THE INFLUENCE OF HABITUAL EXERCISE ON CENTRAL HAEMODYNAMICS AND PERIPHERAL BLOOD PRESSURE REGULATION IN NORMOTENSIVE MIDDLE-AGED MEN

Denis James Wakeham
BSc (Hons) MSc

Submitted in accordance with the requirements for the

Degree of Doctor of Philosophy

Cardiff School of Sport and Health Sciences

Cardiff Metropolitan University

August 2020

Abstract

Central (aortic) haemodynamics change markedly with age, as aortic stiffness and systolic pressure augmentation increase thereby elevating aortic blood pressure. Furthermore, the autonomic regulation of vascular sympathetic activity also changes; whereby the level of sympathetic nerve activity to the skeletal muscle vasculature, termed muscle sympathetic nerve activity (MSNA), increases, which can elevate peripheral blood pressure. The increase in blood pressure with age increases cardiovascular risk. Notably, exercise training is one recommended strategy to reduce this elevated risk. Although habitual endurance exercise is well known to elicit a range of physiological adaptations, the effects on aortic haemodynamics and peripheral blood pressure regulation are not well described. Therefore, the primary aim of this thesis was to investigate the effects of chronic habitual endurance exercise on aortic (central) haemodynamics and the autonomic regulation and neural control of brachial (peripheral) blood pressure in healthy ageing. To achieve this aim, normotensive middle-aged and young men were recruited into separate groups of chronically endurance-trained runners or recreationally-active nonrunners. Young men were studied to determine the effects of habitual exercise independently of age. Collectively, this thesis shows that in middle-aged men habitual exercise has no significant effects on aortic haemodynamics or the ability of MSNA to effect vascular responses during sympathoexcitation. However, the main finding from this thesis is that habitual endurance exercise, until middle-age, alters the autonomic regulation of MSNA at rest. Specifically, for a similar blood pressure, the arterial baroreflex is set higher in runners, which is associated with a greater likelihood of MSNA burst occurrence. However, there were no between-group differences in young men. Therefore, this greater likelihood of MSNA burst occurrence in middle-aged male runners may represent a fundamental physiological adaptation to chronic (~30 years) habitual endurance exercise to support resting peripheral blood pressure in an expanded cardiovascular system with greater reserve.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank those involved, both directly and indirectly, with helping me complete this thesis. First and foremost, my supervisory team Dr. Jonathan Moore, Prof. Rob Shave and Dr. Chris Pugh. Your guidance throughout my PhD has taught me an awful lot, both professionally and personally, and I thank you for the opportunity to complete my PhD training with you. Second, I would like to thank Dr. Lewis Fall and Prof. Bruce Davies. Lew, you believed in me and gave me my first opportunity to be involved in research during my undergraduate degree. Bruce, since the final year of my BSc you have been a great mentor and friend who continues to teach and test me, no matter what we discuss. Long may our discussions continue. Without you both I would not be in Science and this thesis would not exist. Third, to all of those in the Physiology and Health research group and research house at Cardiff Met, as well as the members of the Centre for Heart, Lung and Vascular Health at UBC Okanagan, I have thoroughly enjoyed getting to know you all over the years. You have all been a great support network, both as friends and colleagues. Fourth, my parents; your support to date has been unwavering and has got me through so much. Last, but by no means least, this thesis would not have been possible without the willing participants who volunteered their precious time. Your willingness to participate has helped both my personal and professional development; getting to know you all has been a real highlight of my PhD.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACh - Acetylcholine

aDBP – Aortic diastolic blood pressure

AIx – Augmentation index

aMAP – Aortic mean arterial pressure

AP – Augmentation pressure

aPP – Aortic pulse pressure

aPWV – Aortic pulse wave velocity

aSBP – Aortic systolic blood pressure

AVP – Arginine vasopression

bpm – Beats per minute

bPWV – Brachial pulse wave velocity

BRG – Baroreflex gain

CPT – Cold pressor test

CVLM – Caudal ventrolateral medulla

DBP – Brachial diastolic blood pressure

FVC – Forearm vascular conductance

FVR – Forearm vascular resistance

IML - Intermediolateral cell column

LBNP – Lower body negative pressure

MAP – Brachial mean arterial pressure

MNR – Middle-aged nonrunner

MR – Middle-aged runner

ms – milliseconds

MSNA – Muscle sympathetic nerve activity

MVC – Maximal voluntary contraction

NA – Nucleus ambiguous

NTS - nucleus of the tractus solitarius

PEMI – Post-exercise muscle ischaemia

PP – Brachial pulse pressure

PPA – Pulse pressure amplification

PWV – Pulse wave velocity

RTF – Return to flow

RVLM – Rostral ventrolateral medulla

SBP – Brachial systolic blood pressure

SHG – Static hand grip exercise

YNR – Young nonrunner

YR – Young runner

CHAPTER 1. GENERAL INTRODUCTION

1.0 Introduction

Ageing is associated with cardiac (Arbab-Zadeh *et al.*, 2004) and central (aortic) elastic artery stiffening (McEniery *et al.*, 2005), as well as changes in the autonomic regulation and neural control of the circulation (Iwase *et al.*, 1991; Ebert *et al.*, 1992). Age-related aortic stiffening and elevations in the level of vascular sympathetic nervous system activity to skeletal muscle (termed muscle sympathetic nerve activity [MSNA]) contribute to elevations in central and peripheral blood pressure with advancing age, respectively. This elevation in blood pressure increases cardiovascular risk (Benetos *et al.*, 2010), which contributes to age being the leading risk factor for the development of cardiovascular diseases (Benjamin *et al.*, 2018). The ability to distinguish the effects of healthy versus pathophysiological ageing on cardiovascular structure, function and regulation is, therefore, of clear clinical relevance. Accordingly, strategies to offset age-related changes to the cardiovascular system are an area of intense research focus.

Exercise training is recommended as one strategy to increase health span, the proportion of a person's lifespan in which they are in good health (Seals *et al.*, 2018), and to reduce cardiovascular risk (Eckel *et al.*, 2014). Notably, the cardiovascular effects of chronic habitual endurance exercise training are typically considered in cross-sectional observational studies by comparing endurance-trained and sedentary individuals. There are numerous effects of sedentarism on cardiovascular structure and function, including higher circulating inflammatory cytokines and left ventricular stiffness as well as lower vascular endothelial function (Lavie *et al.*, 2019). Furthermore, low physical activity levels (i.e. sedentarism) independently increases the risk of cardiovascular disease and all-cause mortality (Dempsey *et al.*, 2020). Thus, appropriately selecting groups of individuals dependent on their level of physical activity is of prime importance when determining the effects of habitual exercise cross-sectionally. Indeed, it may be more appropriate to compare endurance-trained with recreationally-active, rather than sedentary, individuals. Recruiting these groups may better

enable the determination of the effects of healthy ageing without the confounding effects of sedentarism. In addition, some authors even suggest that endurance-trained individuals should be considered as the control group in studies of ageing as they best reflect "*the inherent ageing process*" (Lazarus and Harridge, 2010) or "*primary ageing*" (Tanaka *et al.*, 2019).

To date, the effects of chronic habitual endurance exercise on the heart have been well described, reporting that well-trained individuals have a lower (intrinsic) heart rate (Katona *et al.*, 1982) and a greater left ventricular stroke volume and compliance (Bhella *et al.*, 2014). However, the effects of habitual exercise on central (aortic) stiffness, systolic pressure augmentation and pressure, have not been studied widely. This is despite these indices of aortic haemodynamics being predictive of cardiovascular morbidity and/or mortality (Roman *et al.*, 2007; Roman *et al.*, 2009; Vlachopoulos *et al.*, 2010; Wang *et al.*, 2010; Ben-Shlomo *et al.*, 2014). The majority of previous reports have focused upon the effects of habitual exercise on aortic stiffness alone and compared endurance-trained and sedentary individuals, as reviewed recently (Seals *et al.*, 2019). Thus, more studies are warranted to determine the effects of habitual exercise on the aortic haemodynamic profile, that is aortic stiffness, systolic pressure augmentation and pressure, together, especially in healthy human ageing. In addition, the influence of habitual endurance exercise on the autonomic regulation and neural control of peripheral blood pressure is not well characterised. This is despite the primary role vascular sympathetic activity plays in the support of peripheral blood pressure. It is often believed that the level of vascular sympathetic activity decreases similarly in clinical and healthy populations following short-term endurance training. However, a comprehensive review of the literature showed that MSNA was reduced following short-term exercise training in 15 of 15 (100%) studies in clinical populations. This contrasts the data in healthy individuals where only 1 of 9 (11%) studies reported a reduction in MSNA with short-term training (Carter and Ray, 2015). Thus,

these data refute the conventional wisdom that short-term exercise training effects vascular sympathetic activity similarly in health and disease. This conclusion is, however, based upon the responses to short-term exercise training. Whether chronic habitual endurance exercise in healthy human ageing is associated with changes in vascular sympathetic activity is unclear.

Due to the ageing population (Beard *et al.*, 2016), understanding the effects of chronic habitual endurance exercise in healthy human ageing on central haemodynamics and peripheral blood pressure regulation are of importance. Moreover, the recent physical activity guidelines from the United Kingdom Chief Medical Officers (2019) recommend exercise throughout the lifespan. Therefore, given the aforementioned gaps within the literature, the aims of this thesis were to investigate the effects of chronic habitual endurance exercise in middle-age on i) aortic haemodynamics and, ii) the autonomic regulation of vascular sympathetic activity and peripheral blood pressure and iii) the neural control of the peripheral vasculature (i.e. sympathetic vascular transduction). To address these aims, all data were collected using a cross-sectional study design in normotensive males, categorized according to training status as either endurance-trained runners or recreationally-active nonrunners. In addition, young runners and nonrunners were studied to discern the effects of habitual endurance exercise independently from the effects of age. This study design enabled an integrative assessment of the determinants of central and peripheral blood pressure, in the same individuals, and at one time, to provide novel insight into the cardiovascular effects of habitual endurance exercise in healthy human ageing.

CHAPTER 2. REVIEW OF LITERATURE

This chapter provides an overview of central (aortic) haemodynamics (Section 2.2) and peripheral blood pressure regulation (Section 2.3), with specific attention paid to the techniques utilised within the experimental chapters of this thesis (Chapters 4-6). Also, within each section, the known effects of age and habitual endurance exercise are discussed and the gaps within the literature base are highlighted. It is these gaps in the literature which are addressed in the following experimental chapters (Chapters 4-6).

2.1 Arterial Blood Pressure

The heart, blood and blood vessels make up the mammalian cardiovascular system, which primarily functions to provide convective transport of oxygen and nutrients to tissues (Herring and Paterson, 2018). The distribution of blood flow, and therefore delivery of oxygen, is dependent on the pressure gradient between the arterial and venous components of the circulation. Mean arterial blood pressure (MAP) is kept relatively similar throughout the circulation, via pressure sensitive neural reflexes eliciting changes in regional vascular tone (i.e. vascular resistance) (Herring and Paterson, 2018). In line with this, MAP is determined by the balance between flow (i.e. cardiac output) and vascular resistance; a relationship similar to Ohm's law, which describes that electrical current (i.e. pressure) is dependent on the balance between the voltage (i.e. flow) and resistance.

Calculation of Mean arterial pressure:

Mean Arterial Pressure = Cardiac Output x Total Peripheral Resistance

Where: Cardiac output is the sum of heart rate (in beats per minute) and stroke volume (in millilitres). Total peripheral resistance is a calculated variable from the division of mean arterial pressure (in mmHg) and cardiac output (in litres per minute).

The above equation highlights that the relationship between cardiac output and vascular resistance is fundamental to the regulation of blood pressure. Routine assessment of cardiac output and total peripheral resistance in the laboratory is not common, therefore, MAP is more commonly calculated from the measurement of peripheral blood pressure, in mmHg above atmospheric pressure (via sphygmomanometry or auscultation). As blood

pressure is not a static variable across the cardiac cycle three measures of blood pressure are reported and calculated which are used to derive MAP, namely: systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP). Notably, the factors which influence SBP, DBP and MAP differ. SBP is primarily influenced by the interaction of stroke volume (cardiac output) and vascular stiffness and DBP is largely influenced by peripheral vascular tone (resistance). As mentioned above, MAP is relatively similar throughout the arterial tree, despite a small change (decrease) in DBP but a relatively, compared to the change in DBP, larger change (increase) in SBP from central to peripheral blood vessels (Peng *et al.*, 2017). The increase in blood pressure from central to peripheral arteries is termed pulse pressure amplification (PPA; Figure 1). PPA is mediated by the decrease in vessel diameter and increases in vascular stiffness and relative wall thickness which contribute to the increase in excess pressure from the central to peripheral vasculature (Peng *et al.*, 2017). Notably, PPA is an important haemodynamic variable as it independently predicts cardiovascular mortality (Benetos *et al.*, 2010). Accordingly, the central and peripheral circulation play different roles in haemodynamic regulation; this likely relates to the differences in the determinants of blood pressure between these different vascular regions. Thus, in this thesis the determinants of central and peripheral blood pressure were assessed to provide novel insight into the effects of chronic habitual endurance exercise.

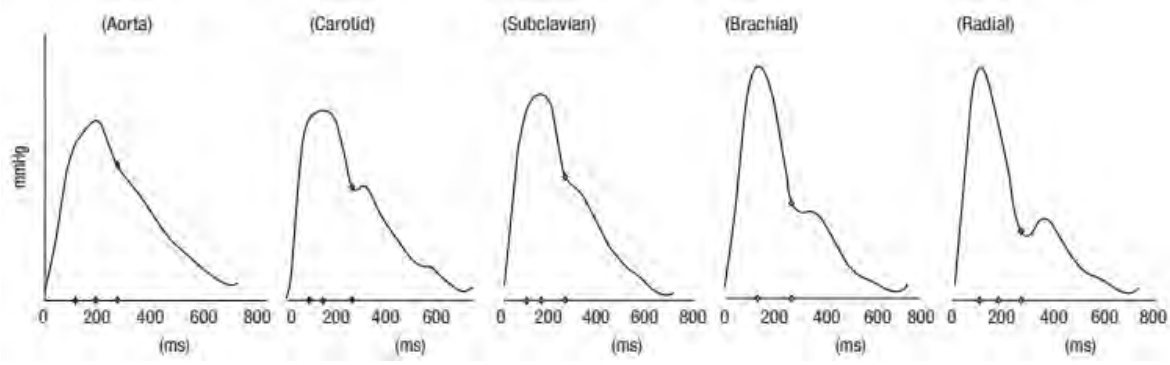


Figure 1 – Pulse pressure amplification. Arterial pressure waveforms from the aorta through to peripheral blood vessels highlighting the physiological phenomenon of pulse pressure amplification. The increase in pulse pressure is likely mediated by a greater involvement of the backward-travelling pressure wave augmenting systolic pressure in the peripheral blood vessels (Peng *et al.*, 2017). *Figure from Nichols et al., (2011).*

2.2 Central Haemodynamics

Ever since blood pressure was first recorded using the cuff sphygmomanometer by Riva-Rocci in the 19th century, a technique improved upon by Korotkoff in 1905, the aim has been to record blood pressure as close to the aorta as possible (Booth, 1977). With technological advancement in the last 30 years, it is now possible to routinely estimate ascending aortic pressure via applanation tonometry of the peripheral (e.g. brachial or radial) arterial pulse waveform. Due to the phenomenon of PPA, individuals with similar aortic blood pressures can have markedly different brachial artery pressures, and vice-versa (McEniery *et al.*, 2008). Accordingly, peripheral arterial blood pressure is a poor surrogate for central blood pressure, as aortic (central) pressure is often lower than the corresponding brachial (peripheral) values. This limits the utilisation of brachial blood pressure to index the pressure load on the left ventricle, as well as other central organs (e.g. the brain, eyes and kidneys) which are susceptible to target organ damage, thereby increasing the risk of end-organ disease. As outlined by Sharman and Laurent (2013), the primary lines of evidence which support the (clinical) assessment of central blood pressure include: i) large intra-individual variability in PPA; ii) central blood pressure is independently related to end-organ damage; iii) central blood pressure independently predicts cardiovascular morbidity and mortality; iv) divergent central and peripheral blood pressure responses to antihypertensive medications; and v) reductions in end-organ damage with therapy have stronger relations to central compared to peripheral blood pressure. However, it is important to emphasise that brachial blood pressure is also an important cardiovascular variable and that both central and brachial arterial pressure should be collected and reported together.

2.2.1 Assessment of aortic haemodynamics

The gold-standard method to assess (ascending) aortic pressure is via the direct insertion of a pressure and velocity transducer into the ascending aorta (Nichols *et al.*, 2011). However, this is a significant barrier to routinely determining aortic blood pressure in the clinic or

research laboratory. The development of a non-invasive method to estimate aortic blood pressure was therefore of significant importance for the study of aortic haemodynamics. Indeed, Chen and colleagues (1997) showed it was possible to estimate ascending aortic waveforms via the use of a generalised inverse (i.e. in opposition to the direction of pressure propagation) transfer function applied to radial artery waveforms, as collected via the technique of applanation tonometry (Figure 2A). The generalised transfer function used to estimate aortic blood pressure was first described by Karamanoglu and colleagues (1993) utilising automated radial tonometry and invasive aortic catheterisation. Utilising Fourier frequency analyses, the transfer function is determined by the ratio of amplitude and phase of the harmonics within the radial and aortic waveforms (see below). Notably, when compared to the generalised inverse transfer function, applying an individualised inverse transfer function, for each participant separately, only led to a 3% improvement in the fit between estimated and measured ascending aortic waveforms (Chen *et al.*, 1997). Since then, a version of this transfer function has been incorporated into numerous devices (including the SphygmoCor from AtCor Medical as used in this thesis) to enable the routine estimation of ascending aortic blood pressure from radial artery applanation tonometry.

Transfer function:

$$H_{(A\text{-}B)} = P_B(\omega)/P_A(\omega)$$

Where: $H_{(A-B)}$ represents the transfer function; $P_A(\omega)$ and $P_B(\omega)$ represent the frequency domain of the pressure wave at sites A and B, respectively (More completely: $P_A(\omega) = M_A(\omega)e^{j\varphi}$ and $P_B(\omega) = M_B(\omega)e^{j\varphi}$); P represents pressure; site A is the ascending aorta and site B is the radial artery; ω is the angular frequency; $M_A(\omega)$ and $M_B(\omega)$ are the moduli of the respective pressure wave; $\varphi_A(\omega)$ and $\varphi_B(\omega)$ are the phases of each pressure wave; e is Eulers number; j is the imaginary number, as used in electronics; φ is the inverse golden ratio. As described by Karamanoglu and colleagues (1993).

As well as being used to estimate the ascending aortic waveform, applanation tonometry is used to determine the pulse wave velocity in different arterial segments, including the aorta. The measurement of aortic pulse wave velocity (aPWV, Figure 2B) is

the current gold-standard method for the non-invasive assessment of aortic stiffness (Townsend *et al.*, 2015). Pulse wave velocity is assessed due to the important role arterial wall elasticity plays in the propagation of pulse waves (Nichols *et al.*, 2011), which can be measured between arterial sites, utilising techniques such as applanation tonometry (Figure 2A). Thus, applanation tonometry enables the characterisation of the aortic haemodynamic profile, that is aortic stiffness, systolic pressure augmentation and pressure.

