plots represent mean difference and 95% limits of agreement, respectively

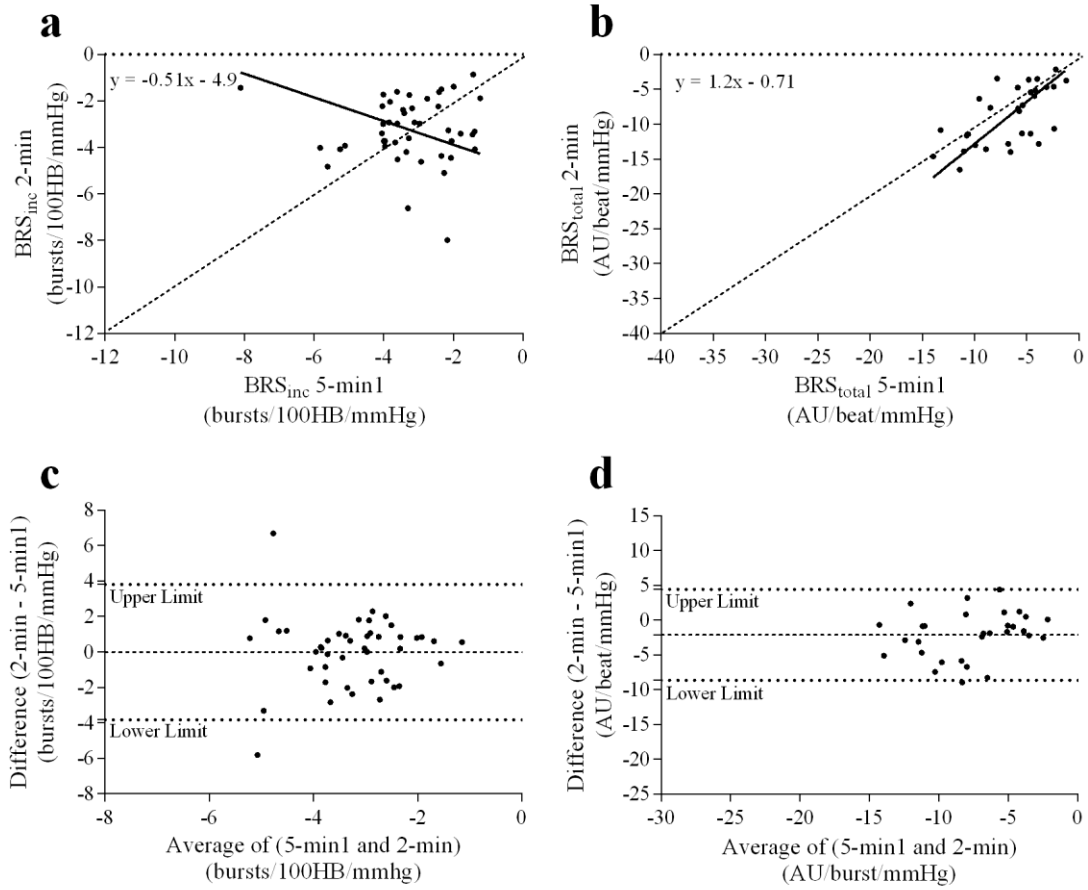

Figure 3.3. Sympathetic BRS quantified using the first 5-min period (5-min1) and 2-min period in males. Ordinary least products (OLP) regression analysis using a) BRS_{inc} and b) BRS_{total} and Bland-Altman plots c) BRS_{inc} and d) BRS_{total}. The solid and dashed lines in the OLP plots represent the line of regression and line of unity, respectively. The dashed and dotted lines in the Bland-Altman plots represent mean difference and 95% limits of agreement, respectively

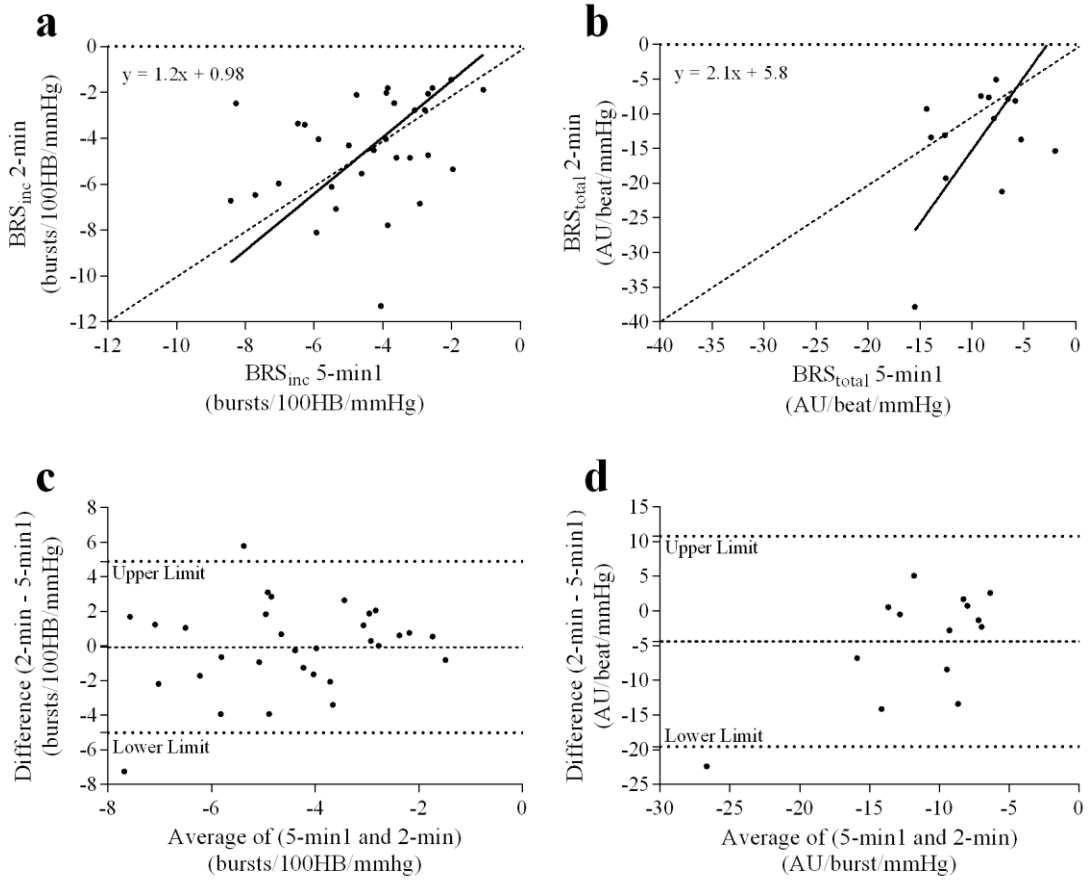

Figure 3.4. Sympathetic BRS quantified using the first 5-min period (5-min1) and 2-min period in females. Ordinary least products (OLP) regression analysis using a) BRS_{inc} and b) BRS_{total} and Bland-Altman plots c) BRS_{inc} and d) BRS_{total}. The solid and dashed lines in the OLP plots represent the line of regression and line of unity, respectively. The dashed and dotted lines in the Bland-Altman plots represent mean difference and 95% limits of agreement, respectively

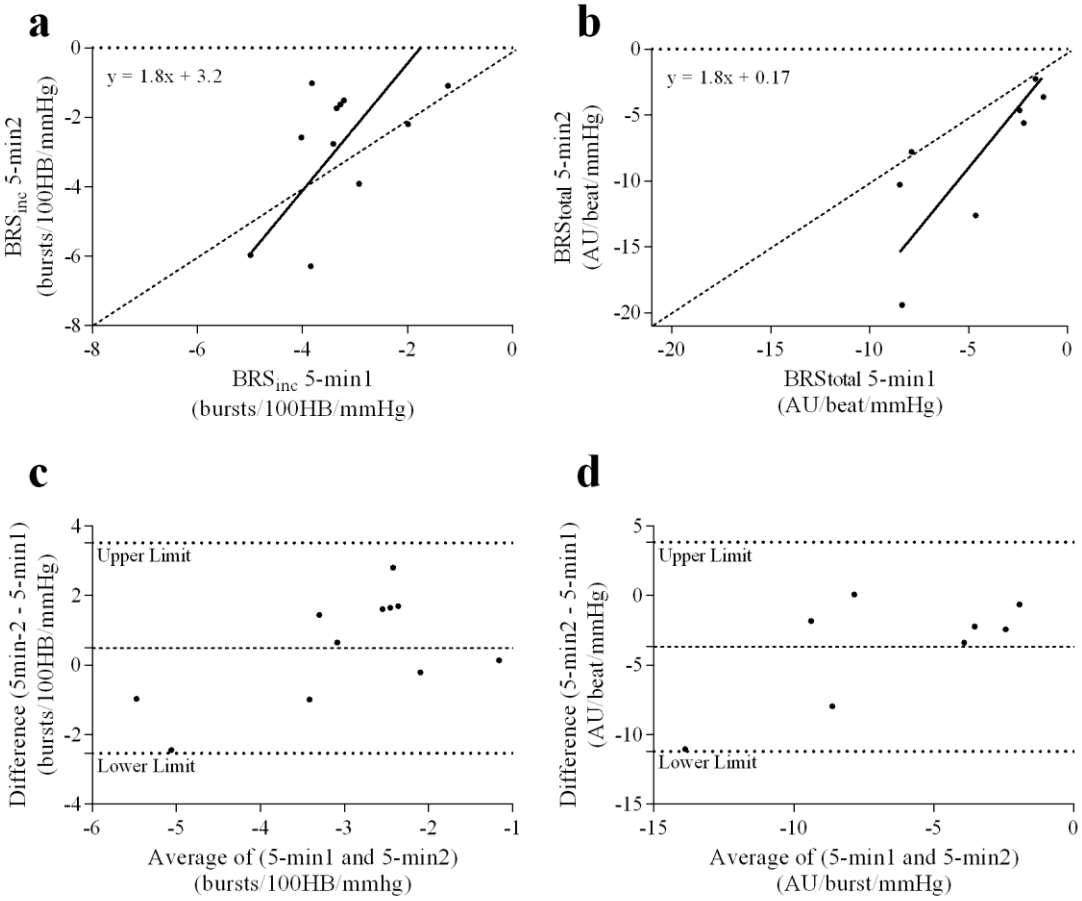

Figure 3.5. Sympathetic BRS quantified using two 5-min periods recorded on separate days. Ordinary least products (OLP) regression using a) BRS_{inc} and b) BRS_{total} and Bland-Altman plots using c) BRS_{inc} and d) BRS_{total}. The solid and dashed lines in the OLP plots represent the line of regression and the line of unity, respectively. The dashed and dotted lines in the Bland-Altman plots represent mean difference and 95% limits of agreement, respectively

3.6.5 Diastolic pressure ranges

In males, the diastolic pressure range was significantly greater in the 5-min recording period (21 ± 6 mmHg) when compared with the 2-min recording period (16 ± 6 mmHg, $p < 0.01$). Similarly, in females, the diastolic pressure range was significantly greater in the 5-min recording period (17 ± 6 mmHg) compared with the 2-min recording period (14 ± 6 mmHg, $p = 0.01$). In males, the diastolic pressure range was inversely related to the BRS; the greater the diastolic pressure range, the lower the individual's BRS. This was demonstrated for both the 5 and 2-min recordings when BRS was expressed as BRS_{inc} (5-min $r = 0.44$, $p < 0.01$; 2-

min r = 0.47, p < 0.01) and BRS_{total} (5-min r = 0.68, p < 0.01; 2-min r = 0.54, p < 0.01). In females, the diastolic pressure range was also inversely related to BRS in the 5-min period when expressed as BRS_{inc} (r = 0.55, p < 0.01) but not BRS_{total} (r = 0.03, p = 0.92). Similarly, when quantifying BRS using 2-min recording, diastolic pressure range was inversely related with BRS_{inc} (r = 0.57, p < 0.01) and trended towards significance with BRS_{total} (r = 0.45, p = 0.10).

3.7. Discussion

When performing baroreflex assessments the results are only meaningful if we understand the stability of baroreflex sensitivity and if the analytical technique used to quantify spontaneous sympathetic BRS is repeatable. This is the first study to employ appropriate statistical tests to examine the stability and repeatability of spontaneous sympathetic BRS techniques. The main findings of this study are i) both BRS_{inc} and BRS_{total} are moderately stable within a recording session when using periods of the same duration, with no evidence of fixed or proportional bias in males, but proportional bias in females; ii) there is generally poor repeatability when comparing recording periods of differing length (5-min vs. 2-min) with evidence of fixed and proportional bias in BRS_{total} values; and iii) when measured on separate days, BRS is moderately repeatable with evidence of proportional bias in BRS_{inc} and fixed bias in BRS_{total}.

3.7.1 The stability and repeatability of baroreflex sensitivity

Sympathetic baroreflex sensitivity is reportedly lower in certain populations, such as those with hypertension (Greaney et al., 2017), heart failure (Ferguson et al., 1992) and obstructive sleep apnoea (Carlson et al., 1996). However, evidence on the repeatability of sympathetic BRS is limited. The quantification of BRS has predominantly been performed using the cardiac arm of the baroreflex as it is easily obtained non-invasively via ECG and beat-to-beat measurements of systolic blood pressure (Parati et al., 1988). Previous studies have shown cardiac BRS, quantified using analytical techniques such as the sequence method and spectral analysis, to be repeatable within the same session and over a period of weeks to months (Dawson et al., 1997, Herpin and Ragot, 1997, Davies et al., 1999, Johnson et al., 2006). The quantification of sympathetic BRS involves techniques similar to that of cardiac BRS.

However, due to the invasive nature of microneurography, less research has been conducted on sympathetic BRS. These experiments are more technically demanding and require significant expertise. Moreover, the amplitude of a burst of MSNA depends on the proximity of the microelectrode tip to the active sympathetic axons, so absolute amplitudes cannot be directly compared across experimental sessions, though relative amplitudes can be. There has been only one other study where the repeatability of sympathetic BRS was examined. In this study, Kienbaum and colleagues (2001) reported spontaneous sympathetic BRS to be repeatable within the same session in healthy adults. However, the only statistical analysis employed was the Student's paired t-test. It is unlikely that this approach is sensitive enough to reveal systematic differences in BRS values obtained from the two recordings. Nevertheless, it does not provide information on the correlation between repeat tests and, in particular, whether there are large intra-individual differences. In the current study, there were moderate intra-class correlations for BRS_{inc} and BRS_{total} between the two 5-min periods in both males and females. There was also moderate repeatability in these variables when assessed on different days. The fact that these relationships are not stronger may reflect the dynamic nature of the baroreflex, indicating that BRS is not fixed, even under resting conditions. By evaluating sympathetic BRS from two segments in the same recording period we can gain an appreciation of the stability of BRS. The data suggest that the baroreflex may be constantly adapting and reacting to small changes in the internal environment despite no changes in the experimental conditions. Furthermore, what constitutes a meaningful difference in sympathetic BRS has yet to be established. In previous studies statistically significant changes in sympathetic BRS have been reported in the range of 1.5 to 2.91 bursts/100HB/mmHg following various interventions, such as heat stress (Keller et al., 2006), renal denervation (Hart et al., 2013) and insulin (Young et al., 2010), as well as between phases of the menstrual cycle (Carter et al., 2009). In the current study the mean difference in BRS_{inc} between the two 5-min recording periods was 0.07 ± 1.5 bursts/100HB/mmHg for males, and 0.65 ± 1.8 bursts/100HB/mmHg for females. Based on the previous literature, the mean difference may be deemed too small to be meaningful, thus supporting the view that BRS is relatively stable at rest. However, the variation suggests that for some individuals the differences were comparable to the changes in sympathetic BRS observed in intervention studies. Nevertheless, statistically significant changes do not necessarily reflect meaningful ones and there is currently no consensus on what constitutes a low sympathetic BRS value or a meaningful change. Further research is needed to establish clinical thresholds associated

with elevated cardiovascular risk and thus meaningful changes in sympathetic BRS following interventions. Further research is also required to determine the normal ranges of within- and between-subject variability in sympathetic BRS in healthy populations.

Although intra-class correlations provide some evidence of repeatability in measures of BRS, this approach does not offer any indication of the error or bias in the data. To explore the systematic differences between repeat baroreflex assessments, we performed OLP regression and Bland-Altman analysis and found no fixed or proportional bias when comparing BRS values using two consecutive 5-min recordings in males. This suggests that spontaneous sympathetic BRS analysis is not susceptible to bias within the same session. However, there was evidence of proportional bias in females when BRS was quantified using MSNA burst incidence. Moreover, when comparing between two recording periods of different lengths, fixed and proportional bias were evident when BRS was quantified using total MSNA. It is therefore recommended that studies involving repeat measurements of BRS include intervals of the same duration. Previous studies have reported spontaneous sympathetic BRS using intervals of 1-min (Ichinose et al., 2006), 4-min (Ogoh et al., 2007, Ichinose et al., 2004b), 5-min (Hinojosa-Laborde et al., 2014, Ichinose et al., 2004a, Keller et al., 2006, Kienbaum et al., 2001, Hart et al., 2010) and 10-min (Hissen et al., 2017, Hissen et al., 2015, Taylor et al., 2015). The current study suggests that care should be taken when comparing BRS values between studies when different time periods have been used.

3.7.2 Sex differences on sympathetic baroreflex sensitivity

Previous research indicates that several aspects of cardiovascular control differ between males and females. For instance, studies have shown that females have lower blood pressure, CO and vascular transduction when compared with males (Joyner et al., 2015, Briant et al., 2016). Furthermore, resting MSNA in males is inversely related to CO (Hart et al., 2009a), which may explain how young healthy males can have similar resting blood pressure levels despite differing levels of MSNA. Conversely, this relationship is not apparent in premenopausal females (Hart et al., 2011a). Previous evidence suggests this may be due to enhanced ß-adrenergic sensitivity in females, which therefore balances the vasoconstrictor effects of sympathetic outflow (Hart et al., 2011a, Kneale et al., 2000). In the current study,

baroreflex control of MSNA, quantified using both MSNA burst incidence and total MSNA was greater in females, as has been reported previously (Hogarth et al., 2007). However, others have reported no differences between males and females (Hart et al., 2011b, Tank et al., 2005). Although there is evidence to suggest females have higher sympathetic BRS, it may not necessarily demonstrate enhanced baroreflex buffering of arterial pressure as ß-adrenergic receptors counteract the vasoconstrictor nature of MSNA (Hart et al., 2011a, Taylor et al., 2015).

3.7.3 Indices of sympathetic baroreflex sensitivity

In this study, spontaneous sympathetic BRS was quantified using both MSNA burst incidence and total MSNA and thus can contribute to debates around the most appropriate characteristics of MSNA for baroreflex analysis. When using a 5-min recording, there was a higher success rate for acquiring an acceptable baroreflex slope ($r > 0.5$) using MSNA burst incidence (82 out of 84) than when using total MSNA (42 out of 84). Kienbaum et al. (2001) previously showed that the quantification of spontaneous BRS was more successful when using MSNA burst incidence (referred to as threshold analysis) than MSNA burst amplitude which gave rise to the hypothesis that there are two central nervous system pathways of MSNA. The results indicated that the baroreflex modulation of MSNA is more closely related to the occurrence of MSNA bursts than the strength of MSNA bursts. This may be due to non-baroreflex inputs such as respiration dominating over the baroreflex to determine the size of a sympathetic burst (Hart et al., 2010). However, previous studies suggest that the amplitude of MSNA bursts is an important and influential factor in the changes in arterial pressure (Vianna et al., 2012, Fairfax et al., 2013b). While it may be argued that methods involving total integrated MSNA provide a more comprehensive assessment of baroreflex modulation of MSNA, the current study supports previous evidence that the MSNA burst incidence approach provides the most robust BRS slopes (Kienbaum et al., 2001, Taylor et al., 2015).

The lack of significant BRS_{inc} and BRS_{total} slopes in some individuals may be due to an insufficient blood pressure range within the 2-min recording period. A 2-min time interval may not contain sufficient spontaneous fluctuations in diastolic pressure and the

corresponding MSNA. Longer recording periods may allow enough time for a larger diastolic blood pressure range and therefore explain why in the current study there was a higher success rate of acquiring a sympathetic BRS slope with a linear relationship of $r > 0.5$ using the 5-min period (82/84 for BRS_{inc} and 42/84 for BRS_{total}). In this study, the 5-min recording period was associated with a significantly larger diastolic blood pressure range when compared with the 2-min recording period in both males and females. When comparing the 5-min recording with the 2-min recording, intra-class correlation coefficients indicated poor to moderate repeatability. The Bland-Altman analysis also revealed fixed bias for BRS_{total} in males and proportional bias for BRS_{total} in females. The results suggest that, in males, BRS_{total} values quantified using the 2-min recording period were higher by a constant amount when compared with values from the 5-min period. In females, BRS_{total} values from the 2-min recording increased in proportion to the BRS values in the 5-min recording. Regression analyses revealed that larger diastolic blood pressure ranges are associated with lower BRS values. This suggests that the use of very short recordings, such as 2 minutes, is associated with limited pressure ranges and artificially high BRS values. The results also highlight the importance of comparing BRS values using recording periods that are of the same duration to ensure that the intervention, stressor or change in environment is the actual cause of the shift in BRS.

3.7.4 Methodological considerations

To date, the quantification of sympathetic BRS has been performed predominantly through spontaneous techniques (Hart et al., 2010, Hart et al., 2011b, Hissen et al., 2015, Ichinose et al., 2008). However, methods involving active perturbation of blood pressure, such as the modified Oxford method, have also been applied. This technique involves bolus injections of sodium nitroprusside and phenylephrine to drive decreases and increases in blood pressure, respectively. This is advantageous as it partially opens the closed-loop system of the baroreflex, allowing estimates of the ratio of the inputs and outputs of the baroreflex to be observed (Lipman et al., 2003, Diaz and Taylor, 2006). Although the modified Oxford method is regarded as the gold standard for quantifying cardiac BRS, its use in determining sympathetic BRS can raise some technical issues (Dutoit et al., 2010, Taylor et al., 2014). For instance, when assessing cardiac BRS, there is an RR-interval for every cardiac cycle plotted against systolic pressure. However, when determining sympathetic BRS, not every cardiac

cycle is associated with a MSNA burst, and it is not uncommon for the administration of phenylephrine to cause rapid and severe inhibition of MSNA bursts, making it difficult to plot MSNA/diastolic pressure relationships (Taylor et al., 2014). Spontaneous methods are therefore often preferred for the sympathetic baroreflex, but future research on the repeatability of approaches involving active perturbations is important as these techniques provide more rapid changes in pressure, typically over a broader range.

When comparing measurements of MSNA and sympathetic BRS between segments of data, it is important to take heart rate into account. The more cardiac cycles within the segment, the greater the probability of a burst of MSNA and thus the more data points available for assessing sympathetic BRS. In this study there were no significant differences in the number of cardiac cycles in segments of the same duration, largely due to all comparisons being made at rest. However, in studies where sympathetic BRS is compared between different conditions, such as rest and mental stress, differences in heart rate may be present. In these cases, it may be more appropriate to control for the number of cardiac cycles per segment, rather than segment duration.

3.8. Conclusion

This study demonstrates, for the first time, that sympathetic BRS is moderately stable within a single recording session. When quantified using both MSNA burst incidence and total MSNA there is no fixed or proportional bias present in males. There is, however, proportional bias for BRS_{inc} in females. When data segments of different durations are used, intra-class correlations generally indicate poor repeatability with both fixed and proportional bias. Recordings of a shorter duration were associated with small diastolic blood pressure ranges and artificially elevated BRS values. When sessions are performed on separate days the analytical techniques used to quantify spontaneous sympathetic BRS are associated with moderate repeatability in healthy young males and females. Results from this study indicate that measures of spontaneous sympathetic BRS are moderately repeatable but only when the duration of recording periods is the same. Future research is required to examine the interactions between sex and ageing, and to establish clinical thresholds in sympathetic BRS.

Chapter 4: Study 2

SEX DIFFERENCES IN SYMPATHETIC VASCULAR TRANSDUCTION

4.1. Abstract

Indirect evidence suggests that individual variability exists in sympathetic vascular transduction at rest, especially between young males and females. The aim of this study was to assess vascular transduction directly using Doppler ultrasound and spontaneously occurring bursts of MSNA in healthy young males and females. It is hypothesised that vascular transduction is lower in young premenopausal females compared with young males. A secondary aim of this study is to compare two methods recently presented in the literature for quantifying beat-to-beat vascular transduction at rest. Sympathetic vascular transduction was quantified in 39 young healthy individuals (19 males). Beat-by-beat SFA diameter and blood velocity were measured using Doppler ultrasound to calculate blood flow. Sympathetic vascular transduction was quantified by calculating the mean percent change in leg vascular conductance (blood flow/mean arterial pressure) and mean arterial pressure for 10 cardiac cycles following MSNA bursts. Vascular transduction was represented by the nadir change in leg vascular conductance (VT_{CON}) and the peak increase in mean arterial pressure (VT_{MAP}). Vascular transduction was also measured by plotting diastolic pressure against MSNA burst area (over two cardiac cycles) 8-6 cardiac cycles preceding each diastolic pressure. The regression slope of this relationship represented an individual's vascular transduction (VT_{DBP}). Unpaired t-tests were used to compare cardiovascular variables, VT_{CON}, VT_{MAP} and VT_{DBP} between males and females. Pearson correlation coefficient was performed to examine whether VT_{CON} was correlated with VT_{MAP}. Lastly, VT_{CON} and VT_{DBP} values were compared using correlation analyses to confirm whether the VT_{DBP} approach can be used in place of the VT_{CON} method in instances where blood flow cannot be measured. VT_{CON} was significantly lower in males compared with females (-4.86 ± 4.1% Δ $ml/min^{-1}/mmHg^{-1}$ vs. -7.82 ± 4.4% Δ $ml/min^{-1}/mmHg^{-1}$, $p = 0.04$). There was no significant difference in VT_{MAP} between males and females (2.26 ± 1.2% Δ mmHg vs. 2.41 ± 1.5% Δ mmHg, $p = 0.74$). VT_{CON} was positively correlated with VT_{MAP} in males only ($r = 0.47$, $p = 0.05$). There was no significant difference in VT_{DBP} between males and females (0.10 ± 0.06 mmHg $(\% s)^{-1}$ vs. 0.09 ± 0.04 mmHg $(\% s)^{-1}$, $p = 0.53$). VT_{CON} was not correlated with VT_{DBP} ($p > 0.05$). It is concluded that sympathetic vascular transduction is greater in healthy young females when Doppler ultrasound is used for direct assessment of the vascular responses to MSNA. However, sex differences are not apparent when examining the transduction of MSNA to changes in blood pressure. These approaches may provide insight into different aspects of the transduction of MSNA to vasoconstriction and the regulation of arterial pressure.