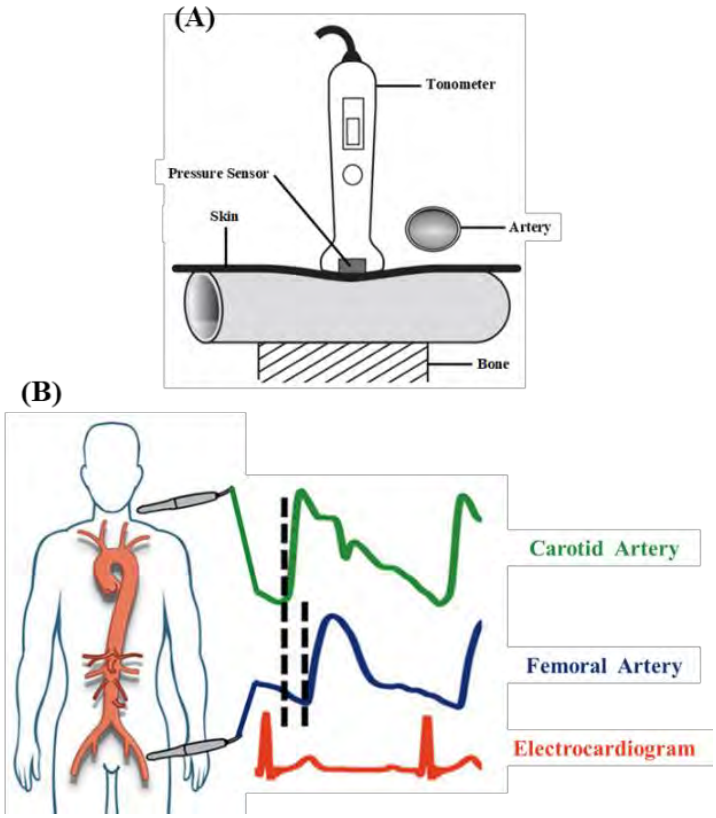


Figure 2 – Applanation tonometry and aortic pulse wave velocity. (A) This technique flattens the artery between the pressure sensor and the bone and collects high fidelity waveforms. For the estimation of ascending aortic blood pressure radial artery applanation tonometry is most often used as it can be assured that the artery is being flattened with bone underneath, which is not the case in the brachial artery. These high-fidelity radial artery waveforms (see Figure 1) are then analysed using a generalised inverse transfer function, for example within the SphygmoCor device (AtCor Medical), to estimate the ascending aortic waveform for subsequent determination of aortic pressure. **(B)** The measurement of carotid to femoral pulse wave velocity, often termed aortic pulse wave velocity (aPWV), is the gold-standard method to assess aortic stiffness. To do this, the technique of applanation tonometry is used to record arterial waveforms from the carotid and femoral arteries, respectively. With subsequent determination of the time between the peak of the R wave and the foot of the carotid and femoral waveforms, it is possible to calculate the time difference between the two waveforms. Along with the measured distance between the two arterial sites it is possible to calculate the velocity of the pulse waveform through the aorta using the following equation: Velocity = Distance/Time. Pulse wave velocity (PWV) is inversely related to blood vessel distensibility, therefore the higher the PWV the higher the blood vessel stiffness (See further details in Figure 4). *Panels A and B are adapted from Nichols et al., (2011) and Chirinos et al. (2019), respectively.*

2.2.2 The aortic pressure waveform

Aortic blood pressure is influenced by two principal interactions which occur during cardiac systole. In a compliant aorta there is one pressure peak in systole (Figure 3A); however, with increasing aortic stiffness a second clear systolic peak occurs (Figure 3B). These pressures are referred to Pressure 1 (P1) and P2, respectively. This difference in aortic waveform morphology with arterial stiffening occurs due to systolic pressure augmentation (McEniery *et al.*, 2005), as measured by augmentation pressure (AP) and index (AIx). These indices quantify the magnitude of the increase in aortic systolic blood pressure (aSBP) caused by systolic pressure augmentation.

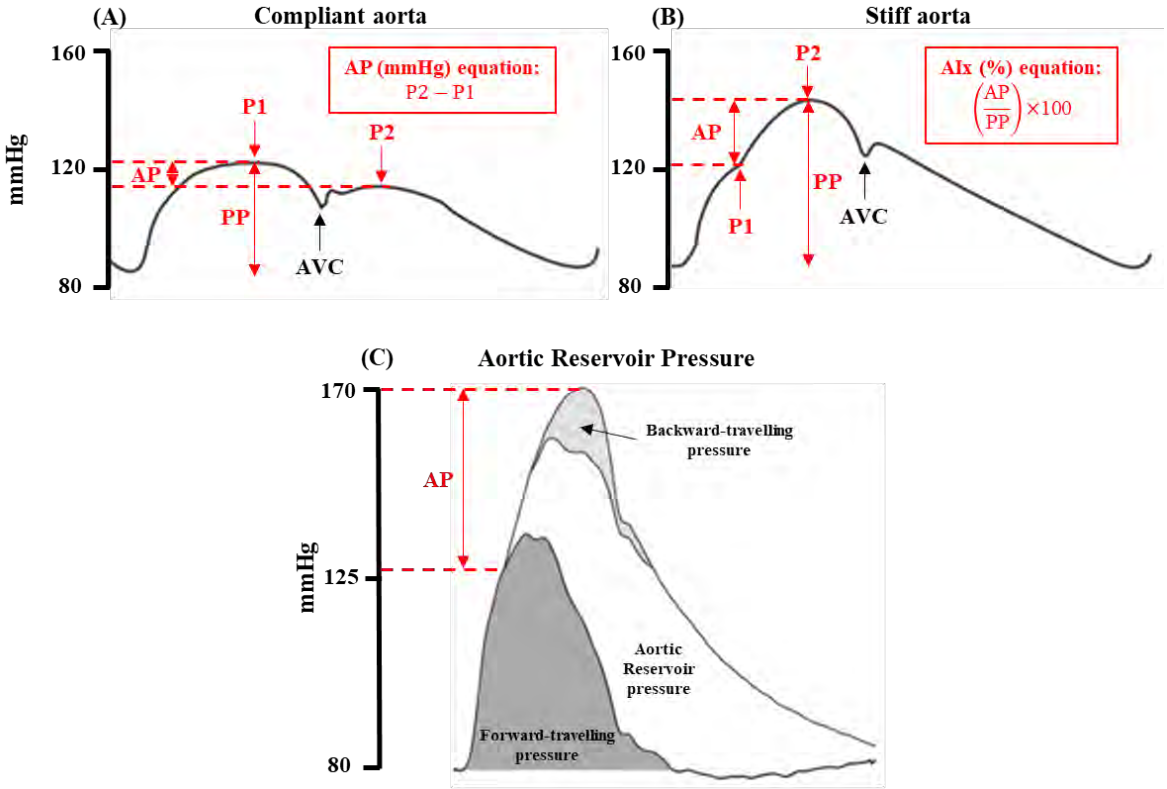


Figure 3 – Ascending aortic blood pressure waveform. (A-B) These panels represent the effects of differences in aortic stiffness on aortic blood pressure and systolic pressure augmentation. Augmentation pressure (AP) and index (AIx) quantify systolic pressure augmentation; the equations to calculate AP and AIx are shown in boxes within panels A and B, respectively. As outlined in text, P1 is taken as aortic systolic blood pressure (aSBP) in panel A, whereas P2 is recorded as aSBP in panel B, due to positive systolic pressure augmentation. Aortic diastolic pressure is determined from the nadir of the waveform immediately prior to ventricular ejection which leads to the upstroke of the pressure waveform. Aortic valve closure (AVC) is noted at the incisura, which represents the end of ventricular ejection. **(C)** This panel outlines the contribution of aortic reservoir pressure and backward-travelling pressure to systolic pressure augmentation in a patient with high aortic systolic blood pressure. *Abbreviations: P, pressure. Panels A-B are redrawn from Nichols et al. (2011) and panel C is redrawn from Davies et al. (2010).*

In a compliant aorta there is often little or no systolic pressure augmentation and P1 is recorded as aSBP (Figure 3A). P1 is primarily influenced by the interaction between the forward-travelling pressure (Figure 3C), from left ventricular ejection, and ascending aortic stiffness (Nichols *et al.*, 2008) and is referred to as non-augmented aSBP in this thesis. In a compliant (young) aorta, the second pressure peak, P2, is recorded in diastole (Figure 3A). However, with aortic stiffening P2 occurs in systole, and only in this instance is recorded as aSBP (Figure 3B). P2 is primarily influenced by aortic reservoir pressure (Figure 3C); accordingly, the aortic reservoir pressure is the major determinant of systolic pressure augmentation (Davies *et al.*, 2010). The aorta acts as a reservoir which fills and empties with each cardiac cycle. Specifically, during systole stroke volume is ejected from the left ventricle which distends the aorta thereby increasing aortic volume as, within systole, aortic inflow is greater than aortic outflow. Approximately 40% of this ejected stroke volume is stored in the distended central elastic arteries (Wang *et al.*, 2003); this ability to distend and store stroke volume characterises the function of the aortic reservoir. The increase in aortic volume increases the reservoir volume and pressure until aortic valve closure. Thereafter, the aorta recoils during diastole which lowers reservoir volume and pressure, respectively, via the outflow of the stored stroke volume (Schultz *et al.*, 2014). Indeed, aortic stiffening, which reduces reservoir function, leads to a faster increase in the aortic reservoir pressure for a similar stoke volume, which shifts P2 from diastole (Figure 3A) into systole (Figure 3B). This shift of P2 into systole leads to systolic pressure augmentation (Davies *et al.*, 2010). When the forward-travelling left ventricular ejection wave arrives at sites of impedance mismatch, which include arterial branch points or changes in artery wall composition (e.g. elastic aorta to muscular femoral artery), the backward-travelling pressure returns towards the heart arriving in systole (Baksi *et al.*, 2009). This backward-travelling pressure exceeds the reservoir pressure thereby increasing P2 further (Figure 3C) which again increases aSBP.

2.2.3 Considerations when assessing aortic haemodynamics

When assessing aortic stiffness it is important to adjust aPWV for major confounding variables, especially blood pressure (Townsend *et al.*, 2015). Statistical adjustment of aPWV for blood pressure is necessary due to the relationship between blood pressure and pulse wave velocity (Figure 4). As outlined recently (Van Bortel *et al.*, 2020), not appropriately adjusting aPWV can lead to "erroneous conclusions" regarding the effects of an intervention or the presence of between-group differences. With higher blood pressure the arteries distend, due to the pressure-area relationship (Figure 4), which increases arterial wall tension thereby increasing the velocity at which the pulse wave travels through the artery (Lacolley *et al.*, 2017). Thus, adjusting aPWV for the static component of blood pressure, that being MAP, as opposed to the dynamic components of arterial pressure, SBP and DBP, is most appropriate as MAP is not principally determined by arterial stiffness itself (Van Bortel *et al.*, 2020). In addition, independently of changes in blood pressure, increases in heart rate elicit significant increases in aPWV (0.16 $m \cdot s^{-1}$ per 10bpm) and has therefore been recommended to be included as a covariate when comparing aPWV between groups (Tan *et al.*, 2016a; Tan *et al.*, 2016b). The correction for heart rate is especially relevant when heart rate differs between groups. Together, the appropriate adjustment of aPWV for blood pressure and heart rate are of real importance so "erroneous conclusions" are not made.

In addition to adjusting aPWV, it is also important to adjust AP and AIx. Both measures of systolic pressure augmentation are adjusted to a heart rate of 75bpm by the SphygmoCor device (AtCor Medical); this device is focused upon as it is the method utilised in this thesis. This adjustment is made based upon the findings from Wilkinson and colleagues (2000; 2002), whereby increases in heart rate, achieved via right atrial pacing, decreased AIx. The adjustment which the SphygmoCor makes for heart rate is based upon the linear regression slopes for heart rate and AIx generated from the two previous studies by Wilkinson and colleagues.

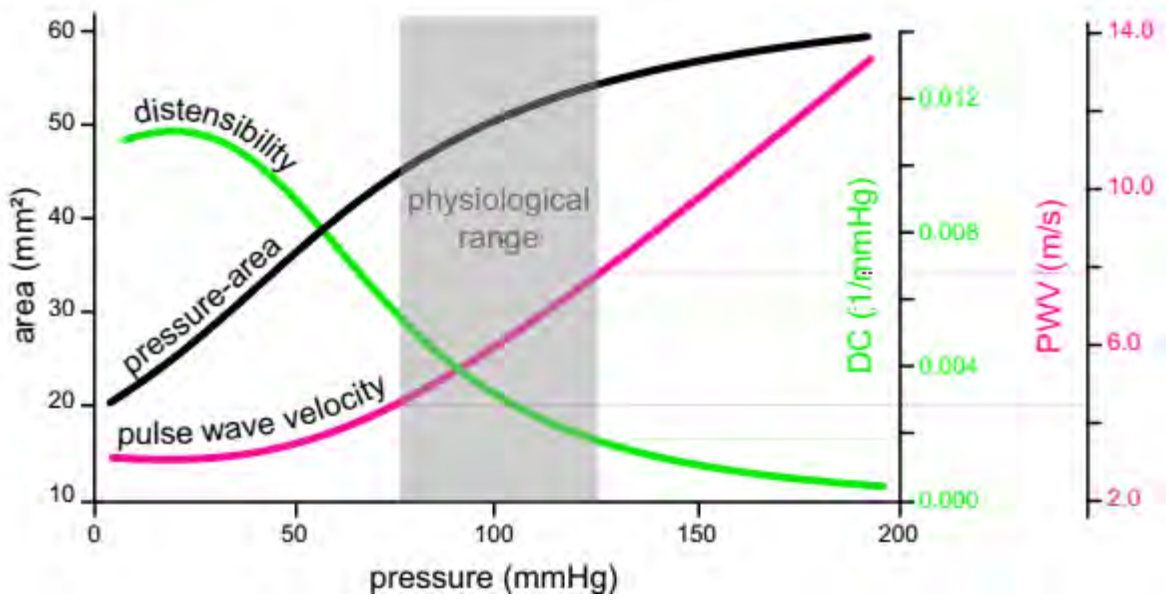


Figure 4 – Mean arterial blood pressure relation to artery cross-sectional area, distensibility and pulse wave velocity. This figure depicts the influence of blood pressure on the assessment on arterial stiffness. The change in slope of the pressure-area relationship with increasing blood pressure is mediated by the transfer of arterial load from the elastin to the less compliant collagen within the arterial wall. Accordingly, when assessing aortic pulse wave velocity, which is related to the pressure-area curve, it is necessary to adjust aPWV for the mean arterial pressure. *Figure adapted from Lacolley et al., (2017).*

However, these studies included individuals with *in situ* cardiac pacemakers (Wilkinson *et al.*, 2000) or patients prior to investigation for cardiac dysrhythmia or after diagnostic coronary angiography (Wilkinson *et al.*, 2002). Accordingly, the data used in this adjustment may not be appropriate for the apparently healthy individual (Stoner *et al.*, 2014). Another limitation of this method when using the SphygmoCor is that the adjustment of AP and AIx for heart rate can only be calculated if the heart rate is between 40-110bpm. In the general population this is likely not a limitation; however, when studying those with bradycardia (< 40 bpm), for example endurance-trained individuals, this adjustment of AP and AIx to 75 bpm cannot be carried out. Therefore, in line with recommendations of Stoner and colleagues (2014), a more appropriate method may be to adjust AP and AIx by including heart rate as a covariate in a statistical model. Furthermore, body height (Smulyan *et al.*, 1998), and MAP (Wilkinson *et al.*, 2001) also influence AP and AIx. Accordingly, including these parameters

as covariates in a statistical model is likely the most appropriate method to appropriately adjust AP and AIx (Stoner *et al.*, 2014) for between-group comparisons.

Interim summary

Together, aortic stiffness plays a primary role in determining aortic reservoir function, which, along with backward-travelling pressure, influences aortic blood pressure. The assessment of the aortic haemodynamic profile, that is aortic stiffness, systolic pressure augmentation and pressure, provides a comprehensive understanding of central haemodynamics. Aortic stiffness (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014) and systolic pressure augmentation (Wang *et al.*, 2010) and pressure (Roman *et al.*, 2007; Roman *et al.*, 2009; Vlachopoulos *et al.*, 2010) are all predictive of cardiovascular morbidity and/or mortality. Due to the ability to predict cardiovascular risk, it is important to further understand the effect of lifestyle factors, such as habitual exercise, on aortic haemodynamics which could reduce cardiovascular risk. Thus, in this thesis, aortic stiffness, aortic systolic pressure augmentation and aortic blood pressure will be assessed and reported together to provide insight into the effects of chronic habitual endurance exercise on central haemodynamics. The known effects of age and habitual exercise in middle-aged and older individuals are outlined below; attention is drawn to whether the appropriate adjustments were conducted in these studies. Some studies to date have assessed the central arterial waveform "directly" at the carotid artery, but this waveform differs to that of the ascending aorta with greater systolic pressure augmentation at the carotid artery (Nichols *et al.*, 2011). Therefore, focus will be primarily drawn to studies assessing the ascending aortic waveform and associated aortic haemodynamics.

2.2.4 Effects of age and habitual exercise on aortic haemodynamics

Age

The effects of age on aortic haemodynamics have been well studied and the profile of "normal" vascular ageing has been described previously, see Figure 5 (McEniery *et al.*, 2005). One key change that occurs in middle-age (40-65 years of age) is the shift towards a reversal of the arterial stiffness gradient. The change in artery wall composition (i.e. lower elastic content in peripheral vessels) along with the decrease in arterial calibre explain the increase in arterial stiffness from central to peripheral blood vessels (Fortier and Agharazii, 2016). With ageing aortic stiffness increases but muscular artery stiffness remains relatively similar (McEniery *et al.*, 2005); this reduction in the arterial stiffness gradient contributes to the reduction in PPA with age (Herbert *et al.*, 2014). Although suggested to play an important role in the generation of reflected waves, via increasing backward-travelling pressure (Mitchell *et al.*, 2004; Vyas *et al.*, 2007; Nichols *et al.*, 2011), recent evidence shows that the arterial stiffness gradient plays little role in systolic pressure augmentation (Schultz *et al.*, 2015; Hickson *et al.*, 2016). However, with age-related aortic stiffening the reservoir pressure increases as the aorta is less able to distend and store left ventricular stroke volume in systole, thereby increasing aortic blood pressure. In other words, for the same left ventricular stroke volume the aortic reservoir pressure will be higher with increasing aortic stiffness, which increases systolic pressure augmentation (Davies *et al.*, 2010). Specifically, the age-related increase in aortic stiffness (i.e. aPWV; Figure 5A) increases the first (P1) and second (P2) systolic shoulders of the aortic waveform (Figure 3B). P1 increases due to the faster velocity of the left ventricular ejection wave due to increased ascending aortic stiffness. Whereas, P2 increases (i.e. increased systolic pressure augmentation; Figure 5B) due to higher aortic reservoir pressure. Together, aSBP and aPP increase with age whilst aDBP and aMAP remain constant until older age (65+ years) where they begin to decrease

(Herbert *et al.*, 2014). Together, ageing exerts marked effects on both aortic stiffness and systolic pressure augmentation, which contribute to the increases in aSBP and aPP.

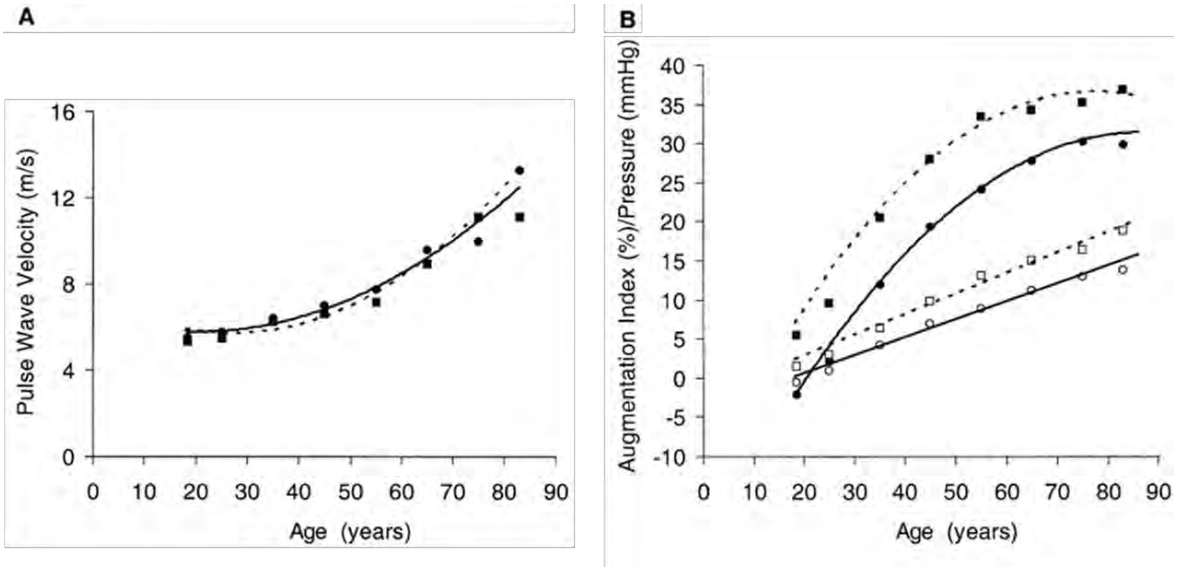


Figure 5 – The effects of age on aortic pulse wave velocity and systolic pressure augmentation. **(A)** With age aortic pulse wave velocity (aPWV) remains relative stable until ~55 years of age, thereafter the increase becomes more linear. However, **(B)** systolic pressure augmentation increases linearly with age, as indexed by augmentation pressure (open shapes) rather than augmentation index (closed shapes). *Figure adapted from McEniery et al. (2005).*

Habitual Endurance Exercise

Despite the well described benefits of chronic habitual endurance exercise on physiological function (Tanaka *et al.*, 2019), the effects on the aortic haemodynamic profile remains relatively understudied. To date, most research studies investigating the effects of habitual exercise have focused upon aortic stiffness (Seals *et al.*, 2019). Vaitkevicius and colleagues (1993) first reported that middle-aged/older endurance-trained athletes (> 54 years of age) had lower aPWV (assessed via doppler flow probes) and AIx (determined from the carotid arterial waveform) compared to age-matched sedentary individuals (Figure 6). However, in this study (Vaitkevicius *et al.*, 1993), neither aPWV nor AIx were appropriately adjusted for the respective confounding factors mentioned above.