4.2. Introduction

The sympathetic arm of the autonomic nervous system contributes to the regulation of vascular tone by activating or inhibiting sympathetic outflow to the vasculature supplying skeletal muscle. Vascular tone is regulated by noradrenaline released from nerve terminals and its co-transmitters, NPY and ATP (Burnstock, 2009). A contributing factor that aids in the regulation of vascular tone is the baroreflex modulation of MSNA. This essential mechanism enables the body to maintain perfusion of blood to tissue at rest and also during manoeuvres such as changes in posture, exercise and mental stress (Deley et al., 2009, Durocher et al., 2011, Laterza et al., 2007, O'Leary et al., 2003). This beat-to-beat control relies upon effective transduction of MSNA. The term 'vascular transduction' describes the vasoconstrictor effect MSNA has on the peripheral vasculature in order to maintain arterial pressure. Many investigators have made attempts at quantifying vascular transduction during manoeuvres that drive increases in MSNA, such as lower body negative pressure (Ray and Monahan, 2002), isometric handgrip exercise (Tan et al., 2013a) and post-exercise ischaemia (Minson et al., 2000a). Although these approaches have improved our understanding of vascular transduction in humans, they do not provide insight into beat-to-beat vascular transduction at rest.

Initial investigations of vascular transduction at rest focused on the blood pressure response to sympathetic nerve activity. Wallin and Nerhed (Wallin and Nerhed, 1982) reported that the increase in vascular resistance in response to MSNA is not instant and occurs with a delay, as demonstrated by a peak increase in mean arterial pressure approximately 7 cardiac cycles (or 5.5 s) following a burst of MSNA. Furthermore, there is evidence that the increase in arterial pressure in response to MSNA bursts is dictated by burst amplitude; bursts with greater amplitudes cause a greater increase in blood pressure when compared with smaller bursts (Vianna et al., 2012). More recently, Briant and colleagues (2016) developed a technique for quantifying vascular transduction by plotting the relationship between MSNA burst area and diastolic blood pressure. It was reported that premenopausal females had significantly lower vascular transduction when compared with young males. This approach is useful in instances where measurements of blood flow cannot be acquired. However, because this technique uses arterial pressure as a proxy, it may be limited for determining individual variability in the vascular responses to MSNA, including whether this mechanism differs between males and

females. With the use of Doppler ultrasound, the beat-to-beat vascular responses to MSNA can be quantified.

Fairfax and colleagues (2013b) utilised Doppler ultrasound to measure blood flow through the common femoral artery to directly observe the end organ response to MSNA. To achieve this, the mean percent change in leg vascular conductance was plotted following all MSNA bursts in a recording period at rest. Following MSNA bursts, leg vascular conductance decreased to a nadir of -7.7 ± 1.1% (6 cardiac cycles) (Fairfax et al., 2013b). Evidence suggests that significant inter-individual variability exists in the magnitude of the vascular response to spontaneous MSNA (Fairfax et al., 2013b). Previous investigators (but not all (Vianna et al., 2012)) have reported sex differences in vascular transduction with lower transduction in young females compared with young males (Briant et al., 2016, Hart et al., 2011a, Hogarth et al., 2007). The technique described by Fairfax et al., (Fairfax et al., 2013a, Fairfax et al., 2013b, Fairfax et al., 2013c) has only been performed on young normotensive males. It is not known if vascular transduction, characterised using this method, provides results consistent with those of Briant et al. (2016), whereby vascular transduction was greater in males than females. If diastolic pressure is to be used as proxy for the vascular responses to sympathetic nerve activity, the two approaches need to provide the same information.

The aim of this chapter is to examine whether sympathetic vascular transduction, quantified using Doppler ultrasound and spontaneously occurring MSNA bursts (Fairfax et al., 2013b), differs between healthy young males and females. It is hypothesised that vascular transduction is lower in young premenopausal females, than when compared with young males. A secondary aim is to compare this method with an alternative method that does not involve ultrasound (Briant et al., 2016) in which transduction of MSNA to changes in blood pressure is quantified. It is hypothesised that sex differences are apparent using both methods of vascular transduction analysis.

4.3. Methods

4.3.1 Participants

Data from 39 (19 males) young healthy individuals were used in the following analysis. Exclusion criteria were those who smoked or took regular medication or had a known history of cardiovascular, respiratory, or endocrine disease. Table 4.1 provides detail on participant characteristics.

Table 4.1 Participant characteristics in 19 males and 20 females (mean ± SD)

	Males	Females	p-value
Age (years)	21 ± 3	24 ± 5	0.04
Height (cm)	173 ± 8	163 ± 5	<0.0001
Weight (kg)	71 ± 8	62 ± 14	0.01
BMI (ml/kg)	24 ± 3	23 ± 5	0.82
Oral contraceptive use		11 out of 20	
Training status (min/week)	418 ± 171	211 ± 149	0.0003

4.3.2 Experimental setup

Refer to Chapter 2, section 2.3 for the experimental setup.

4.3.3 Measurements

Beat-to-beat changes in heart rate, blood pressure, MSNA and SFA diameter and velocity were measured during 5-min of rest. Refer to Chapter 2, section 2.4 for all variables and how they were measured.

4.3.4 Data analysis

Sympathetic vascular transduction using Doppler ultrasound

During 5-min of rest, beat-to-beat sympathetic vascular transduction was quantified using a method as previously described (Fairfax et al., 2013a, Fairfax et al., 2013b, Vranish et al., 2018). The 'Fairfax method' can be used to assess the transduction of MSNA to changes in both vascular conductance and mean arterial pressure. For each participant, ECG, blood pressure and MSNA were extracted from LabChart as a text file. A text file was also extracted for each participant containing the SFA diameter and velocity output from the edge tracking software as described in Chapter 2, section 2.4.4. Both files were put through a custom designed LabView program that aligned the two files for the transduction analysis. Blood flow from the SFA was calculated using the following equation:

V_{mean} (in cm/s)$\cdot\pi\cdot$[mean SFA diameter (in cm) $\div$ 2]$^2\cdot$60 s/min

Leg vascular conductance was calculated as SFA blood flow divided by mean arterial pressure for each cardiac cycle containing a MSNA burst and also 10 cardiac cycles following each MSNA burst. Vascular transduction was represented by the nadir of the mean percentage change in leg vascular conductance following all MSNA bursts. The nadir was chosen at approximately 4-10 cardiac cycles following MSNA bursts. This range was chosen as previous work has shown that the transduction of MSNA to changes in arterial pressure is approximately 5.5 s (Wallin and Nerhed, 1982). Vascular transduction was also quantified by calculating the mean percent change in mean arterial pressure 10 cardiac cycles following all MSNA bursts. Vascular transduction quantified using leg vascular conductance will be referred to as VT_{CON} and vascular transduction quantified using mean arterial pressure will be referred to as VT_{MAP}. An example of the time course in the percent change in a) leg vascular conductance and b) mean arterial pressure following MSNA bursts in an individual is shown in figure 4.1.

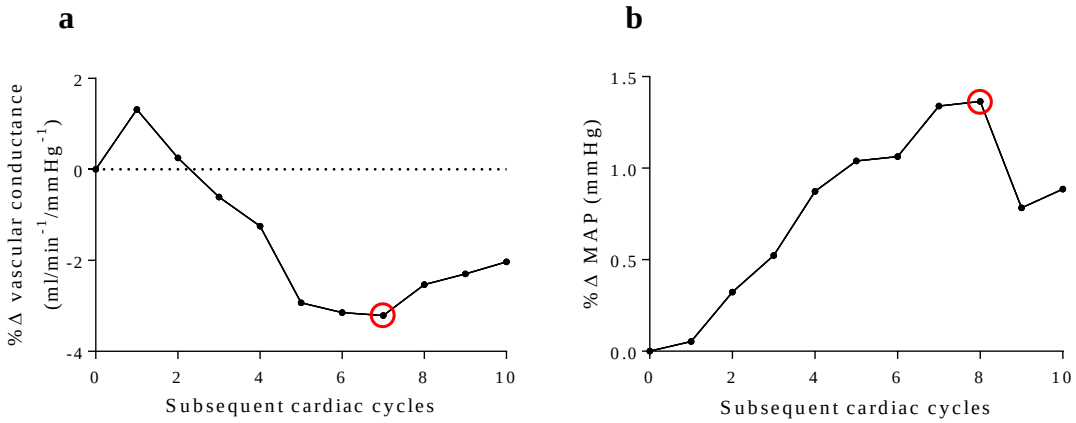

Figure 4.1. a) Mean percent change in leg vascular conductance and b) Mean percent change in mean arterial pressure following MSNA bursts in a healthy 18-year-old male. Red circle shows nadir/peak of the response and represents the individual's vascular transduction

Sympathetic vascular transduction using diastolic pressure

Sympathetic vascular transduction using diastolic pressure was calculated using the method described by Briant et al. (2016) and performed using the neurovascular transduction toolkit of Ensemble (Elucimed Ltd, Wellington, New Zealand). During the 5-min baseline recording, MSNA burst area was normalised to the MSNA burst with the largest area. MSNA burst area was calculated over a 2 cardiac cycle window 8-6 cardiac cycles preceding each diastolic blood pressure. MSNA burst area was binned into 1% s bins and plotted against the corresponding diastolic pressure. A weighted linear regression was then performed, the slope of which provided the measurement of vascular transduction (units of mmHg (% s)$^{-1}$). Vascular transduction quantified using the 'Briant method' will be referred to as VT_{DBP} to distinguish from the methods above.

4.3.5 Statistics

Unpaired t-tests were used to compare cardiovascular and vascular transduction variables between males and females. These variables include heart rate, mean arterial pressure, systolic pressure, diastolic pressure, MSNA burst frequency, MSNA burst incidence, mean vascular conductance, VT_{CON}, VT_{MAP} and VT_{DBP}. Pearson's correlations coefficient was used

to examine the strength of the relationship between VT_{CON} and VT_{MAP}. Pearson's correlation coefficient was used to determine whether VT_{CON} is related to VT_{DBP}.

4.4. Results

4.4.1 Participants

Two males and two females had an increase instead of a decrease in leg vascular conductance following MSNA bursts. Since their results do not reflect the transduction of MSNA to vasoconstriction, data from these participants were not included in further analysis. Therefore, the comparisons reported below were made in 17 males and 18 females. Mean cardiovascular values for males and females are displayed in table 4.2. Males exhibited a greater systolic blood pressure, MSNA burst incidence and mean leg vascular conductance, whilst heart rate was significantly greater in females ($p < 0.05$). All other variables were not significantly different between males and females.

Table 4.2 Cardiovascular variables in 17 males and 18 females at rest (mean ± SD)

Cardiovascular variable	All	Males	Females	p-value
Systolic blood pressure (mmHg)	115 ± 12	120 ± 12	110 ± 10*	0.01
Diastolic blood pressure (mmHg)	70 ± 8	68 ± 8	72 ± 7	0.11
Mean arterial pressure (mmHg)	85 ± 8	85 ± 9	85 ± 8	0.79
Heartrate (bpm)	67 ± 9	64 ± 9	70 ± 7*	0.05
Burst incidence (bursts/100HB)	34 ± 13	38 ± 16	29 ± 9*	0.04
Burst frequency (bursts/minute)	22 ± 8	24 ± 9	20 ± 6	0.11
Mean SFA diameter (cm)	0.57 ± 0.09	0.60 ± 0.07	0.54 ± 0.10	0.04
Mean SFA velocity (cm/s)	5.4 ± 2.7	6.1 ± 3.3	4.8 ± 1.9	0.15
Mean SFA blood flow (ml/min^{-1})	83 ± 45	102 ± 52	65 ± 29	0.01
Mean conductance ($ml/min^{-1}/mmHg^{-1}$)	1.04 ± 0.61	1.3 ± 0.72	0.80 ± 0.34*	0.01

* represents a significant difference between males and females, $p < 0.05$

4.4.2 Sex differences in vascular transduction

The average decrease in leg vascular conductance following MSNA bursts reached a nadir of -4.39 ± 5.0%. Individual VT_{CON} values are provided in figure 4.5a. On average, the nadir in leg vascular conductance following MSNA bursts occurred at 8 cardiac cycles in males and 6 cardiac cycles in females. Males displayed a significantly lower VT_{CON} when compared with females (-4.86 ± 4.1 % Δ ml/min^{-1}/mmHg^{-1} vs. -7.82 ± 4.4 % Δ ml/min^{-1}/mmHg^{-1}, p = 0.04). Figure 4.2 illustrates the individual time course of the percent change in leg vascular conductance following MSNA bursts in a) males and b) females.

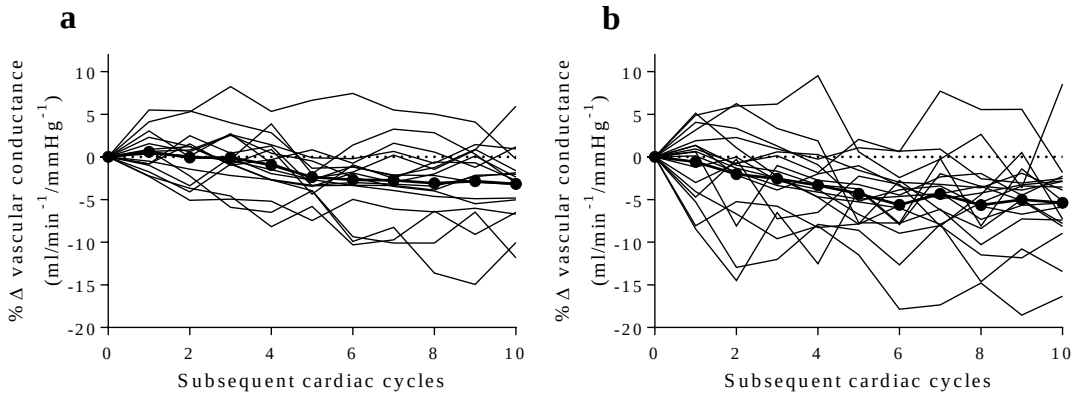

Figure 4.2. Individual time course of mean percent change in leg vascular conductance 10 cardiac cycles following MSNA bursts in a) 17 males and b) 18 females

The increase in mean arterial pressure following MSNA bursts peaked at approximately 2.18 ± 1.3%. Individual VT_{MAP} values are shown in figure 4.5b. On average, the peak percent increase in mean arterial pressure occurred after approximately 7 cardiac cycles in males and 6 cardiac cycles in females. There was no significant difference in VT_{MAP} between males and females (2.26 ± 1.2% Δ mmHg^{-1} vs. 2.41 ± 1.5% Δ mmHg^{-1}, p = 0.74). Figure 4.3 illustrates the individual time course of the percent change in mean arterial pressure following MSNA bursts in a) males and b) females. There was a positive correlation between VT_{CON} and VT_{MAP} in in males (r = 0.47, p = 0.054) but not in females (r = 0.19, p = 0.44).

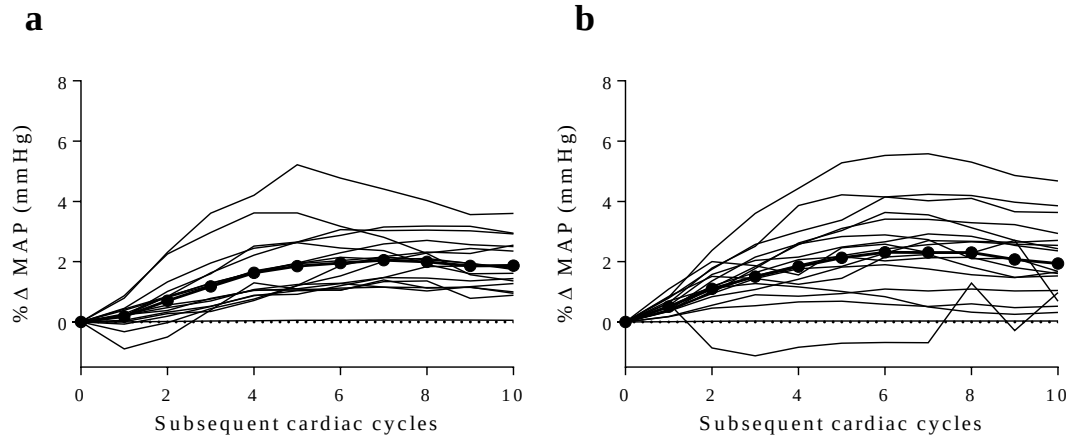

Figure 4.3. Individual time course of mean percent change in mean arterial pressure 10 cardiac cycles following MSNA bursts in a) 17 males and b) 18 females

Vascular transduction quantified using diastolic blood pressure (VT_{DBP}) was not significantly different between males and females (0.10 ± 0.06 mmHg (% s)$^{-1}$ vs. 0.09 ± 0.04 mmHg (% s)$^{-1}$, p = 0.53). Figure 4.4 provides of an example of VT_{DBP} in a) one male and b) one female. Individual VT_{DBP} values are shown in figure 4.5c.

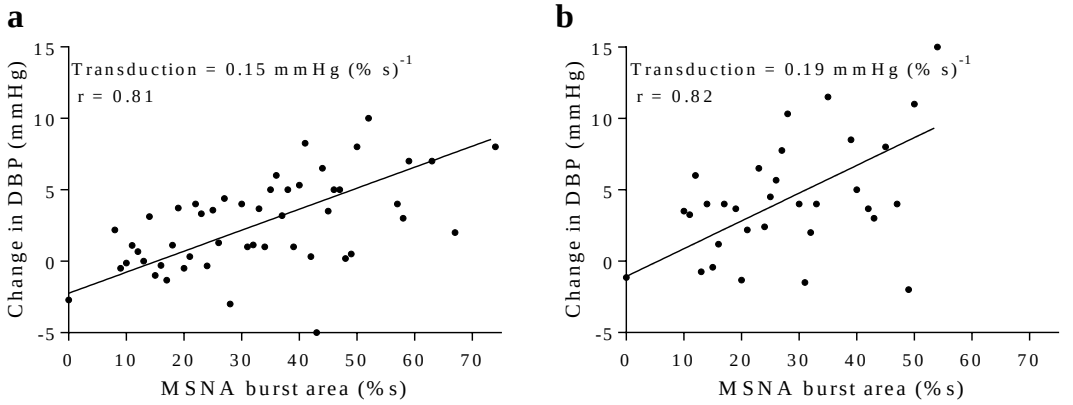

Figure 4.4. Vascular transduction characterised as the relationship between MSNA burst area and its effect on diastolic pressure in a) one male and b) one female

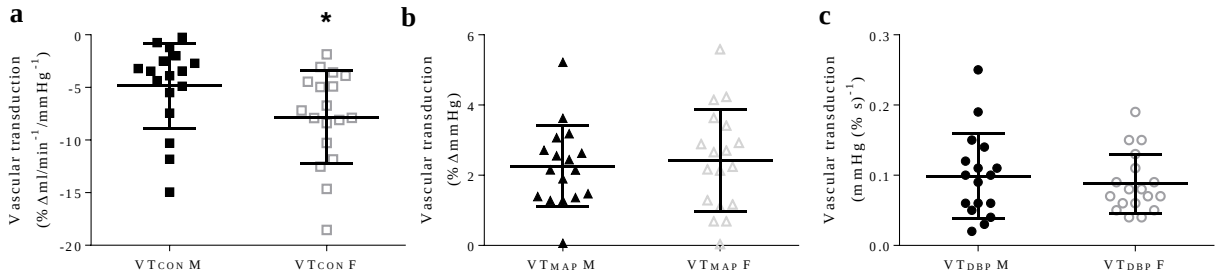

Figure 4.5. Vascular transduction in males and females quantified as a)VT_{CON}, b) VT_{MAP} and c) VT_{DBP}. a) Vascular transduction is greater in females than males when characterised as the nadir of the percent change in leg vascular conductance following MSNA bursts. Transduction is not significantly different between males and females when characterised using b) mean arterial pressure and c) diastolic blood pressure

4.5. Discussion

The main finding of this study is that beat-to-beat transduction of MSNA to changes in leg vascular conductance (VT_{CON}), assessed directly using Doppler ultrasound, is greater in young females than young males. In contrast, methods involving changes in blood pressure to quantify vascular transduction did not reveal sex differences. These approaches may provide insight into different aspects of the transduction of MSNA to vasoconstrictor drive and the regulation of arterial pressure.

4.5.1 Approaches for sympathetic vascular transduction assessment

In this study, sympathetic vascular transduction was quantified on a beat-by-beat basis in three ways. The first two (i.e., the Fairfax method) involved calculating the mean peak percent change in leg vascular conductance (VT_{CON}) and mean arterial pressure (VT_{MAP}) for 10 cardiac cycles following all MSNA bursts. The third method (Briant method) involved assessing the relationship between diastolic pressure and MSNA burst area (over 2 cardiac cycles) with a fixed lag of 8-6 cardiac cycles between MSNA and diastolic pressure (VT_{DBP}). In this study, the average decrease in leg vascular conductance following MSNA bursts occurred at approximately 7 cardiac cycles and the increase in mean arterial pressure peaked at approximately 7 cardiac cycles. This is in agreement with previous reports that explored

vascular transduction on a beat-to-beat basis at rest (Fairfax et al., 2013b, Vianna et al., 2012, Wallin and Nerhed, 1982). When quantified directly using Doppler ultrasound, vascular transduction (VT_{CON}) was greater in females than males. Charkoudian et al. (2006) previously reported that resting MSNA is inversely related to the forearm vasoconstrictor response to noradrenaline and tyramine, suggesting that higher levels of MSNA may blunt the level of vasoconstriction. In the current investigation, males had greater levels of resting MSNA than females when expressed as bursts per 100 heartbeats. It is possible that greater transduction in young females compensates for lower levels of sympathetic outflow. Alternatively, the lower levels of transduction in males may reflect a limitation in the approach. A potentially limiting factor of the technique may be when applying it to individuals with high levels of resting MSNA. This is because the method depends on locating MSNA bursts that are followed by cardiac cycles not associated with a burst. In individuals with high MSNA, it may be difficult to ascertain a true baseline level of conductance, i.e. without bursts of MSNA, and so tracking the beat-to-beat vasoconstrictor effects of sympathetic outflow to the vasculature may be problematic. The greater number of MSNA bursts in males, resulting in fewer silent periods (i.e., cardiac cycles following MSNA bursts not containing a MSNA burst), may explain why males had significantly lower transduction when compared with females. The effects of resting MSNA on vascular transduction will be explored in Chapter 6.

One of the aims of this study was to determine whether VT_{CON} and VT_{DBP} provide similar information on the vasoconstrictor responses to MSNA. Pearson's correlation analyses revealed that results gained from these two approaches are not related, and therefore the two approaches may not be used in place of one another when measures of blood flow cannot be acquired. The discrepancies between the two methods may be due to the metrics used to characterise MSNA (burst incidence versus burst area) or the cardiac cycle window used to observe the vasoconstrictor responses to MSNA. The Briant method controls for individual variability of MSNA burst incidence by taking into account the sympathetic outflow for every cardiac cycle and thus the size of the MSNA bursts. The Fairfax method did explore the effects of MSNA burst amplitude and burst clusters on the vascular response to MSNA. However, unlike the Briant method, this does not provide a single value of transduction that can be used to compare between groups over time. In the Fairfax method, vascular

transduction is determined by the individual vascular response to MSNA bursts. Evidence suggests that the effect of MSNA on the vasculature occurs at a peak response time of approximately 5.5 s, which typically equates to around 6 cardiac cycles following a burst of MSNA (Wallin and Nerhed, 1982). However this window is also dependent on an individual's heart rate. For example, based on a response time of 5.5 s, an individual with a heart rate of 50 bpm would have a peak response 4-5 cardiac cycles following MSNA bursts, whereas an individual with a heart rate of 80 bpm would have a peak response 7-8 cardiac cycles following MSNA bursts. With the Fairfax method (VT_{CON}) the timing of the peak response can be determined and thus variability in the lag between MSNA bursts and changes in vascular conductance can be taken into account. Conversely, the Briant method involves a fixed cardiac cycle window for MSNA of 8-6 cardiac cycles prior to each diastolic pressure, regardless of an individual's heart rate. However, in their investigation, Briant et al. (2016) did compare different cardiac cycle windows ranging from 3-1 to 10-8 cardiac cycles and reported, through cross-correlation analysis, that the peak correlation of beat-to-beat MSNA burst area and diastolic pressure occurred within the 8-6 cardiac cycle window in both young males and premenopausal females.

4.5.2 Sex differences in vascular transduction

It is well known that sex differences exist in cardiovascular control. What is unclear is whether there are sex differences in sympathetic vascular transduction. The vasoconstrictor effect of noradrenaline and its co-transmitters, ATP and NPY, compete with the vasodilatory effects of ß-adrenergic activity and circulating levels of nitric oxide. Previous reports have demonstrated that males have greater autonomic support of blood pressure regulation than females (Christou et al., 2005, Narkiewicz et al., 2005, Wehrwein et al., 2010). In young males MSNA is positively related to TPR, but this relationship is not apparent in young females (Charkoudian et al., 2005). Current evidence suggests that a greater ß-adrenergic receptor activity offsets α-adrenergic vasoconstriction in young females (Hart et al., 2011a). Hart and colleagues (2011a) reported these findings after observing the effects of administering noradrenaline in the forearm before and after ß-blockade with propranolol. Noradrenaline induced marked vasoconstriction in the forearm in males but not in females. However, following the administration of propranolol, vasoconstriction was observed in females and the degree of vasoconstriction did not change in males (Hart et al., 2011a).

Greater ß-adrenergic receptor activity may be an important factor contributing to the blunting of sympathetic vascular transduction in premenopausal females, and reduce the prevalence of hypertension in this population. Once females reach menopause, the relationship between MSNA and TPR becomes more apparent (Hart et al., 2011a). This change in blood pressure regulation in females suggests an important role for sex hormones, in particular oestrogen, in cardiovascular control (Hart et al., 2011a). In support of this, Vongpatanasin and colleagues (2001) reported a reduction in MSNA following 8-weeks of transdermal oestrogen administration in postmenopausal females. Furthermore, once women reach menopause, the prevalence of hypertension increases and at a greater rate than older males (Barnes et al., 2014).

The reports discussed above provide strong evidence for a blunted vascular transduction in young females when compared with males. However, in the current study, vascular transduction was significantly greater in females than males when expressed as the percent decrease in leg vascular conductance following MSNA bursts (VT_{CON}) and was not significantly different between males and females when derived using arterial pressure (VT_{MAP} and VT_{DBP}). The conflicting results in the current study to previous reports may be due to the methods used to examine the transduction of MSNA to vascular tone. Briant et al. (2016) quantified vascular transduction by using diastolic blood pressure as a proxy for the vascular response to sympathetic outflow and showed that vascular transduction was greater in young males when compared with premenopausal females. This method of analysis is useful in instances where measurements of blood flow cannot be acquired. However, this technique bypasses the end organ response of MSNA and therefore may not provide the same depth of information on the vasoconstrictor effects of MSNA as those obtained through direct measures of blood flow using Doppler ultrasound. Nevertheless, Briant method does provide consistent results with previous reports of sex differences in vascular transduction. For instance, vascular transduction, when measured by the decrease in forearm blood flow and vascular conductance in response to noradrenaline, is greater in males when compared with females (Hart et al., 2011; Kneale et al., 2000). Vianna et al. (2012) quantified vascular transduction as the beat-to-beat increases in mean arterial pressure following MSNA bursts and showed that vascular transduction is attenuated as males age. Additionally, following ß-blockade the vascular responses to MSNA increase to a greater extent in young females

compared with males (Hart et al., 2011a, Kneale et al., 2000), suggesting that ß-adrenergic vasodilation may counteract the vasoconstrictor effects of MSNA more in females than in males. When vascular transduction was quantified using the Briant method (VT_{DBP}) in the current study, there was no significant difference between males and females. Whilst the VT_{DBP} in young males was similar between the two studies, VT_{DBP} in females was greater in the current study (0.09 ± 0.04 mmHg (% s)$^{-1}$) when compared with the cohort presented by Briant (2016) (0.03 ± 0.01 mmHg (% s)$^{-1}$). A potential reason for inconsistent findings in VT_{DBP} between the two investigations may be the posture of the participants. In the current study participants were in a semi-recumbent posture with their left leg supported in an extended position whereas the participants in the study by Briant (2016) were supine. Low resting MSNA, which may be associated with the supine position, may not provide sufficient data to quantify vascular transduction. However, the level of resting MSNA in young females was in fact similar between the two studies despite the differences in posture, and therefore postural differences cannot explain the contrasting results. Whilst the Briant and Fairfax methods both have their strengths and limitations, the discrepancies in the results regarding sex differences (VT_{CON} was greater in females and no sex differences were observed for VT_{DBP}) suggest that the two methods may provide different information on the vascular response to MSNA. For instance, following MSNA bursts, the change in vascular conductance is specific to only one vascular bed (i.e., the leg), whereas the change in diastolic blood pressure is influenced by many vascular beds; including the splanchnic and capacitance vessels (such as those in the skin) (Magder, 2014).

4.5.3 Limitations

Since MSNA can occur in isolation or in a sequence of bursts, the vascular transduction technique developed by Fairfax et al. (2013b) also takes into account the effects of burst pattern and size on the magnitude of the vascular response to MSNA. Higher levels of MSNA (as indicated by larger bursts or greater number of consecutive bursts), and therefore, greater amount of noradrenaline released, are associated with more significant reductions in vascular conductance (Fairfax et al., 2013b). When clusters of bursts are made up of similar total MSNA but different numbers of bursts, the clusters with fewer (but larger) bursts were the most effective in decreasing leg vascular conductance. This was not performed in the current study, as it does not provide a single value to represent sympathetic vascular transduction for

each individual. Future research is needed to determine whether the effect of burst pattern and size on the magnitude of change in the vascular response to MSNA occurs in females as it does in males.

4.6. Conclusion

The results of this study demonstrate that sympathetic vascular transduction is greater in young females than young males when Doppler ultrasound is used for direct measurement of the vascular response to MSNA. The higher resting MSNA in males may limit the ability to quantify a true baseline level of vascular conductance (i.e. conductance when there are no bursts of MSNA), which could mask the full extent of the change in vascular conductance. The methods used to assess vascular transduction have a significant impact on investigations of sex differences; differences between young males and females were not apparent when examining the transduction of MSNA to changes in blood pressure. These discrepancies may also be attributed to other methodological differences, such as the metrics used to measure MSNA (burst incidence versus burst area) and/or the cardiac cycle window chosen to measure the response. The approaches presented in this chapter may provide insight into different aspects of the transduction of MSNA to vasoconstrictor drive and regulation of arterial pressure.

Chapter 5: Study 3

INTERACTIONS BETWEEN SYMPATHETIC BAROREFLEX SENSITIVITY AND VASCULAR TRANSDUCTION

5.1. Abstract

Sympathetic BRS is a tool used to quantify how effectively the baroreflex buffers beat-to-beat changes in blood pressure. However, current methods of assessment do not take into account sympathetic BRS in conjunction with vascular transduction; i.e. the response of the peripheral vasculature to vasoconstrictor drive. Having high sympathetic BRS does not necessarily mean that the transduction of MSNA to the peripheral vessels is also efficient. Evidence suggests that compensatory interactions exist between factors involved in cardiovascular control. Whether such interactions exist between sympathetic BRS and vascular transduction has yet to be investigated. Therefore, the aim of this study is to examine the relationship between sympathetic BRS and sympathetic vascular transduction. A secondary aim is to determine whether resting MSNA influences an individual's vascular transduction. Heart rate, blood pressure, MSNA and SFA blood flow (Doppler ultrasound) were recorded at rest during a 5-min recording period in 35 (17 males) healthy adults. Sympathetic BRS was quantified by plotting MSNA burst incidence against mean diastolic pressure (3 mmHg bins) using linear regression. Vascular transduction was quantified as the mean percent change in leg vascular conductance for 10 cardiac cycles following each MSNA burst, with the nadir of this response providing an estimate of each individual's vascular transduction (VT_{CON}). Linear regression analysis was used to examine the relationships between sympathetic BRS and vascular transduction. Subgroup analysis was performed to assess these relationships for males and females separately. Linear regression analyses were also performed to determine whether resting MSNA is a significant predictor of vascular transduction and/or sympathetic BRS. Sympathetic BRS was significantly greater in females when compared with males (-3.5 ± 1.3 bursts/100HB/mmHg vs. -2.4 ± 1.3 bursts/100HB/mmHg; $p = 0.02$). In males, sympathetic BRS was inversely related to vascular transduction ($r = 0.52$; $p = 0.03$). However, this relationship was not present in females ($r = 0.14$; $p = 0.59$) or when data from males and females were pooled ($r = 0.0004$; $p = 0.99$). Resting MSNA was not related to vascular transduction when expressed as both bursts/100HB ($r = 0.29$; $p = 0.1$) and bursts/min ($r = 0.31$; $p = 0.08$). Similarly, resting MSNA was not related to sympathetic BRS when expressed as both bursts/100HB ($r = 0.09$; $p = 0.60$) and bursts/min ($r = 0.19$; $p = 0.27$). Subgroup analysis revealed no significant relationship between resting MSNA and vascular transduction or sympathetic BRS in both males and females ($p > 0.05$). To conclude, sympathetic BRS is inversely related to vascular transduction in healthy males, such that males with high sympathetic BRS have low vascular

transduction, and vice versa. This may represent a compensatory effect to ensure that blood pressure is regulated effectively. Further research is needed to determine why this relationship is not apparent in females.

5.2. Introduction

It is well understood that differences in cardiovascular control exist between males and females. So far in this thesis, sex differences have been investigated in the baroreflex control of MSNA, and the transduction of MSNA to vasoconstriction. The previous chapters in this thesis demonstrate the variability of sympathetic BRS and vascular transduction. The results of Studies 1 and 2 illustrate that sex differences in sympathetic baroreflex function are largely dependent on the methodological approach and analysis technique. For instance, in Study 2, the method chosen to quantify vascular transduction provided different results regarding sex differences. When using the Fairfax method vascular transduction was enhanced in females when compared with males, whereas the Briant method revealed no differences between males and females. The conflicting results between the two approaches are not because one is invalid but that each provide different information on the vascular response to MSNA. Baroreflex sensitivity and vascular transduction are two components of baroreflex function. Ogoh et al. (2009) have defined BRS and vascular transduction as the neural and peripheral arcs of the sympathetic baroreflex, respectively. Baroreflex sensitivity reflects the reflex changes in MSNA in response to arterial pressure, whereas vascular transduction is the response of the peripheral vasculature, assessed via beat-to-beat changes in vascular conductance, to sympathetic outflow (Ogoh et al., 2009). Whilst previous research reveals significant inter-individual variability in these two components of sympathetic baroreflex function, how they interact with one other in relation to arterial pressure regulation is unclear.

There is evidence to suggest that the body provides compensatory interactions in an attempt to maintain blood pressure homeostasis. Charkoudian et al. (2005) reported that resting MSNA is directly related to TPR and inversely related to CO. Individuals with high MSNA have low CO, more specifically stroke volume, and those with low levels of MSNA have high levels of CO and stroke volume (Charkoudian et al., 2005). However, these relationships have only been established in young normotensive males; the relationship between CO and MSNA does not exist in females (Hart et al., 2009a). Previous evidence suggests this may be due to enhanced ß-adrenergic sensitivity in females, which therefore balances the vasoconstrictor effects of sympathetic outflow (Hart et al., 2011a, Kneale et al., 2000). This highlights the fact that there are variables other than MSNA that control TPR in premenopausal females.

Compensatory interactions may also exist between resting MSNA and sympathetic vascular transduction. There is an inverse relationship between resting MSNA and adrenergic responsiveness in the forearm (Charkoudian et al., 2006). Resting levels of MSNA are also correlated with the level of nitric oxide production (Skarphedinsson et al., 1997). Furthermore, the change in leg vascular conductance following MSNA bursts at rest is inversely related to MSNA burst frequency accounting for 35% of the variation in resting MSNA (Fairfax, 2013). It was reported that those with low resting MSNA have greater vascular transduction, and those with high resting MSNA have lower vascular transduction. These findings suggest that individuals with higher levels of MSNA have blunted vasoconstrictor responses to sympathetic activity. It is also speculated that impairments in sympathetic vascular transduction may be counterbalanced by higher sympathetic BRS (Fairfax et al., 2013b). Baroreflex sensitivity provides a measure of an individual's blood pressure buffering capability, but current methods of assessment do not take into account sympathetic BRS in conjunction with the direct effects of MSNA on the vasculature. Establishing whether there is a relationship between sympathetic BRS and vascular transduction will confirm whether or not an enhanced sympathetic BRS does lead to more effective control of blood pressure.

It is hypothesised that sympathetic BRS and sympathetic vascular transduction, acquired using Doppler ultrasound, are negatively correlated under resting conditions in young healthy individuals. This would mean that individuals with high sympathetic BRS have less effective vascular transduction during spontaneous changes in blood pressure. Therefore, the aim of this study is to examine the relationship between sympathetic BRS and sympathetic vascular transduction. This will be assessed at rest during spontaneous changes in MSNA, leg vascular conductance and blood pressure. As there are sex differences in cardiovascular control, these relationships will be examined in males and females together and also separately. In Chapter 4, it was reported that males have lower levels of vascular transduction when assessed using the Fairfax method. This was an unexpected result as previous research suggests that vascular transduction is lower in females due to the counteracting effects of β-adrenergic vasodilation to MSNA (Hart et al., 2011a, Kneale et al., 2000). This may be explained by the greater levels of resting MSNA in males and might influence the values of transduction using the Fairfax method. Therefore, a secondary aim of this study is to determine whether levels of

resting MSNA predict vascular transduction. It is hypothesised that resting MSNA is inversely related to vascular transduction.

5.3. Methods

5.3.1 Participants

The cohort of participants in this chapter is the same as those included in Chapter 4 (17 males and 18 females). Table 5.1 provides detail on participant characteristics.

Table 5.1 Participant characteristics in 17 males and 18 females (mean ± SD)

	Males	Females	p-value
Age (years)	21 ± 3	24 ± 5	0.04
Height (cm)	174 ± 8	162 ± 6	<0.0001
Weight (kg)	72 ± 8	62 ± 14	0.02
BMI (m/kg)	24 ± 3	24 ± 6	0.99
Oral contraceptive use		9 out of 18	
Training status (min/week)	414 ± 181	190 ± 138	0.0002

5.3.2 Experimental setup

Refer to Chapter 2, section 2.3 for the experimental setup.

5.3.3 Measurements

ECG, blood pressure, MSNA and SFA diameter and velocity were measured on a beat-to-beat basis and recorded during a 5-min baseline period. Refer to Chapter 2, section 2.4 for each variable and how they were measured.

5.3.4 Data analysis

Sympathetic baroreflex sensitivity (BRS_{inc}) and vascular transduction (VT_{CON}) were quantified for each participant under resting conditions. Refer to Chapter 2, section 2.5.1 for how sympathetic baroreflex sensitivity (BRS_{inc}) was quantified. Refer to Chapter 4, section 4.3.4 for how sympathetic vascular transduction (VT_{CON}) was calculated.

5.3.5 Statistics

Linear regression analysis was used to examine the relationships between sympathetic BRS_{inc} and vascular transduction. Subgroup analysis was performed to assess these relationships for males and females separately. Linear regression analyses were also performed to investigate whether resting MSNA (burst incidence and burst frequency) predicts sympathetic BRS and/or vascular transduction. Statistical analyses were performed using Prism v6.00 for Windows (GraphPad software, San Diego, California, USA). For all statistical tests, a probability level of $p < 0.05$ was regarded as significant. All values are expressed as mean ± SD.

5.4. Results

In one individual (female), her BRS_{inc} value was large (-16.53 bursts/100HB/mmHg). In this case, their baroreflex slope was made with only 3 diastolic pressure bins and resulted in an overestimation of sympathetic baroreflex sensitivity. The participant was therefore excluded from analysis and the results reported in this chapter are for 17 females. Mean cardiovascular values for males and females are displayed in table 5.2. Males exhibited a greater systolic blood pressure, MSNA burst incidence and mean leg vascular conductance whereas heart rate was greater in females ($p < 0.05$). Sympathetic BRS_{inc} was significantly lower in males when compared with females (-2.4 ± 1.3 bursts/100HB/mmHg vs. -3.5 ± 1.3 bursts/100HB/mmHg; $p = 0.02$, Fig 5.1a). Similarly, sympathetic VT_{CON} was significantly lower in males when compared with females (-4.86 ± 4.07 $\%\Delta ml/min^{-1}/mmHg^{-1}$ vs. -8.07 ± 4.39 $\%\Delta ml/min^{-1}/mmHg^{-1}$; $p = 0.03$, Fig 5.1b).

Table 5.2 Cardiovascular variables in 17 males and 18 females at rest (mean ± SD)

	All	Males	Females	p-value
Systolic blood pressure (mmHg)	115 ± 12	120 ± 12	110 ± 10*	0.01
Diastolic blood pressure (mmHg)	70 ± 8	68 ± 8	73 ± 7	0.08
Mean arterial pressure (mmHg)	85 ± 8	85 ± 9	85 ± 8	0.93
Heart rate (bpm)	67 ± 9	64 ± 9	70 ± 7*	0.04
Burst incidence (bursts/100HB)	34 ± 13	38 ± 16	28 ± 9*	0.03
Burst frequency (bursts/minute)	22 ± 8	24 ± 9	20 ± 6	0.09
Mean conductance ($ml/min^{-1}/mmHg^{-1}$)	1.04 ± 0.61	1.3 ± 0.72	0.82 ± 0.34*	0.02

* represents a significant difference between males and females, $p < 0.05$

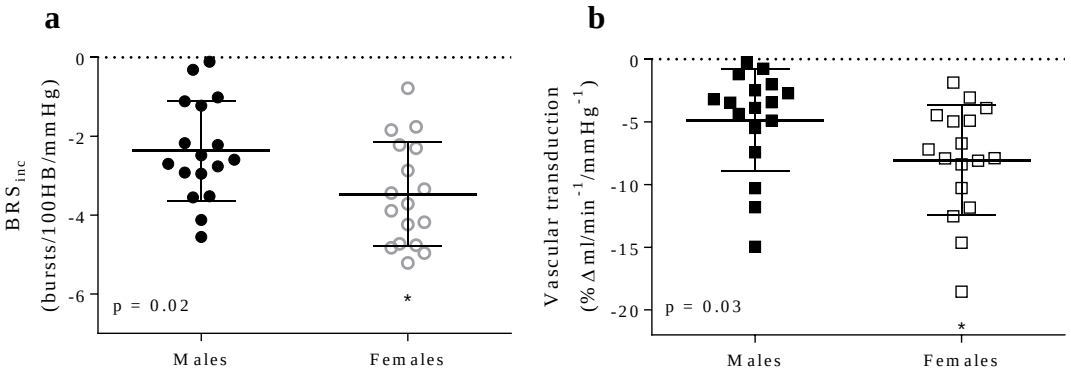

Figure 5.1. a) Spontaneous sympathetic BRS_{inc} and b) sympathetic vascular transduction (VT_{CON}) are significantly greater in young females when compared with males

5.4.1 Relationship between sympathetic baroreflex sensitivity, resting MSNA and vascular transduction

Linear regression analysis showed that sympathetic BRS_{inc} was inversely related to VT_{CON} in males ($r = 0.52$; $p = 0.03$, Fig 5.2a). However, this relationship was not present in females ($r = 0.14$; $p = 0.59$, Fig 5.2b) or when data from males and females were pooled ($r = 0.0004$; $p = 0.99$). Resting MSNA was not significantly related to BRS_{inc} when characterised using MSNA burst incidence ($r = 0.09$; $p = 0.60$) or MSNA burst frequency ($r = 0.19$; $p = 0.27$). Similarly, resting MSNA was not significantly related to VT_{CON} when characterised using MSNA burst incidence ($r = 0.29$; $p = 0.10$) or MSNA burst frequency ($r = 0.31$; $p = 0.08$).

Subgroup analysis based on sex revealed no relationship between resting MSNA and BRS_{inc} (Fig 5.3), and between MSNA and VT_{CON} (Fig 5.4) in either males or females ($p > 0.05$).

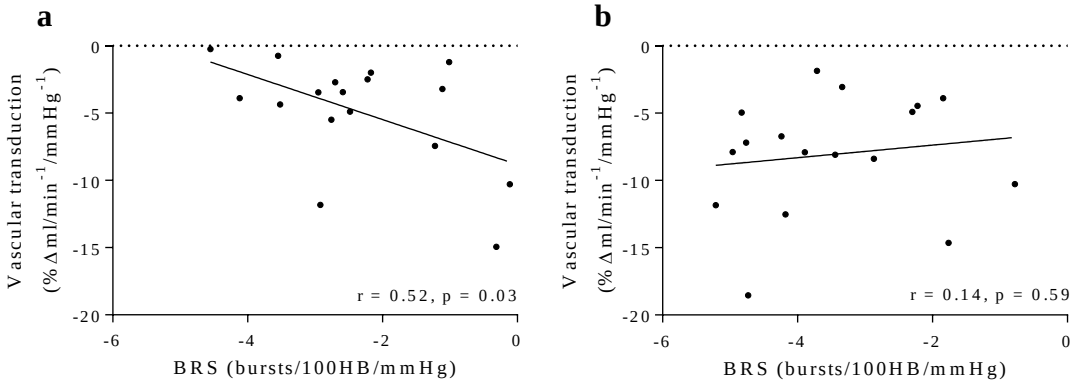

Figure 5.2 Relationship between sympathetic baroreflex sensitivity and vascular transduction in a) males and b) females

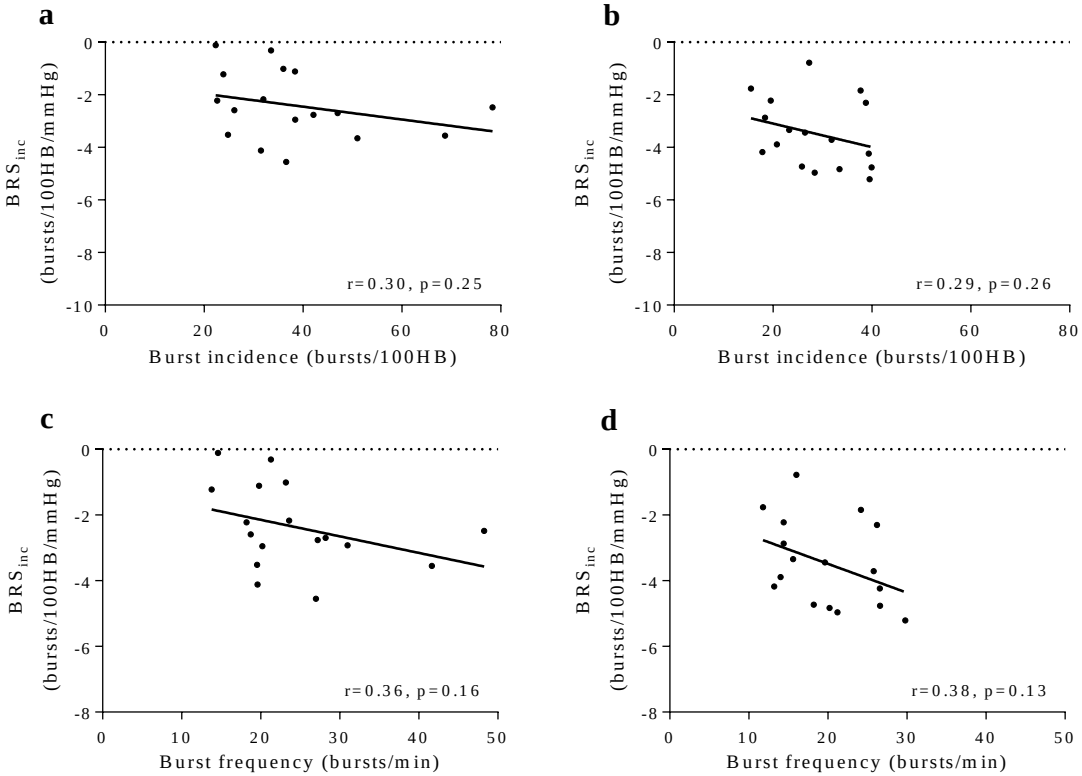

Figure 5.3. Relationship between resting MSNA and sympathetic baroreflex sensitivity, when MSNA is quantified using a) MSNA burst incidence in males, b) MSNA burst incidence in females, c) MSNA burst frequency in males and d) MSNA burst frequency in females

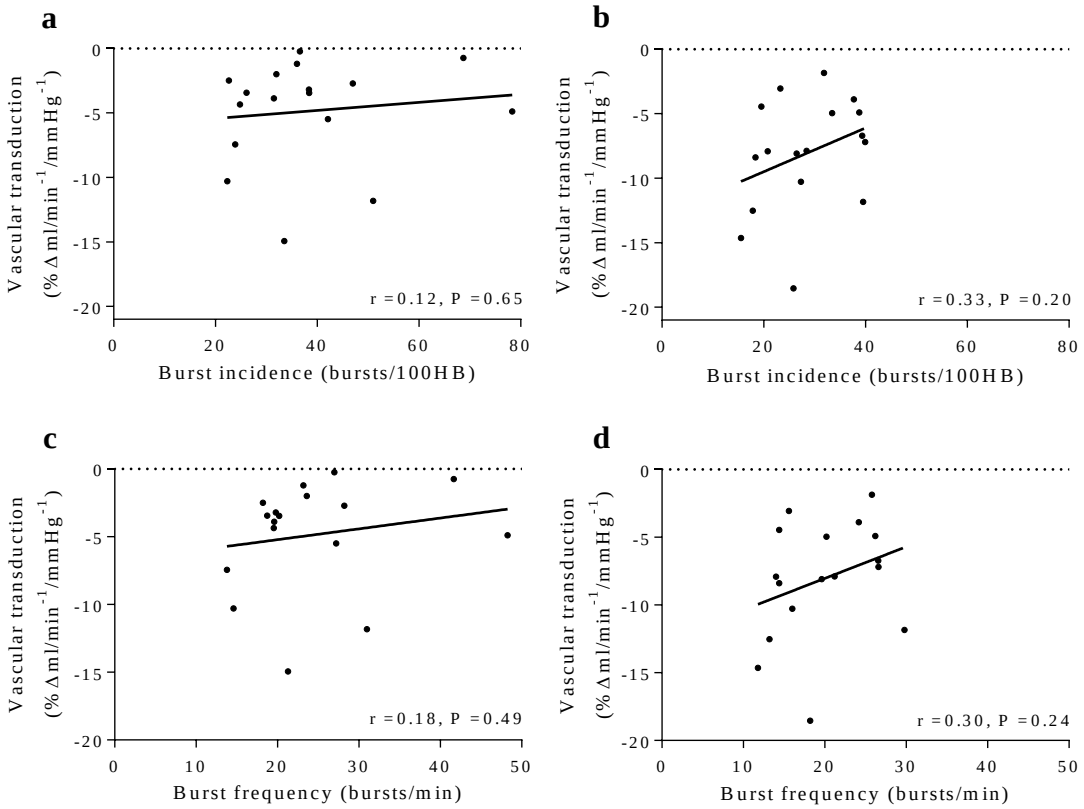

Figure 5.4. Relationship between resting MSNA and vascular transduction, when MSNA is quantified using a) MSNA burst incidence in males, b) MSNA burst incidence in females, c) MSNA burst frequency in males and d) MSNA burst frequency in females

5.5. Discussion

This is the first study to explore the relationship between sympathetic BRS and vascular transduction at rest in young healthy adults. The findings of this study show that sympathetic BRS is inversely related to sympathetic vascular transduction in young males but not in young females. This concurs with previous reports of compensatory interactions that occur in cardiovascular control and their differences between males and females to maintain blood pressure homeostasis. Resting MSNA does not predict sympathetic baroreflex sensitivity or vascular transduction in either males or females.

5.5.1 Compensatory interactions in cardiovascular control

The baroreflex is a negative feedback loop that ensures the maintenance of arterial pressure. With regards to the sympathetic arm, the baroreflex is composed of a neural and peripheral arc, whereby the neural arc (BRS_{inc}) involves the reflex response of MSNA to changes in arterial pressure and the peripheral arc (VT_{CON}) is the vascular response to sympathetic outflow (Ogoh et al., 2009). Current methods of sympathetic baroreflex assessment only take into account sympathetic BRS and not vascular transduction so it is unclear if an enhanced BRS does in fact lead to effective buffering of changes in blood pressure. The current findings suggest that, in young healthy males, poor sympathetic BRS does not necessarily mean that the baroreflex buffering ability is inhibited as it is compensated for by greater transduction of sympathetic outflow to the vasculature. However, in females, although sympathetic BRS and vascular transduction are greater when compared with males, there is no apparent relationship between BRS and vascular transduction. These results align with previous research in which compensatory interactions between cardiovascular variables have been observed at rest, which may serve to ensure blood pressure homeostasis. As with the current study, these relationships have predominantly been shown in males only. For instance, in young males, but not young females, MSNA is positively related to TPR and inversely related to CO (Charkoudian et al., 2005, Hart et al., 2009a). Evidence suggests that the lack of compensatory interactions in neural control in females is predominantly due to sex hormones, such as circulating oestradiol, which increase nitric oxide synthesis and increase ß-adrenergic sensitivity thereby offsetting the vasoconstrictor effect of α-adrenergic receptor activity (Hart et al., 2011a, Kneale et al., 2000). The results of this study may have implications for previous research in which diminished sympathetic BRS has been reported in certain population groups, such as individuals who are obese (Grassi et al., 2004) and those with obstructive sleep apnoea (Carlson et al., 1996). It is possible that in some population groups, impaired sympathetic baroreflex function is compensated for by greater vascular transduction. Further research would be required to investigate these relationships.

5.5.2 Does resting MSNA determine vascular transduction?

An acute increase in MSNA causes an increase in arterial pressure, and it therefore plays an important role in maintaining homeostasis. In young males and females the level of resting

MSNA is not correlated with mean arterial pressure (Narkiewicz et al., 2005). However, resting levels of MSNA have been shown to influence other components of cardiovascular control. For instance, Charkoudian et al. (2006) previously reported an inverse relationship between resting MSNA and forearm vasoconstrictor responses to noradrenaline and tyramine. This suggests higher levels of resting MSNA may blunt the level of vasoconstriction. Moreover, resting levels of MSNA are also correlated with nitric oxide production (Skarphedinsson et al., 1997). As reported in Chapter 4, sympathetic vascular transduction was significantly greater in females when compared with males when assessed using the Fairfax method. It was speculated that a greater vascular transduction in females might compensate for lower resting MSNA. However, it was also proposed that the attenuated vascular transduction observed in males might reflect a limitation in the approach used. The Fairfax method might underestimate vascular transduction in individuals with particularly high levels of MSNA, as it is difficult to ascertain a true baseline level of vascular conductance as there are fewer silent periods (i.e., cardiac cycles following MSNA bursts that are not associated with a MSNA burst). Therefore, any changes in conductance that are calculated following MSNA bursts might not be as great as they do not represent changes from a true baseline. To address whether resting MSNA predicts vascular transduction, regression analyses were performed.