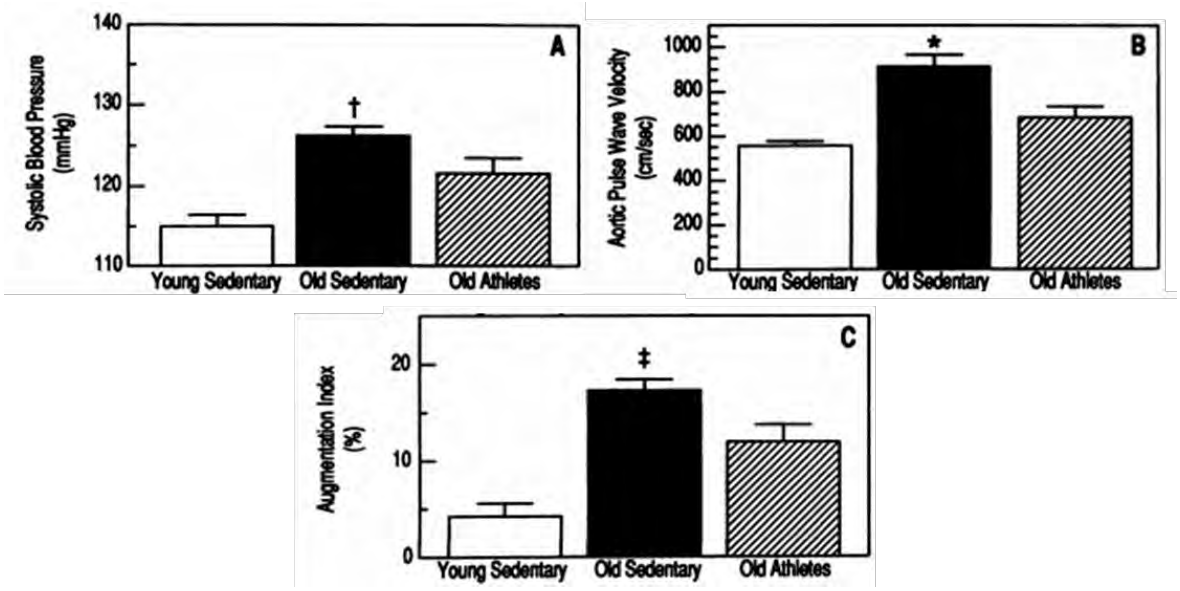


Figure 6 – The effects of chronic endurance training on brachial systolic blood pressure, aortic pulse wave velocity and augmentation index. Group mean data are presented with error bars representing the standard error of the mean (SEM). **(A)** Brachial systolic blood pressure was higher in old compared to young sedentary men ($P < 0.01$), with no other between-group differences. **(B)** Aortic pulse wave velocity was higher in old sedentary men compared to both other groups ($P < 0.0001$). **(C)** Augmentation index was different between all groups ($P < 0.0001$). *Figure adapted from Vaitkevicius et al., (1993).*

Subsequent studies have confirmed the findings of Vaitkevicius and colleagues (1993), showing lower aPWV in middle-aged/older endurance-trained compared to untrained individuals (Gates *et al.*, 2003; McDonnell *et al.*, 2013; Tarumi *et al.*, 2013). Notably, only one of these studies adjusted aPWV for blood pressure (brachial MAP) (McDonnell *et al.*, 2013). However, this study compared groups of active and sedentary middle-aged/older individuals, whom had high-normal blood pressure (average SBP of > 130 mmHg) in both groups. Furthermore, the adjustment for brachial MAP may not be appropriate in the presence of a significant difference between aMAP and MAP; furthermore, adjusting aPWV for aMAP is likely more physiologically appropriate. However, as outlined above, it is necessary to adjust aPWV for both aMAP and heart rate.

Systolic pressure augmentation (AP and AIx) has been shown to be lower (Vaitkevicius *et al.*, 1993) or similar between middle-aged/older endurance-trained and sedentary individuals when assessed at the carotid artery (Tarumi *et al.*, 2013). Only one

study to date has assessed aortic systolic pressure augmentation and reported that AIx (adjusted for heart rate and height) was similar between middle-aged/older active and sedentary individuals (McDonnell *et al.*, 2013). This study reported that aSBP was lower in active individuals (McDonnell *et al.*, 2013), however both groups included individuals with high-normal blood pressure. Together, there is no study to date which comprehensively characterises the effects of chronic habitual endurance exercise on the aortic haemodynamic profile in normotensive middle-aged individuals. Furthermore, no study has appropriately adjusted aPWV and AP/AIx for confounding factors. Despite this, it is currently well accepted that habitual endurance exercise lowers aortic stiffness throughout the lifespan. Also, the majority of studies to date have compared endurance-trained and sedentary individuals, notwithstanding the negative cardiovascular consequences of sedentarism (Lavie *et al.*, 2019). The effects of habitual endurance exercise on aortic haemodynamics may therefore have been exaggerated previously. Whether habitual endurance exercise effects the aortic haemodynamic profile when compared to recreationally-active individuals, is currently unclear.

2.2.4 Summary on aortic haemodynamics

This section of the literature review highlights the clinical relevance of aortic haemodynamics and outlines that the aortic haemodynamic profile needs to be reported. In addition, studies should complete the appropriate adjustments of aortic stiffness and systolic pressure augmentation. Due to the aortic haemodynamic indices being predictive of cardiovascular morbidity and/or mortality, it is important to determine whether, or not, habitual endurance exercise effects the aortic haemodynamic profile in healthy middle-age. This is due to the increase in aortic stiffness and pressure becoming more linear thereafter, thereby increasing cardiovascular risk. Selecting the appropriate control group and appropriately adjusting aPWV and AP and AIx will provide novel information regarding the effects of chronic habitual endurance exercise on the aortic haemodynamic profile.

2.3 The regulation of peripheral blood pressure

As outlined in section 2.1, blood pressure differs between the central elastic arteries and the peripheral vasculature due to PPA. Central blood pressure is influenced by the interaction of stroke volume and the aorta; however, the autonomic regulation and neural control of the heart and peripheral vasculature determine peripheral blood pressure with each cardiac cycle. The control of peripheral blood pressure primarily occurs within the muscular resistance vessels, via changes in vascular sympathetic activity.

The autonomic nervous system is the primary controller of the cardiovascular system on a beat-by-beat basis. Through reflex-mediated changes in efferent outflow of the sympathetic and parasympathetic limbs of the peripheral nervous system, the cardiovascular control centres within the brainstem regulate peripheral blood pressure via changes in total peripheral resistance and cardiac interval, respectively. These reflex (i.e. feedback) arcs include: i) the arterial baroreflex; ii) the arterial chemoreflex; iii) baroreflexes from within the heart and pulmonary circulation (erroneously referred to as the "cardiopulmonary baroreflex"); and iv) the skeletal muscle mechanoreflex and metaboreflex (Kaufmann *et al.*, 2020). Furthermore, central command, a feedforward control mechanism (i.e. not a reflex responding to a peripheral error signal) controlled by the brainstem, contributes to the regulation of cardiac interval immediately prior to and during exercise (Williamson, 2010), thereby influencing the level of sympathetic nervous system activity quantified per unit time (as discussed later). Of these autonomic regulatory pathways, the arterial baroreflex is the primary reflex arc involved in the regulation of arterial pressure (Benarroch, 2008). Both at rest and during physiological stress, these neural arcs work to "fine-tune" blood pressure via changes in total peripheral resistance and cardiac output. The main efferent pathway that contributes to blood pressure control is the sympathetic nervous system (SNS), which innervates the arterial and venous circulation; reflex changes in vascular resistance contribute most to changes in blood pressure at rest (Herring and Paterson, 2018). The SNS

also innervates the heart. However, at rest it is primarily changes in cardiac parasympathetic (cardiovagal) nervous system activity which influence cardiac interval; changes in cardiac interval play a secondary role in the modulation of blood pressure at rest and during physiological stress (Ogoh *et al.*, 2003a).

The appropriate reflex control of vascular sympathetic activity and the ability of sympathetic nerves to elicit vascular smooth cell contraction (i.e. sympathetic vascular transduction) are fundamental to the autonomic regulation and neural control of peripheral blood pressure. The focus of this thesis, in relation to peripheral blood pressure regulation, is on: i) the arterial baroreflex control of vascular sympathetic activity and, ii) efferent neurotransmission in the vasculature, termed sympathetic vascular transduction. An overview of these two key contributors to the regulation and control of peripheral blood pressure is provided below, before the known effects of age and habitual exercise are reviewed.

2.3.1 Assessment of the autonomic nervous system in humans

Vascular sympathetic activity

It has been possible to directly record efferent sympathetic activity to the skeletal muscle vasculature in conscious humans since the technique of sympathetic microneurography was developed by Vallbo and Hagbarth (1967). The microneurographic technique involves the insertion of tungsten microelectrodes into peripheral nerves to record sympathetic action potentials. Recording sympathetic outflow to the skeletal muscle circulation (termed muscle sympathetic nerve activity [MSNA]) is important as it is the largest vascular bed in the body and therefore plays a major role in the control of blood pressure (Dornhorst, 1963). Microneurography was already an established technique used to investigate the fusimotor system, which is the neural arc that controls muscle spindles (Hagbarth and Macefield, 1995). The initial recordings of vascular sympathetic activity were thought to be "artefact" or "gamma efference" within the signal (Vallbo *et al.*, 2004). However, following further

investigation in the same laboratory, it was determined that this "artefact" was indeed efferent sympathetic outflow in unmyelinated C-fibres (Torebjork and Hallin, 1974). Therefore, it was possible to record efferent sympathetic outflow directed to the vasculature within the skin or skeletal muscle (Hagbarth and Vallbo, 1968), termed skin or muscle sympathetic nerve activity, respectively. Skin sympathetic nerve activity is the primary regulator of cutaneous vasomotor tone, which has a marked influence on sweating and therefore thermoregulation (Greaney and Kenney, 2017). MSNA is principally vasoconstrictor and plays a pivotal role in blood pressure control, due to its influence on vascular resistance. Herein, as the skeletal muscle vasculature plays a greater role in blood pressure regulation compared to the cutaneous circulation, this thesis will only focus upon MSNA. Notably, the term "burst(s)" will be used throughout this thesis when referring to the interpretation of MSNA data. The technique of sympathetic microneurography and associated data analyses are outlined in further detail within Chapter 5. Briefly, however, raw MSNA neurograms are recorded and then, following amplification and integration, MSNA bursts are the peaks identified within the integrated (i.e. mean voltage) neurogram. MSNA bursts represent the sum of all sympathetic action potentials occurring during each cardiac cycle, as recorded within the raw neurogram (see Chapter 5, Figure 22C). To quantify vascular sympathetic activity, MSNA bursts are counted and indexed as a percentage of the number of cardiac cycles (MSNA burst incidence or probability) and per unit time (MSNA burst frequency) (Charkoudian and Wallin, 2014).

Parasympathetic (cardiovagal) nerve activity

The first direct microneurographic recordings of cardiovagal outflow in conscious humans were published by Professor Vaughan Macefield and colleagues in 2020 (Ottaviani *et al.*, 2020). This is a significant advance for the study of integrative human cardiovascular control, as it is now possible to conduct simultaneous direct recordings of cardiovagal and vascular sympathetic activity in conscious humans. To date, however, several other indices

of cardiovagal activity have been used. These include: i) pharmacological blockade of cardiac muscarinic receptors to subsequently determine cardiovagal tone (i.e. the increase in heart rate from baseline on blockade; see Figure 7), ii) beat-to-beat heart rate variability (HRV) to determine the cardiovagal modulation of heart rate, iii) the recovery of heart rate post-exercise to determine cardiovagal reactivation and iv) reflex-mediated changes in heart rate or cardiac interval in response to changes in SBP, to determine cardiovagal responsiveness (Chapleau and Sabharwal, 2011). These indices all provide different information on cardiovagal activity, and the methods and measurements of all are reviewed extensively elsewhere (Chapleau and Sabharwal, 2011; Gourine and Ackland, 2019). As the focus of this thesis is on the autonomic regulation of blood pressure, cardiovagal responsiveness will be assessed. In this thesis cardiovagal responsiveness will be determined using the sequence method which quantifies the arterial baroreflex control of cardiac interval. This technique was first utilised in humans by Parati and colleagues (1988) and has subsequently been used extensively within the literature to interrogate arterial baroreflex control of the heart both at rest and in response to various acute and chronic interventions (Chapleau and Sabharwal, 2011). Furthermore, cardiovagal responsiveness has been associated with adverse clinical outcomes in clinical groups, as reviewed previously (La Rovere *et al.*, 2013; Pinna *et al.*, 2015).

Although both limbs of the peripheral nervous system exert control over cardiac interval, it is important to note that the heart has an intrinsic ability to control cardiac interval (and therefore heart rate), at ~600 milliseconds (ms; ~100 bpm) (Katona *et al.*, 1982). This intrinsic cardiac rhythm is primarily regulated by the "funny" (I_f) current within the sinoatrial node which influences the slope of sinoatrial node depolarisation during diastole (Tan and Verrier, 2013). Neither cardiovagal nor cardiac sympathetic activity set cardiac interval *per se*; rather, they exert external influence over the slope of diastolic sinoatrial node depolarisation via inhibition or activation of the I_f current, respectively (DiFrancesco, 2010).

From the intrinsic rate at rest, tonic cardiovagal activity alone reduces heart rate by ~50%; whereas, tonic cardiac sympathetic activity alone can only influence heart rate by ~17% (Katona *et al.*, 1982), as shown in Figure 7. Thus, compared to cardiac sympathetic activity, cardiovagal activity has a larger influence on resting heart rate. However, during physiological stress (e.g. exercise) cardiac sympathetic activity increases, with reciprocal decreases in cardiovagal activity, and becomes the primary modulator of cardiac interval and therefore heart rate (White and Raven, 2014).

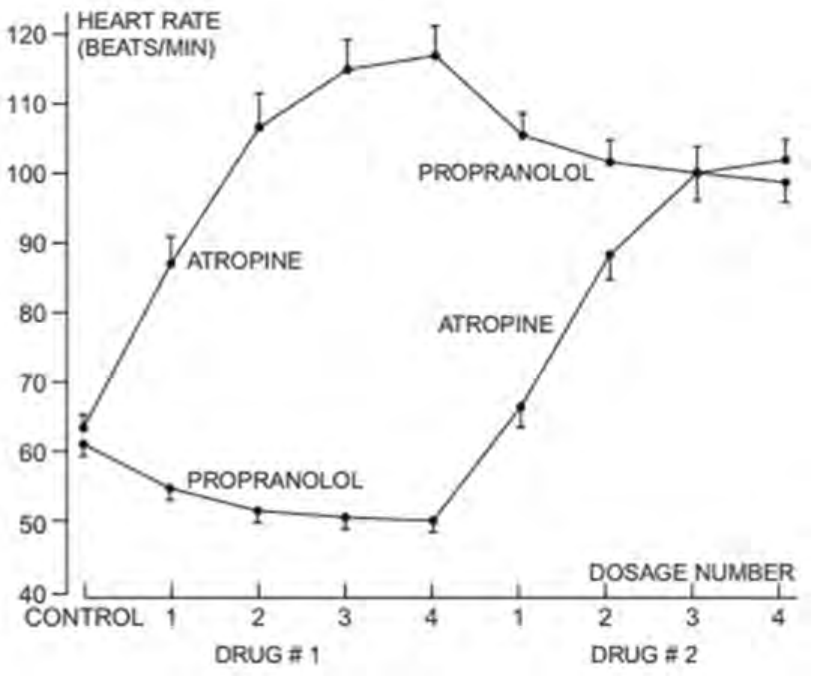


Figure 7 – Intrinsic heart rate. Resting heart rate, independent of cardiac autonomic tone, is termed the intrinsic heart rate and can be assessed following pharmacological blockade of both the parasympathetic and sympathetic limbs of the autonomic nervous system. Following incremental doses of atropine (parasympathetic/cardiovagal blockade) heart rate increases; whereas, with incremental doses of propranolol (cardiac sympathetic blockade) heart rate decreases. This figure demonstrates that, at rest, parasympathetic tone exerts a greater influence on heart rate, via the mechanisms explained within text. *Figure adapted from Katona et al. (1982).*

In this thesis MSNA and cardiovagal responsiveness will be assessed to index the vascular sympathetic and cardiovagal regulation of blood pressure. How these indices relate to the arterial baroreflex are outlined in the next section.

2.3.2 The arterial baroreflex

Baroreceptors are located throughout the central circulation to "sense" and "defend" arterial blood pressure (Figure 8), but all act via different pathways exerting principle influence on either vascular sympathetic or cardiovagal activity (Hainsworth, 2014). The arterial baroreflex is principally a negative-feedback (i.e. an increase in one signal leads to a decrease in another) controller of the cardiovascular system (Chapleau, 2012) and is best described as a multi-input, multi-output and multilevel control system (Sagawa, 1983). There has been some suggestion that this reflex arc contributes to the long-term control of blood pressure (Thrasher, 2004; 2005); however, much more is known about the short-term, beat-by-beat control of blood pressure by the arterial baroreflex. Only the short-term arterial baroreflex control is focused upon within this thesis.

There are three principle components of the arterial baroreflex arc that are essential to ensuring appropriate regulation of blood pressure: (1) Mechanical deformation of barosensory arteries leading to changes in afferent nerve activity, termed mechanosensory transduction; (2) central processing (described in Figure 8); and (3) transduction of efferent autonomic outflow to end-organ responses (i.e. heart and vasculature), termed efferent neurotransmission. Upon left ventricular ejection the stroke volume distends the aortic arch, and shortly thereafter, the carotid sinus; the two primary baroreceptors regions. Upon vascular stretch in systole and recoil in diastole, there are respective increases and decreases in afferent nervous system activity mediated by mechanically sensitive ion channels (Zeng *et al.*, 2018). An increase in baroreceptor afferent activity leads to vascular sympathetic neural silence and a concomitant increase in cardiovagal activity, and vice-versa. As outlined in Figure 8, there is complex circuitry within the brainstem which processes the afferent signal and then determines the reflex response. This reflex response involves changes in vascular sympathetic and cardiovagal activity to elicit the appropriate reflex change in blood pressure.