The results of the current study suggest that resting MSNA does not determine sympathetic BRS or vascular transduction in either males or females. Conversely, others have reported an inverse relationship between MSNA burst incidence and vascular transduction (Tan et al., 2013a, Wallin and Nerhed, 1982). A potential reason for this lack of relationship is the range of resting MSNA in the cohort. There was a trend towards an inverse relationship between resting MSNA and vascular transduction ($p \leq 0.1$). In this study, there were two males with very high resting MSNA (69 and 78 bursts/100HB) but the rest of the cohort had low to moderate levels of MSNA (19-51 bursts/100HB). The relationship between MSNA and vascular transduction and/or BRS may become apparent when using a more balanced range of resting MSNA (i.e., more people with high resting MSNA) and would provide a better understanding of the effects of resting muscle sympathetic activity on vascular transduction. Furthermore this relationship may become apparent when examining older individuals as resting MSNA is greater when compared with younger males and females (Hart et al., 2009b,

Hart et al., 2011a, Peinado et al., 2017). Previous reports reveal an attenuation of sympathetic vascular transduction in older males when compared with younger males (Briant et al., 2016, Tan et al., 2013a, Vianna et al., 2012). Vianna et al. (2012) showed that older males are highly reliant on sympathetic outflow for beat-to-beat maintenance of arterial pressure, which may help to explain the greater resting MSNA. However, there is confounding evidence on the age-related changes in vascular transduction in females. Vianna et al. (2012) report an attenuated vascular transduction in postmenopausal females, where Briant et al. (2016) reported greater vascular transduction in postmenopausal females when compared with young premenopausal females. The reason for the different findings in postmenopausal females may be due to the method employed in quantifying sympathetic vascular transduction, which, as demonstrated in Chapter 4, can lead to marked differences in results.

5.5.3 Methodological considerations

The Fairfax method allows the quantification of sympathetic vascular transduction on a beat-to-beat basis at rest. As indicated above, the Fairfax approach may be limited in individuals with high levels of resting MSNA. A way to combat this limitation is to utilise the MSNA burst amplitude component of the Fairfax method, which considers how variations in burst size can influence the vascular response to sympathetic outflow. As reported by Fairfax et al. (2013b), higher levels of MSNA (as indicated by larger bursts or greater number of consecutive bursts), and therefore, greater amount of noradrenaline released, are associated with more significant reductions in vascular conductance. Incorporating the effects of burst strength on the vascular response may therefore provide a more informative method of assessing vascular transduction in individuals with high resting MSNA. However, MSNA burst amplitude was not used in the current study as it does not provide a single value for vascular transduction. Another way to ensure a true baseline vascular conductance is captured is by performing the analysis using longer recording periods.

5.6. Conclusion

Sympathetic BRS is inversely related to vascular transduction in healthy males. Those with high sympathetic BRS have low vascular transduction, and vice versa. This may represent a

compensatory effect to ensure that blood pressure is regulated effectively. However, the relationship between sympathetic BRS and vascular transduction is not present in females. Further research is needed to identify the factors involved in determining vascular transduction in healthy and clinical populations.

Chapter 6: Study 4

SEX DIFFERENCES IN VASCULAR TRANSDUCTION DURING SYMPATHOEXCITATION

6.1. Abstract

Although previous studies have reported attenuated sympathetic vascular transduction in young females at rest, evidence of sex differences in vascular transduction during manoeuvres that drive increases in MSNA is limited. Therefore, the aim of this study is to examine sympathetic BRS and vascular transduction during physiological stressors that drive increases in MSNA. It is hypothesised that vascular transduction is greater in males when compared with females during physiological stress. It is also hypothesised that the sympathetic baroreflex is reset to higher blood pressures during physical stress. Heart rate, blood pressure, MSNA and SFA blood flow (Doppler ultrasound) were measured in 25 (12 males) participants during isometric handgrip to fatigue at 35% of maximum voluntary contraction and in 22 (13 males) participants during a 6-min cold pressor test. Vascular transduction was quantified by plotting mean arterial pressure against the corresponding MSNA burst count for each 30 s interval during the task and the rest period prior to the task. Vascular transduction was also derived using leg vascular conductance obtained from the Doppler ultrasound measurements. Vascular transduction slopes were also constructed using total MSNA to take into account both the frequency and strength of bursts during the tasks. Cardiovascular responses to stressor tasks were compared to data collected during a rest period immediately prior to each task. Spontaneous sympathetic BRS was quantified during the last 5-min of the cold pressor test and the 5-min rest period preceding the task. Unpaired t-tests were performed to determine whether vascular transduction during isometric handgrip and the cold pressor test differs between males and females. In addition, a two-way ANOVA was performed for each variable to examine the main effects of the stressor and sex. Unpaired t-tests were performed to test whether there were significant differences in the percent changes in each variable when comparing males and females. Finally, a two-way ANOVA was performed for BRS_{inc} and T50 to examine the main effects of the cold pressor test and sex. Vascular transduction was significantly greater in males when quantified as the relationship between MSNA burst count and vascular conductance ($p = 0.004$) and between total MSNA and vascular conductance ($p = 0.01$) during isometric handgrip. During the cold pressor test, vascular transduction was greater in males when expressed as the relationship between MSNA burst count and vascular conductance ($p = 0.04$) but not when using total MSNA ($p = 0.43$). There were no sex differences in vascular transduction when derived using mean arterial pressure during both isometric handgrip and cold pressor test ($p > 0.05$). Sympathetic baroreflex sensitivity was not different between males and females during the cold pressor test but was reset to a higher blood pressure range. The absolute and percent

increase in MSNA during isometric handgrip task and cold pressor test were not significantly different between males and females. Vascular transduction when quantified as the relationship between MSNA and vascular conductance during sympathoexcitation is greater in young males than young females. Whilst increases in MSNA during sympathoexcitatory manoeuvres are comparable between males and females, vascular transduction is reduced in females as evidenced by the attenuated decrease in vascular conductance when compared with males.

6.2. Introduction

The study Chapters presented in this thesis have demonstrated sex differences in the baroreflex control of MSNA under resting conditions. The results of Study 1 indicate that, in young, healthy individuals, sympathetic BRS is greater in females when compared with males. Study 2 showed that vascular transduction directly measured on a beat-by-beat basis is greater in females when compared with males. Study 3 revealed there is an inverse relationship between sympathetic BRS and vascular transduction in males but not in females. Previous studies related to sex differences in vascular transduction have reported attenuated sympathetic vascular transduction in young females both at rest and during physiological stress (Briant et al., 2016, Hogarth et al., 2007). However, other reports have shown no significant sex differences at rest and also during sympathetic activation such as the cold pressor test (Jarvis et al., 2011, Vianna et al., 2012). Evidence of sex differences in vascular transduction during manoeuvres that drive increases in MSNA is limited and inconsistent. Previous reports have shown that cardiovascular control during physical stressor tasks are associated with similar patterns between males and females where arterial pressure, MSNA and heart rate increase when exposed to a physical stimulus (Dishman et al., 2003, Hogarth et al., 2007, Jones et al., 1996, Shoemaker et al., 2001). However, the pressor response to sympathetic activation during physiological stress such as isometric handgrip was shown to be attenuated in females when compared with males (Jarvis et al., 2011). Hogarth et al. (2007) reported smaller increases in arterial pressure in response to isometric handgrip in females, while absolute increases in MSNA were similar to males. As young females tend to have lower resting levels of MSNA, the percent increase in MSNA was greater in females (Hogarth et al., 2007). Despite this, the change in calf vascular resistance was blunted in females when compared with males. This suggests that the transduction of MSNA to vasoconstrictor drive is attenuated in females during sympathetic activation (Hogarth et al., 2007).

Physical stressor tasks enable investigators to quantify vascular transduction during increases in MSNA and provide a non-pharmacological means of increasing the range of sympathetic outflow and arterial pressure. Examples include the cold pressor test (Fagius et al., 1989, Victor et al., 1987) and isometric handgrip (Halliwill et al., 1996, Mark et al., 1985, Minson et al., 2000a, Seals, 1989b) where the increase in MSNA during these tasks correlates

strongly with an increase in blood pressure. Thus, both of these tasks provide suitable manoeuvres to increase vasoconstrictor drive to the muscle vascular bed. During both isometric handgrip and the cold pressor test, sympathetic BRS resets to a higher blood pressure range (Cui et al., 2002, Ichinose et al., 2006). Resetting can be monitored by determining the diastolic pressure at which 50% of cardiac cycles are associated with a burst. This T50 value provides information on the average setting of the baroreflex over the range of blood pressures in a recording (Sundlof and Wallin, 1978, Wallin et al., 1974). Although the cold pressor test and isometric handgrip task induce significant increases in heart rate, blood pressure and MSNA, it is unclear how the inter-individual differences in vascular transduction may influence the magnitude of the blood pressure response and whether these inter-individual differences are, in part, related to sex. Therefore, the aim of this study is to examine sympathetic BRS and vascular transduction during physiological stressors that drive increases in MSNA. It is hypothesised that vascular transduction is greater in males when compared with females during physiological stress. It is also hypothesised that the sympathetic baroreflex is reset to higher blood pressures during physical stress in both males and females.

6.3. Methods

6.3.1 Participants

Refer to Chapter 2, section 2.2 for participant characteristics. The data presented in this chapter are based on 25 (12 males) participants who completed isometric handgrip, and 22 (13 males) participants who completed the cold pressor test. Table 6.1 provides detail on participant characteristics.

Table 6.1 Participant characteristics in 13 males and 13 females (mean ± SD)

	Males	Females	p-value
Age (years)	22 ± 3	23 ± 4	0.48
Height (cm)	172 ± 8	163 ± 5	0.004
Weight (kg)	71 ± 8	61 ± 13	0.03
BMI (m/kg)	24 ± 3	23 ± 4	0.41
Oral contraceptive use		7 out of 13	
Training status (min/week)	432 ± 203	207 ± 157	0.004

6.3.2 Experimental setup

ECG, blood pressure, MSNA and SFA diameter and velocity were measured on a beat-to-beat basis and recorded during two physical stressor tasks. Refer to Chapter 2, section 2.4 for each variable and how they were measured.

6.3.3 Blood pressure reactivity to stressor tasks

Cardiovascular responses to stressor tasks were compared to data collected during a rest period immediately prior to each task. There was a minimum of 5-min between tasks to allow for recovery and to ensure that cardiovascular variables had returned to resting levels. Additional rest was given if required, until blood pressure returned to baseline. The following stressor tasks were performed:

Isometric Handgrip Exercise: Following 2-min of rest, participants were instructed to grip a dynamometer to fatigue using 35% of their previously recorded maximum voluntary contraction (MVC). Cessation of the task occurred when the participant reduced the force applied to less than 30% of their MVC for 5 s despite verbal encouragement.

Cold Pressor Test: After a 5-min period of rest, participants placed their right hand in a container of ice and water (0 ± 1^{o}C) for 6 min. The cold pressor test was performed for 6-min to allow sufficient time for blood pressure to reach steady state (~1min) so that sympathetic BRS could be calculated (5-min). The cold pressor test was chosen because it is associated with robust increases in MSNA through which to assess vascular transduction. In addition, the duration of the cold pressor test allowed for more insight into the cardiovascular responses over the time course of the test.

6.3.4 Data analysis

Beat-to-beat values were extracted from LabChart (ADInstruments, Dunedin, New Zealand) for systolic blood pressure, diastolic blood pressure, mean arterial pressure and MSNA. The

detection and area of each MSNA burst was performed using the Peak Parameters module of LabChart (ADInstruments). The MSNA burst with the greatest amplitude during the baseline period prior to each task was given a value of 100 and all other bursts were normalised against this. The edge tracking output of the SFA diameter and velocity was used to calculate blood flow using the following equation:

V_{mean} (in cm/s)·π·[mean artery diameter (in cm) ÷ 2]^2·60 s/min

Leg vascular conductance was calculated as SFA blood flow divided by mean arterial pressure. For each stressor task, systolic blood pressure, diastolic blood pressure, mean arterial pressure, heart rate, MSNA burst count (bursts/30 s), total MSNA (mean MSNA burst amplitude * MSNA burst count, expressed as AU/30 s), blood flow and vascular conductance were determined across 30 s intervals throughout rest and task. Absolute changes in each variable were calculated by subtracting the peak 30 s interval during the task by the first 30 s interval at baseline. The percent change in each variable during isometric handgrip and cold pressor test was calculated using the following formula:

Peak 30 s – first 30 s baseline ÷ first 30 s baseline *100

The first 30 s baseline interval was used to account for any changes to baseline activity due to an anticipatory response that may have occurred prior to each task.

Vascular transduction: Vascular transduction was characterised by plotting mean arterial pressure against the corresponding MSNA burst count for each 30 s interval during the isometric handgrip and the 2-min rest period prior to the task. The relationship between mean arterial pressure and MSNA was determined via ordinary least squares regression. This method of assessing vascular transduction has been used previously as a valid method of evaluating the end organ response of sympathetic nerve activity (Halliwill et al., 1996, Seals, 1989b, Tan et al., 2013a, Tan et al., 2013b). This approach was also used for the 6-min cold pressor test and the 5-min rest period prior to the task. Linear regression analysis was also performed between the mean vascular conductance and MSNA burst count for each 30 s interval during both isometric handgrip and cold pressor test. The slope of the linear regression provided the vascular transduction for each individual during each task. Vascular transduction slopes were also performed with total MSNA to take into account not only the

frequency of bursts but also the strength of the bursts during the tasks. No threshold was set for the acceptance of vascular transduction slopes as a low r value may indicate poor vascular transduction for that individual, and thus provide important information.

Sympathetic BRS_{inc}: Refer to Chapter 2, section 2.5.1 for quantification of sympathetic BRS_{inc}. Sympathetic BRS_{inc} was calculated during the last 5-min of the cold pressor test and separately during the previous 5-min rest period. Sympathetic BRS was only quantified during the cold pressor test as it provides sufficient data under steady state conditions, whereas arterial pressure does not reach steady state during isometric handgrip. As shown in Chapter 3, Study 1, when comparing recordings of different durations, 2-min recordings do not provide a substantial blood pressure range and can lead to an overestimation of an individual's sympathetic BRS. It is therefore important that a 5-min period be used to quantify sympathetic BRS. To assist in confirming whether sympathetic BRS_{inc} was reset during the cold pressor test, the T50 threshold was calculated both at rest and during the cold pressor test. The T50 threshold is defined as the diastolic pressure at which 50% of cardiac cycles are associated with a burst of MSNA (Kienbaum et al., 2001, Wehrwein et al., 2010). The T50 value was calculated using Ensemble (Elucimed Ltd, Wellington, New Zealand).

6.3.5 Statistical analysis

Unpaired t-tests were performed to determine whether vascular transduction during isometric handgrip and the cold pressor test differs between males and females. A two-way ANOVA was performed for each variable to examine the main effects of the stressor (rest vs. peak) and sex (males vs. females). Additionally, unpaired t-tests were performed to test whether there were significant differences in the percent changes in each variable when comparing males and females. Finally, a two-way ANOVA was performed for BRS_{inc} and T50 to examine the main effects of the cold pressor test (rest vs. task) and sex (males vs. females).

6.4. Results

6.4.1 Sex differences in vascular transduction

Isometric handgrip: When assessed using the increase in MSNA during isometric handgrip, the difference in vascular transduction between males and females was dependent on whether it was derived from changes in mean arterial pressure or leg vascular conductance (Fig. 6.1). When expressed using the relationship between mean arterial pressure and MSNA, the range of r values was 0.15 to 0.97, with 20 out of 25 individuals demonstrating at least a moderate relationship ($r \geq 0.5$). When expressed using the relationship between leg vascular conductance and MSNA, the range of r values was 0.07 to 0.97, with 15 out of 25 individuals demonstrating at least a moderate relationship. When expressed as the relationship between total MSNA and mean arterial pressure, the range of r values was 0.21 to 0.98, with 21 out of 25 individuals demonstrating a moderate relationship. When expressed as the relationship between total MSNA and leg vascular conductance, the range of r values was 0.01 to 0.89, with 13 out of 25 individuals demonstrating a moderate relationship. Figure 6.2 displays examples of both strong and weak relationships between MSNA burst count with mean arterial pressure and MSNA burst count with leg vascular conductance. When expressed using the relationship between MSNA burst count and mean arterial pressure, vascular transduction was similar in males and females (2.0 ± 0.9 mmHg/burst vs. 1.6 ± 1.1 mmHg/burst; $p = 0.30$). Vascular transduction, when expressed as the relationship between MSNA burst count and leg vascular conductance, was significantly greater in males when compared with females (-0.06 ± 0.06 $ml/min^{-1}/mmHg^{-1}$/burst vs. 0 ± 0.02 $ml/min^{-1}/mmHg^{-1}$/burst; $p = 0.004$). When vascular transduction was expressed via the relationship between total MSNA and mean arterial pressure, there was no significant difference between males and females (0.01 ± 0.02 mmHg/AU vs. 0.02 ± 0.01 mmHg/AU; $p = 0.18$). When expressed as the relationship between total MSNA and leg vascular conductance, vascular transduction was greater in males when compared with females (-0.001 ± 0.001 $ml/min^{-1}/mmHg^{-1}$/AU vs. 0.00003 ± 0.0008 $ml/min^{-1}/mmHg^{-1}$/AU; $p = 0.01$).

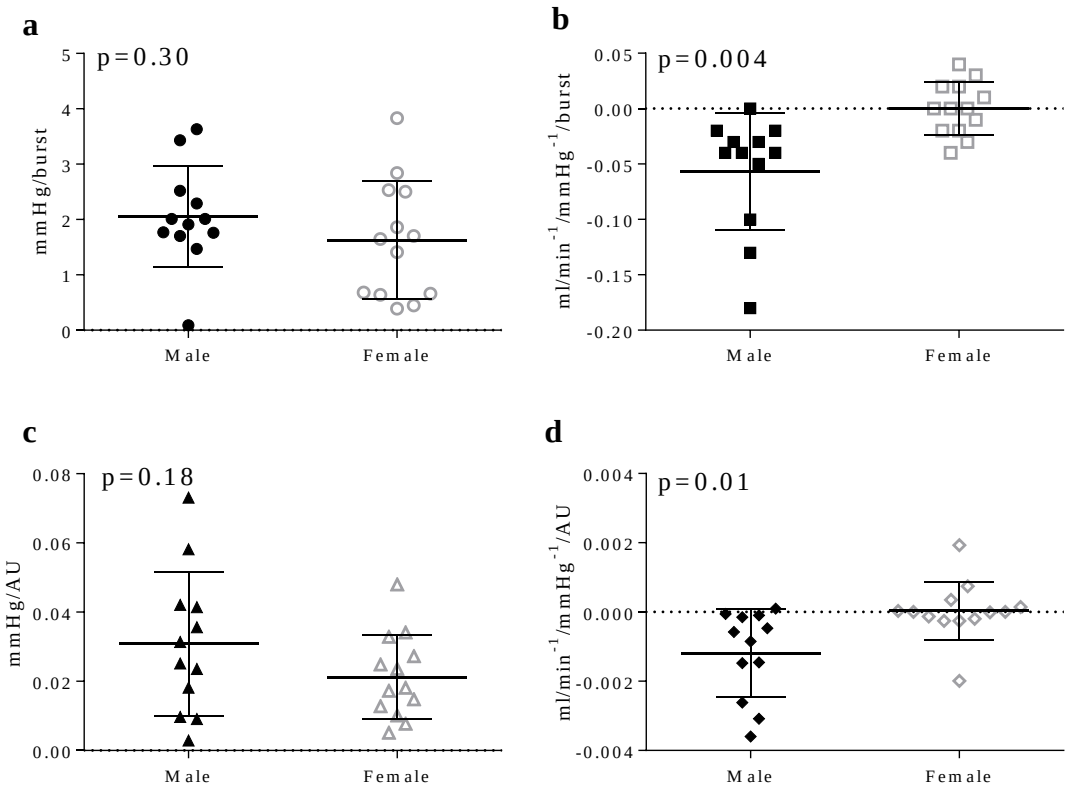

Figure 6.1. Group data displaying mean ± SD of vascular transduction (derived from handgrip exercise) in males and females when expressed using the relationships between a) mean arterial pressure and MSNA burst count b) leg vascular conductance and MSNA burst count, c) mean arterial pressure and total MSNA and d) leg vascular conductance and total MSNA

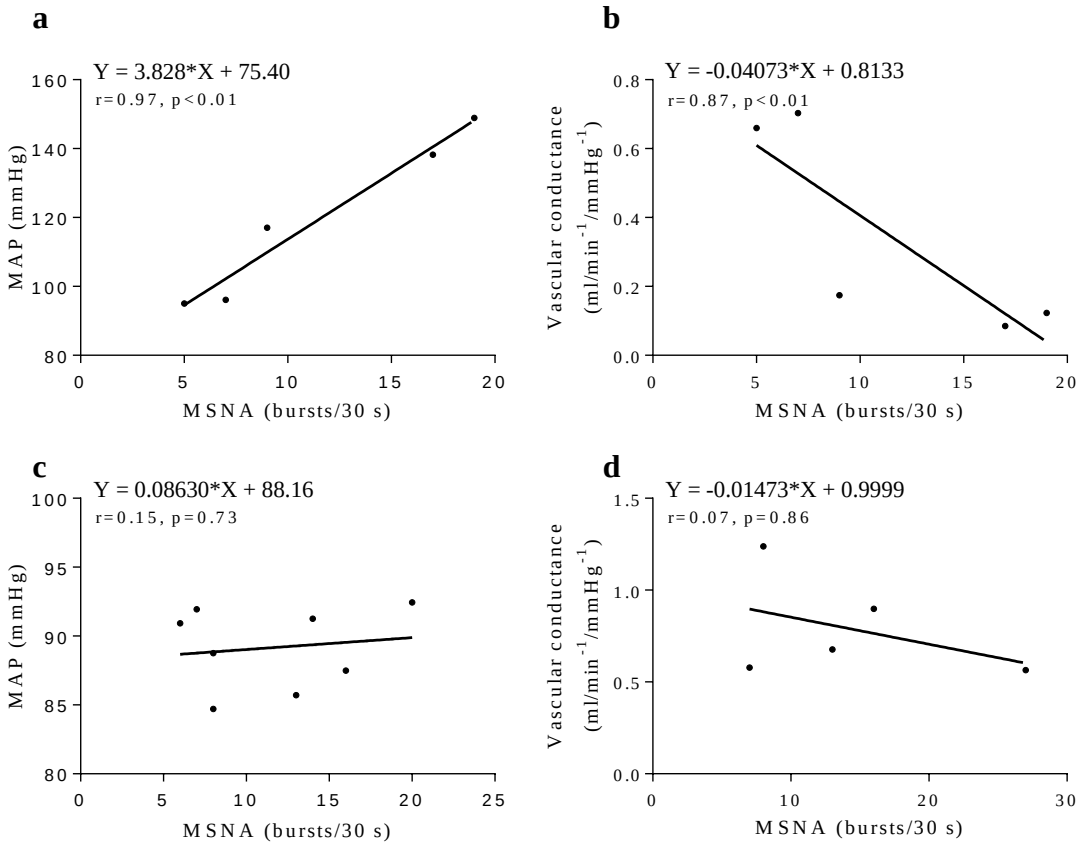

Figure 6.2. Individual vascular transduction slopes derived from isometric handgrip exercise illustrating a) a strong relationship between MSNA burst count and mean arterial pressure, b) a strong relationship between MSNA burst count and leg vascular conductance, c) a weak relationship between MSNA burst count and mean arterial pressure and d) a weak relationship between MSNA burst count and leg vascular conductance

Cold pressor test: When assessed using the increase in MSNA during the cold pressor test, vascular transduction was only significantly different between males and females when quantified using MSNA burst count and leg vascular conductance (Fig. 6.3). When expressed using the relationship between MSNA burst count and mean arterial pressure, the range of r values was 0.23 to 0.88, with 15 out of 22 individuals demonstrating a moderate relationship ($r \geq 0.5$). When expressed using the relationship between MSNA burst count and leg vascular conductance, the range of r values was 0.01 to 0.83, with 8 out of 22 individuals demonstrating a moderate relationship. When expressed as the relationship between total MSNA and mean arterial pressure, the range of r values was 0.27 to 0.73, with 18 out of 22

individuals demonstrating a moderate relationship. When vascular transduction was expressed as the relationship between total MSNA and leg vascular conductance, the range of r values was 0.04 to 0.81, with 10 out of 22 individuals demonstrating a moderate relationship. When expressed using the relationship between MSNA burst count and mean arterial pressure, vascular transduction was comparable between males and females (1.5 ± 0.6 mmHg/burst vs. 1.6 ± 0.4 mmHg/burst; p = 0.51). When calculated from the relationship between MSNA burst count and leg vascular conductance, vascular transduction was significantly greater in males (-0.03 ± 0.03 ml/min^{-1}/mmHg^{-1}/burst vs. -0.005 ± 0.01 ml/min^{-1}/mmHg^{-1}/burst; p = 0.04). When vascular transduction was quantified using total MSNA, there was no significant difference between males and females when using mean arterial pressure (0.03 ± 0.02 mmHg/AU vs. 0.04 ± 0.02 mmHg/AU) or leg vascular conductance (-0.001 ± 0.001 ml/min^{-1}/mmHg^{-1}/AU vs. -0.0003 ± 0.0004 ml/min^{-1}/mmHg^{-1}/AU; p = 0.43).