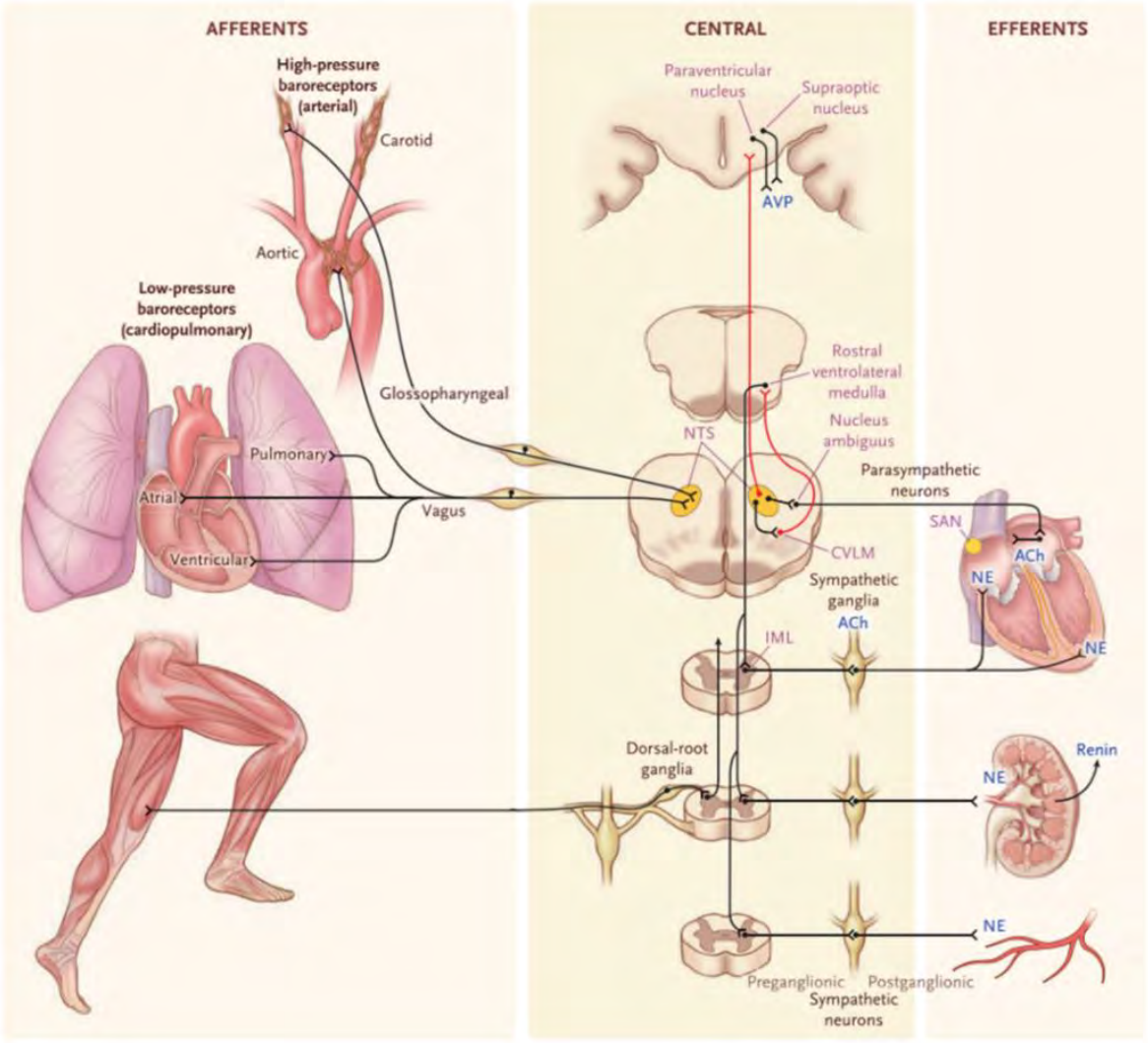


Figure 8 – Autonomic reflex arcs. Arterial, cardiac and pulmonary (erroneously termed cardiopulmonary in the figure) baroreceptors send afferent signals via the glossopharyngeal and vagus nerves, respectively. These nerves synapse at the nucleus of the tractus solitarius (NTS) within the brainstem. Upon receiving this afferent feedback, the NTS elicits three primary responses: i) The first is the reduction of efferent vascular sympathetic activity; this is the vascular sympathetic limb of the arterial baroreflex. This reflex acts via signalling through the caudal (CVLM) and rostral ventrolateral medulla (RVLM) regions. The RVLM is the principle controller of efferent sympathetic outflow. Axons descend from the RVLM through the intermediolateral cell column (IML) in the spinal cord and signal through the preganglionic and postganglionic nerves whereby the reduction in vascular sympathetic activity will elicit vasodilation (via reductions in noradrenaline release) within the skeletal muscle, mesenteric or renal vascular beds. In turn, vascular resistance and blood pressure will fall. ii) The second response to increased afferent baroreceptor activity is to increase efferent cardiovagal activity which acts to lengthen the cardiac interval, thereby reducing heart rate; this is the cardiovagal limb of the arterial baroreflex. This efferent response is transmitted through to the nucleus ambiguous (NA) along preganglionic neurons which subsequently increase postganglionic parasympathetic efferent activity to the sinoatrial node (SAN) which, via increasing acetylcholine (ACh), lengthens cardiac interval. iii) The third mechanism by which the baroreflex modulates blood pressure is via reducing circulating arginine vasopressin (AVP; a potent vasoconstrictor hormone) released from the pituitary, via a direct connection between the NTS and the supraoptic and paraventricular nuclei in the hypothalamus. Separate to the baroreflexes, thinly myelinated group III and IV muscle afferents, which are mechanically and metabolically sensitive, respectively, are also shown here. They synapse in the dorsal root ganglia and travel through the spinal cord as excitatory inputs to increase vascular sympathetic activity and decrease cardiac interval. These muscle afferents are of relevance for study 3 in this thesis. *Figure adapted from Kaufmann and colleagues (2020).*

2.3.3 Assessment of arterial baroreflex function

The function of the arterial baroreflex is characterised by three main features: 1) operating pressure, 2) operating point and 3) reflex gain (Figure 9). The resting operating peripheral blood pressure (i.e. the input variable) and point (i.e. the output variable) are determined across a recording period (between 5-10 minutes). DBP and MSNA burst incidence (i.e. burst probability) are the input and output variables, respectively, of the vascular sympathetic baroreflex (Sundlof and Wallin, 1978). For the cardiovagal baroreflex, SBP and R-R interval are the input and output variables, respectively (Smyth *et al.*, 1969). These values show the level of autonomic support of blood pressure at that time; for example, with increasing MSNA burst probability the sympathetic support of blood pressure is greater. Vascular sympathetic and cardiovagal baroreflex gain can be determined via multiple methods. Reflex gain is a measure of how effective that limb of the baroreflex is at regulating blood pressure; in other words, reflex gain provides insight into how well the three principle components of the baroreflex arc (see above) are working. Reflex gain can be assessed for both the vascular sympathetic and cardiovagal limbs of the arterial baroreflex by the relationship between changes in DBP and MSNA burst probability or SBP and R-R interval, respectively (Figure 9).

The most common methodology employed to assess arterial baroreflex gain in the literature, is termed the spontaneous method. This assesses the relationship between spontaneous fluctuations in arterial pressure at rest. Utilising this method enables assessment of arterial baroreflex responsiveness within an individual's resting operating blood pressure range. This differs to the other methods used to determine arterial baroreflex function, including the modified oxford baroreceptor test, the Valsalva manoeuvre, the use of neck pressure/suction or tilt testing, as all other tests incorporate challenges which elicit changes in blood pressure (Carter, 2019). The modified oxford technique is the gold-standard technique to assess arterial baroreflex gain as it can partially open the baroreflex loop

allowing for assessment of baroreflex gain across a wider range of pressures (Charkoudian and Wallin, 2014). The modified oxford technique employs intravenous infusion of sequential doses, separated by 60 seconds, of sodium nitroprusside and phenylephrine which cause a fall and rise in arterial blood pressure, respectively (Ebert and Cowley, 1992). However, both sodium nitroprusside (Hogan *et al.*, 1999; Kienbaum and Peters, 2004) and phenylephrine (Bonyhay *et al.*, 1997) have independent effects on the arterial baroreflex, which are significant limitations of this technique, as reviewed previously (Taylor *et al.*, 2014). Despite this, however, sympathetic baroreflex gain values determined via the spontaneous and modified oxford methods are strongly correlated (Hart *et al.*, 2010). In this thesis vascular sympathetic and cardiovagal baroreflex gain will be determined during spontaneous fluctuations in arterial pressure.

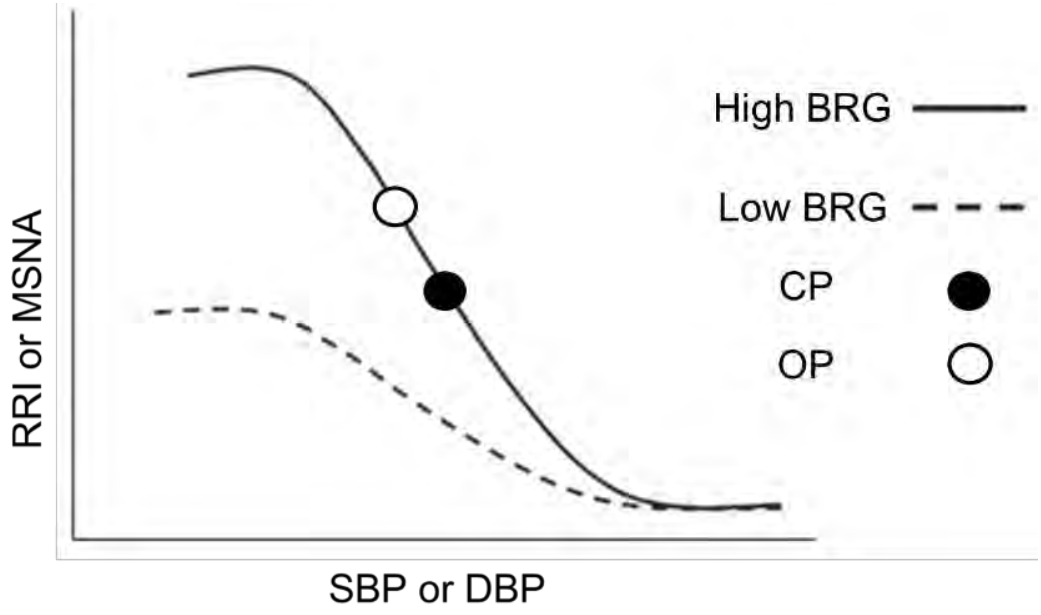


Figure 9 – Assessment of arterial baroreflex function. The slope of the relationship between changes in either systolic (SBP) or diastolic blood pressure (DBP) and R-R interval (RRI) or muscle sympathetic nerve activity (MSNA) is used to index baroreflex gain (BRG). The steeper the slope, the more effective the arterial baroreflex is at regulating the output variable. The centring point (CP) of this relationship is where the gain is the highest; thus, gain is measured in the linear portion of this slope away from the top (saturation) and bottom (threshold) of the sigmoidal baroreflex relationship. The operating point (OP) figuratively indexes the average input and output variables for either the cardiovagal or vascular sympathetic baroreflexes. *Figure adapted from Wehrwein and Joyner (2013).*

Vascular sympathetic baroreflex

When assessing the arterial baroreflex control of MSNA, it has been shown that the vascular sympathetic baroreflex has a stronger influence over the probability of MSNA burst occurrence rather than burst frequency and size, assessed as either burst amplitude or area (Kienbaum *et al.*, 2001). In other words, the vascular sympathetic baroreflex acts as a "gate" which controls whether, or not, bursts of MSNA occur (Keller *et al.*, 2006); thus, when the baroreflex is engaged the "gate" is closed. Furthermore, the occurrence of bursts of MSNA have marked cardiac rhythmicity due to their control by the vascular sympathetic baroreflex. MSNA bursts occur ~1.3 seconds following the R wave of the electrocardiogram (Delius *et al.*, 1972a) as each cardiac cycle involves engagement and disengagement of the arterial baroreflex (Charkoudian and Wallin, 2014). During systole, pressure rises during left ventricular ejection, which engages the arterial baroreflex and inhibits MSNA (i.e. closes the gate); whereas, during diastole pressure falls which disengages the arterial baroreflex and the inhibition of bursts of MSNA is removed, which allows a burst to occur (i.e. the gate opens) (Charkoudian and Wallin, 2014). During spontaneous falls in blood pressure, baroreflex-mediated increases in MSNA act to increase total peripheral resistance and therefore blood pressure.

To index vascular sympathetic baroreflex gain diastolic pressure is most often grouped (or binned) into increments ranging between 1-3 mmHg and plotted against the corresponding MSNA burst probability (Hart *et al.*, 2010). Accordingly, vascular sympathetic baroreflex gain is reported in % per mmHg (%·mmHg^{-1}). Within an individual, lower diastolic blood pressures are associated with higher MSNA burst probability, therefore the relationship is negative (i.e. inverse).

Cardiovagal baroreflex

When assessing cardiovagal baroreflex function both R-R interval and heart rate can be used as the output variable. However, the relationship between R-R interval and heart rate is

inverse and curvilinear (Draghici and Taylor, 2016), which can lead to differences in interpretation of gain especially when heart rate is changing (O'Leary, 1996). Notably, it is R-R interval that is the regulated variable of the cardiovagal limb of the arterial baroreflex. Thus, to determine spontaneous cardiovagal baroreflex gain at rest the sequence method (Parati *et al.*, 1988) is most often used with R-R interval as the output variable. This method identifies three or more sequential baroreflex sequences, where SBP and R-R interval change in the same direction, and regression analyses are performed for each sequence to quantify baroreflex gain. The SBP is regressed against the R-R interval of the same or subsequent cardiac cycle (Smyth *et al.*, 1969), dependent on the baroreflex delay and the prevailing R-R interval. Indeed, if the R-R interval is greater than 800 msec (heart rate of >75 bpm) then the baroreflex exerts its effects on SBP within the same cardiac cycle, or the following cardiac cycle if the R-R interval is lower than 800 msec (heart rate of <75 bpm) (Eckberg and Eckberg, 1982). The average of all slopes recorded is taken to index cardiovagal baroreflex gain and is presented in milliseconds per mmHg ($ms \cdot mmHg^{-1}$). The cardiovagal baroreflex slope is positive, due to the cardiovagal baroreflex sequences being assessed when both SBP and R-R interval change in the same direction.

2.3.4 Efferent neurotransmission in the vasculature

The regulation of vascular tone is dependent on the balance between intra- and extra-vascular control. Circulating vasodilator (e.g. nitric oxide, prostaglandin and adenosine triphosphate) and vasoconstrictor (e.g. endothelin-1, peroxynitrite and thromboxane A_2) factors, and vascular sympathetic activity are the principle intra- and extra-vascular control mechanisms, respectively. When vascular sympathetic activity increases there is a release of noradrenaline, amongst other vasoactive co-transmitters (Burnstock, 2013), which acts to elicit vascular smooth muscle cell contraction thereby increasing vascular resistance and blood pressure. This process is termed sympathetic vascular transduction. Therefore, quantifying the relationship between changes in MSNA and vascular resistance/conductance

or blood pressure provides important insight into efferent neurotransmission in the vasculature, the third principle component of the vascular sympathetic baroreflex arc.

The assessment of sympathetic vascular transduction is now being conducted more often due to increased technical ability with high-resolution vascular ultrasonography and/or greater software processing to analyse the relationship between changes in MSNA and the chosen vascular outcome. Sympathetic vascular transduction has been assessed both at rest and in response to various physiological stimuli. Initially, sympathetic vascular transduction was assessed at rest with the vascular outcome being the time taken from a spontaneous burst to the peak change in DBP; an increase of 2-3 mmHg occurred ~5.5 seconds following sympathetic burst occurrence in healthy individuals (Wallin and Nerhed, 1982). 30 years later, Vianna and colleagues (2012) reassessed sympathetic vascular transduction at rest using a similar approach but used MAP and total vascular conductance as the vascular outcomes. Subsequently many groups have employed this approach to determine the effects of various factors/interventions on sympathetic vascular transduction (Fairfax *et al.*, 2013; Briant *et al.*, 2016; Vranish *et al.*, 2018; Babcock *et al.*, 2019; Hissen *et al.*, 2019; Robinson *et al.*, 2019; Steinback *et al.*, 2019). The assessment of sympathetic vascular transduction at rest enables the assessment of the relationship between temporal changes in MSNA and vascular tone.

Another approach to determine sympathetic vascular transduction is to elicit larger increases in MSNA and study the vascular response. This method enables the determination of the relationship between changes in mass sympathetic discharge and vascular responses, as first conducted using linear regression analyses by Halliwill and colleagues (1996), see Figure 10. To elicit large increases in MSNA, groups have used the following stimuli: i) static hand grip exercise (SHG) (Halliwill *et al.*, 1996; Jarvis *et al.*, 2011; Tan *et al.*, 2013; Hissen, 2019; Engelland *et al.*, 2020); ii) the cold pressor test (CPT) (Jarvis *et al.*, 2011; Usselman *et al.*, 2015) and, iii) lower body negative pressure (LBNP) (Ray and Monahan,

2002; Notarius *et al.*, 2012; Stuckless *et al.*, 2020). When assessing sympathetic vascular transduction in this manner (Figure 10), MSNA is indexed using either burst frequency (number of bursts per minute), burst amplitude/area, or total activity (bursts per minute x average amplitude/area). This is due to the time-dependent manner of the responses being assessed, as these stressors lead to graded increases in MSNA throughout the stimulus. As well as using the correct index of MSNA (i.e. not MSNA burst incidence which is used to study the vascular sympathetic baroreflex) it is important to use the appropriate index of the vascular and/or pressure response when quantifying sympathetic vascular transduction. In the vasculature, local (forearm) and systemic (total) vascular resistance and conductance can be calculated from arterial pressure and flow. From simultaneous recording of MAP and forearm blood flow (FBF) both forearm vascular resistance (FVR; [MAP/FBF]*100, $100mmHg^{-1}\cdot ml\cdot min^{-1}$) and conductance (FVC; [FBF/MAP]*100, $ml\cdot min^{-1}\cdot 100mmHg^{-1}$) can be calculated.

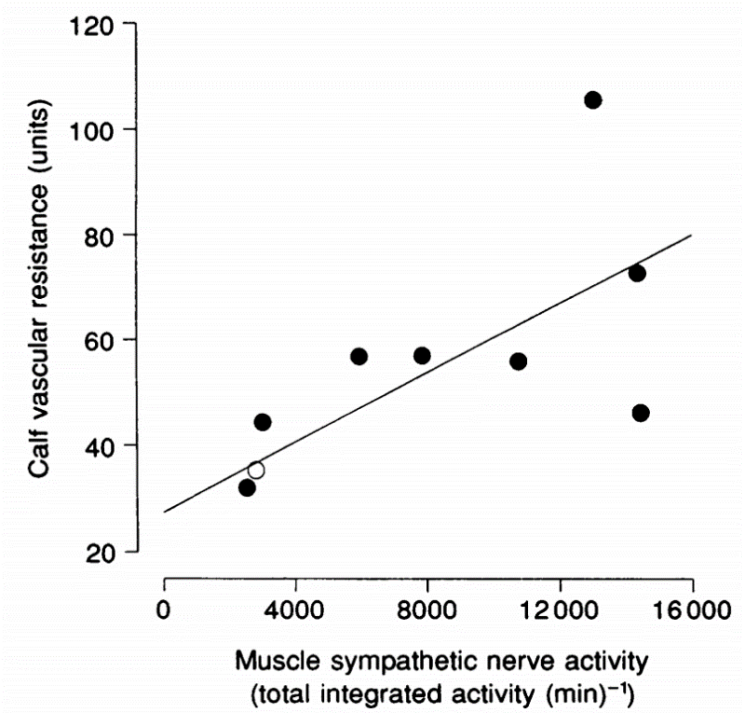
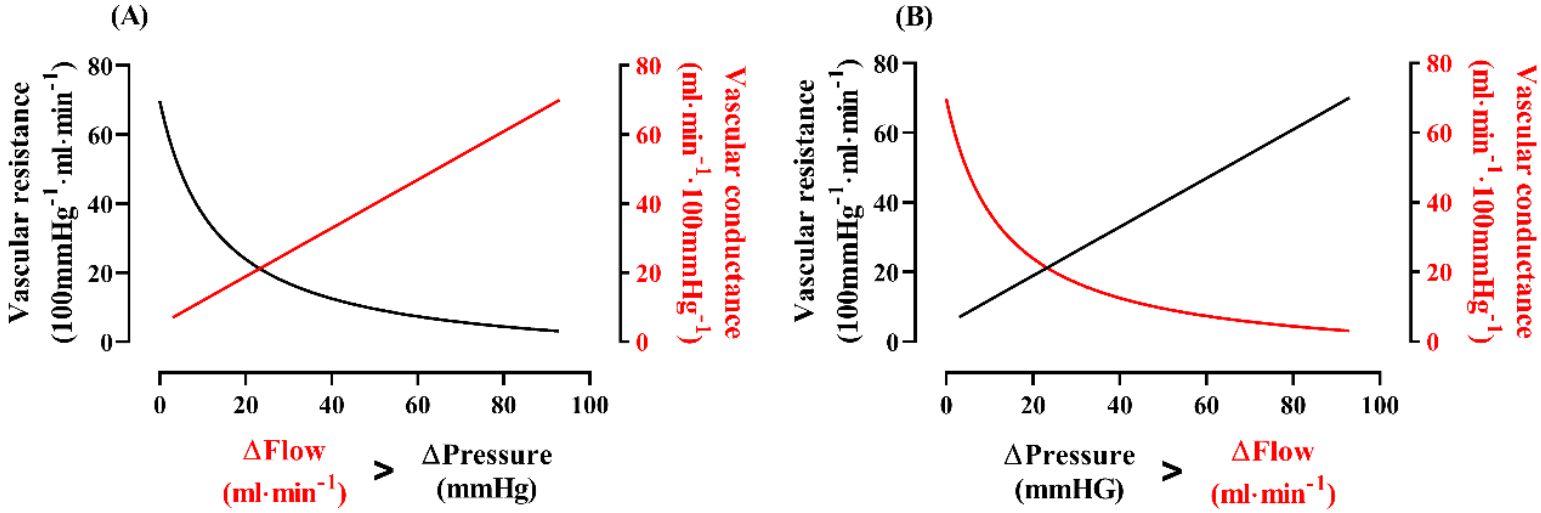


Figure 10 – Assessment of sympathetic vascular transduction. For each participant a transduction slope was generated, constructed using the 30 second average of MSNA (input variable) and calf vascular resistance (outcome variable) during handgrip exercise to fatigue. *Figure from Halliwill et al., (1996).*

To calculate total peripheral resistance (TPR) or total vascular conductance (TVC), FBF is substituted with cardiac output without multiplication by 100 (e.g. TPR = MAP/Cardiac Output, $mmHg \cdot L \cdot min^{-1}$). The use of either resistance or conductance depends on the physiological situation under assessment. Most importantly, despite it being well documented that it is not the case, it is still often believed that vascular resistance and conductance are interchangeable indices of vascular tone, especially in response to physiological stress. Although mathematically vascular resistance is the reciprocal of vascular conductance, these outcomes should not be used interchangeably (Lautt, 1989; O'Leary, 1991; Buckwalter and Clifford, 2001; Joyce *et al.*, 2019; Craig *et al.*, 2020; Limberg *et al.*, 2020). This is due to the ratio used to calculate these variables; whereby the numerator will have a greater influence on the response. As outlined by Lautt (1989), vascular resistance should be used when pressure is changing most and vascular conductance should be used when flow changes most (Figure 11). In other words, it is most appropriate to select the vascular outcome which is linearly related to the variable which is changing most, whether that be flow or pressure.