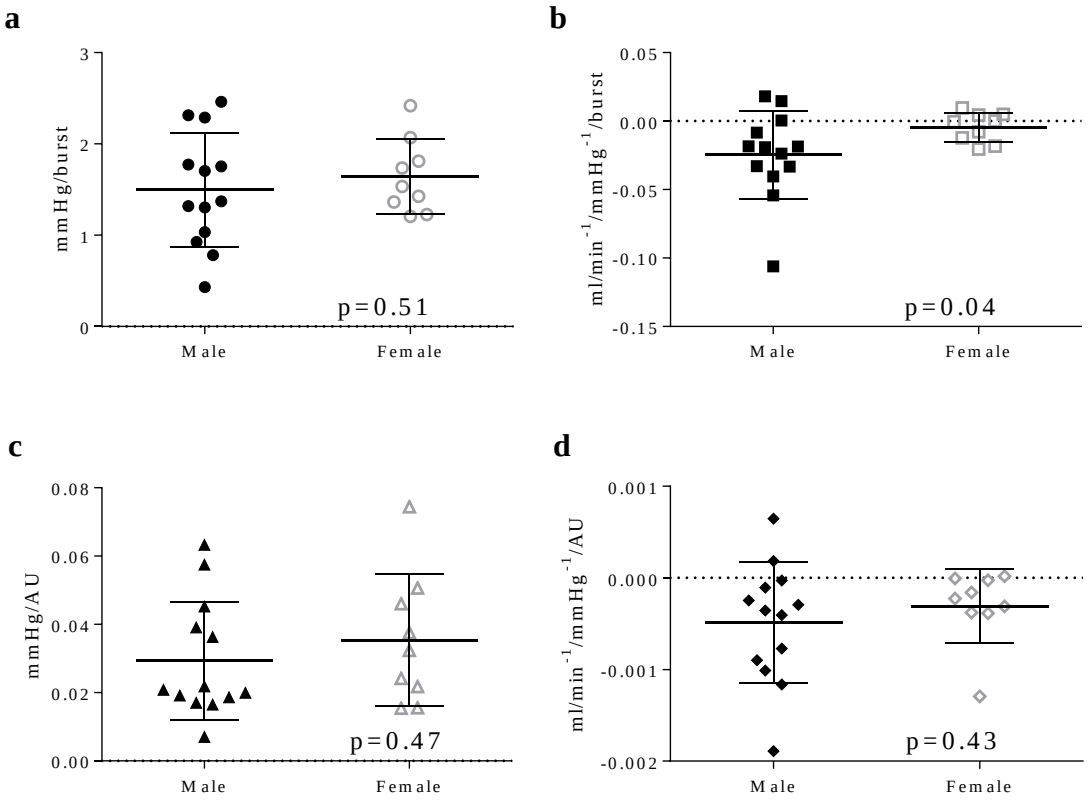

Figure 6.3. Grouped data displaying mean ± SD of vascular transduction (derived from cold pressor test) in males and females when expressed using the relationship between a) mean arterial pressure and MSNA burst count, b) leg vascular conductance and MSNA burst count, c) mean arterial pressure and total MSNA and d) leg vascular conductance and total MSNA

6.4.2 Sex differences in sympathetic BRS during the cold pressor test

Acceptable sympathetic BRS_{inc} slopes ($r \geq 0.5$) during both baseline and the cold pressor test were acquired in 9 males and 8 females. At rest, BRS_{inc} was significantly greater in females than males (-3.9 ± 1.3 bursts/100HB/mmHg vs. 2.5 ± 1.2 bursts/100HB/mmHg; $p = 0.03$). There was no significant change in BRS_{inc} during the cold pressor test ($p = 0.30$). Subgroup analysis, with results for males and females reported separately, resulted in no significant differences in sympathetic BRS_{inc} during cold pressor test when compared with rest ($p > 0.05$). The T50 was significantly higher during the cold pressor test compared with baseline in both males (71 mmHg vs. 91 mmHg) and females (64 mmHg vs. 91 mmHg; $p < 0.001$) and were comparable between groups ($p > 0.05$). This means that 50% of bursts occurred at a higher diastolic blood pressure during the cold pressor test, confirming the resetting of the baroreflex during the cold pressor test.

6.4.3 Sex differences in cardiovascular and sympathetic responses to isometric handgrip

The rationale behind using physical stressors in this study was to drive an increase in muscle vasoconstrictor drive to enable calculation of vascular transduction during a wider sympathetic range. Table 6.2 displays each cardiovascular variable at rest and the peak response during the isometric handgrip task. MSNA burst count and total MSNA significantly increased in both males and females during isometric handgrip (< 0.01). Importantly, there were no significant differences in the absolute or percent changes in MSNA burst count or total MSNA between males and females ($p > 0.05$). Heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure significantly increased in both males and females during isometric handgrip ($p < 0.0001$). Blood flow did not significantly increase in males ($p = 0.56$) whereas leg vascular conductance significantly decreased ($p = 0.04$). Conversely, blood flow in females significantly increased ($p = 0.01$) during isometric handgrip and leg vascular conductance did not significantly change ($p = 0.58$). When expressed in absolute terms, males had a greater decrease in leg vascular conductance ($p = 0.02$) when compared with females. Absolute increases in systolic pressure, diastolic pressure, mean arterial pressure and heart rate were not significantly different between males and females ($p > 0.05$). When comparing the percent change from rest to peak during the isometric handgrip, systolic pressure, diastolic pressure and mean arterial pressure

were not significantly different between males and females. Males had a significantly greater percent increase in heart rate during isometric handgrip than females ($p = 0.04$). There was no significant difference in the peak percent change in blood flow or leg vascular conductance between males and females ($p > 0.05$).

Table 6.2. Cardiovascular variables in males (n = 12) and females (n = 13) at rest and peak response during isometric handgrip (mean ± SD)

	Rest		Peak		Percent change	
	Males	Females	Males	Females	Males	Females
Systolic blood pressure (mmHg)	129 ± 14	113 ± 11 †	170 ± 24*	141 ± 21* †	32 ± 15	25 ± 12
Diastolic blood pressure (mmHg)	68 ± 11	69 ± 9	95 ± 18*	91 ± 12*	41 ± 22	33 ± 15
Mean arterial pressure (mmHg)	88 ± 11	83 ± 9	120 ± 18*	108 ± 14*	37 ± 18	30 ± 15
Heart rate (bpm)	66 ± 11	69 ± 8	84 ± 9*	81 ± 10*	30 ± 19	18 ± 9 †
Blood flow (ml/min^{-1})	105 ± 79	46 ± 24 †	92 ± 103	76 ± 51	-2.4 ± 100	68 ± 101
Conductance ($ml/min^{-1}/mmHg$)	1.20 ± 0.98	0.58 ± 0.31 †	0.80 ± 0.90*	0.62 ± 0.41*	-26 ± 82	14 ± 69
MSNA burst count (bursts/30s)	10± 5	9 ± 4	22 ± 9*	21 ± 4*	144 ± 44	169 ± 127
Total MSNA (AU/ 30 s)	478 ± 284	412 ± 229	1578 ± 1284*	1631 ± 1077*	246 ± 185	371 ± 363

† significantly different between males and females * significantly different from resting baseline

6.4.4 Sex differences in cardiovascular and sympathetic responses to the cold pressor test

Table 6.3 displays each cardiovascular variable at rest and the peak response during the cold pressor test. During the cold pressor test, MSNA burst count and total MSNA significantly increased in both males and females ($p < 0.01$). When expressed in absolute terms, females had a greater increase in MSNA burst count ($p = 0.03$) but the percent increase in MSNA burst count was comparable between males and females. There was no significant difference in the absolute or percent change in total MSNA between males and females. Heart rate,

systolic blood pressure, diastolic blood pressure and mean arterial pressure significantly increased in both males and females ($p < 0.05$). There was no difference in the absolute increases in heart rate, systolic blood pressure, diastolic blood pressure or mean arterial pressure between males and females during the cold pressor test. When comparing the percent change from rest to peak, females had a significantly greater increase in systolic blood pressure when compared with males ($p = 0.02$). There was no difference in the percent increase in heart rate, diastolic blood pressure or mean arterial pressure between males and females. In absolute terms, females had a significantly greater increase in blood flow ($p = 0.02$) during the cold pressor test whereas males had an insignificant absolute decrease in blood flow ($p = 0.50$). Leg vascular conductance did not significantly change in either males or females during the cold pressor test. There was no significant difference in the absolute or percent change in leg vascular conductance between males and females.

Table 6.3. Cardiovascular variables in males (n = 13) and females (n = 9) at rest and peak response during cold pressor test (mean ± SD)

	Rest		Peak		Percent change	
	Males	Females	Males	Females	Males	Females
Systolic blood pressure (mmHg)	127 ± 13	113 ± 15 †	159 ± 19*	149 ± 17*	23 ± 9	32 ± 9 †
Diastolic blood pressure (mmHg)	69 ± 11	69 ± 5	89 ± 15*	94 ± 5*	31 ± 13	37 ± 9
Mean arterial pressure (mmHg)	87 ± 11	85 ± 6	111 ± 14*	115 ± 8*	28 ± 11	35 ± 7
Heart rate (bpm)	63 ± 9	69 ± 9	72 ± 10*	82 ± 10* †	16 ± 9	20 ± 17
Blood flow (ml/min^{-1})	97 ± 91	54 ± 41	77 ± 95	83 ± 58*	-0.63 ± 82	62 ± 64
Conductance ($ml/min^{-1}/mmHg$)	1.1 ± 0.96	0.63 ± 0.45	0.67 ± 0.90	0.59 ± 0.54	-24 ± 73	-8.5 ± 67
MSNA burst count (bursts/30 s)	13 ± 7	10 ± 4	22 ± 6*	24 ± 6*	126 ± 161	247 ± 332
Total MSNA (AU/ 30 s)	576 ± 398	360 ± 242	1252 ± 644*	1205 ± 701*	219 ± 241	312 ± 242

† significantly different between males and females *significantly different from resting baseline

6.5. Discussion

Sympathetic vascular transduction, when derived from changes in leg vascular conductance during sympathoexcitatory manoeuvres, is greater in males when compared with females. However, when assessed using increases in arterial pressure during such manoeuvres, there are no significant sex differences. During isometric handgrip and the cold pressor test, changes in heart rate, arterial pressure and MSNA increase similarly in males and females. Furthermore, resetting of the sympathetic baroreflex to higher pressures during sympathoexcitation occurs to a similar extent in males and females.

Studies of sex differences in vascular transduction during increases in MSNA are few and inconsistent. Previous reports have shown that cardiovascular control during physical stressors, such as the isometric handgrip and cold pressor test, is associated with similar patterns between males and females (Dishman et al., 2003, Hogarth et al., 2007, Jones et al., 1996, Shoemaker et al., 2001). However, others have reported attenuated cardiovascular and sympathetic responses during isometric handgrip in young females when compared with their male counterparts (Ettinger et al., 1996, Jarvis et al., 2011, Minahan et al., 2018). In the current study, increases in heart rate and arterial pressure during the cold pressor test were similar between males and females but the absolute increase in MSNA was greater in females. This is most likely due to lower levels of resting MSNA in females than males. During the isometric handgrip task, increases in arterial pressure, MSNA and heart rate were comparable between males and females. However, leg vascular conductance significantly decreased in males but did not significantly change in females suggesting a blunted vascular transduction in females during sympathoexcitation. This is consistent with previous reports of blunted calf vascular resistance during isometric handgrip in females despite similar increases in MSNA to their male counterparts (Hogarth et al., 2007). Additionally, when quantified as the relationship between MSNA and leg vascular conductance during isometric handgrip and the cold pressor test, vascular transduction was greater in males when compared with females. The results of this study are in agreement with previous investigations that indicate that the transduction of MSNA into vasoconstrictor drive is greater in males when compared with females (Briant et al., 2016, Hart et al., 2011a). These sex differences are in contrast to when sympathetic vascular transduction is directly quantified on a beat-to-beat basis via changes in leg vascular conductance. As shown in Chapter 4, vascular transduction was

greater in females when characterised by the beat-to-beat changes in leg vascular conductance following MSNA bursts at rest.

The sex differences in vascular transduction observed in the current study, particularly when derived from changes in leg vascular conductance, may be due to differences in direction of change in leg vascular conductance during sympathoexcitation in females. During the isometric handgrip task, some females had an increase (6/13) in leg vascular conductance where others had a decrease (7/13). Only one male demonstrated an increase in leg vascular conductance during the isometric handgrip task. During the cold pressor test, vascular transduction was greater in males when characterised as the relationship between MSNA burst count and leg vascular conductance. However, there were no significant sex differences in the changes in leg vascular conductance, where 3 out of 13 males presented with an increase in leg vascular conductance and 2 out of 9 females had an increase in conductance. It is unclear what caused the divergent changes in leg vascular conductance in females. A potential factor may be individual variability in β-adrenergic receptor sensitivity. Some females may have greater β-adrenergic sensitivity than others, or a greater proportion of β-adrenoreceptors to α-adrenoreceptors resulting in sympathetically mediated vasodilation as shown through increases in leg vascular conductance. However, these conclusions are speculative as individual variability in β-adrenoreceptor sensitivity was not measured in this study. Evidence suggests that greater β-adrenergic sensitivity offsets the vasoconstrictor responses to noradrenaline in young females and is influenced by circulating oestrogen (Hart et al., 2011a, Kneale et al., 2000). Oestrogen and possibly progesterone affect β-adrenergic receptor sensitivity in young females. Circulating levels of oestrogen in female stimulate nitric oxide synthesis resulting in increased nitric oxide availability (Miller and Duckles, 2008, Sudhir et al., 1996). Furthermore β-adrenoreceptor vasodilation occurs in part by a nitric oxide mechanism (Jordan et al., 2001, Queen et al., 2006). The lack of sex differences in cardiovascular reactivity to sympathoexcitation observed in current study may be due to not controlling for contraceptive use. Although females were tested in the low hormone phase of their menstrual cycle, approximately half of the females (7/13) were taking oral contraceptives. Minahan et al. (2018) previously reported greater increases in arterial pressure in response to isometric handgrip in females who take oral contraceptives when compared with normally menstruating females. This greater response in females taking oral

contraceptives was similar to that observed in their male counterparts. This is likely due to a suppression of endogenous oestrogen in females who take oral contraceptives and resulted in attenuated sex-related differences in the metaboreflex during isometric handgrip (Minahan et al., 2018).

6.5.1 Sympathetic baroreflex control during the cold pressor test

The baroreflex control of arterial pressure has been shown to reset to a greater operating point during excitatory manoeuvres to accommodate for the increase in demand of oxygen to cells for energy production and removal of metabolic waste. Such excitatory manoeuvres include isometric handgrip (Ichinose et al., 2006, Kamiya et al., 2001), post exercise ischaemia (Cui et al., 2001, Ichinose et al., 2006, Ogoh et al., 2009), dynamic exercise (Kim et al., 2012, Ogoh et al., 2007) and the cold pressor test (Cui et al., 2002). In the current study, sympathetic BRS_{inc} was examined both at rest and during the cold pressor test. Sympathetic BRS_{inc} was significantly greater in females at rest. These results were also observed in Chapters 3 and 6. However, during the cold pressor test, BRS_{inc} did not significantly change from rest in either males or females and no sex differences in BRS_{inc} were apparent. Conversely, in a report by Cui et al. (2002), although sympathetic BRS was reset to a greater blood pressure during the cold pressor test, sympathetic BRS increased. The differences in findings may be due to the analytical approach in quantifying BRS. Cui et al. (2002) calculated sympathetic BRS via the modified Oxford method whereas in the current study BRS was quantified spontaneously via the burst incidence method (i.e., threshold method). Literature on sex differences in sympathetic baroreflex resetting during sympathoexcitatory manoeuvres is limited. Through the use of carotid neck pressure and suction, Kim and colleagues (2012) were the first to examine sex differences in blood pressure control during dynamic exercise. It was found that the carotid baroreflex control of mean arterial pressure and heart rate is reset similarly between males and females. The current investigation is the first study to examine sex differences relating to the resetting of sympathetic BRS during excitatory manoeuvres. The baroreflex control of MSNA was reset to a greater diastolic blood pressure range as indicated by a greater T50 value during the cold pressor test when compared with rest in both males and females. The results of this study suggest that sympathetic BRS during sympathetic activation is preserved in both males and females but is reset to a higher blood pressure range.

6.5.2 Methodological considerations

The method employed in this study provides further insight into the transduction of MSNA to vasoconstrictor drive as it allows us to investigate the vasoconstrictor effect of MSNA during a greater sympathetic range. This is in contrast to the other methods employed in this thesis where vascular transduction is examined through beat-to-beat changes in leg vascular conductance and arterial pressure at rest. The approach utilised in this study allows us to quantify sympathetic vascular transduction during large increases in MSNA by plotting the relationship between MSNA and arterial pressure or leg vascular conductance. The reason this method was chosen was based on previous studies that demonstrated a strong relationship between MSNA and the vascular response during excitatory manoeuvres (Halliwill et al., 1996, Kim et al., 2012, Minson et al., 2000a, Seals, 1989b). However, the relationship between MSNA and arterial pressure or leg vascular conductance in the current study was poor in some individuals. The strength of the relationship may be influenced by the measurement chosen to characterize vascular transduction. Achieving a moderate relationship ($r \geq 0.5$) during isometric handgrip and the cold pressor test was more successful when using mean arterial pressure than when using leg vascular conductance. In studies by Halliwill et al. (1996) and Seals (1989b) the relation between MSNA and vascular response to isometric handgrip was assessed using changes in calf vascular resistance. Seals (1989b) reported regressions (r) ranging from 0.80 to 0.99, indicating consistently strong relationships. However, Tan et al. (2013a) reported a larger variation in the relationship between changes in MSNA and vascular resistance/conductance during isometric handgrip (r^2 0.01-0.91), which is similar to what was observed in the current investigation. More work is needed to examine why the relationship is strong in some people and not in others and whether this approach is repeatable within individuals.

6.6. Conclusion

The results of this study indicate that cardiovascular and muscle sympathetic reactivity to isometric handgrip and cold pressor test is comparable between males and females. Despite this, vascular transduction of MSNA during sympathoexcitatory manoeuvres is greater in males when compared with females. The sex differences observed in vascular transduction during sympathoexcitation is reflected by the attenuated decrease in leg vascular conductance

in females when compared with males. The individual variability in the direction of change in vascular conductance, particularly in females, may in part be why sex differences occur during activation of the sympathetic nervous system.

Chapter 7: GENERAL DISCUSSION

The studies in this thesis were conducted to further our understanding of the regulatory mechanism of the sympathetic arm of the baroreflex, from initial changes in blood pressure to the end-organ response. It was proposed that, in young healthy adults, having high levels of sympathetic BRS does not necessarily mean that the transduction of MSNA to peripheral vasculature is also efficient at regulating acute changes in arterial pressure. To explore this, the aim of this thesis was to investigate the interactions between sympathetic BRS and direct measures of sympathetic vascular transduction at rest and during sympathoexcitation in young males and females. The purpose of this chapter is to summarise the main findings of this thesis, discuss how these findings contribute to the current body of knowledge and provide recommendations for future research.

7.1. Summary of main findings

Before exploring how vascular transduction relates to sympathetic BRS, it is important to establish the repeatability of sympathetic BRS and its stability within a recording session. Therefore, in Chapter 3, the first study of this thesis aimed to test the hypothesis that spontaneous sympathetic BRS is stable within the same recording period and repeatable when recorded on separate days in both males and females. It was also hypothesised that recording periods of longer durations are associated with greater diastolic pressure ranges at rest and that poor correlations exist between sympathetic BRS values derived from recording periods of different durations. The results of Study 1 show that sympathetic BRS is moderately stable within a recording session when using periods of the same duration. When quantified using both MSNA burst incidence and total MSNA, there is no evidence of fixed or proportional bias in males. There is however, proportional bias for BRS_{inc} in females. Recording periods of at least 5 min should be used when quantifying BRS as shorter durations can lead to an overestimation of BRS values. When measured on separate days, BRS is moderately repeatable with evidence of proportional bias in BRS_{inc} and fixed bias in BRS_{total}.

The beat-to-beat assessment of vascular transduction represents a recent advancement in the field. Whilst lower levels of vascular transduction have been demonstrated in young females using the Briant method, the Fairfax method had not previously been used to examine sex

differences. In Chapter 4, the aim of the second study of this thesis was to examine whether vascular transduction using the Fairfax method differs between healthy young males and females, and to compare the Fairfax method with the Briant method. It was hypothesised that males have greater vascular transduction than females when quantified using both the Fairfax and Briant methods. For the Fairfax method, leg vascular conductance was measured directly using Doppler ultrasound. Sympathetic vascular transduction was expressed as the beat-to-beat changes in leg vascular conductance following MSNA bursts. Contrary to the hypothesis, it was found that vascular transduction quantified using the Fairfax method was significantly *greater* in young females compared with young males. In contrast, the Briant method, which involves using changes in diastolic blood pressure to quantify vascular transduction, did not reveal sex differences. These approaches may provide insight into different aspects of transduction of MSNA to vasoconstriction and the regulation of arterial pressure.

As there are many compensatory interactions within the cardiovascular system to maintain homeostasis, Study 3 (Chapter 5) was conducted to determine the interactions between sympathetic BRS and vascular transduction. It was hypothesised that sympathetic BRS is inversely related to sympathetic vascular transduction at rest during spontaneous changes in MSNA, blood pressure and SFA blood flow. In the conclusions of Chapter 4 it was speculated that greater resting MSNA might explain the blunted vascular transduction that was observed in males when using the Fairfax method. Therefore, a second aim of this study was to determine whether resting MSNA predicts vascular transduction. The results of this study indicate that sympathetic BRS and vascular transduction are negatively correlated under resting conditions, thus supporting the hypothesis that a compensatory interaction exists. This means that individuals with high sympathetic BRS have less effective vascular transduction during spontaneous changes in blood pressure. However, this was only apparent in young males; there was no relationship observed in females. Furthermore, resting MSNA did not predict sympathetic BRS or vascular transduction in either males or females.

The assessment of vascular transduction at rest is reliant upon spontaneous fluctuations in MSNA, and is therefore limited by the relatively small changes experienced under resting

conditions. In order to examine vascular transduction during more substantial increases in MSNA, classic sympathoexcitatory manoeuvres (the cold pressor test and static handgrip exercise) were employed in Chapter 6. It was hypothesised that vascular transduction during physiological stress was significantly greater in males when compared with females. The results support this hypothesis, as sympathetic vascular transduction during sympathoexcitation was significantly greater in males when vascular transduction was derived from changes in leg vascular conductance. These results are in direct contrast to those reported under resting conditions using beat-to-beat methods of assessing transduction, as discussed in more detail below.

This thesis highlights the limitations of reporting sympathetic baroreflex sensitivity alone as a measure of an individual's capacity to regulate their blood pressure on a beat-to-beat basis. In this thesis the sympathetic arm of baroreflex was examined via spontaneous methods for quantifying sympathetic BRS. This analytical technique measures the reflex changes in MSNA in response to changes in diastolic pressure. However, it does not take into account the end-organ response of sympathetic outflow to the peripheral vasculature. Therefore, the overarching aim of this thesis was to examine the interaction between sympathetic BRS and vascular transduction. A number of techniques were utilised to estimate sympathetic vascular transduction, each with its own strengths and weaknesses, and the results clearly demonstrate how the method chosen can have a profound effect on the findings regarding sex differences and the interaction between vascular transduction and sympathetic BRS. The strengths and limitations of each method, and the implications of the findings will be discussed in the following sections.

7.2. The dynamic nature of the sympathetic baroreflex

The sympathetic baroreflex is a dynamic mechanism that is constantly adapting and reacting to small changes in the internal environment to ensure arterial pressure is maintained. The results of the studies presented in this thesis illustrate the dynamic nature of the sympathetic baroreflex in regulating arterial pressure. In Study 1 (Chapter 3), despite comparing sympathetic BRS values that were calculated within the same recording session, intra-class

correlations revealed that sympathetic BRS was moderately stable. The fact that the relationship between the two BRS values was not stronger indicates that the baroreflex is not fixed, even under resting conditions. It is unknown whether the interaction between sympathetic BRS and vascular transduction is static or if it changes with ageing, or in response to different environments. The pregnancy case study presented in Appendix B further highlights the fact that these mechanisms are not static, but rather values for sympathetic BRS and vascular transduction (and the relationship between the two) may change over time. The sympathetic baroreflex also displays a dynamic process in an acute setting. The resetting of the baroreflex is not fixed but is constantly adjusted to regulate arterial pressure over a range that suits a given situation such as exercise or mental stress (Dampney, 2017). This was demonstrated in Study 4 (Chapter 6), where sympathetic BRS was reset to a greater blood pressure range during the cold pressor test in both males and females. The dynamic nature of the sympathetic baroreflex is not only observed in an acute setting but also chronically as shown by the effects of ageing. For example, sympathetic vascular transduction is blunted in older adults when compared with younger individuals (Vianna et al., 2012). Furthermore, age-associated reductions in the mechanical component of sympathetic BRS in older adults is counteracted by an augmented neural component when quantified during both increases and decreases in arterial pressure (Studinger et al., 2009).

7.3. Sex differences in sympathetic baroreflex sensitivity

In this thesis, sympathetic BRS was greater in females when compared with males, as has been reported previously (Hogarth et al., 2007). However, others have reported no differences in sympathetic BRS between males and females (Hart et al., 2011a, Tank et al., 2005), and others have reported greater BRS in males (Christou et al., 2005). Although the results in this thesis suggest females have greater sympathetic BRS, this does not necessarily mean they demonstrate greater baroreflex buffering capacity. Evidence suggests that males have greater autonomic support of arterial pressure regulation than females as males experience greater decreases in arterial pressure following autonomic blockade when compared with females (Christou et al., 2005). Although endogenous sex hormones were not measured in this thesis, current evidence suggests that they play an important role in blood pressure control. Oestrogen has a significant impact on central autonomic regulation of arterial pressure by attenuating central sympathetic outflow (Saleh and Connell, 2000). Moreover, Minson et al.

(2000a) reported greater sympathetic BRS during the mid-luteal phase of the menstrual cycle, where circulating levels of oestrogen are greater when compared with the early follicular phase. Furthermore, circulating levels of oestrogen have been found to increase nitric oxide synthesis, resulting in increased nitric oxide availability (Miller and Duckles, 2008, Sudhir et al., 1996). Young females also have greater β-adrenergic receptor sensitivity, which offsets the vasoconstrictor effect of MSNA (Hart et al., 2011a, Kneale et al., 2000). This body of evidence suggests that female sex hormones have a great influence on the regulation of arterial pressure. Wehrwein et al. (2010) explored sex differences in sympathetic BRS by determining the differences in the threshold at which a burst of sympathetic activity is initiated in males and females. This was performed by calculating the error signal (T50 minus mean diastolic pressure). The T50 is defined as the diastolic pressure at which 50% of cardiac cycles are associated with a MSNA burst (Kienbaum et al., 2001). Despite similar sympathetic BRS, females exhibited a greater negative error signal than their male counterparts (Wehrwein et al., 2010). This means that the mean diastolic pressure in females was substantially higher than their T50 value, which reflects a relatively greater suppression of MSNA. These findings further support the idea that females have less autonomic support of blood pressure than males. Furthermore, the autonomic support of arterial pressure increases once females reach menopause as the error signal becomes positive – i.e., the mean diastolic pressure is lower than the T50 so the likelihood of a burst occurrence increases (Peinado et al., 2017). Although the literature suggests reduced autonomic support of blood pressure in young women, the elevated sympathetic BRS in females in this thesis may be a surprising result. Consequently, we might expect this to be counteracted by attenuated vascular transduction. However, this thesis highlights the variability in measures of vascular transduction when using different methods and, in particular, how this may lead to different conclusions regarding sex differences.

7.4. Sex differences in vascular transduction

Previous reports have shown that males have more effective vascular transduction than females (Briant et al., 2016, Hogarth et al., 2007). The results of Study 4 (Chapter 6) are consistent with these: vascular transduction was greater in males when quantified as the relationship between MSNA and leg vascular conductance during manoeuvres that cause a drive in MSNA. Conversely, in Study 2 (Chapter 4), females demonstrated a greater vascular

transduction when characterised as the beat-to-beat changes in leg vascular conductance following MSNA bursts at rest (Fairfax method, VT_{CON}). There were no differences in vascular transduction between males and females when characterised as the beat-to-beat increases in mean arterial pressure following MSNA bursts (Fairfax method, VT_{MAP}). The same was true in a previous report on sex differences in vascular transduction in young adults using the same method (Vianna et al., 2012). Finally, when characterised using the Briant method, vascular transduction was not significantly different between males and females. These results do not agree with those of Briant et al. (2016), where vascular transduction was shown to be significantly lower in young females when compared with young males, older males and postmenopausal women. In Study 2, comparisons were made between the Fairfax method and Briant method to see whether they can be used interchangeably in instances where muscle blood flow cannot be acquired. The Fairfax method involves calculating beat-to-beat decreases in leg vascular conductance in response to individual MSNA bursts, while the Briant method uses diastolic blood pressure as a proxy marker of the vascular response to MSNA. Correlation analysis indicated that the two methods cannot be used in place of one another. Therefore, caution should be used when comparing studies relating to sex differences that have used different methods to estimate vascular transduction. The two methods appear to provide insight into different aspects of sympathetic vascular transduction, as discussed in more detail below.

7.5. Methodological considerations for the assessment of sympathetic vascular transduction

The greater vascular transduction (VT_{CON}) observed in females in Study 2 was an unexpected result and may reflect a limitation in the approach used. The Fairfax method may underestimate vascular transduction in individuals with particularly high levels of MSNA, as it is difficult to establish a true baseline level of leg vascular conductance. In these individuals there were fewer silent periods following MSNA bursts (i.e. cardiac cycles following MSNA bursts that are not associated with a burst). This may have implications for using this approach in populations with elevated levels of resting MSNA, such as individuals with obstructive sleep apnoea (77 ± 6 bursts/100HB) (Lundblad et al., 2014), heart failure (64.7 ± 3.2 bursts/100HB), heart failure and metabolic syndrome (80.9 ± 3.2 bursts/100HB)

(Grassi et al., 2007), and central obesity (65.4 ± 2.0 bursts/100HB) (Grassi et al., 2004). Previous reports using the Fairfax method have only examined vascular transduction in young healthy individuals with low to moderate levels of resting MSNA (~29 bursts/100HB) (Fairfax et al., 2013a, Fairfax et al., 2013b, Fairfax et al., 2013c). The Fairfax approach has yet to be tested extensively in population groups with high levels of resting MSNA.

The results of Study 3 suggest that resting MSNA does not predict sympathetic BRS or vascular transduction in young males and females. However, this is in contrast with previous reports where resting MSNA is inversely related to vascular transduction (Tan et al., 2013a). In this thesis, males had significantly greater levels of resting MSNA when expressed as burst incidence where two males had particularly high levels of MSNA (69 and 78 bursts/100HB) compared with the rest of the males (22-51 bursts/100HB). Repeating this analysis in individuals with a more balanced range of MSNA may provide greater insight into the effects of resting MSNA on baroreflex function. These relationships may also become apparent in older adults. The age-related increase in MSNA has been shown to compensate for an attenuated vascular transduction (Tan et al., 2013a, Vianna et al., 2012). The results from Tan et al. (2013a) suggest that the blunted vascular transduction associated with ageing may lead to greater resting MSNA to support the maintenance of arterial pressure. In males, ageing is associated with a reduction in forearm post-junctional α_1-adrenergic responsiveness to endogenous noradrenaline release (Dinenno et al., 2002), and elevated sympathetic nerve activity in older males is associated with reduced basal leg arterial blood flow and vascular conductance (Dinenno et al., 1999). It has also been shown that vasoconstrictor responses to increases in MSNA in response to lower body negative pressure are lower in older males (Davy et al., 1998). Vianna et al. (2012) propose that older males are highly reliant on sympathetic outflow for the maintenance of arterial pressure, whereas in older females MSNA has minimal influence on resting beat-to-beat fluctuations in blood pressure. This was revealed by looking at the beat-to-beat responses of arterial pressure following muscle sympathetic bursts and following the arterial pressure response to cardiac cycles not associated with MSNA bursts. When cardiac cycles did not contain sympathetic bursts, the decrease in mean arterial pressure was greater in older males when compared with older females and younger males and females. This may help to explain the greater levels of MSNA in older males.

The Briant method was designed to cater for clinical population groups with elevated levels of MSNA (Briant et al., 2016). In their analysis, MSNA burst area was calculated over a two-cardiac cycle window at a fixed lag of 8-6 cardiac cycles preceding each diastolic pressure. The relationship between MSNA burst area and the corresponding diastolic pressure provided a measure of vascular transduction (Briant et al., 2016). However, as Young et al. (2016) point out, the small transduction slopes from this relationship indicate that MSNA has little influence on resting diastolic pressure. This may be because in the Briant method, every cardiac cycle is used in the analysis regardless of whether those cardiac cycles contain MSNA bursts or not. In the study by Briant et al. (2016), each group (i.e., older and younger males and females) had different levels of resting MSNA but similar heart rates. Including diastolic pressures not associated with MSNA bursts in the analysis may have minimised the transduction slopes (Young et al., 2016). Young et al. (2016) propose that the difference in transduction between the groups presented in the study by Briant et al. (2016) may be due to varying levels of resting MSNA. In Studies 2 and 3, vascular transduction was quantified at rest on a beat-to-beat basis and enabled us to determine differences between males and females during spontaneously occurring MSNA bursts. Conversely, in Study 4 (Chapter 6), sex differences in vascular transduction were examined by relating MSNA with arterial pressure or conductance during manoeuvres that caused an increase in muscle vasoconstrictor drive. This method enables us to quantify the transduction of MSNA to vasoconstriction over a wider range of muscle sympathetic outflow. It was demonstrated that vascular transduction was greater in males than females during both isometric handgrip exercise and the cold pressor test. However, the relationship between MSNA and arterial pressure/vascular conductance was not strong in everyone. It is not clear whether the poor relationship in some individuals reflects poor vascular transduction in those individuals, or is due to limitations in the approach. It is possible that other factors, such as changes in CO or vasodilatory mechanisms, are masking the influence of MSNA on blood pressure. An example of a vasodilatory mechanism includes greater ß-adrenergic sensitivity in females which has been shown to offset the vasoconstrictor effects of MSNA to the peripheral vasculature (Hart et al., 2011a).

Although the Fairfax method may be limited in populations with high MSNA, there are ways in which it can be modified for use in these individuals. Bursts of MSNA can occur in

isolation or in clusters of bursts, each varying in amplitude. Fairfax et al. (2013b) describe an extension of their method in which the vascular response to different MSNA burst clusters and size is assessed. Higher levels of MSNA (as indicated by larger bursts or greater number of consecutive bursts), and therefore greater amount of noradrenaline released, are associated with more significant reductions in leg vascular conductance (Fairfax et al., 2013b). When clusters of bursts are made up of similar total MSNA but different numbers of bursts, the clusters with fewer (but larger) bursts are most effective in decreasing leg vascular conductance. This has been utilised to examine ethnic differences in vascular transduction. In each cluster Vranish and colleagues (2017) show that the decrease in vascular conductance following MSNA bursts was greater in African-American males when compared with Caucasian males. Furthermore, the bursting pattern observed in these studies illustrates how the central nervous system can modulate the rate and amount of noradrenaline released to provide a variety of durations and strengths of vascular responses (Fairfax et al., 2013a, Fairfax et al., 2013b). However, this component of the Fairfax method does not provide a single vascular transduction value as there are four cluster sizes (i.e. clusters of 1, 2, 3 or 4+ MSNA bursts) and each cluster is separated into 4 quartiles according to MSNA burst amplitude. Future research is needed to adapt this approach to control for individual variability in resting MSNA and include the effects of both burst incidence and burst strength on the end organ response of MSNA.

The results relating to sex differences in vascular transduction may be different according to the methods used as they are influenced by different vascular beds. For instance, the Fairfax method explores the changes in vascular conductance in only one vascular bed (i.e. the leg) whereas the Briant method, in which changes in blood pressure are used, is influenced by vasoconstriction of many vascular beds, including the splanchnic, heart and capacitance (cutaneous) vessels as well as those in skeletal muscle. However, Fairfax et al. (2013b) also quantified vascular transduction using total vascular conductance, which provided a similar response to that observed using leg vascular conductance. Total vascular conductance (CO/MAP) was calculated by estimating stroke volume from the blood pressure waveform, using the ModelFlow software (Finapres Medical Systems) to calculate cardiac output. While total vascular conductance was not measured in this thesis, Vianna et al. (2012) report similar decreases in total vascular conductance following MSNA bursts between young males and

females. This suggests that there are no sex differences in relation to the whole body response to MSNA bursts and reflects the different vascular beds that are sympathetically innervated. Each method employed in this thesis provides useful but slightly different information regarding the transduction of MSNA to vasoconstrictor drive. In the Fairfax method, the timing of the peak response of leg vascular conductance following MSNA bursts is determined and controls for individual heart rate. Thus, variability in the lag between MSNA bursts and changes in vascular conductance can be taken into account. Conversely, the Briant method involves a fixed lag between MSNA and the diastolic pressure response. However, in their investigation, Briant et al. (2016) did compare different cardiac cycle windows and reported that the peak correlation of beat-to-beat MSNA burst area and diastolic pressure occurred within the 8-6 cardiac cycle window in both young males and premenopausal females.

For Studies 2 and 3 of this thesis, 27 males and 25 females were recruited. Of these, 19 males and 20 females were included based on MSNA and Doppler recordings being of acceptable quality. However, two males and two females had an increase instead of a decrease in leg vascular conductance following MSNA bursts. These participants were excluded from further analyses as their results did not reflect the transduction of MSNA to vasoconstriction. This decrease in sample size may have limited the power with which to detect a meaningful difference in vascular transduction between males and females. Retrospective power calculations have been used to determine the statistical power based on the standard deviations of the current data sets. As with sympathetic BRS, it is still unclear what is regarded as a meaningful difference in vascular transduction. However, based on the study by Vranish et al. (2018) comparing vascular transduction between Caucasian and African American males, a difference in VT_{MAP} of 1.5% may be considered meaningful. Post hoc power calculations revealed that with the sample size of 17 males and 18 females, and standard deviations of 1.2 for males and 1.5 for females, there was 90.6% power to detect a meaningful difference of 1.5%, in VT_{MAP}, assuming a 0.05 two-sided significance level. For VT_{DBP} sex differences of 0.08 may be deemed significant, based on sex differences reported by Briant et al. (2016). With a sample size of 17 males and 18 females, and standard deviations of 0.06 for males and 0.04 for females, there was 99.6% power to detect a meaningful difference of 0.08. Therefore, we believe that the hypotheses tested in this thesis

were sufficiently powered to detect meaningful differences in vascular transduction between males and females.

7.6. Interaction between sympathetic BRS and vascular transduction

Evidence suggests that compensatory interactions exist in cardiovascular control for the maintenance of arterial pressure. Ogoh et al. (2009) reported that the increase in sympathetic BRS during post-exercise ischaemia compensates for attenuated vascular transduction in young healthy adults. In Study 3 (Chapter 5), there was further evidence of compensatory interactions as sympathetic BRS was inversely related to vascular transduction in young males at rest. This may have implications for the use of sympathetic BRS as a marker of cardiovascular risk. It may be difficult to ascertain what would be considered a clinically low sympathetic BRS if it is potentially compensated for by greater vascular transduction. Conversely, there was no relationship between sympathetic BRS and vascular transduction in females. Based on these results, if we take two females with similar sympathetic BRS, they will have different levels of vascular transduction and thus different capacities for buffering changes in arterial pressure. Therefore, it is unclear whether the application of sympathetic BRS alone in young females provides a good indication of the effectiveness of their sympathetic baroreflex. The results further support the theory that circulating levels of sex hormones have an important role in arterial pressure regulation in premenopausal females. It is possible that the relationship between sympathetic BRS and vascular transduction may become apparent during chronic sympathetically-mediated vasoconstriction, such as during pregnancy. The results of Appendix B suggest that the attenuated sympathetic vascular transduction during pregnancy offsets the increase in sympathetic nerve activity, as has also been observed in previous studies (Reyes et al., 2018, Usselman et al., 2015c). Furthermore, although variable, overall, sympathetic BRS was greater during pregnancy than pre-pregnancy levels and may also be compensating for the decrease in vascular transduction. It is difficult to make conclusions based on these results as data were only collected from one individual. However, it does raise questions regarding the alterations in baroreflex control during pregnancy. The inverse relationship between sympathetic BRS and vascular transduction observed in Study 3 (Chapter 5) may have implications for previous research that reported attenuated sympathetic BRS in certain population groups. The lower sympathetic BRS in clinical populations may have been compensated for by greater vascular

transduction. Future research is needed to investigate the relationship between sympathetic BRS and vascular transduction in different population groups.

7.7. Methodological recommendations

Based on the current literature of vascular transduction and results of this thesis, the approach used in future studies is dependent on the research question, resources and technical skills of future investigators carrying out the research. Although each measure of vascular transduction used in this thesis has strengths and weaknesses, it is recommended that in future studies the method chosen should be the one deemed most appropriate for the particular research question. For example, if wanting to compare different vascular beds, such as the arm and leg, the Fairfax method may be beneficial in determining the difference between body regions. The Briant method may be more suitable for assessing vascular transduction in clinical populations or when measurements of blood flow are not possible, as it provides more of an integrated measure. Furthermore, if the research question is related to wider ranges of sympathetic outflow, the relationship between leg vascular conductance and MSNA during physical stressor tasks may be more appropriate than during spontaneous changes at rest.

It is recommended that studies involving repeat measurements of sympathetic BRS include intervals of the same duration. As demonstrated in Study 1, when comparing recordings periods of different durations, fixed and proportional bias was evident. Furthermore, the length of the recordings may have serious implications for the assessment of sympathetic BRS where shorter recording periods (2-min) result in an overestimated sympathetic BRS value. As this thesis has shown, compensatory interactions in sympathetic baroreflex function are evident. Therefore, future research on sympathetic baroreflex function should take into account both sympathetic BRS and vascular transduction as one may be compensating for the other and can therefore provide information on the whole baroreflex loop.

7.8. Conclusions and future research

The control of MSNA via the baroreflex is an important mechanism of blood pressure control, but this mechanism is reliant upon effective transduction of MSNA at the level of the vasculature. It was hypothesised that poor baroreflex sensitivity is compensated for by enhanced vascular transduction, and vice versa. The findings of this thesis support this hypothesis: in young males sympathetic BRS is inversely correlated with vascular transduction. However, this relationship is not apparent in young females. Whilst this thesis provides evidence of sex differences in sympathetic BRS and vascular transduction, it also highlights the differences between the various approaches available for quantifying vascular transduction. As the studies in this thesis have shown, the way in which the baroreflex controls arterial pressure is different in males and females. Future research on sympathetic baroreflex function should examine both sympathetic BRS and vascular transduction when drawing conclusions on the effectiveness of the sympathetic baroreflex. The development of a method that provides detail of the balance between sympathetic BRS and vascular transduction in a single value may be beneficial in understanding the effectiveness of baroreflex function on an individual basis. Furthermore, what constitutes a meaningful difference in sympathetic BRS has yet to be established so there is no consensus on what constitutes a low BRS value or a meaningful change. Further research is needed to establish clinical thresholds associated with elevated cardiovascular risk and thus meaningful changes in sympathetic BRS following interventions. Longitudinal studies from young adulthood to older age may be useful to determine if having low sympathetic BRS at a young age would predispose individuals to cardiovascular disease as they age. Further research is also required to determine the normal ranges of within and between-subject variability in sympathetic BRS in healthy populations. A limitation proposed in the Fairfax method was that it may not cater to individuals with high levels of MSNA. However, previous studies that have utilised this technique have only collected data in young healthy males with low to moderate levels of MSNA (Fairfax et al., 2013a, Fairfax et al., 2013b, Fairfax et al., 2013c, Vranish et al., 2017). The studies conducted in this thesis were the first to measure vascular transduction using this approach in young females. However, it is unknown whether the effects of burst size and cluster are similar between males and females or whether it is suitable in ageing or clinical populations.

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APPENDICES

Appendix A

BAROREFLEX MODULATION OF MUSCLE SYMPATHETIC NERVE ACTIVITY AT REST DOES NOT DIFFER BETWEEN MORNING AND AFTERNOON

Appendix A is published as:

Hissen, S. L., Macefield, V. G., Brown, R., Witter, T., & Taylor, C. E. (2015). Baroreflex modulation of muscle sympathetic nerve activity at rest does not differ between morning and afternoon. *Front Neurosci, 9*, 312. doi:10.3389/fnins.2015.00312

A.1. Abstract

The incidence of cardiovascular events is significantly higher in the morning than other times of day. This has previously been associated with poor blood pressure control via the cardiac baroreflex. However, it is not known whether diurnal variation exists in vascular sympathetic baroreflex function, in which blood pressure is regulated via muscle sympathetic nerve activity (MSNA). The aim of this study was to compare vascular sympathetic baroreflex sensitivity (BRS) in the same participants between the morning and afternoon. In 10 participants (mean age 22 ± 2.9 years), continuous measurements of blood pressure, heart rate and MSNA were made during 10 min of rest in the morning (between 0900 and 1000 h) and afternoon (between 1400 and 1500 h). Spontaneous vascular sympathetic BRS was quantified by plotting MSNA burst incidence against diastolic pressure (vascular sympathetic BRS_{inc}), and by plotting total MSNA against diastolic pressure (vascular sympathetic BRS_{total}). Significant vascular sympathetic BRS_{inc} and vascular sympathetic BRS_{total} slopes were obtained for 10 participants at both times of day. There was no significant difference in sympathetic BRS_{inc} between morning (-2.2 ± 0.6% bursts/mmHg) and afternoon (-2.5 ± 0.2% bursts/mmHg; P=0.68) sessions. Similarly, vascular sympathetic BRS_{total} did not differ significantly between the morning (-3.0 ± 0.5 AU/beat/mmHg) and afternoon (-2.9 ± 0.4 AU/beat/mmHg; P=0.89). It is concluded that in healthy, young individuals baroreflex modulation of MSNA at rest does not differ between the morning and afternoon. The results indicate that recording MSNA at different times of the day is a valid means of assessing sympathetic function.

A.2. Introduction

The incidence of cardiovascular and cerebrovascular events is higher in the morning than at any other time of day (Elliott, 1998; Muller et al., 1989). The morning is associated with a surge in blood pressure alongside elevated heart rate, blood viscosity and platelet aggregability, which are thought to increase the risk of transient ischaemic events (Muller et al., 1989). Acute increases in blood pressure can cause rupture of atherosclerotic plaques from the arterial wall and arterial thrombosis, leading to myocardial infarction and stroke. Poor blood pressure control in the morning may therefore play a role in the elevated risk of such events.

The baroreflex provides the principle means of buffering acute changes in blood pressure. It operates as a negative feedback loop responding to the activation of stretch sensitive receptors in the carotid sinus and aortic arch (baroreceptors), which project to the nucleus tractus solitarious (NTS) in the medulla via the glossopharyngeal and vagus nerves (Andresen & Kunze, 1994). The baroreflex can be described as having two distinct arms; the cardiac and sympathetic baroreflexes, through which heart rate and sympathetic vasoconstrictor drive are modulated, respectively. We have previously demonstrated a diminished cardiac baroreflex response to changes in blood pressure in the morning compared with the afternoon (Taylor et al., 2011). It is currently not known whether diurnal variation exists in sympathetic baroreflex function, and thus whether diminished blood pressure control in the morning may also be attributed to poor control of sympathetic outflow to the peripheral vasculature. The hypothalamic paraventricular nucleus (PVN), which directly innervates sympathetic preganglionic neurones in the intermedioloateral cell column of the spinal cord, as well as supplying the rostral ventrolateral medulla (RVLM), receives input from the master body clock (suprachiasmatic nuclei), NTS and RVLM (Blair et al., 1996). The RVLM is the primary output nucleus for sympathetic vasoconstrictor drive (Dampney et al., 2003; Macefield & Henderson, 2010; James et al., 2013) and this pathway may therefore provide a means for the body clock to influence the modulation of muscle sympathetic nerve activity (MSNA) and sympathetic baroreflex function, although no evidence of coupling between PVN and MSNA has been found in humans (James et al., 2013).

The aim of this study is to investigate diurnal variation in vascular sympathetic BRS in young healthy adults. When examining cardiac BRS we have previously employed the modified Oxford method, which is a pharmacological method for assessing baroreflex function (Taylor et al., 2011; Taylor et al., 2013). Whilst it is considered the gold standard technique for assessing cardiac BRS (Diaz & Taylor, 2006; Taylor et al., 2014; Dutoit et al., 2010), this pharmacological approach has limitations when used for the sympathetic baroreflex, particularly with regards to increases in arterial pressure following the bolus injection of phenylephrine - when MSNA bursts can be almost entirely inhibited (Dutoit et al., 2010). For the current research question, the baroreflex response to rising pressures is important, given the heightened risk of cardiovascular events linked with acute increases in blood pressure in the morning (Muller et al., 1989). Spontaneous techniques allow baroreflex responses to both rising and falling pressures to be incorporated under resting, physiological conditions. Therefore, in the current study spontaneous methods of assessing sympathetic BRS, previously described by Kienbaum et al. (2001), will be used. It is hypothesized that sympathetic BRS is lower in the morning than in the afternoon, such that increases in blood pressure are subject to less damping.

A.3. Methods

A.3.1 Participants

The study was conducted with the approval of the Human Research Ethics committee, University of Western Sydney, and satisfied the Declaration of Helsinki. Based on the information presented by Keller et al. (2006), a meaningful difference in vascular sympathetic BRS of -2.0 bursts/mmHg was identified. Previous pilot work performed by our group has provided a standard deviation of differences of 1.6 bursts/100heartbeats/mmHg in vascular sympathetic BRS. From this, it is estimated that a sample size of eight participants will have >80% power to detect a meaningful difference in vascular sympathetic BRS of 2.0 bursts/mmHg, using a paired t-test with a 0.05 two-sided significance level. In order to account for unsuccessful experiments and insignificant baroreflex slopes, 12 healthy participants, aged between 19 and 27 years, were recruited. Exclusion criteria included diagnosed cardiovascular, respiratory or endocrine disease, hypertension (>140 mmHg systolic and/or >90 mmHg diastolic blood pressure) and those who smoked or took regular

medication. Participants were instructed to abstain from alcohol or vigorous exercise 24 h prior and to not consume any caffeine on the day of both morning and afternoon experiments. Diet was otherwise uncontrolled; subjects studied in the morning had eaten their normal breakfast and those in the afternoon their typical lunch. The changes in hormone levels during the menstrual cycle have been shown to affect MSNA and sympathetic BRS (Minson et al., 2000); accordingly, females were tested in the low hormone (early follicular) phase of their menstrual cycle to minimize the effects of sex hormones on BRS. Written informed consent was obtained from all participants prior to conducting the experiment, who were reminded that they could withdraw at any time.

A.3.2. Measurements

Participants were studied in an upright-seated position in a comfortable chair, with the legs supported in the extended position. Continuous MSNA recordings were made from muscle fascicles of the common peroneal nerve through tungsten microelectrodes (FHC, Bowdoin, ME, USA) inserted percutaneously at the level of the fibular head. Multi-unit neural activity was amplified (gain 20,000, bandpass 0.3–5.0 kHz) using an isolated amplifier (Neuroamp EX, ADInstruments, Sydney, Australia) and stored on computer (10-kHz sampling) using a computer-based data acquisition and analysis system (Powerlab 16SP hardware and LabChart 7 software; ADInstruments, Sydney, Australia). A root-mean-square (RMS) processed version of this signal was computed, with a moving average of 200 ms. Blood pressure was recorded non-invasively via a finger cuff (Finometer; Finapres Medical System, Amsterdam, the Netherlands). Heart rate was recorded via electrocardiogram (0.3-1.0kHz, Ag-AgCl surface electrodes, sampled at 2kHz). Respiration was measured via a strain-gauge transducer (Pneumotrace, UFI, Morro Bay CA, USA) wrapped around the chest.

A.3.4. Experimental protocol

Participants completed two trials, one beginning at 0800 h and one at 1300 h on two separate days. This was to ensure that, based on time to set up and obtain high quality nerve recordings (approximately 60-90 min), data collection coincided with the times of day associated with high (0900-1000 h) and low occurrence (1400-1500 h) of cardiovascular

events during daylight hours (Muller et al., 1989). The order of the two trials was randomised. A minimum of 10 min of resting data was recorded in order to record spontaneous fluctuations in blood pressure and the corresponding changes in MSNA. Participants were not instructed about their breathing.

A.3.5. Data analysis

Beat-to-beat values were extracted from LabChart (ADInstruments, Sydney, Australia) for systolic pressure, diastolic pressure, R-R interval, and MSNA. A custom-written LabView program was used to detect and measure the area of individual bursts of MSNA. The numbers of bursts per minute (MSNA burst frequency) and per 100 heartbeats (MSNA burst incidence) were determined for each individual.

Vascular sympathetic baroreflex sensitivity: burst incidence method

Vascular sympathetic BRS was quantified using methods previously described by Kienbaum et al. (2001). For all methods of assessing sympathetic BRS, the nerve trace was shifted to account for the delay in conduction, and this was adjusted for each participant to account for inter-individual differences in sympathetic burst latency. The average shift applied was 1.24 ± 0.02s. For each participant, the diastolic pressure values for each cardiac cycle throughout the 10-min rest period were assigned to 3 mmHg bins, removing potential non-baroreflex stimuli (Ebert et al., 1992; Tzeng et al., 2009). For each bin the corresponding MSNA burst incidence (number of bursts per 100 cardiac cycles) was determined. Vascular sympathetic BRS was quantified by plotting MSNA burst incidence against the mean diastolic blood pressure for each bin. Each data point was weighted according to the number of cardiac cycles because the bins at the highest and lowest diastolic pressures contain fewer cardiac cycles (Kienbaum et al., 2001). The value of the slope, determined via linear regression analysis, provided the vascular sympathetic BRS for the individual, which will be referred to as 'vascular sympathetic BRS_{inc}' in order to differentiate it from other methods of determining vascular sympathetic BRS.

Vascular sympathetic baroreflex sensitivity: total MSNA method

The largest MSNA burst during the 10-min rest period was assigned a value of 1000 and the remaining MSNA bursts were calibrated against this to allow measures of MSNA to be normalized to individual resting values (Halliwill et al., 2000). The relationship between diastolic blood pressure and total MSNA was assessed using 3 mmHg bins. Total integrated MSNA was determined for each bin using a segregated signal averaging approach described by Halliwill (2000) and expressed as arbitrary units (AU) per beat. Linear regression was used to determine the relationship between total MSNA and diastolic blood pressure with the application of the weighting procedure described above to account for the number of cardiac cycles per bin. If threshold or saturation regions were identified, i.e. the presence of 3 or more pressure bins across which there was a plateau in MSNA, then these bins were removed leaving the linear portion of the slope. These baroreflex values will be referred to as 'vascular sympathetic BRS_{total}' in order to differentiate them from the MSNA burst incidence method for assessing vascular sympathetic BRS.