In addition to using vascular resistance or conductance, DBP has been suggested as a vascular outcome which can be used to determine sympathetic vascular transduction (Briant *et al.*, 2016). As noted above, DBP is a target variable of vascular sympathetic activity and changes in DBP (input variable) correlate with changes in MSNA (output variable), as used to determine vascular sympathetic baroreflex gain. Thus, the inverse of this relationship can also be used to determine the effectiveness of MSNA in eliciting changes in DBP. The use of DBP as the vascular outcome is recommended due to the ease of measurement, the relation to changes in MSNA, and the linear relationship between sympathetic vascular transduction assessed with DBP or TPR (Briant *et al.*, 2016).



From Lautt (1989), "Thus, in vivo, where local changes in vascular tone result primarily in blood flow changes, the use of vascular conductance to accurately reflect vascular tone is clearly superior to the traditional method of data expression using resistance. Under some limited and very specific situations, where changes in vascular tone produce changes in perfusion pressure rather than blood flow, resistance remains the appropriate means of data expression.".

Figure 11 – Vascular resistance or conductance to express changes in vascular tone. (A) When the change in blood flow is greater than the change in arterial pressure, it is most appropriate to use vascular conductance, as in this instance, conductance is linearly related to the variable which is changing most and therefore better represents the change in vascular tone. However, **(B)** when pressure increases to a greater extent that blood flow, vascular resistance is the most appropriate index of vascular tone. This is highlighted in the quote from Lautt (1989), within the figure. Thus, the selection of the appropriate index of vascular tone is important in the study of sympathetic vascular transduction, either assessed at rest or during physiological stress. *Figure is using hypothetical data, but based upon previous reports, including that from Lautt (1989).*

Buckwalter and Clifford (2001) showed that quantifying vascular responses as a delta or percentage change also provide a sensitive index of sympathetic vasoconstriction. This analysis method has been used widely within the literature in studies without the simultaneous recording of MSNA (Smith *et al.*, 2007; Crecelius *et al.*, 2015; Hearon *et al.*, 2017; Bunsawat *et al.*, 2019; Hearon *et al.*, 2019; Craig *et al.*, 2020; Hearon *et al.*, 2020; Terwoord *et al.*, 2020). Thus, the assessment of the relative forearm vascular response to a stress provides insight into neural control of the vasculature; this index will be used within this thesis.

Interim summary

The arterial baroreflex is the primary controller of resting peripheral blood pressure via modulation of MSNA and R-R interval. Through effective transduction of efferent outflow to end organ responses, the cardiovagal and vascular sympathetic limbs of the arterial baroreflex function together to control systolic and diastolic, and therefore mean, blood pressure. The known effects of age and habitual exercise on the autonomic regulation (baroreflex function) and neural control (sympathetic vascular transduction) of peripheral blood pressure are outlined below.

2.3.5 Effects of age and habitual exercise on arterial baroreflex function

Age

Vascular sympathetic baroreflex function

Many studies have reported age-related increases in resting MSNA burst incidence and frequency (Sundlof and Wallin, 1978; Iwase *et al.*, 1991; Ng *et al.*, 1993; Matsukawa *et al.*, 1994; Ng *et al.*, 1994b; Esler *et al.*, 1995; Matsukawa *et al.*, 1996; Jones *et al.*, 1997; Davy *et al.*, 1998b; Davy *et al.*, 1998c; Matsukawa *et al.*, 1998b; a; Esler *et al.*, 2002; Narkiewicz *et al.*, 2005; Hart *et al.*, 2009b; Studinger *et al.*, 2009; Vianna *et al.*, 2012; Greaney *et al.*, 2013; Hart *et al.*, 2013; Best *et al.*, 2014; Greaney *et al.*, 2014; Hart *et al.*, 2014; Shantsila *et al.*, 2015), see Figure 12.

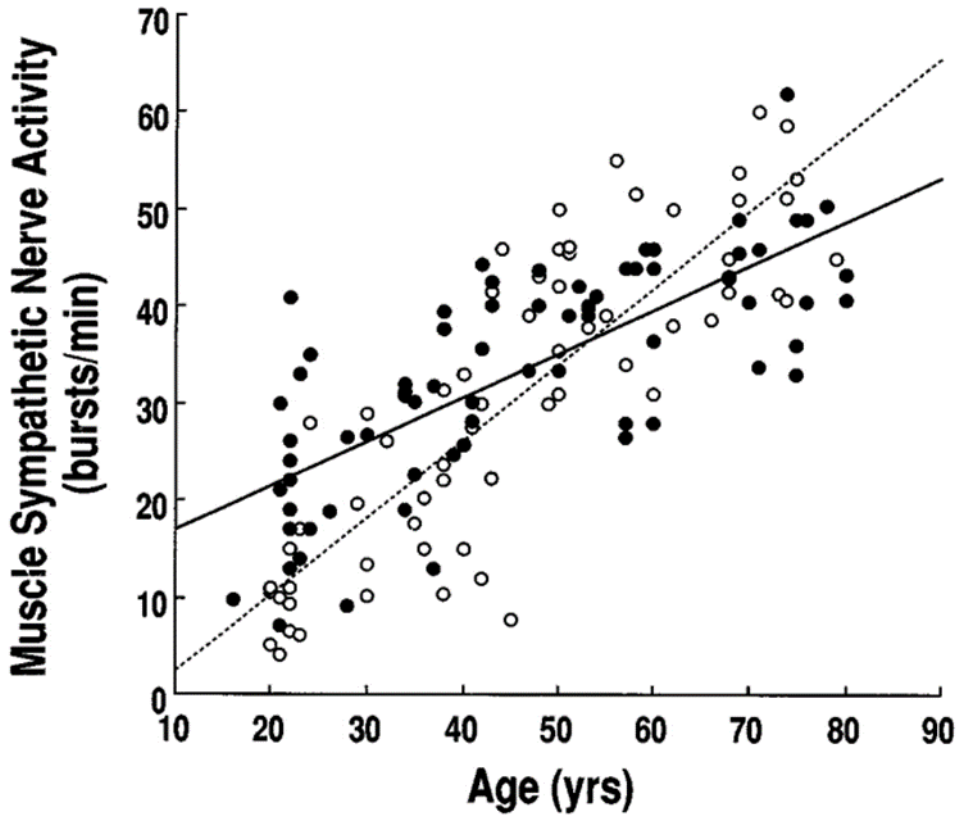


Figure 12 – Relationship between age and muscle sympathetic nerve activity (MSNA) burst frequency. These relationships between age and MSNA were significant in 76 men (closed circles; r = 0.751, $P < 0.0001$) and 69 women (open circles; r = 0.846, $P < 0.0001$). There was no effect of age on resting HR; therefore, the age-related increase in MSNA burst frequency is mediated by gradual upward resetting of the vascular sympathetic baroreflex (i.e. increases in MSNA burst incidence) with age. *Figure from Matsukawa et al. (1998b).*

Seven of these aforementioned studies also characterised the effects of age on arterial baroreflex function, reporting no effects on vascular sympathetic baroreflex gain, despite the higher operating pressure and point (Ebert *et al.*, 1992; Matsukawa *et al.*, 1994; Matsukawa *et al.*, 1996; Davy *et al.*, 1998b; Davy *et al.*, 1998c; Matsukawa *et al.*, 1998a; Studinger *et al.*, 2009; Greaney *et al.*, 2013). Ebert and colleagues (1992) performed the first comprehensive study to determine the effects of age on arterial baroreflex function by applying the modified oxford technique they had recently developed (Ebert and Cowley, 1992). These authors reported distinct differences in the age-related changes in the vascular sympathetic compared to cardiovagal limbs of the arterial baroreflex (Figure 13). With increasing age, Ebert and colleagues (1992) found that the operating pressure (DBP) and point (in this study MSNA total activity) of the vascular sympathetic baroreflex were higher with no difference in reflex gain between groups (Figure 13A). However, the operating pressure (in this study mean arterial pressure) of the cardiovagal baroreflex was higher in older, but not middle-aged, individuals when compared to the young group. The operating point (R-R interval) was not different between groups. Cardiovagal baroreflex gain was found to be lower in middle-aged and older adults when compared to the young group (Figure 13B). These findings led Ebert and colleagues (1992) to suggest that the higher operating point of vascular sympathetic baroreflex with age may compensate for the reduction in cardiovagal baroreflex gain. To the best of the authors knowledge, whether changes in vascular sympathetic baroreflex function influence the cardiovagal baroreflex gain is still not known.

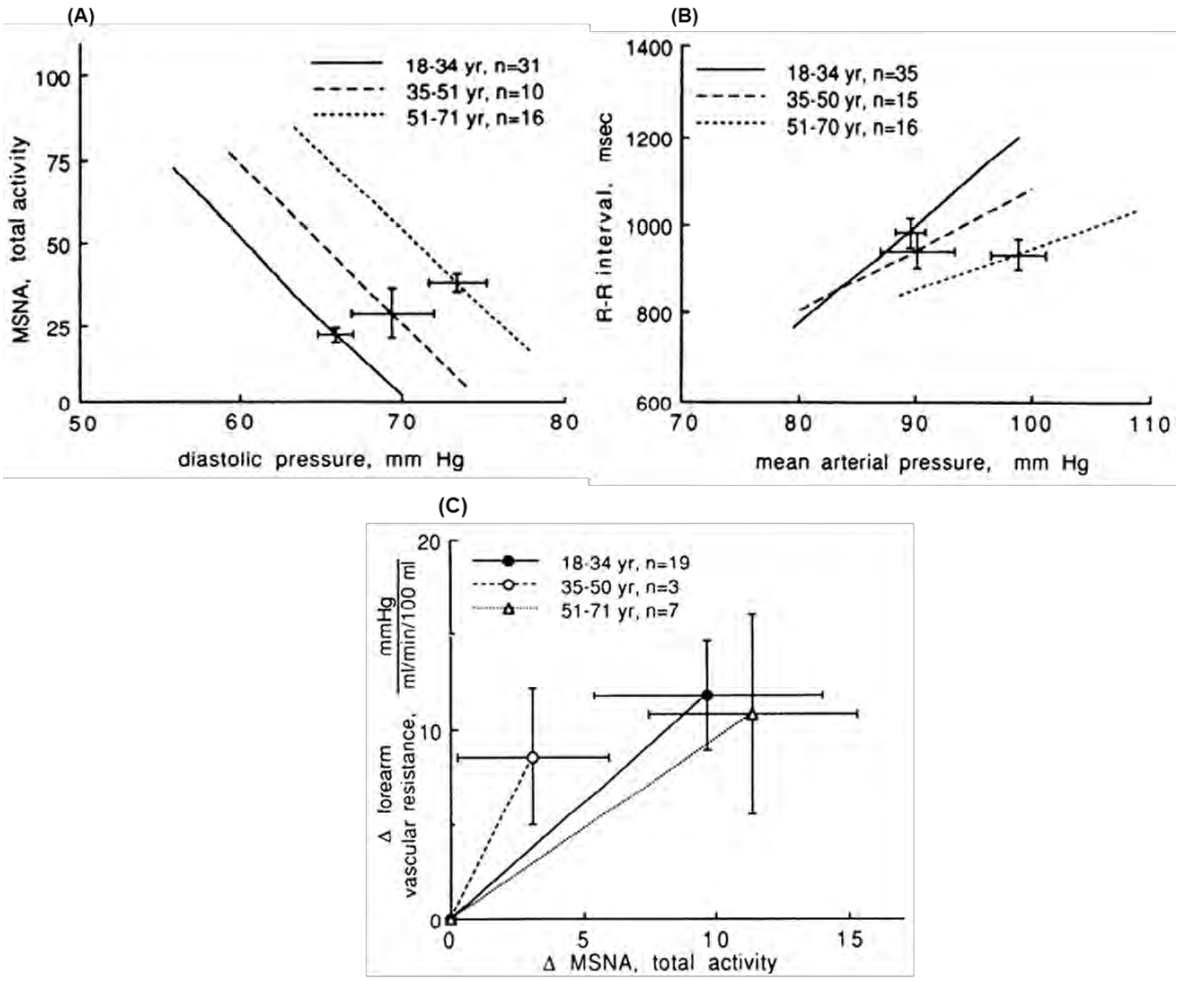


Figure 13 – The effects of age on vascular sympathetic and cardiovagal baroreflex function and sympathetic vascular transduction. (A) Vascular sympathetic baroreflex gain was not different between young, middle-aged and older adults; despite the slopes being set further rightward (higher pressure) and upward (higher muscle sympathetic nerve activity [MSNA]) with increasing age ($P < 0.05$). **(B)** Cardiovagal baroreflex gain (using MAP in this instance) was lesser in middle-aged and older groups when compared to the young; the slopes were set rightward (i.e. to a higher pressure; $P < 0.05$) with ageing. **(C)** There were no effects of age on the peak change in MSNA or forearm vascular resistance at the end of a 60 second cold pressor test; an index of sympathetic vascular transduction. *Figure adapted from Ebert et al. (1992).*

Cardiovagal baroreflex function

The original Oxford method (infusion of phenylephrine to increase blood pressure), developed by Smyth and colleagues (1969), was first used to characterise the effects of age on cardiovagal baroreflex function. This study reported that cardiovagal baroreflex gain was lower with advancing age (Bristow *et al.*, 1969). Many subsequent studies support this finding and also show that the lower gain occurs alongside a higher operating systolic pressure with no difference in operating cardiac interval (Gribbin *et al.*, 1971; Ebert and

Cowley, 1992; Parati *et al.*, 1995; Davy *et al.*, 1998a; Laitinen *et al.*, 1998; Rudas *et al.*, 1999; Monahan *et al.*, 2000; Hunt *et al.*, 2001; Monahan *et al.*, 2001a; Monahan *et al.*, 2001c; Monahan *et al.*, 2004). The age-related reduction in cardiovagal baroreflex gain has been suggested to be mediated by increased barosensory artery stiffness with age (Monahan *et al.*, 2001a). This vascular stiffening influences the ability of the baroreceptors to "sense" changes in vascular distention, thereby reducing mechanosensory transduction. Indeed, Hunt and colleagues (2001) showed that the mechanical (i.e. mechanosensory transduction) and neural (i.e. central processing and efferent neurotransmission in the heart) components of the cardiovagal baroreflex are lower with age. Despite these studies reporting no influence of age on resting heart rate, intrinsic heart rate has been shown to be lower with age (Jose and Collison, 1970; Opthof, 2000), which, alongside reduced cardiovagal baroreflex function, may explain why the vascular sympathetic support of blood pressure is greater with age in humans (Jones *et al.*, 2001).

Sympathetic vascular transduction

Sympathetic vascular transduction has been shown to be similar (Ebert *et al.*, 1992) (Figure 13C) or reduced with age (Davy *et al.*, 1998c; Vianna *et al.*, 2012; Tan *et al.*, 2013) in sedentary individuals. This effect of age is likely mediated by decreases in vascular alpha-adrenergic sensitivity (Dinenno *et al.*, 2002; Smith *et al.*, 2007). The increase in the operating point of the vascular sympathetic baroreflex has been suggested to offset this decrease in sympathetic vascular transduction (Taylor and Tan, 2014). In other words, due to the blood vessels being less responsive to noradrenaline with age, it may be necessary for the vascular sympathetic baroreflex to increase the level of MSNA to maintain appropriate neural control of blood pressure.

Habitual Endurance Exercise

Vascular sympathetic baroreflex function

Ng and colleagues (1994a) conducted the first study to compare the level of MSNA between older endurance-trained and untrained individuals (Figure 14). These authors identified that both MSNA burst frequency and burst incidence were higher in a mixed-sex sample of older endurance-trained individuals. When the effects of habitual exercise were considered alongside the effects of sex, it was clear that the finding of higher MSNA (burst frequency and incidence) in older endurance-trained individuals was due to the inclusion of women; there was no difference between endurance-trained and untrained older men for either index of MSNA (Ng *et al.*, 1994a). The authors of this study did not interpret their data in relation to the arterial baroreflex regulation of MSNA. However, upon inspection these data suggest habitual endurance exercise is associated with a higher MSNA operating point in women, but not men.