Cardiac baroreflex sensitivity: sequence method

Cardiac BRS was assessed using the sequence method, in which "up" and "down" sequences are identified. "Up" sequences consisted of three or more consecutive cardiac cycles for which there is a sequential rise in both systolic blood pressure and R-R interval. "Down" sequences consisted of three of more cardiac cycles for which there is a sequential fall in systolic blood pressure and R-R interval (Parati et al., 1988). Baroreflex sensitivity was quantified by plotting R-R interval against systolic blood pressure for each sequence ($r \geq 0.8$ acceptance level) and taking the average slope value for "up" sequences (cardiac BRS_{up}), "down" sequences (cardiac BRS_{down}), and all sequences pooled (cardiac BRS_{pooled}). Values of cardiac BRS were accepted when the number of sequences was ≥ 3 for both up and down sequences.

A.3.6. Statistical analysis

Vascular sympathetic BRS values were compared between morning and afternoon using Student's t-tests for paired data. All statistical analyses were performed using Prism v6.00 for

Mac OS X (GraphPad software, San Diego, California, USA). For all statistical tests, a probability level of $p < 0.05$ (two-tailed) was regarded as significant. All values are expressed as means and standard error (SE).

A.4. Results

A.4.1. Participants

Twelve participants were recruited for the study. One participant, who reported a family history of hypertension, had a resting blood pressure of 150/82 mmHg and was therefore excluded from the study. Nerve recordings were successfully obtained in all experiments bar one afternoon experiment; this participant was excluded from the analysis. Baroreflex sensitivity was therefore assessed on two occasions for 10 participants. Significant vascular sympathetic BRS_{inc}, vascular sympathetic BRS_{total} and cardiac BRS values for both the morning and afternoon were acquired for all 10 participants. The mean age of these young, healthy participants was 22 ± 1 year and mean body mass index (BMI) was 23.8 ± 1.0 kg/m^2.

A.4.2. Resting cardiovascular variables

Resting cardiovascular variables for the 10 participants at both times of day are presented in Table A.1. Resting systolic pressure was significantly higher in the afternoon (129 ± 2 mmHg) compared with the morning (120 ± 3 mmHg; $p = 0.02$). However, there were no significant differences in resting diastolic pressure, heart rate or MSNA between the morning and afternoon ($p > 0.05$). Figure A.1. shows raw data recordings from one individual in the morning and afternoon.

Table 1. Resting cardiovascular variables in the morning and afternoon (n=10).

Variable	Morning	Afternoon	Mean difference	p
Systolic pressure (mmHg)	120 ± 3	129 ± 2	9 ± 3*	0.02
Diastolic pressure (mmHg)	69 ± 2	70 ± 3	1 ± 2	0.62
Mean arterial pressure (mmHg)	86 ± 2	90 ± 2	3 ± 2	0.11
Heart rate (beats/min)	66 ± 3	68 ± 2	2 ± 2	0.52
MSNA burst frequency (bursts/min)	38 ± 4	37 ± 2	-1 ± 3	0.73
MSNA burst incidence (bursts/100heartbeats)	57 ± 5	58 ± 3	1 ± 5	0.82

MSNA = muscle sympathetic nerve activity. * Significant difference between morning and afternoon ($p < 0.05$).

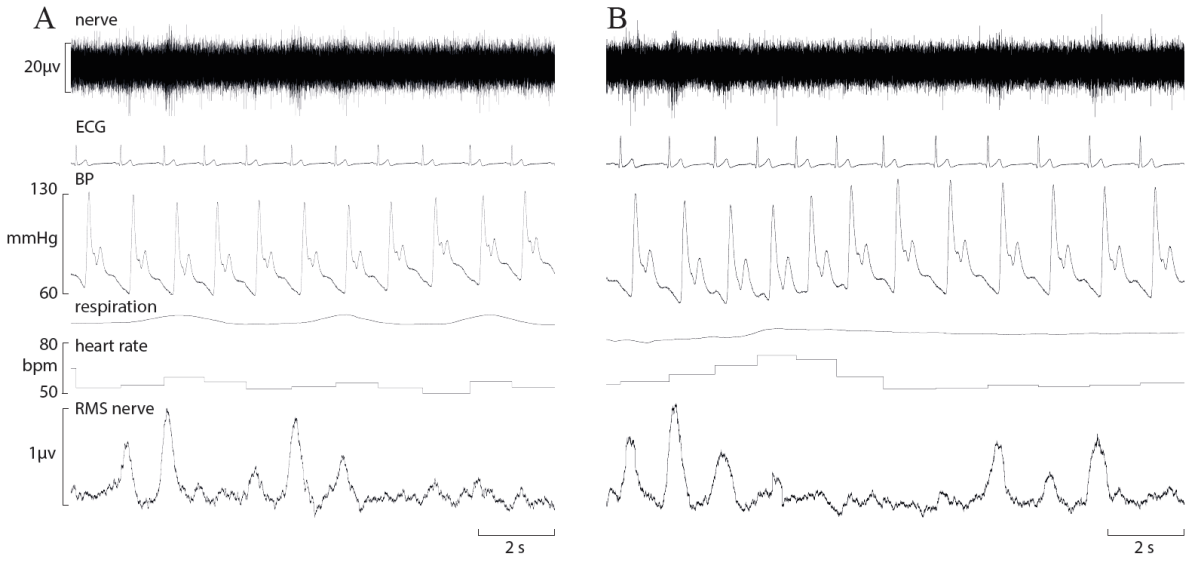

Figure A.1. Raw data recordings of MSNA, ECG, blood pressure and respiration in a 25-year old male in the morning A) and afternoon B). A fall in diastolic pressure is associated with a baroreflex-driven increase in MSNA, and a rise in diastolic pressure causes inhibition of MSNA bursts.

A.4.3. Vascular sympathetic baroreflex sensitivity

There was no significant difference in vascular sympathetic BRS_{inc} between the morning and afternoon sessions (p = 0.68). Similarly, there was no significant difference in vascular

sympathetic BRS_{total} between the morning and afternoon (p = 0.89). These results are summarized in Table 2. Figure A.2. illustrates vascular sympathetic baroreflex slopes in one individual, studied in the morning and in the afternoon on separate days.

Table 2. Sympathetic and cardiac baroreflex sensitivities in the morning and afternoon (n = 10).

Baroreflex sensitivity	Morning	Afternoon	Mean difference	*P*
Sympathetic BRS_{inc} (bursts/100heartbeats/mmHg)	-2.2 ± 0.6	-2.5 ± 0.2	0.2 ± 0.6	0.68
Sympathetic BRS_{total} (AU/beat/mmHg)	-3.0 ± 0.5	-2.9 ± 0.4	0.1 ± 0.6	0.89
Cardiac BRS_{pooled} (ms/mmHg)	15.2 ± 1.6	12.5 ± 1.6	-2.7 ± 2.2	0.26
Cardiac BRS_{up} (ms/mmHg)	15.3 ± 1.4	12.0 ± 1.6	-3.2 ± 1.9	0.12
Cardiac BRS_{down} (ms/mmHg)	15.9 ± 2.3	12.6 ± 1.8	-3.3 ± 2.9	0.29

BRS = baroreflex sensitivity; AU = arbitrary units.

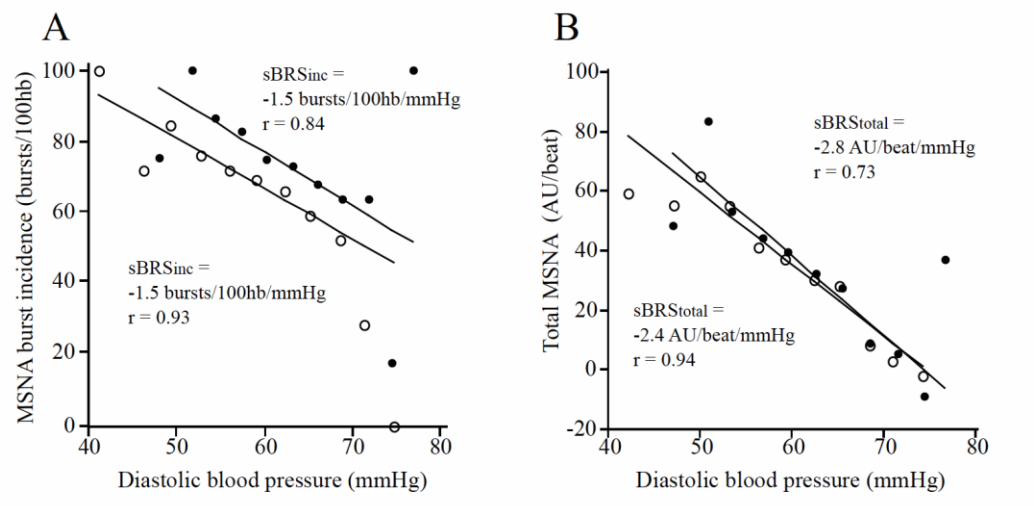

Figure A.2. Sympathetic baroreflex slopes for a 21-year old male in the morning (closed circles) and afternoon (open circles) using A) the sympathetic BRS_{inc} method, and B) the sympathetic BRS_{total} method

A.4.4. Cardiac baroreflex sensitivity

There was no significant difference in cardiac BRS_{pooled}, cardiac BRS_{up}, or cardiac BRS_{down} between morning and afternoon sessions ($p > 0.05$; Table 2). Moreover, there was no significant difference between morning and afternoon sessions in the number of cardiac BRS sequences for pooled (31 ± 5 vs. 42 ± 7; $p = 0.24$), 'up' sequences (16 ± 3 vs. 20 ± 4; $p = 0.31$) or 'down' sequences (16 ± 3 vs. 22 ± 4; $p = 0.23$).

A.5. Discussion

In this study diurnal variation in vascular sympathetic baroreflex sensitivity has been examined for the first time. Previous research indicates that there are two sites for modulation of MSNA: one responsible for burst incidence and the other burst amplitude (Kienbaum et al., 2001). We report that baroreflex modulation of MSNA burst incidence and total MSNA is not significantly different between the morning and afternoon, suggesting that neither site exhibits diurnal variation in the modulation of MSNA.

A.5.1. Baroreflex sensitivity and time of day

We have previously demonstrated reduced cardiac BRS in the morning compared with the afternoon (Taylor et al., 2011), as assessed using the modified Oxford method. The cardiac and vascular sympathetic baroreflexes share a common afferent arm and therefore we predicted similar diurnal variation in sympathetic BRS. However, no significant difference in vascular sympathetic BRS was observed between the morning and afternoon sessions. Different approaches for assessing the baroreflex were employed for the two studies, which may explain these differences. In our previous work on the cardiac baroreflex we have used the modified Oxford method, which is considered the gold standard technique for studying this arm of the baroreflex (Diaz & Taylor, 2006; Taylor et al., 2014; Dutoit et al., 2010). As previously discussed, this pharmacological approach has potential limitations when used for the vascular sympathetic baroreflex, particularly with regards to increases in arterial pressure (Taylor et al., 2014). Spontaneous techniques allow baroreflex responses to both rising and falling pressures to be incorporated but do not provide the rapid changes in pressure

associated with techniques, such as the modified Oxford method, in which blood pressure is actively perturbed (Diaz & Taylor, 2006). Kienbaum et al. (2004) showed that vascular sympathetic BRS at rest differs from sympathetic BRS quantified during pharmacologically-driven hypotension. It is possible that the baroreflex requires testing under greater and more rapid changes in pressure to reveal significant effects of time of day.

The use of spontaneous techniques may explain why we revealed no significant differences for 'up', 'down' or pooled cardiac baroreflex sequences between the morning and afternoon. There was also no significant difference in the number of sequences that occurred at the two times of day. The sequence method is arguably one of the most commonly used spontaneous methods for assessing cardiac baroreflex function. Using this method, Parati et al., (1988) also reported no significant differences in cardiac BRS or number of sequences between the morning (0900–1100 h) and afternoon (1600–1800 h), despite significantly higher values at night (2300-0300 h). Similar findings have been reported in hypertensive patients (Toshikubo et al., 1997). Hossman et al. (1980) used infusions of noradrenaline to assess cardiac BRS over 24 hours. Although this is a pharmacological approach, it is argued that the baroreflex challenge provided by noradrenaline infusions (as opposed to bolus injections) is too gradual, allowing the baroreflex to respond with sufficient changes in heart rate to maintain steady state blood pressure and therefore prevent useful baroreflex slopes from being attained (Taylor et al., 2014; Diaz & Taylor, 2006). Noradrenaline infusions do not provide the rapid changes in blood pressure associated with the bolus injections of sodium nitroprusside and phenylephrine used in the modified Oxford method. Interestingly, the study by Hossman et al. (1980) revealed significantly higher cardiac BRS at 0300 and 1200 h, and significantly lower values at 0900 and 1500 h. This is consistent with the studies involving spontaneous techniques in which cardiac BRS was high at night and low in the morning and afternoon (Parati et al., 1988; Toshikubo et al., 1997). Our study suggests that both cardiac and vascular sympathetic baroreflex sensitivities, measured under physiological conditions at rest, are not significantly different between the morning and afternoon.

A negative correlation between cardiac BRS and blood pressure responses to stressors has previously been reported (Lipman et al., 2002), suggesting that low BRS is associated with a

poor capacity for buffering stress-induced increases in blood pressure. Should diurnal variation in baroreflex modulation of MSNA exist we might expect this to be reflected in the magnitude of the blood pressure response to the cold pressor test, a classic sympathoexcitatory manoeuvre. However, we previously demonstrated no significant differences in systolic or diastolic pressure responses to a cold pressor test in the morning and afternoon (Dunn & Taylor, 2014), which is consistent with the lack of diurnal variation in vascular sympathetic BRS in the current study. To our knowledge, no other studies have been performed to assess diurnal variation in vascular sympathetic baroreflex function. However, Nakazato et al. (1998) studied nocturnal variation in vascular sympathetic BRS using a spontaneous approach similar to the sequence method. The method involved identifying sequences of three or more cardiac cycles in which there were sequential increases or decreases in diastolic pressure. Only sequences associated with a negative correlation (regression coefficient <0) between diastolic pressure and total MSNA were accepted and then entered into a linear regression model to determine the overall baroreflex slope. The authors reported that vascular sympathetic BRS is high at 2300 h but declines during nocturnal sleep, remaining low in the morning (0700 h). However, due to the focus on nocturnal variation, it is not clear from this study when a daytime rise might occur that leads to high vascular sympathetic BRS in the evening. Furthermore, it was reported that microelectrodes had to be re-inserted at least once per participant during the night and it is not clear if this was taken into account by normalising the MSNA values to the new recording site. Future studies of diurnal variation in vascular sympathetic BRS could incorporate active perturbations in blood pressure, although issues of quantifying responses to rapid rising pressures would need to be considered.

A.5.2. Resting MSNA

The present study indicates that resting MSNA, when expressed as both burst incidence and burst frequency, does not differ between morning and afternoon. It has been proposed that the morning represents a transition period from low to high sympathetic activity (Panza et al., 1991; Scheer et al., 2010; Somers et al., 1993). It has been shown that MSNA is lower during sleep than wakefulness, except during REM sleep when it exceeds that of wakefulness (Hornyak et al., 1991; Somers et al., 1993). However, no direct comparisons were made between specific times of day so this does not offer insight into variation within daylight

hours. In an earlier study, Linsell et al. (1985) reported that whilst noradrenaline exhibits a circadian rhythm this is driven by posture and sleep, with greater levels when individuals are upright and awake. Therefore, we may not expect to observe large differences between the morning and afternoon, but predominantly between periods of sleep and wakefulness. Scheer et al. (2010) later demonstrated circadian variation in sympathetic outflow with a peak in plasma noradrenaline at 0900 h. However, measurements of plasma noradrenaline cannot offer the rapid time resolution that can be achieved with microneurography, which provides a direct measure of sympathetic outflow (Vallbo et al., 2004). Middlekauff & Sontz (1994) used microneurography to measure MSNA in the morning (0630-0830 h) and afternoon (1400-1600 h) and reported no significant effect of time of day on MSNA at rest or in response to lower body negative pressure or handgrip exercise. While the current findings support this previous research, we have further shown that baroreflex modulation of MSNA at rest does not differ between the morning and afternoon.

Finally, Panza et al. (1991) found that forearm vascular resistance was higher and blood flow lower in the morning compared with the afternoon and evening, and that infusions of phentolamine (α-adrenergic antagonist) eliminated the time-of-day differences in vascular resistance. The authors therefore concluded that greater sympathetic vasoconstriction in the morning is responsible for the elevated vascular resistance. This may suggest that, whilst MSNA has been shown to be consistent between morning and afternoon, the end-organ response may be greater in the morning. To date there have been no studies of diurnal variation in neurovascular transduction. Greater vascular transduction of MSNA in the morning may explain elevated vascular resistance and contribute to the higher incidence of cardiovascular events at this time of day, though we cannot provide any mechanistic insight into how this augmented vascular transduction comes about.

A.5.3. Limitations

The findings of the current study are limited to healthy young populations and may not be extrapolated to older and/or hypertensive populations. Future research is required to assess diurnal variation in vascular sympathetic BRS in ageing populations and those at risk of cardiovascular events. Although the higher resting systolic pressure in the afternoon was

surprising, importantly resting diastolic pressure was not significantly different between the two times of day. It is the changes in diastolic pressure that drive MSNA, and thus diastolic pressure is more closely correlated with MSNA (Sundlof & Wallin, 1978). The current findings indicate that diastolic pressure, resting MSNA burst incidence and the relationship between the two are consistent between the morning and afternoon. This is in contrast to our previous findings in cardiac BRS, in which the morning was associated with diminished cardiac baroreflex function (Taylor et al., 2011). This may be explained by the use of the modified Oxford method in the previous study; the current findings may be limited by the use of spontaneous techniques for assessing vascular sympathetic BRS.

A.6. Conclusion

In this study diurnal variation in vascular sympathetic baroreflex sensitivity was examined for the first time. The findings indicate that baroreflex modulation of MSNA burst incidence and total MSNA does not differ between morning and afternoon at rest. Future research using methods to actively perturb blood pressure would allow diurnal variation in vascular sympathetic baroreflex control during rapid changes in pressure to be explored. Further research is required to determine whether vascular transduction of MSNA differs with time of day.

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Appendix B

MUSCLE SYMPATHETIC NERVE ACTIVITY PEAKS IN THE FIRST TRIMESTER IN HEALTHY PREGNANCY: A LONGITUDINAL CASE STUDY

Appendix B is published as:

This chapter was published in the Journal *Clinical Autonomic Research* on the 3rd of July 2017. The authors of this article were Sarah L Hissen, Khadigeh El Sayed, Vaughan G Macefield, Rachael Brown and Chloe E Taylor. *Clin Auton Res;* 27(6): 401-406. doi 10.1007/s10286 017 0439 1

Vascular transduction analyses have been added to the appendix for the purpose of this thesis.

B.1. Abstract

Previous research indicates that MSNA is elevated during normotensive pregnancy; with some reports indicating increases in the first trimester while others indicate increases towards the end of the gestational period. Despite sympathetic activation during pregnancy, vascular transduction and BRS are blunted. In order to further our understanding of sympathetic activation during normal pregnancies, it is important to define the levels of MSNA and its modulation via the baroreflex throughout pregnancy using a longitudinal study design. Therefore, MSNA, vascular transduction and, BRS were examined during 10-min of rest in a healthy 28-year old female before, during (weeks 6, 11, 17, 22, 25, 33 and 36) and after her first normotensive pregnancy. Muscle sympathetic nerve activity was elevated during pregnancy with a large peak in the first trimester (Δ 17 bursts/min) and a secondary peak in the third trimester (Δ 11 bursts/min). Vascular transduction decreased during pregnancy and remained blunted post-partum. Cardiac BRS peaked in the first trimester (10 ms/mmHg vs. 6ms/mmHg pre-pregnancy) and then gradually decreased to below pre-pregnancy, whereas sympathetic BRS was greater throughout pregnancy compared with pre-pregnancy levels. The increased MSNA early in pregnancy cannot be explained by a reduction in BRS, while the secondary increase in burst frequency in the third trimester may, in part, be explained by the elevated heart rate.

B.2. Introduction

Pregnancy comes with a number of physiological adaptations to support the growth and development of the foetus and to protect both mother and foetus. This includes an increase in blood volume associated with activation of the renin-angiotensin-aldosterone-system (RAAS) (Fu and Levine, 2009). According to Chapman et al. (1998), hormonal changes associated with pregnancy result in renal vasodilation, overriding vasoconstriction elicited by the RAAS system. A simultaneous increase in systemic vasodilation occurs, possibly driven by increased endothelial nitric oxide release stimulated by elevated circulating oestradiol (Fu and Levine, 2009). Therefore, despite an increase in CO and blood volume, mean arterial pressure is reduced (Chapman et al., 1998). Elevated MSNA may represent a compensatory mechanism employed to balance these vasodilator effects and to maintain blood pressure (Fu and Levine, 2009). However, it is theorised that if the increase in sympathetic activity dominates over the increase in systemic vasodilation, the elevated blood pressure that ensues, via increased vasoconstriction, may lead to pregnancy-induced hypertension, or preeclampsia (Fischer et al., 2004).

Literature on the role of the sympathetic nervous system during pregnancy is limited. In some studies, MSNA has been reported to increase as early as 6 weeks into the gestation period (Jarvis et al., 2012, Okada et al., 2015), and in others, MSNA is elevated during the later stages (Fischer et al., 2004, Greenwood et al., 2001, Greenwood et al., 1998, Okada et al., 2015). Our current knowledge of sympathetic activation during pregnancy is constrained by study design, likely due to the level of difficulty associated with conducting repeated experiments with pregnant women. Studies comparing levels of MSNA during pregnancy have been performed longitudinally in either the first half or second half of pregnancy, or have compared pregnant women with non-pregnant controls (Fischer et al., 2004, Greenwood et al., 2001, Greenwood et al., 1998, Jarvis et al., 2012, Okada et al., 2015). In order to increase our understanding of sympathetic activation throughout normal pregnancies, it is essential to define the levels of MSNA throughout pregnancy using a longitudinal study design. To date, only two longitudinal studies have been published that examine the sympathetic nervous system before, during and after pregnancy (Okada et al., 2015, Reyes et al., 2018). Okada and colleagues (2015) reported that when compared with pre-pregnancy, MSNA increased early in the first trimester and increased further into the third trimester.

Reyes et al. (2018) conducted a longitudinal case series on two females throughout their pregnancy and reported that the increase in sympathetic outflow during pregnancy is strongly correlated with the increased levels of circulating oestradiol and progesterone. However, despite sympathetic activation during pregnancy, vascular transduction was blunted. Attenuated vascular transduction during pregnancy has also been reported by others in as early as 6 weeks of gestation (Jarvis et al., 2012) and also during the last trimester of pregnancy (Usselman et al., 2015b). It has been proposed that the attenuated vascular transduction offsets the increase in sympathetic nerve activity throughout pregnancy (Reyes et al., 2018, Usselman et al., 2015b). It is unclear why vascular transduction is blunted during pregnancy, but it may help to prevent a rise in blood pressure. Furthermore, it is unknown what impact pregnancy has on the baroreflex control of MSNA.

In previous studies, attenuated cardiac and sympathetic BRS have been reported during the third trimester of pregnancy (Moertl et al., 2009, Usselman et al., 2015a), with further reductions in cardiac BRS associated with gestational hypertension and preeclampsia (Silver et al., 2001). Even though cardiac BRS has been assessed longitudinally from early pregnancy to the post-partum period (Moertl et al., 2009), studies of sympathetic BRS have been limited to a single assessment in the third trimester of pregnancy in a cross-sectional design with non-pregnant controls (Usselman et al., 2015a). This does not tell us how MSNA or sympathetic BRS are affected throughout pregnancy in the same individual. Therefore, it is hypothesised that during a normotensive pregnancy, MSNA is elevated, whilst vascular transduction and sympathetic BRS are attenuated. The aim of this case study is to examine MSNA, vascular transduction and baroreflex modulation of both MSNA and heart rate at rest throughout a normal, healthy pregnancy in a single subject. Data were obtained before she became pregnant, during all three trimesters of her pregnancy, and following the birth of her child.

B.3. Methods

B.3.1.Participant

A healthy 28-year old Caucasian female (height 161 cm; pre-pregnancy weight 58 kg; weight following childbirth 72 kg; weight 16 weeks post-partum 69 kg) was serially assessed prior to and throughout her first normotensive pregnancy. This included one pre-pregnancy assessment (5 months before conception), seven during pregnancy, and two post-partum. At the pre-pregnancy time point, the participant was tested in the mid-luteal (high hormone) phase of her menstrual cycle to ensure that any changes observed during pregnancy were due to pregnancy and not because of comparisons with the low hormone phase of the menstrual cycle (Jarvis et al., 2012, Minson et al., 2000). The study was conducted with the approval of the Human Research Ethics Committee of Western Sydney University and in accordance with the Declaration of Helsinki. The participant provided written informed consent before taking part in each experiment.

B.3.2. Measurements and experimental protocol

For each experiment, the participant sat in a semi-recumbent posture with the left leg supported in an extended position in a temperature-controlled laboratory (22–23º _C). Beat-to-beat measurements of blood pressure were continuously recorded via finger pulse plethysmography (NOVA; Finapres Medical System, Amsterdam, the Netherlands), sampled at 400 Hz. An electrocardiogram measured heart rate through Ag–AgCl surface electrodes on the chest sampled at 2 kHz. Respiration was measured via a strain-gauge transducer (Pneumotrace II; UFI, Morro Bay, CA, USA) wrapped around the chest, sampled at 0.4 kHz. Muscle sympathetic nerve activity was directly recorded from a muscle fascicle of the common peroneal nerve at the level of the fibular head via tungsten microelectrodes (Frederick Haer, Bowdoin, ME, USA). Multi-unit neural activity was amplified (gain 20,000, band pass 0.3–5.0 kHz) using an isolated head stage (NeuroAmpEX; ADInstruments, Sydney, Australia) and stored on a computer (10 kHz sampling) using a computer-based data acquisition system (PowerLab 16SP hardware and LabChart 7 software; ADInstruments). A root-meansquare- processed version of the signal was computed with a moving average of 200 ms. Recording of the data began once spontaneous MSNA was found, and was recorded

for 10 min to obtain spontaneous fluctuations in MSNA, together with continuous blood pressure and R–R interval.

B.3.3. Data analysis

Beat-to-beat values were extracted for blood pressure, R–R interval and MSNA. An established custom-written Lab-View program (National Instruments, Austin, TX, USA) was used to detect individual bursts of MSNA. The number of bursts per minute (MSNA burst frequency), and per 100 heartbeats (MSNA burst incidence) was determined over 10 min for each time point during pregnancy.

Vascular transduction

Sympathetic vascular transduction was calculated using the Fairfax method described in Chapter 4, section 4.3.4. Doppler ultrasound was not used in this study so vascular transduction was quantified using the beat-to-beat changes in mean arterial pressure following each MSNA burst.