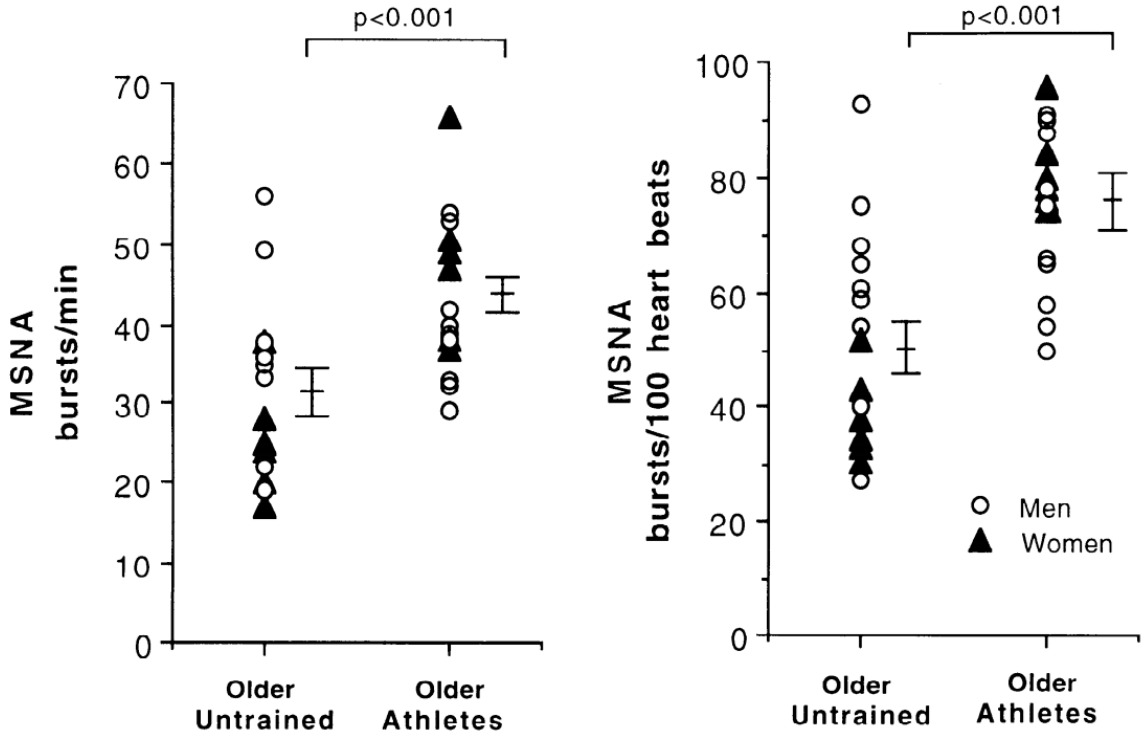


Figure 14 – The effect of habitual exercise on muscle sympathetic nerve activity (MSNA) in older adults. MSNA was higher, expressed as either MSNA burst frequency (left panel) or burst incidence (right panel), in older athletes compared to age-matched untrained individuals. *Figure adapted from Ng et al., (1994a).*

Two subsequent studies investigated the effects of habitual endurance exercise on MSNA in middle-aged men and have reported conflicting results. In the only study to characterise the effects of habitual exercise on vascular sympathetic baroreflex function, Stüdinger *et al*., (2009) found no significant influence on baroreflex gain. Furthermore, there was no significant difference in vascular sympathetic baroreflex operating pressure or point between middle-aged endurance-trained and sedentary men. However, due to the lower heart rate in the trained men and a similar MSNA burst incidence, MSNA burst frequency was lower in the trained group (Studinger *et al.*, 2009). In the other study, Notarius and colleagues (2012) found that MSNA burst incidence was higher in endurance-trained compared to sedentary men, with no difference in MSNA burst frequency; however, the untrained sample in this study had high-normal blood pressure. Collectively, there is only one report on the effects of habitual exercise on vascular sympathetic baroreflex function in healthy normotensive middle-aged men with inconsistencies between all other studies on resting MSNA alone. Further research is warranted to better characterise this limb of the arterial baroreflex as the vascular sympathetic baroreflex is the primary controller of peripheral blood pressure. This research may provide further insight into the effects of chronic endurance exercise training on cardiovascular regulation.

Cardiovagal baroreflex function

The operating point (R-R interval) of the cardiovagal baroreflex is higher in endurance-trained compared to sedentary individuals, due to exercise-induced bradycardia (Hellsten and Nyberg, 2015). Exercise-induced bradycardia could be mediated via two primary mechanisms: 1) a reduction in intrinsic heart rate, or 2) a greater level of cardiovagal tone; there is no consensus within the literature regarding which mechanism mediates this response, as both mechanisms likely contribute, as reviewed in a recent point:counterpoint series (Billman, 2017; Boyett *et al.*, 2017). Nevertheless, exercise-induced bradycardia is a well described physiological characteristic of habitually endurance-trained individuals.

Relative to the vascular sympathetic baroreflex, the effects of habitual exercise on cardiovagal baroreflex function are well characterised (Davy *et al.*, 1996; Davy *et al.*, 1998a; Monahan *et al.*, 2000; Hunt *et al.*, 2001; Monahan *et al.*, 2001a; Pierce *et al.*, 2016). The most comprehensive study performed to date was conducted in men by Monahan and colleagues (2000). Focusing on the results in middle-age, this study found that the operating pressure was similar between endurance-trained, moderately active and sedentary men but the operating point was only different between endurance-trained and sedentary men (Monahan *et al.*, 2000). As shown in Figure 15, there were no significant differences in cardiovagal baroreflex gain between endurance-trained and moderately active men, but gain was higher in these groups when compared to sedentary men. This effect was suggested to be mediated by lower arterial stiffness in the exercising groups (Monahan *et al.*, 2001c). However, this hypothesis was subsequently disproven (Hunt *et al.*, 2001), as the higher level of cardiovagal baroreflex gain in older trained compared to sedentary men is mediated by a maintained neural component of this baroreflex arc with age. The other studies to date support these findings related to the effects of habitual exercise on cardiovagal baroreflex function (Davy et al., 1996; Davy et al., 1998a; Hunt et al., 2001; Monahan et al., 2001a; Monahan et al., 2001b; Pierce et al., 2016).

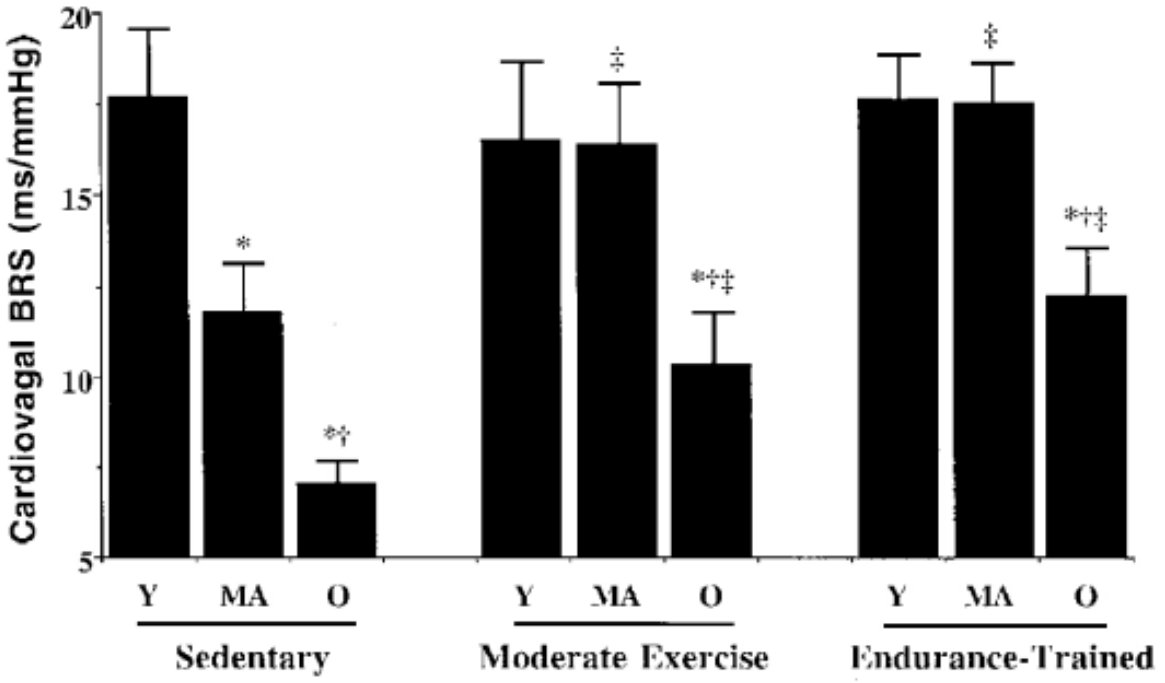


Figure 15 – The effects of age and habitual exercise on cardiovagal baroreflex sensitivity (i.e. gain). A marked effect of age on cardiovagal baroreflex sensitivity (BRS) is observed in sedentary men, with differences between each age-group (Young, 18-37 years; middle-aged, 38-56 years; and older, 57-79 years). Whereas, in the moderate exercise and endurance-trained groups there was no effect of age between young and middle-aged groups. However, cardiovagal BRS was significantly lower in the older group compared to the young and middle-aged groups in the moderate exercise and endurance-trained groups. *Symbols: * $P < 0{\cdot}05$ vs. young subjects within same activity group; † $P < 0{\cdot}05$ vs. middle-aged subjects within same activity group; ‡ $P < 0{\cdot}05$ vs. sedentary subjects of same age group. Abbreviations: Y, young; MA, middle age; O, older. Figure adapted from Monahan and colleagues (2000).*

Sympathetic vascular transduction

Short-term exercise training in healthy middle-age has been shown to reduce vascular alpha-adrenergic sensitivity (Mortensen *et al.*, 2014). Alongside remodelling of the macro- and microvasculature (Hellsten and Nyberg, 2015), chronic endurance exercise training may therefore lower sympathetic vascular transduction. Indeed, this could explain the previous finding by Notarius and colleagues (2012). These authors reported that in moderately endurance-trained middle-aged men, there was no relationship between MSNA and FVR during sympathoexcitation induced by LBNP (Figure 16). In contrast, the sedentary middle-aged group displayed a positive association between MSNA and FVR across LBNP. Together, the lack of relationship in trained men led the authors to suggest that endurance

training alters sympathetic vascular transduction (Notarius *et al.*, 2012). Notably, however, the sedentary men had high-normal blood pressure; thus, it is unclear whether this difference is mediated by chronic endurance training or by the higher arterial pressure in sedentary men. In other words, it is unclear whether sympathetic vascular transduction was exaggerated in the sedentary individuals in this study. Together, the effects of habitual endurance exercise on sympathetic vascular transduction remains unclear. Understanding the effects of habitual exercise on efferent neurotransmission in the vasculature is important as it will provide new information on the effects of chronic endurance exercise training on the neural control of blood pressure. This is an area relatively understudied to date despite its importance in the control of arterial pressure.

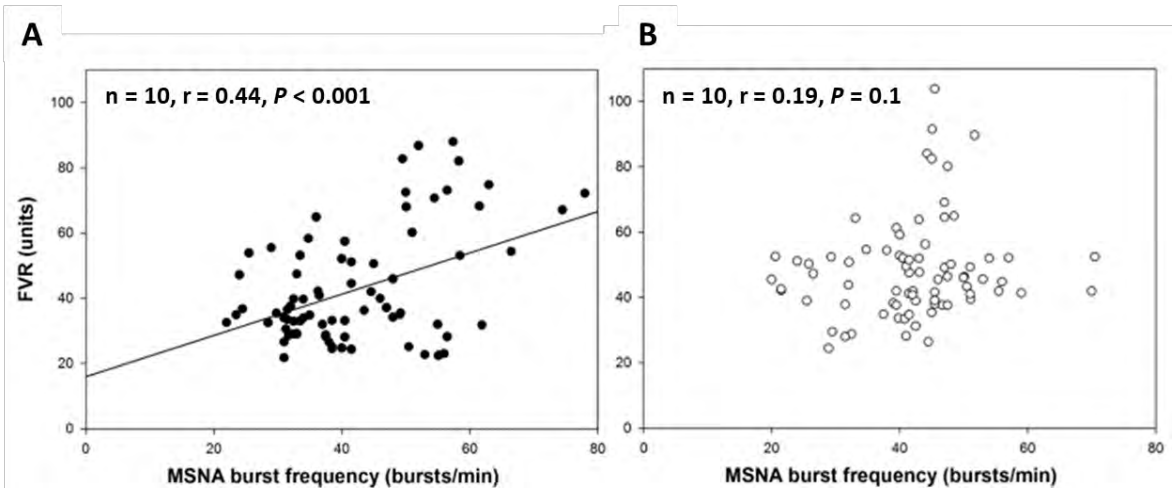


Figure 16 – The effect of fitness on reflex sympathetic neurovascular transduction in middle-aged men. (A) There was a significant relationship between MSNA burst frequency and forearm vascular resistance in sedentary men (categorised by $\dot{V}O_2$ Peak < 100% predicted). **(B)** However, no significant relationship was observed for endurance-trained men (categorised by $\dot{V}O_2$ Peak > 100% predicted), which led the authors to suggest that, "... *fitness uncouples the vasoconstrictor response to reflex increases in sympathetic nerve firing rate present in SED men*". All data were collected during lower body negative pressure at -5, -10, -20 and -40 mmHg. *Figure redrawn from Notarius and colleagues (2012).*

2.4 Overall Summary

This chapter has provided an overview of the primary factors which determine aortic and peripheral blood pressure. The age-related increase in aortic stiffness, systolic pressure augmentation and pressure lead to an increase in cardiovascular risk. Studies investigating the effects of habitual endurance exercise to date have primarily focused on the effects on aortic stiffness; no study to date has systematically assessed aortic stiffness, aortic systolic pressure augmentation and aortic pressure, to characterise the aortic haemodynamic profile, in normotensive middle-age. Whether, or not, habitual exercise influences aortic haemodynamics, thereby reducing cardiovascular and all-cause mortality risk, is of clinical relevance. Similarly, the effects of age on the autonomic regulation (arterial baroreflex function) and neural control (sympathetic vascular transduction) of blood pressure are well characterised. However, there has only been one study to date to investigate the effects of chronic habitual endurance exercise training on all aspects of arterial baroreflex function (operating pressure, operating point and baroreflex gain). Furthermore, only one study has assessed the effects of habitual exercise on sympathetic vascular transduction in middle-age, despite evidence suggesting that vascular alpha-adrenergic sensitivity is lower following short-term exercise training. Therefore, based upon these gaps in the literature, three primary research questions are addressed in this thesis to determine the effects of chronic habitual endurance exercise training on central haemodynamics and peripheral blood pressure regulation.

2.5 Aims of thesis

The primary aim of this thesis is to characterise the effects of chronic habitual endurance exercise on central haemodynamics and peripheral blood pressure regulation in normotensive middle-aged men. Three discrete research questions were posed (Figure 17) as part of one large cross-sectional study and the findings are presented in the three

experimental chapters herein (Chapters 4-6). Hypotheses are presented within each of the following experimental chapters. The primary aim of each study is outlined below:

Study (Question) 1 - Chapter 4:

Primary aim: To determine the effects of chronic habitual endurance exercise on the aortic haemodynamic profile in normotensive middle-aged men.

Study (Question) 2 - Chapter 5:

Primary aim: To determine the effect of chronic habitual endurance exercise on the autonomic regulation of resting brachial blood pressure by the vascular sympathetic baroreflex in normotensive middle-aged men.

Study (Question) 3 - Chapter 6:

Primary aim: To determine the effect of chronic habitual endurance exercise on sympathetic vascular transduction in normotensive middle-aged men.

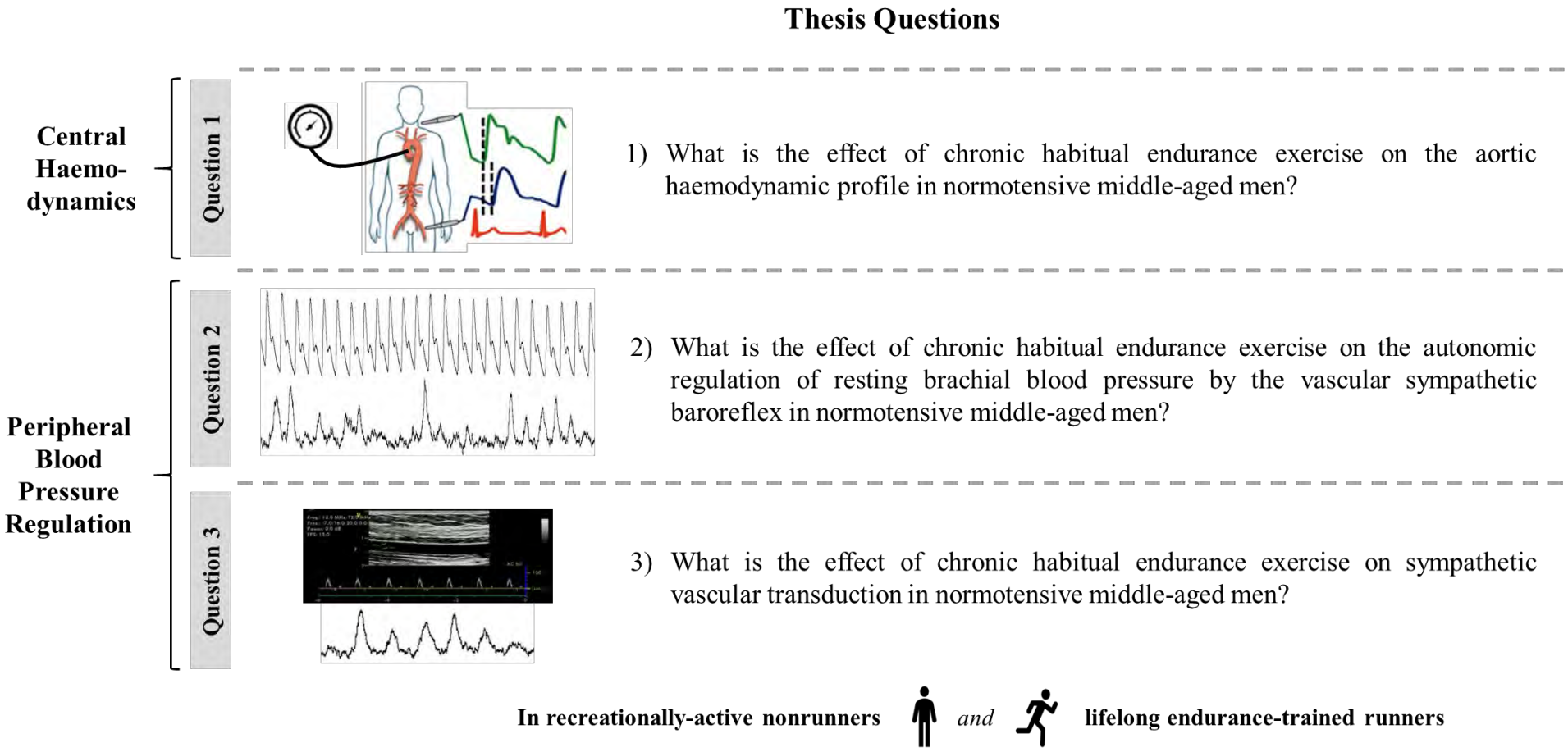


Figure 17 – The three primary research questions addressed in this thesis.

CHAPTER 3. METHODOLOGY AND PARTICIPANT CHARACTERISTICS

This chapter outlines the study design and the participant screening procedures and characteristics. Each experimental chapter contains the relevant methodology with further detail on the statistical analyses used.

3.1 Study Overview

To determine the effects of habitual exercise on central haemodynamics and peripheral blood pressure regulation, in line with the three discrete aims (and therefore studies) of this thesis, a four-group cross sectional design was employed. To address the primary aim, groups of middle-aged recreationally-active nonrunners (MNR) and middle-aged endurance-trained runners (MR) were recruited. A separate, but secondary, aim was to discern the effects of chronic habitual exercise from the effects of age by also studying young recreationally-active nonrunners (YNR) and young endurance-trained runners (YR). As outlined within the literature review, men only were studied in this thesis for the following reasons. The effects of habitual exercise on central haemodynamics (Tanaka *et al.*, 1998) and resting vascular sympathetic activity (Ng *et al.*, 1994a) have been previously reported in women. Furthermore, there are marked sex differences in sympathetic vascular control (Casey *et al.*, 2011; Joyner *et al.*, 2015; Briant *et al.*, 2016) and autonomic support of blood pressure (Christou *et al.*, 2005).

All participants were asked to complete two experimental visits in a temperature-controlled laboratory (~22°C). The first visit involved the assessment of body mass, stature and supine BP, followed by the measurement of the aortic haemodynamic profile at rest (question 1), prior to the assessment of $\dot{V}O_2$ Peak on a cycle ergometer. For the remaining visit, participants fasted for six hours prior to their arrival to the laboratory, before haemodynamics and vascular sympathetic activity were recorded at rest (question 2) and in response to physiological stress (question 3), see Figure 18.

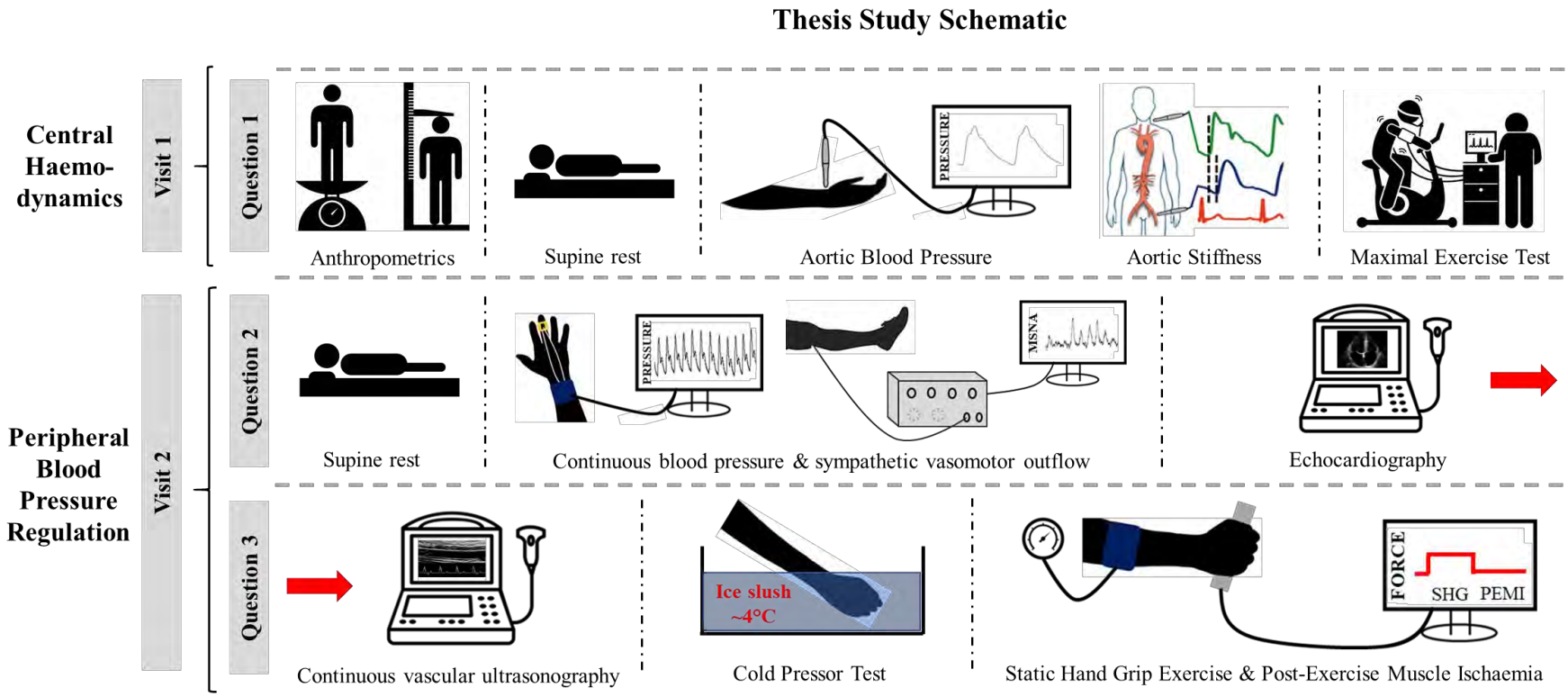


Figure 18 – Thesis Schematic. All participants completed two study visits. The first visit included assessments of anthropometrics, peripheral blood pressure and the aortic haemodynamic profile (Question 1), which was followed by an incremental maximal exercise test on a cycle ergometer. Participants then completed the remaining study visit, which addressed Questions 2 and 3 of this thesis. For Question 2, following supine rest, participants were instrumented for continuous recording of brachial arterial blood pressure and vascular sympathetic activity (i.e. muscle sympathetic nerve activity [MSNA]), followed by the assessment of left ventricular stroke volume with echocardiography. The red arrow denotes that data collection continued from question 2 to address question 3. To address Question 3, participants were already instrumented to record blood pressure and MSNA. Vascular ultrasonography of the contralateral brachial artery was conducted to determine forearm blood flow, during a cold pressor test and then, following a period of rest, during static hand grip exercise (SHG) and a period of post-exercise muscle ischaemia (PEMI). Left ventricular stroke volume was assessed intermittently during CPT, SHG and PEMI via echocardiography.

Other than the screening procedures which are detailed here, the remaining methods are outlined within each experimental chapter (Chapters 4-6). Due to the number and nature of the techniques involved in this thesis, it took a team of researchers to conduct these experiments (see Appendix III for list of investigators and associated techniques).The coefficients of variation (CoV) for each primary outcome, other than MSNA, are reported within this chapter (Table 2). Other than left ventricular stroke volume, the author of this thesis conducted all remaining analyses.

3.2 Ethics and Participant Consent

This study conformed to the most recent *Declaration of Helsinki* and all procedures were approved the Cardiff Metropolitan University School of Sport Research Ethics Committee (16/7/02R – Appendix I), other than registration in a database. Prior to testing, all participants provided both written (Appendix II) and verbal informed consent following reading the participant information sheet (Appendix II) and completing a screening questionnaire and PARQ+.

3.3 Participant Screening during Visit 1

Participants abstained from caffeine, alcohol and strenuous exercise for the twenty-four hours preceding their arrival to the laboratory on both occasions.

Anthropometrics

All participants underwent the assessment of body mass (SECA, Model 770, Vogel & Halke, Hamburg, Germany), stature (Holtain, Fixed Stadiometer, Pembs, UK) and body fat percentage (via bioelectrical impedance analysis; Bodystat 1500, Bodystat Ltd, Douglas, Isle of Man).

Screening blood pressure

Supine brachial arterial blood pressure was determined, in triplicate, for all participants via manual sphygmomanometry (Welch Allyn Durashock DS66, UK). The assessment of aortic

haemodynamics followed; however, this was not part of participant screening and is detailed in Chapter 4.

Cardiorespiratory fitness

All participants completed an incremental exercise test to exhaustion on a cycle ergometer (Lode Corival, Groningen, The Netherlands) to assess $\dot{V}O_2$ peak. Cycling was chosen for reasons of safety and assessment of the exercise electrocardiogram. Each increment of the exercise test corresponded to an increase of workload by 20 watts per minute (young and middle-aged runners started at 120W and 90W, respectively; middle-aged and young nonrunners started at 30W and 50W, respectively). During the maximal exercise test oxygen consumption was measured continuously via a breath-by-breath analyser (Oxycon Pro, Jaeger, Hoechberg, Germany). Heart rate was measured throughout the exercise test via either a 12-lead electrocardiography (ECG) in middle-aged men (Oxycon Pro, Jaeger, Hoechberg, Germany) or a chest strap in the young groups (Polar Electro, RS400, Finland). A cardiologist oversaw all electrocardiograms conducted in middle-aged men to screen for any ECG anomalies. All middle-aged men (n = 23) presented with a normal ECG, both at rest and during exercise.

3.4 Participant Characteristics

Each participant was categorised according to his age (i.e. middle-aged or young) and training status (i.e. runner or nonrunner). Participants were healthy (free of chronic disease, as assessed via self-report), non-smoking, normotensive (supine resting blood pressure <140/90 mmHg) and non-obese (Body mass index, BMI $\leq$ 30 $kg \cdot m^2$) males who were recruited into four groups (10 young nonrunners, 13 young endurance-trained runners, 10 middle-aged nonrunners and 13 middle-aged endurance-trained runners). All runners had a 5km road running time within the top 30% for their age group in the UK in the year that they were studied; training history for the runners is presented in Table 1. Nonrunners were

recruited based upon self-report of completing at least 150 minutes of moderate to vigorous physical activity (i.e. brisk walking) per week.

Table 1 - Sample characteristics

Variable	YNR	YR	*P*	MNR	MR	*P*
n	10	13		10	13	
Anthropometrics						
Age (years)	23 ± 3	22 ± 3	0.588	53 ± 2	57 ± 5	0.141
Stature (cm)	178.1 ± 5.8	179.9 ± 5.1	0.439	175.6 ± 7.1	174.7 ± 6.3	0.763
Body mass (kg)	80.4 ± 16.2	67.0 ± 5.1	**0.010**	80.9 ± 9.9	66.1 ± 7.9	**0.001**
BMI ($kg \cdot m^2$)	25.4 ± 4.6	20.8 ± 1.3	**0.002**	26.2 ± 3.1	21.6 ± 1.6	**< 0.001**
Body fat (%)	19.7 ± 6.3	10.7 ± 4.6	**0.001**	26.8 ± 9.0	17.5 ± 3.0	**0.002**
Heart rate and Blood Pressure						
Heart rate (bpm)	60 ± 12	46 ± 7	**0.001**	57 ± 11	43 ± 9	**0.003**
SBP (mmHg)	119 ± 13	111 ± 5	0.065	119 ± 7	118 ± 8	0.848
DBP (mmHg)	71 ± 7	66 ± 6	**0.037**	76 ± 5	74 ± 7	0.374
Cardiorespiratory Fitness						
$\dot{V}O_2$ Peak ($mL \cdot min^{-1}$)	2894 ± 508	4018 ± 607	**< 0.001**	2641 ± 726	3349 ± 572	**0.016**
$\dot{V}O_2$ Peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	36.5 ± 6.3	60.6 ± 9.3	**< 0.001**	32.6 ± 8.4	50.7 ± 6.1	**< 0.001**
$\dot{V}O_2$ Peak (% Predicted)	85 ± 14	116 ± 20	**< 0.001**	106 ± 32	143 ± 23	**0.004**
Training History						
Exercise/week (miles)	-	65 ± 14		-	35 ± 10	
Training history (years)	-	8 ± 5		-	29 ± 15	
5000m time (mins:secs)	-	15:22 ± 00:37		-	19:31 ± 00:57	

Data are presented as mean ± standard deviation. P values were determined by independent samples t-tests. *Abbreviations: b, brachial; BMI, body mass index; bpm, beats per minute; DBP, brachial diastolic blood pressure; MNR, middle-aged nonrunner; MR, middle-aged nonrunner; SBP, brachial systolic blood pressure; $\dot{V}O_2$, volume of oxygen; YNR, young nonrunner; YR, young runner.*

3.5 Statistical analyses

In line with the primary and secondary aims of this thesis, separate runner versus nonrunner comparisons were made for middle-aged and young men in all studies. Age was not considered an independent variable in this thesis. Within each experimental chapter, separate statistical analyses sections outline the approach employed to address each research

question. All statistical analyses were conducted using the Statistics Package for Social Sciences (SPSS) software for Windows (version 24, Chicago, IL). The alpha value was set at < 0.05 for all studies. Between-group differences in sample characteristics (Table 1) were determined via independent samples t-tests. For all primary outcomes, hedges *g* is reported as an estimate of effect size ([-]0.2 small effect, [-]0.5 medium effect, [-]0.8 large effect) (Cohen, 1992). Hedges g was calculated using the equation below.

Calculation of effect size (*hedges g*):

$$Hedges\ g = \frac{M_1 - M_2}{SD\ pooled}$$

$$SD\ pooled = \sqrt{\frac{(n_1-1)SD_1^2 + (n_2-1)SD_2^2}{n_1 + n_2 - 2}}$$

Where: M, mean; n, sample size, SD, standard deviation. Sub- and superscript values represent group (1 or 2) for the respective between-group comparison.

3.5.1 Sample Size estimation

Due to the differences in findings between the small number of previous studies on the primary outcomes of each experimental study herein, an a-priori sample size was not conducted. This is because selecting a study to calculate a sample size estimation from would bias this thesis towards finding between-group differences, or not, depending on the results of the chosen study. Accordingly, we recruited a sample size similar to previous studies which have and have not observed differences in the key outcomes for Chapters 4 (Vaitkevicius *et al.*, 1993; Gates *et al.*, 2003), 5 (Ng *et al.*, 1994a; Studinger *et al.*, 2009; Notarius *et al.*, 2012) and 6 (Notarius *et al.*, 2012).

3.5.2 Reliability of primary outcome measures

Multiple assessments of the primary outcomes were conducted within each study enabling the assessment of the reliability of each experimenter of the research team who collected primary outcome data. Accordingly, the coefficient of variation (CoV) data reported were calculated using a within-participant and within-day approach. Thus, aortic haemodynamics

(Chapter 4), stroke volume (Chapter 5 and 6) and forearm vascular parameters (Chapter 6) were assessed twice with participants remaining in the same supine position between assessments; see Appendix III for a list of techniques for each experimenter during each visit. After analysis, the mean, standard deviation and CoV were calculated; a CoV of less than 35% was deemed acceptable (Harris *et al.*, 2007).

Calculation of CoV and absolute difference required:

$$\text{CoV (\%)} = \left(\frac{\text{SD}}{\text{Average}}\right) \times 100$$

Where: CoV, coefficient of variation; SD, standard deviation.

Notably, the reliability of MSNA recordings was not calculated in this thesis, as this would require removing and reinserting the microelectrode, with no guarantee of reacquisition of a stable and appropriate neural recoding. Furthermore, whenever the needle is reinserted there is potential for dysesthesia (Meah *et al.*, 2019); thus, to limit this, once an appropriate neural signal was acquired the study began. Notably, the reliability of MSNA has been reported previously, showing despite marked variability between individuals there is strong intra-individual variability over the short, medium and long-term (Fagius and Wallin, 1993; Grassi *et al.*, 1997; Kimmerly *et al.*, 2004; Fonkoue and Carter, 2015; Notay *et al.*, 2016).

Table 2 – Observer reliability for primary outcomes

Variable	Average	SD	CoV (%)
Aortic Parameter			
aPWV ($m \cdot s^{-1}$)	6.2	0.4	**5.8**
AP (mmHg)	3	0.8	**9.6**
aSBP (mmHg)	102	0.5	**0.4**
Cardiac Parameter			
Stroke Volume (ml)	86	6	**6.4**
Vascular Parameter			
Diameter (cm)	0.39	0.02	**4.2**
Doppler Velocity ($cm \cdot s^{-1}$)	14.6	3.5	**22.1**
Forearm blood flow ($ml \cdot min^{-1}$)	103	30	**27.2**

Abbreviations: aPWV, aortic pulse wave velocity; AP, augmentation pressure; aSBP, aortic systolic blood pressure.

CHAPTER 4. EXPERIMENTAL STUDY 1

THE EFFECT OF CHRONIC HABITUAL ENDURANCE EXERCISE ON THE AORTIC HAEMODYNAMIC PROFILE IN NORMOTENSIVE MIDDLE-AGED MEN

4.1 Introduction

Aortic systolic pressure augmentation (augmentation pressure [AP]) increases throughout human ageing, whereas aortic stiffness (aortic pulse wave velocity [aPWV]) only begins to increase more linearly at ~55 years of age (McEniery *et al.*, 2005). Together, these changes contribute to the elevation in aortic systolic blood pressure (aSBP) with advancing age. The assessment of the aortic haemodynamic profile, that is aortic stiffness, aortic systolic pressure augmentation and aortic blood pressure, is of clinical relevance as these indices are predictive of cardiovascular morbidity and/or mortality (Roman *et al.*, 2007; Roman *et al.*, 2009; Vlachopoulos *et al.*, 2010; Wang *et al.*, 2010; Ben-Shlomo *et al.*, 2014). Whether lifestyle factors, such as habitual endurance exercise, can influence the aortic haemodynamic profile, especially in healthy human ageing, is currently unclear.

The effects of habitual endurance exercise on the aortic haemodynamic profile in middle-age are not well characterised, with studies to date focusing primarily on aortic stiffness (Seals *et al.*, 2019). These studies showed that aortic stiffness and pressure are lower in middle-aged endurance-trained compared to sedentary individuals, with no difference in systolic pressure augmentation measured at either the carotid artery or aorta (Vaitkevicius *et al.*, 1993; Gates *et al.*, 2003; McDonnell *et al.*, 2013; Tarumi *et al.*, 2013). However, sedentarism negatively influences cardiovascular health (Lavie *et al.*, 2019) which may exaggerate the effects of habitual endurance exercise when studied using a cross-sectional design. The effect of chronic endurance exercise training on the aortic haemodynamic profile when compared to a moderately-active, not sedentary, lifestyle is unknown and requires further investigation. Furthermore, the effects of physiological confounders (e.g. aMAP and heart rate) on aPWV and aortic systolic pressure augmentation have not always been performed, potentially leading to authors making “erroneous conclusions” (Van Bortel *et al.*, 2020). Therefore, the primary aim of this study was to determine the effects of chronic habitual endurance exercise on the aortic haemodynamic

profile in normotensive middle-aged men. To achieve this aim, groups of middle-aged male endurance-trained runners (MR) and recreationally-active nonrunners (MNR) were studied. A secondary aim was to compare the effects of chronic habitual endurance exercise in young men; accordingly, the aortic haemodynamic profile was assessed in groups of young endurance-trained runners (YR) and recreationally-active nonrunners (YNR). In line with previous reports in endurance-trained and sedentary individuals, it was hypothesised that adjusted aortic stiffness and aortic blood pressure would be lower in middle-aged runners compared to nonrunners, with no difference in systolic pressure augmentation between groups.

4.2 Methodology

Study Overview

To address this research question, all participants underwent assessments of peripheral blood pressure and aortic haemodynamics (Figure 19).

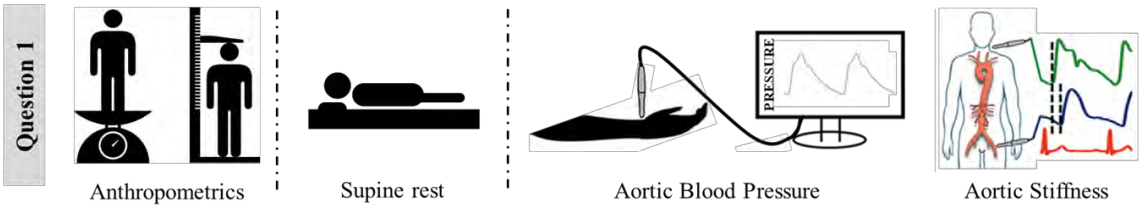


Figure 19 – Study 1 Schematic.

Assessment of aortic haemodynamics

First, brachial arterial blood pressure (manual sphygmomanometry; Welch Allyn Durashock DS66, UK) was measured following 10 minutes of supine rest. Then, to estimate ascending aortic blood pressure and aortic systolic pressure augmentation, radial arterial waveforms were acquired by applanation tonometry from the right wrist at the site of maximal arterial pulsation using a high-fidelity micromanometer tipped probe (Millar Instruments; SphygmoCor, AtCor Medical, Australia). Brachial artery blood pressure values were used to calibrate the radial arterial waveforms within the SphygmoCor software. At least three

waveforms were collected in accordance with the quality criteria within the SphygmoCor software, and an average is reported. Ascending aortic blood pressure was estimated by applying a generalised inverse transfer function, validated by Pauca and colleagues (2001), to radial arterial waveforms (SphygmoCor, AtCor Medical, Australia).

Following this, all participants underwent the assessment of aortic and brachial arterial stiffness. First, the measurement of carotid-femoral path length was assessed via the subtracted method using a tape measure along the body surface. Subsequently, sequential ECG-gated arterial pressure waveforms were recorded from the carotid and femoral arteries, at the site of maximal arterial pulsation, to determine carotid-femoral (aortic) pulse wave velocity (aPWV; expressed as $m \cdot s^{-1}$) (Townsend *et al.*, 2015). Brachial artery stiffness was assessed via determining carotid-radial pulse wave velocity (bPWV), as above, after measuring the carotid-radial path length using the subtracted method. To quantify aortic and brachial arterial stiffness, the SphygmoCor software determined the transit time between the foot of the carotid and the femoral or radial arterial waveforms and divided this by the respective path length. Assessments of aortic stiffness, systolic pressure augmentation and pressure were conducted by one experienced researcher (Appendix III). The coefficient of variation for aPWV, AP and aSBP are 5.8%, 9.6% and 0.4%, respectively (Table 2).

Data Analyses

In order for the aPWV data presented in this thesis to be comparable with previous studies, as well as the published reference values (Reference Values for Arterial Stiffness, 2010), raw aPWV was converted using the equation below (Van Bortel *et al.*, 2012), and are presented as "converted aPWV":

Conversion of raw aPWV:

$$\text{Converted aPWV} = \frac{(\text{Raw carotid to femoral distance} \times 1.12)}{\text{Time}}$$

Where: distance is measured in mm; 1.12 is the conversion factor; time is the absolute difference between the foot of the carotid and femoral pulse waves in seconds (Van Bortel et al., 2012).

This conversion is used so that the PWV distance better reflects true aortic length, as determined from magnetic resonance imaging (MRI) (Huybrechts *et al.*, 2011). However, in line with recent suggestions (Van Bortel *et al.*, 2020), both raw and converted aPWV are reported. Converted aPWV and raw bPWV were statistically adjusted for the respective MAP (Van Bortel *et al.*, 2020) and heart rate (Tan *et al.*, 2016b). The respective MAP adjustment was due to the significant difference between aMAP and MAP in young men (YNR: mean difference -5 [95% confidence interval, -8 to -2], $P = 0.004$; YR: -3 [-5 to -1], $P = 0.013$). This significant pressure gradient was, however, not apparent in middle-aged men (MNR: 0 [-1 to 1], $P = 1.00$; MR: -1 [-4 to -2], $P = 0.420$). The arterial stiffness gradient was also quantified as the difference central and peripheral stiffness (bPWV-aPWV; $m \cdot s^{-1}$).