Sympathetic baroreflex sensitivity

Sympathetic BRS was quantified using methods previously described by Kienbaum et al. (2001). The nerve trace was shifted by ~1.2–1.3 s to account for the baroreflex conduction delay of sympathetic outflow to the peroneal nerve. Diastolic pressures were assigned to 3 mmHg bins, and for each bin the corresponding MSNA burst incidence was determined. Sympathetic BRS was quantified by plotting MSNA burst incidence against the mean diastolic pressure for each bin. Each data point was weighted according to the number of cardiac cycles as the bins at the highest and lowest diastolic pressures contain fewer cardiac cycles (Kienbaum et al., 2001). The value of the slope ($r \geq 0.5$ acceptance level) (Taylor et al., 2015), determined via linear regression analysis, provided the sympathetic BRS

Cardiac baroreflex sensitivity

Cardiac BRS was assessed using the sequence method, in which 'up' and 'down' sequences were identified. 'Up' sequences consisted of three or more consecutive cardiac cycles for which there was a sequential rise in both systolic blood pressure and R-R interval. 'Down' sequences consisted of three or more cardiac cycles for which there was a sequential fall in systolic blood pressure and R-R interval (Parati et al., 1988). Cardiac BRS was quantified by plotting R-R interval against systolic blood pressure for each sequence ($r \geq 0.8$ acceptance level). The average slope values for 'up' and 'down' sequences were combined to get an overall baroreflex slope. Values of cardiac BRS were accepted when the number of sequences was ≥ 3 for both 'up' and 'down' sequences.

B.4. Results

Changes in cardiovascular variables during pregnancy are shown in figure B.1. Diastolic pressure dropped early in pregnancy (6 weeks) and was maintained at this low level for the duration of pregnancy and up to 16 weeks post-partum. Successive increases in heart rate were observed from 17 weeks gestation to birth, with heart rate returning to pre-pregnancy levels post-partum. Resting MSNA peaked at 6 weeks and remained above pre-pregnancy levels throughout pregnancy, with a secondary peak in the third trimester. Figure B.2 shows raw data recordings comparing pre-pregnancy with each trimester of pregnancy. The secondary peak in MSNA was more apparent when expressed as burst frequency.

Changes in vascular transduction during pregnancy are shown in figure B.3. Vascular transduction was variable during the first and second trimester of pregnancy but was blunted in the third trimester of pregnancy. Vascular transduction did increase post-partum but remained lower than when compared with pre-pregnancy.

Changes in cardiac and sympathetic BRS_{inc} before, during and after pregnancy are shown in figure B.4. Cardiac BRS peaked in the first trimester then gradually declined throughout pregnancy to below pre-pregnancy levels by the third trimester, improving again during the

post-partum period. Sympathetic BRS_{inc} was variable during pregnancy, although most values were above that of pre-pregnancy (a more negative value indicates more effective baroreflex buffering of changes in blood pressure).

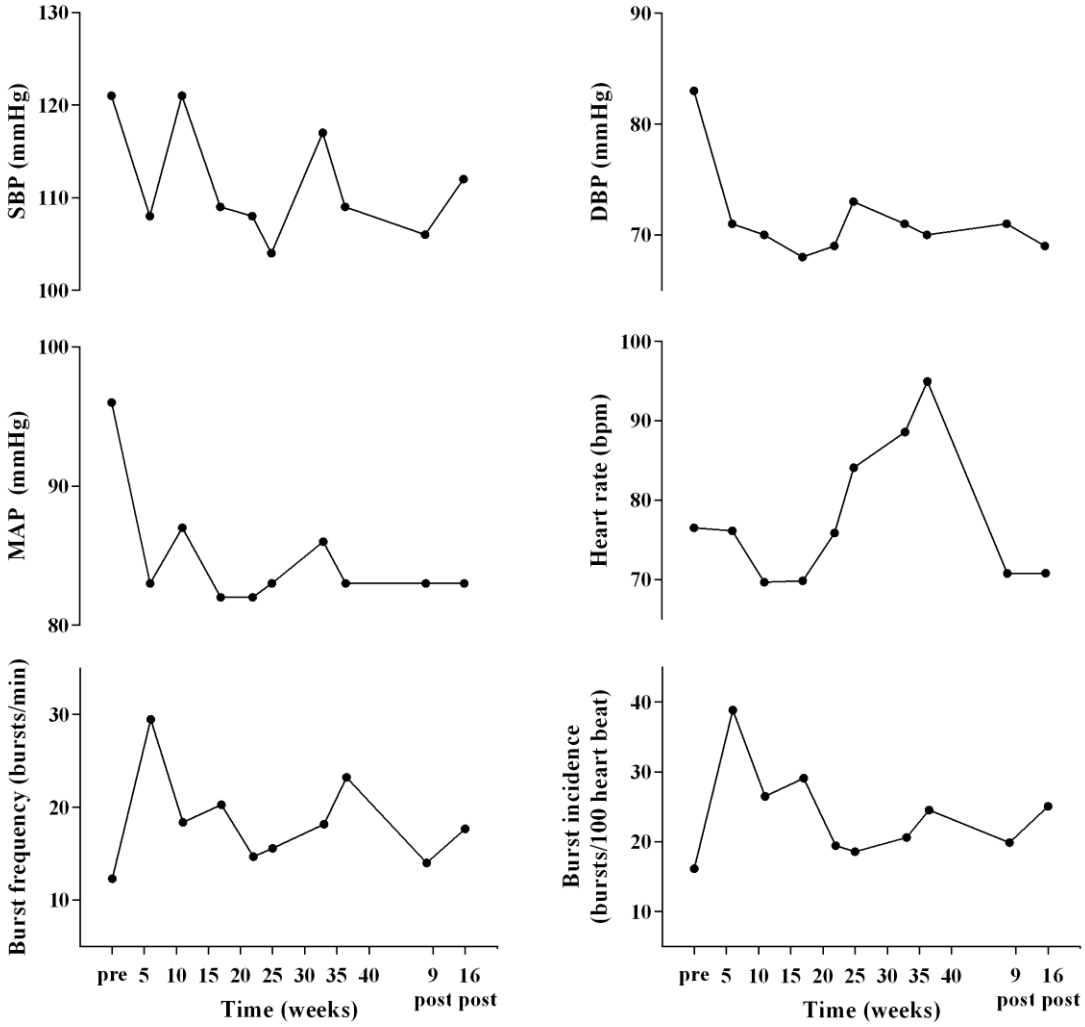

Figure B.1. Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate, MSNA burst frequency and burst incidence at rest before pregnancy, during 6, 11, 17, 22, 25, 33 and 36 weeks of pregnancy, and 9 and 16 weeks post-partum

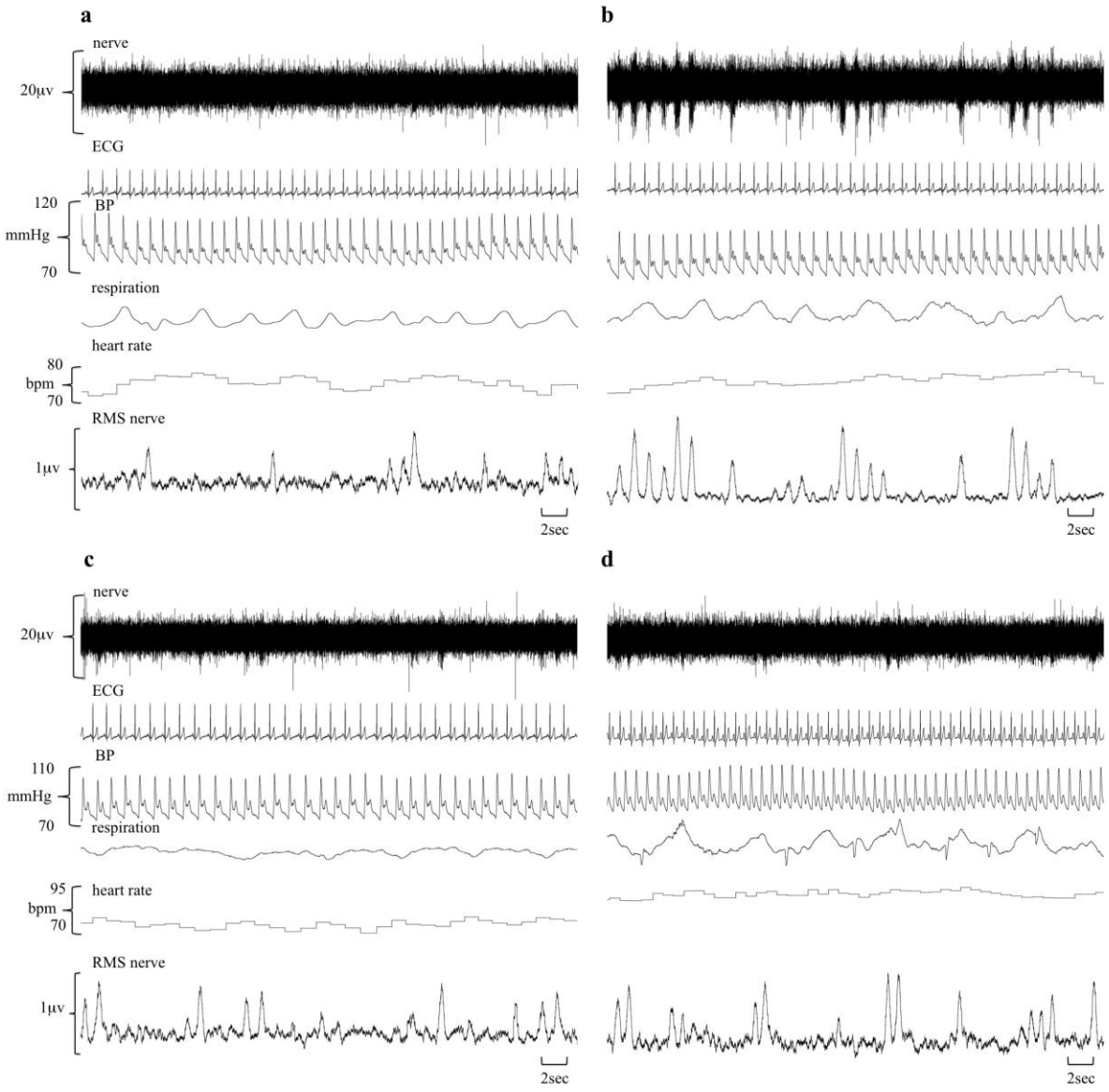

Figure B.2. Raw data recordings of MSNA, ECG, blood pressure and respiration in the 28-year old participant a) before pregnancy, b) during the first trimester (6 weeks pregnant), c) second trimester (17 weeks pregnant), and d) third trimester (36 weeks pregnant)

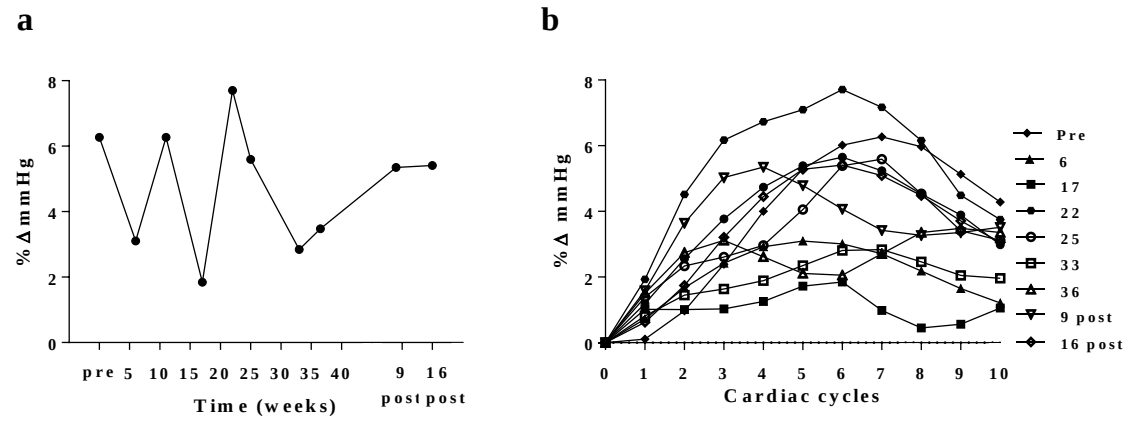

Figure B.3. Changes in a) vascular transduction and b) individual time course of MAP 10 cardiac cycles following MSNA bursts at rest before pregnancy, during weeks 6, 11, 17, 22, 25, 33, 36 of pregnancy, and 9 and 16 weeks post-partum.

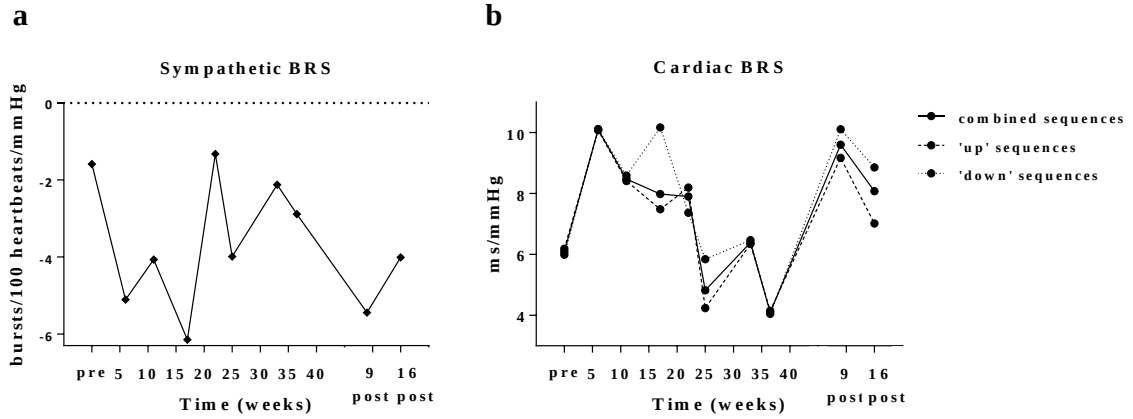

Figure B.4. Changes in a) sympathetic baroreflex sensitivity and b) combined, up and down sequences for cardiac baroreflex sensitivity at rest before pregnancy, during weeks 6, 11, 17, 22, 25, 33 and 36 of pregnancy, and 9 and 16 weeks post-partum

B.5. Discussion

This is the first longitudinal case study in which resting sympathetic activity and baroreflex function have been assessed throughout all three trimesters of a normal, healthy pregnancy. The key findings include i) a spike in resting MSNA in the first trimester, with a secondary

peak in the third trimester, ii) an attenuated vascular transduction in the third trimester of pregnancy and iii) an increase in cardiac BRS in the first trimester of pregnancy, with a drop to below pre-pregnancy levels in the third trimester.

In previous studies, MSNA has been reported to increase during early (Jarvis et al., 2012) and late (Greenwood et al., 2001) pregnancy, with only one study involving a longitudinal examination of the progression of MSNA before, during and after pregnancy (Okada et al., 2015). Okada and colleagues (2015) found that MSNA burst frequency increased early in pregnancy, with further increases towards the end of pregnancy, but they did not examine BRS. In the current case study, MSNA was at its highest in the first trimester (6 weeks gestation) and then dropped for the remainder of gestation - albeit still above pre-pregnancy levels. When MSNA is expressed as burst frequency, the secondary peak in the third trimester is much larger than when expressed as burst incidence. This may be explained by the clear increase in heart rate over the course of pregnancy. With a greater heart rate, the probability of bursts of MSNA over a period of time increases. When MSNA is expressed as burst incidence, heart rate is thus controlled for, and the secondary peak is dampened. Elevated MSNA during the first trimester without a rise in resting blood pressure may be explained by blunted vascular transduction of MSNA during pregnancy (Charkoudian et al., 2017).

In this case study, vascular transduction was variable in the first two trimesters of pregnancy, attenuated in the third trimester of pregnancy and returned close to pre-pregnancy levels post-partum. This is in agreement with previous reports that have observed blunted vascular transduction during early pregnancy (Jarvis et al., 2012, Reyes et al., 2018) and also during the second (Reyes et al., 2018) and third trimesters of pregnancy (Reyes et al., 2018, Usselman et al., 2015a). In the current study, blood flow was not measured so the quantification of vascular transduction was limited by calculating the peak change in mean arterial pressure following MSNA bursts. In Chapter 4 we found that vascular transduction was greater in young females than males when quantified using beat-to-beat changes in leg vascular conductance but no differences were found when vascular transduction was characterised using mean arterial pressure. If Doppler ultrasound had been used in the current

investigation, it may have provided more detail of the vasoconstrictor response to MSNA. Vascular transduction quantified during the cold pressor test also displays an attenuated response in normotensive pregnancy. Usselman and colleagues (2015b) compared vascular transduction during the cold pressor test in females in the third trimester of their pregnancy with non-pregnant controls. Despite an exaggerated sympathetic response to the cold pressor test in pregnant women, the cardiovascular responses such as blood pressure and vascular resistance were comparable to the non-pregnant controls. This demonstrates an attenuated transduction of MSNA to vasoconstrictor drive in pregnant women. Schmidt and colleagues (2018) reported that in pregnant females, the number of action potentials within a burst and the number of active amplitude based clusters of action potentials were similar to non-pregnant controls. However, sympathetic activation was still observed in pregnancy, as the number of multi-unit bursts per minute was greater in pregnant females when compared with non-pregnant controls. This is consistent with the theory that elevated sympathetic discharge is counteracted by mechanisms downstream, such as neurotransmitter release/sensitivity, which are responsible for blunted vascular transduction in pregnancy (Schmidt et al., 2018).

It has been reported that young women operate at a lower baroreflex set point than older women (Peinado et al., 2017). However, during early pregnancy, it appears that the set point is elevated to higher levels of MSNA. In addition to this, there are changes in the baroreflex slope, i.e., in baroreflex sensitivity. Changes in baroreflex modulation of MSNA and heart rate were observed during pregnancy in the current study. Cardiac BRS peaked early in pregnancy and progressively decreased throughout pregnancy, whereas sympathetic BRS fluctuated throughout pregnancy. Previous studies of pregnant women in the third trimester indicate attenuated cardiac (Moertl et al., 2009) and sympathetic BRS (Usselman et al., 2015a). Vagal control of heart rate increases in the first trimester of pregnancy (Kuo et al., 2000). These findings were reflected in the present case study, with improved cardiac BRS in the first trimester when compared with pre-pregnancy levels. In the third trimester of normotensive pregnancy, Usselman et al. (2015a) reported that sympathetic BRS is dampened and the baroreflex slope is shifted upwards as a result of the greater MSNA in pregnant women - despite similar resting blood pressure to non-pregnant controls. It has been proposed that the dampened sympathetic BRS is a consequence of the reduced role of the sympathetic nervous system in regulating blood pressure in pregnancy (Usselman et al.,

2015a). However, in the present case study, although sympathetic BRS was variable throughout pregnancy, the majority of the values indicated a greater sympathetic BRS compared with pre-pregnancy levels. The differences between the current findings and previous studies may be explained by study design. The current study involved a series of repeated measures over time, for which the participant was, therefore, her own 'control', whereas the participants in the study by Usselman et al. (2015a) were compared to non-pregnant controls. More recently in a case study of two participants, Reyes et al. (2018) found that sympathetic BRS was blunted in one participant but did not change in the other. As the current study was conducted on only one participant, and the study presented by Reyes et al. (2018) was on two participants, we are unable to extrapolate to the general population. However, it does give some insight into the variability of cardiovascular control throughout the gestation period.

The changes in sympathetic BRS and cardiac BRS in the present case study display a similar pattern throughout pregnancy. When sympathetic BRS is high, so is cardiac BRS and vice versa. However, the changes in vascular transduction occur in direct opposition to the changes in sympathetic BRS. When BRS increased from one time point to another, vascular transduction decreased. This may suggest a compensatory interaction during pregnancy to ensure appropriate levels of vasoconstriction. However, further research on the relationship between baroreflex sensitivity and vascular transduction throughout pregnancy is required to confirm these observations. This may provide further insight into the mechanisms involved in maintaining baroreflex function throughout pregnancy.

B.6. Conclusion

This case study suggests that muscle sympathetic outflow peaks in the early stages of pregnancy, as early as 6 weeks into the gestation period. This is consistent with the findings from other studies (Jarvis et al., 2012, Okada et al., 2015). However, the increase in MSNA early in pregnancy cannot be explained by a reduction in BRS. Elevations in MSNA burst frequency in the third trimester may be explained, in part, by the increase in resting heart rate. Further research needs to be performed on more individuals, in a longitudinal setting, in order to confirm the time course of MSNA and baroreflex function throughout normal pregnancy.

It is also important to understand, as with many physiological processes, the inter-individual variability of the cardiovascular and autonomic response to pregnancy.

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Appendix C: Subject information statement and consent form

The study information sheet and consent form were provided to each subject who participated in any of the studies

School of Science and Health
University of Western Sydney
Locked Bag 1797
Penrith NSW 2751
Australia
Telephone : 02 4620 3298
e-mail : c.taylor@uws.edu.au

University of Western Sydney
Bringing knowledge to life

Participant Information Sheet (General)

Project Title: Inter-individual differences in vasoconstrictor drive, vascular transduction and blood flow during stress

Project Summary: Some individuals respond to stress with large increases in blood pressure, whilst others do not. Mental stress has been associated with an increase in sympathetic nerve activity, which causes constriction of the blood vessels, and should explain the increases in blood pressure. However, changes in sympathetic nerve activity during mental stress differ significantly between individuals, even in groups where an increase in blood pressure occurs in all participants. We hypothesise that this is due to inter-individual differences in 'vascular transduction', which refers to how much the blood vessels constrict in response to changes in sympathetic nerve activity. To test this hypothesis, we will make beat-to-beat recordings of sympathetic nerve activity whilst imaging the femoral artery (in the upper leg) using ultrasound. This will be done whilst participants perform a series of stressor tasks.

You are invited to participate in a study conducted by Dr. Chloe Taylor (Senior Lecturer in the School of Science and Health) and Professor Vaughan Macefield (Principle Chair of Integrative Physiology in the School of Medicine).

How is this study being paid for?
The study is being sponsored by the School of Science and Health, UWS.

What will I be asked to do?
You will be required to attend the laboratory for a single 2-3 hr session to measure your responses to the stressor tasks (cold water hand-immersion, static handgrip exercise, mental arithmetic and a colour/word mental test). Measurements will include femoral artery diameter and blood flow (via ultrasound), blood pressure, heart rate and respiratory rate (these all are non-invasive). We will also measure sympathetic nerve activity during the stressor tasks. Sympathetic nerve activity is measured in your leg using an invasive technique called microneurography (described below).

How much of my time will I need to give?
The experiment will take a total of 2 to 3 hours.

What specific benefits will I receive for participating?
There are no direct benefits associated with taking part in this study, although you will be reimbursed for your time at a rate of $25 per hour. It should be noted that there are no benefits to students taking part in this study whose teachers are involved as part of the research team.

Will the study involve any discomfort for me? If so, what will you do to rectify it.
The cold water hand-immersion test is associated with brief thermal pain. Although this is generally well-tolerated in young healthy individuals, participants are reminded that they are free to withdraw from the study at any point. All measurements in this study are non-invasive, bar microneurography.

Microneurography involves inserting a fine needle microelectrode into the common peroneal nerve at the knee in order to measure sympathetic nerve activity (which controls the blood vessels). It may take from 10 to 30 minutes to enter the nerve, during which you may experience some transient discomfort. This technique has been used for over 30 years, is tolerated well by normal volunteers and patients alike, and is not associated with any long-term nerve damage. Should you have any further questions regarding the microneurography component of the study, either prior to or after taking part, please feel free to contact Dr. Chloe Taylor via phone (02 4620 3298) or email (C.Taylor@uws.edu.au).

How do you intend on publishing the results.

Please be assured that only the researchers will have access to the raw data you provide. Data will be retained for 5 years following completion of the study. It will be in an identifiable form to allow participants to be identified for potential follow up studies, such as repeatability experiments. Participants are under no obligation to take part in follow up experiments.

The findings of the research will be published in peer-reviewed research journals and conference presentations; but under none of these circumstances will individual participants be identifiable.

Can I withdraw from the study?

Participation is entirely voluntary: and you are not obliged to be involved. If you do participate, you can withdraw at any time without giving any reason.

If you do choose to withdraw, any information that you have supplied will remain strictly confidential and will not be used for any part of the study. Withdrawal from the research will not prejudice your employment or academic progress in any way.

Can I tell other people about the study?

Yes, you can tell other people about the study by providing them with the chief investigator's contact details. They can contact the chief investigator to discuss their participation in the research project and obtain an information sheet.

What if I require further information?

Please contact Dr. Chloe Taylor should you wish to discuss the research further before deciding whether or not to participate.

Dr. Chloe Taylor
Senior Lecturer, School of Science and Health
Tel. 02 4620 3298
Email. C.Taylor@uws.edu.au

What if I have a complaint?

This study has been approved by the University of Western Sydney Human Research Ethics Committee. The Approval number is [enter approval number]

If you have any complaints or reservations about the ethical conduct of this research, you may contact the Ethics Committee through the Office of Research Services on Tel +61 2 4736 0229 Fax +61 2 4736 0013 or email humanethics@uws.edu.au.

Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

If you agree to participate in this study, you may be asked to sign the Participant Consent Form.

Human Research Ethics Committee
Office of Research Services

University of Western Sydney

Participant Consent Form

This is a project specific consent form. It restricts the use of the data collected to the named project by the named investigators.

Note: If not all of the text in the row is visible please 'click your cursor' anywhere on the page to expand the row. To view guidance on what is required in each section 'hover your cursor' over the bold text.

Project Title: Inter-individual differences in vasoconstrictor drive, vascular transduction and blood flow during stress

I, ... , consent to participate in the research project titled 'Inter-individual differences in vasoconstrictor drive, vascular transduction and blood flow during stress'.

I acknowledge that:

I have read the participant information sheet and have been given the opportunity to discuss the information and my involvement in the project with the researcher/s.

The procedures required for the project and the time involved have been explained to me, and any questions I have about the project have been answered to my satisfaction.

I consent to participating in the laboratory-based experiment in which cardiovascular measurements will be made during a series of physical and mental stressor tasks.

I understand that my involvement is confidential and that the information gained during the study may be published but no information about me will be used in any way that reveals my identity.

I understand that I can withdraw from the study at any time, without affecting my relationship with the researcher/s now or in the future.

Signed: _____

Name: _____

Date: _____

Return Address: Dr. Chloe Taylor, School of Science & Health, University of Western Sydney, Locked Bag 1797, Penrith, NSW 2751, Australia

This study has been approved by the University of Western Sydney Human Research Ethics Committee.
The Approval number is: H11138

If you have any complaints or reservations about the ethical conduct of this research, you may contact the Ethics Committee through the Office of Research Services on Tel +61 2 4736 0229 Fax +61 2 4736 0013 or email humanethics@uws.edu.au. Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

Other output during candidature

Hissen, S. L., Macefield, V. G., Brown, R., Witter, T., & Taylor, C. E. (2015). Baroreflex modulation of muscle sympathetic nerve activity at rest does not differ between morning and afternoon. *Front Neurosci, 9*, 312. doi:10.3389/fnins.2015.00312

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