From the ascending aortic waveform the following variables were recorded: non-augmented aortic systolic pressure (P1, the first systolic peak from the aortic waveform), aSBP, aortic diastolic pressure (aDBP), aortic pulse pressure (aPP; aSBP-aDBP) and aortic mean arterial pressure (aMAP). Also, augmentation pressure (AP; the difference between the first and second peaks of the aortic waveform, expressed in mmHg) and aortic augmentation index (AIx; [AP/aPP*100], expressed as a %) were calculated. Both indices are automatically adjusted to a heart rate of 75bpm within the SphygmoCor software; however, this adjustment is not physiological for these groups. Therefore, in line with recent suggestions (Stoner *et al.*, 2014), these data were adjusted in a statistical model. AP and AIx are influenced by aMAP, heart rate and stature. Notably, the primary variable of interest when comparing systolic pressure augmentation is adjusted AP, as AP increases linearly with age (McEniery *et al.*, 2005) and is not influenced mathematically by the concurrent increase in aPP (Namasivayam *et al.*, 2010). Thus, raw and adjusted AP, but only raw AIx, are reported. Pulse pressure amplification was also determined (PP-aPP).

Statistical Analyses

All data were normally distributed and there were no significant outliers (> 3 standard deviations from the mean). Between group comparisons were made via two separate one-way analysis of variance (ANOVA) tests, to address the primary (MNR vs MR) and secondary (YNR vs YR) study aims. ANOVA was used, instead of independent samples t-tests, so that the between group differences were compared using a similar statistical model in both raw and adjusted form. Following this, one-way analysis of covariance (ANCOVA) was used to determine and compare adjusted group mean data. Adjusted group standard deviations (SD) were using the following equation (Atkinson and Batterham, 2013):

Calculation of standard deviation from standard error:

$$SD = \sqrt{n} \times SE$$

Where: SD, standard deviation; n, sample size; SE, the standard error of the estimated marginal mean from each ANCOVA model.

Data were not adjusted for multiple comparisons as the present study completed pre-planned, and not exploratory, statistical analyses (Perneger, 1998; Cramer *et al.*, 2016). All data are presented as mean ± standard deviation or mean difference [95% confidence interval (CI) for difference] and α was set *a-priori* at < 0.05. All statistical analyses were conducted using SPSS for Windows (version 24, Chicago, IL).

4.3 Results

Arterial stiffness, systolic pressure augmentation and blood pressure data are reported in Table 3. Adjusted aPWV, adjusted AP and raw aSBP are also presented in Figure 20. Only primary outcomes have mean differences with 95% CI and *hedges g* noted within text; P values for adjusted aPWV, adjusted AP and aSBP are reported in Figure 20.

Effects of habitual exercise in middle-aged and young men

Heart rate and blood pressure

In middle-aged men, heart rate was lower in middle-aged runners compared to nonrunners, with no significant differences in aortic or brachial pressures between groups. aSBP was not significantly different between middle-aged runners and nonrunners (3 [-3 to 8] mmHg, *hedges g* = 0.3). In young men, heart rate, non-augmented aSBP, aSBP (-8 [-13 to -3] mmHg, *hedges g* = -1.1), aDBP and DBP were lower in runners. There were no other significant differences between young groups.

Arterial Stiffness

Adjusted aPWV (mean difference [95% CI] -0.6 [-1.6 to 0.5] $m \cdot s^{-1}$, *hedges g* = -0.5) and bPWV (0.1 [-0.6 to 0.8] $m \cdot s^{-1}$, *P* = 0.773) were not significantly different between middle-aged runners and nonrunners. Converted aPWV (*hedges g* = -0.8) and raw bPWV were also not significantly different between middle-aged runners and nonrunners. The aortic-brachial stiffness gradient was not different between middle-aged men. In young men, adjusted aPWV was not significantly different between runners and nonrunners (-0.5 [-1.2 to 0.3] $m \cdot s^{-1}$, *hedges g* = -0.7). However, adjusted bPWV was lower in young runners compared to nonrunners (-1.4 [-2.1 to -0.7] $m \cdot s^{-1}$, *P* = 0.001). Without adjustment, converted aPWV (hedges g = -1.2) and raw bPWV were lower in young runners compared to nonrunners. There was no significant difference in the aortic-brachial arterial stiffness gradient between young groups.

Systolic Pressure augmentation

Adjusted AP was not different between middle-aged runners and age-matched nonrunners (2 [-1 to 4] mmHg, *hedges g* = 0.6). However, raw AP (*hedges g* = 1.3) was significantly higher in middle-aged runners compared to middle-aged nonrunners; this was similar for raw AIx. Adjusted AP was not different between young runners and nonrunners (-1 [-4 to 1]

mmHg, *hedges g* = -0.3). Similarly, raw AP (*hedges g* = -0.5) was not different between young groups. These findings were similar when comparing raw AIx between young groups.

Table 3 – Aortic and brachial haemodynamics

Variable	YNR	YR	*P*	MNR	MR	*P*
n	10	13		10	13	
Arterial Stiffness						
Raw aPWV ($m \cdot s^{-1}$)	5.8 ± 0.7	5.1 ± 0.5	**0.009**	7.5 ± 0.8	6.8 ± 0.9	0.062
Converted aPWV ($m \cdot s^{-1}$)	6.5 ± 0.8	5.7 ± 0.5	**0.009**	8.4 ± 1.0	7.6 ± 1.0	0.064
Adjusted aPWV ($m \cdot s^{-1}$)	6.3 ± 0.8	5.8 ± 0.7	0.231	8.3 ± 1.1	7.7 ± 1.1	0.291
Raw bPWV ($m \cdot s^{-1}$)	7.0 ± 0.9	6.2 ± 0.4	**0.014**	7.3 ± 0.7	7.2 ± 0.6	0.703
Adjusted bPWV ($m \cdot s^{-1}$)	7.3 ± 0.7	5.9 ± 0.7	**0.001**	7.2 ± 0.7	7.3 ± 0.7	0.773
bPWV-aPWV ($m \cdot s^{-1}$)	1.2 ± 0.9	1.1 ± 0.7	0.836	-0.2 ± 0.6	0.4 ± 0.9	0.073
Systolic Pressure Augmentation						
Raw AP (mmHg)	-2 ± 2	-3 ± 2	0.301	6 ± 3	10 ± 3	**0.002**
Adjusted AP (mmHg)	-2 ± 3	-3 ± 3	0.217	7 ± 3	9 ± 3	0.179
Raw AIx (%)	-6 ± 9	-11 ± 9	0.178	19 ± 9	28 ± 8	**0.016**
Heart Rate and Blood Pressure						
Heart Rate (bpm)	60 ± 12	46 ± 7	**0.001**	57 ± 11	43 ± 9	**0.003**
Non-augmented aSBP (mm Hg)	99 ± 9	92 ± 4	**0.022**	102 ± 6	100 ± 7	0.606
aSBP (mmHg)	100 ± 9	92 ± 5	**0.014**	108 ± 4	110 ± 8	0.337
aDBP (mmHg)	73 ± 10	66 ± 6	**0.048**	78 ± 5	76 ± 9	0.615
aPP (mmHg)	27 ± 8	26 ± 4	0.822	30 ± 4	34 ± 6	0.054
aMAP (mmHg)	82 ± 5	78 ± 6	0.089	90 ± 5	88 ± 7	0.274
SBP (mmHg)	119 ± 13	111 ± 5	0.065	119 ± 7	118 ± 8	0.848
DBP (mmHg)	71 ± 7	66 ± 6	**0.037**	76 ± 5	74 ± 7	0.374
PP (mmHg)	47 ± 11	45 ± 7	0.609	42 ± 6	44 ± 5	0.471
MAP (mmHg)	87 ± 8	81 ± 5	0.203	90 ± 5	89 ± 7	0.522
PPA (mmHg)	21 ± 7	19 ± 3	0.522	12 ± 4	9 ± 7	0.285

Data are mean ± SD from two one-way analysis of variance tests for middle-aged and young men separately. *Abbreviations: a, aortic; AIx, augmentation index; AP, augmentation pressure; b, brachial; DBP, diastolic blood pressure; MAP, mean arterial pressure; MNR, middle-aged nonrunner; middle-aged runner; PP, pulse pressure; PPA, pulse pressure amplification; PWV, pulse wave velocity; SBP, systolic blood pressure; YNR, young nonrunner; YR, young runner.*

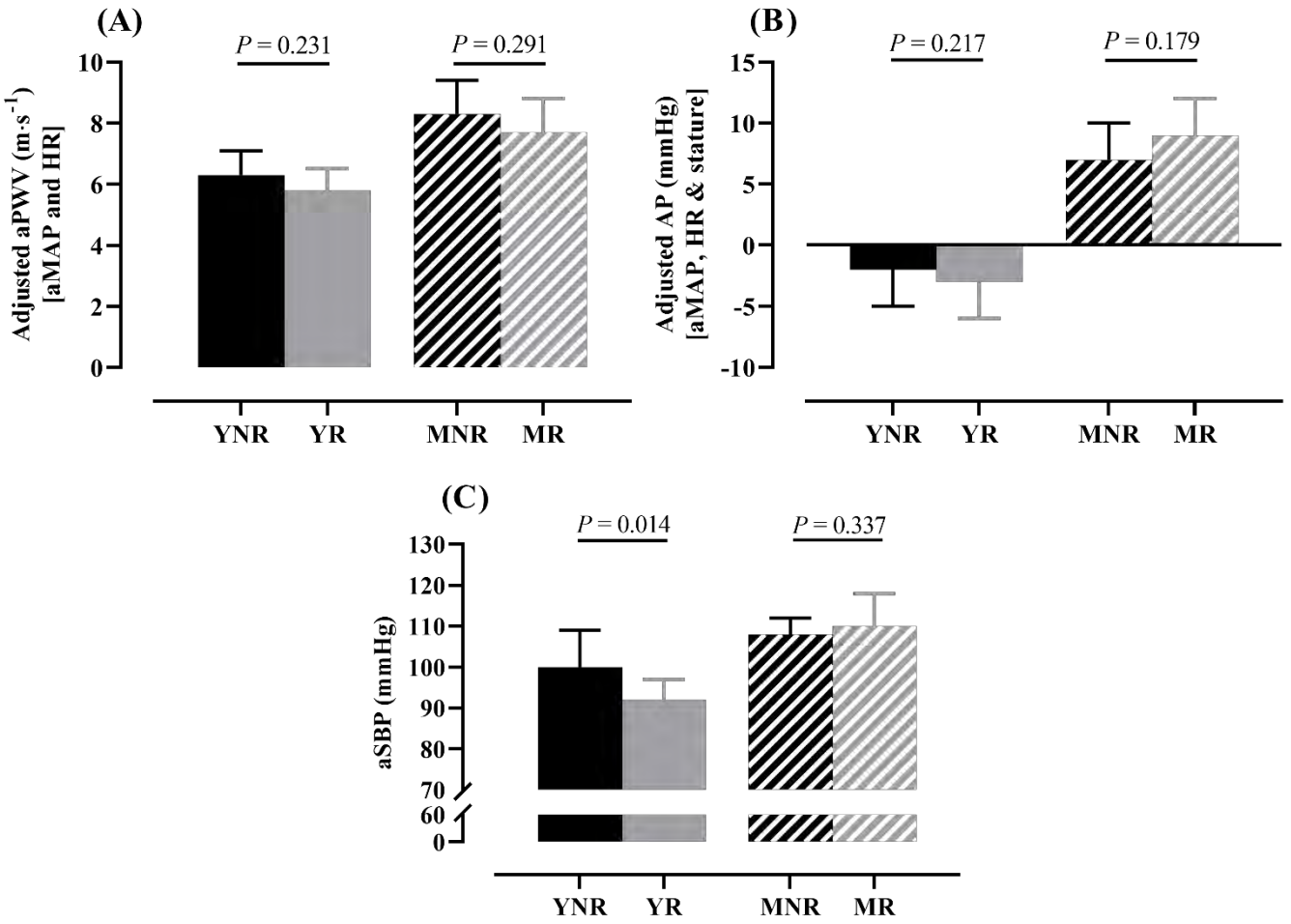


Figure 20 – Effects of habitual exercise on the aortic haemodynamic profile. All data are presented as means with standard deviation error bars. The *P* values presented in panels A & B are from separate ANCOVA analyses to adjust aPWV and AP appropriately. Panel C presents the *P* values as determined by two separate ANOVAs. Adjusted aPWV **(A)** and AP **(B)** were not different between middle-aged or young groups. **(C)** aSBP was not different in middle-aged groups, but was lower in young runners compared to young nonrunners. *Abbreviations: AP, augmentation pressure; aPWV, aortic pulse wave velocity; aMAP, aortic mean arterial pressure; aSBP, aortic systolic blood pressure; HR, heart rate; MNR, middle-aged nonrunners; MR, middle-aged runners; Y, young nonrunners; YR, young runners.*

4.4 Discussion

The primary aim of this thesis chapter was to determine the effects of chronic habitual endurance exercise on the aortic haemodynamic profile in middle-aged men. The principal findings from this chapter are as follows. First, adjusted aPWV, adjusted AP and aSBP were not different between middle-aged runners and nonrunners. Second, adjusted aPWV and adjusted AP were not different between young runners and nonrunners, yet aSBP was lower in young runners. Together, these data suggest that chronic habitual endurance exercise until middle-age does not significantly change the aortic haemodynamic profile when compared to recreationally-active individuals. Furthermore, these data highlight that is necessary to adjust aortic stiffness and systolic pressure augmentation to avoid reporting "erroneous conclusions" regarding the effects of habitual exercise on aortic haemodynamics.

Aortic Stiffness

In the present study, adjusted aPWV was not influenced by habitual exercise in middle-aged men. Notably, converted (and raw) aPWV was also not significantly different between middle-aged endurance-trained runners and recreationally-active nonrunners; the statistical adjustment of aPWV lowered the effect size from a large to a medium effect. These data are in opposition to the previous reports on the effects of habitual exercise in middle-age, which reported lower (raw) aPWV in endurance-trained compared to sedentary individuals (Vaitkevicius *et al.*, 1993; McDonnell *et al.*, 2013; Tarumi *et al.*, 2013). One study also included a recreationally-active group, but only reported a main effect of habitual exercise across groups of young, middle-aged and older men (Gates *et al.*, 2003). Furthermore, in these studies aPWV was not adjusted for aMAP or heart rate (Vaitkevicius *et al.*, 1993; Gates *et al.*, 2003; McDonnell *et al.*, 2013; Tarumi *et al.*, 2013). The inclusion of recreationally-active, but not sedentary, men, alongside the appropriate statistical adjustment, likely explains the disparity between the findings presented here and those reported previously. Including sedentary individuals as a control group may exaggerate the effects of habitual

exercise on aortic stiffness, due to the adverse cardiovascular consequences of sedentarism (Lavie *et al.*, 2019).

Young runners and nonrunners were also compared to discern the effects of habitual exercise from that of age. In younger individuals the effects of habitual exercise on raw aPWV are more equivocal with reports of aortic stiffness being similar (McDonnell *et al.*, 2013; Bjarnegard *et al.*, 2018) or lower (Otsuki *et al.*, 2007a; b) in young trained compared to sedentary individuals, again without appropriate statistical adjustment. When assessed here, adjusted aPWV was not different between young runners and nonrunners. However, unlike in middle-age, habitual exercise was associated with significantly lower converted aPWV in young men. Thus, the adjustment of aPWV may have more importance when comparing younger groups, where, aSBP but not aMAP or aDBP, was lower in runners. Taken together, habitual exercise does not influence aortic stiffness in normotensive middle-aged or young men. In support of these findings, a recent study of older normotensive mixed-sex individuals reported no main effect of habitual exercise on raw aPWV (assessed via applanation tonometry) comparing sedentary, casual, committed and competitive exercisers (Shibata *et al.*, 2018). Nevertheless, future studies should further characterise the effects of habitual exercise on aortic stiffness throughout healthy human ageing.

Aortic systolic Pressure augmentation

Aortic systolic pressure augmentation is also an important determinant of aortic blood pressure, which independently increases cardiovascular risk (Wang *et al.*, 2010). When adjusted for heart rate, aMAP and stature there were no significant effects of habitual endurance exercise on AP (or AIx) in the middle-aged men studied here. Thus, it is unlikely that aortic reservoir pressure, which is the primary determinant of the increase in systolic pressure augmentation (Davies *et al.*, 2010; Schultz *et al.*, 2015), is affected by habitual exercise. Nevertheless, as aortic reservoir pressure was not measured in this thesis chapter, future studies should provide more evidence on this. The finding that habitual exercise does

not significantly influence systolic pressure augmentation is similar to previous reports in middle-aged (McDonnell *et al.*, 2013) and older (Shibata *et al.*, 2018) individuals. In young individuals, however, there are reports that systolic pressure augmentation is similar (Knez *et al.*, 2008; Denham *et al.*, 2016) or lower (Edwards and Lang, 2005; McDonnell *et al.*, 2013; Bjarnegard *et al.*, 2018) in endurance-trained compared to sedentary individuals. In this chapter, there were no differences in adjusted AP in between young groups. As noted above, the comparison of endurance-trained and sedentary individuals may exaggerate the effects of habitual exercise. The appropriate selection of a recreationally-active control group, statistical adjustment of AP and study of men only may explain the difference between the findings presented here and those reported previously. Thus, the current study suggests that habitual endurance exercise exerts no significant influence on adjusted indices of systolic pressure augmentation in middle-aged or young men, when compared to recreationally-active individuals.

Aortic blood pressure

The interaction between aortic stiffness and systolic pressure augmentation largely determines aSBP (Nichols *et al.*, 2008). In the present study, there was no significant effect of habitual endurance exercise on aSBP in middle-aged men. Previously, McDonnell *et al.* (2013) reported that aortic blood pressure was lower in middle-aged/older active compared to sedentary individuals. However, in that study all individuals had high-normal blood pressure or were hypertensive. The authors also compared groups of young individuals, finding that aSBP was lower in the active group (McDonnell *et al.*, 2013). These data are supported by the findings in the current study, whereby aSBP was lower in young runners compared to nonrunners. Thus, at least in normotensive men, there appears to be an age-dependent effect of habitual exercise on aSBP; this occurs despite no effects of habitual exercise on adjusted aortic stiffness or systolic pressure augmentation in either middle-aged or young men. The greater magnitude of difference in non-augmented aSBP between young

(-6 mmHg) compared to middle-aged (-2 mmHg) runners and nonrunners, likely explains this age-dependent effect on aSBP. Non-augmented aSBP (i.e. the first systolic shoulder of the aortic waveform) is determined by the interaction between the left ventricular ejection wave and ascending aortic stiffness (Nichols *et al.*, 2008). This is often the peak pressure reached during systole in young men (Hughes *et al.*, 2013), as was the case for the young men studied here. In this instance, aSBP is largely independent of systolic pressure augmentation. The age-dependent effect of habitual exercise on aSBP is likely influenced by differences in the influence of habitual exercise on ascending aortic stiffness with age; the reason(s) for this are unclear. Short term endurance exercise training has been shown to lower ascending aortic stiffness (Bhuva *et al.*, 2020), as assessed using MRI. The effects of chronic habitual endurance exercise on ascending aortic stiffness are currently unknown but warrant future investigation, especially in relation to the data presented here. The data presented here show that the effect of habitual exercise on aSBP is age-dependent in normotensive men. Notably, therefore, in terms of cardiovascular risk as determined by aortic blood pressure (Vlachopoulos *et al.*, 2010; Kollias *et al.*, 2018), the benefit of habitual exercise is apparent in young but not middle-aged men.

Methodological considerations

There are some limitations of this study which warrant discussion. First, as women were not included in this study, it is not possible to determine the effects of habitual exercise in men and women separately. However, since the collection of these data a study has detailed the effects of habitual exercise on aortic haemodynamics in young women (Bjarnegard *et al.*, 2018) and shows similar findings to those presented here. The effect of habitual exercise on aortic haemodynamics in middle-aged/older women has also been studied previously (Tanaka *et al.*, 1998). This study reported that the age-related (pre- vs post-menopausal) increase in aPWV was absent in physically-active women, unlike the markedly higher aPWV with age in sedentary women (Tanaka *et al.*, 1998).

Second, augmentation pressure was used as an index of systolic pressure augmentation. It is recommended that wave intensity analysis provides better insight into arterial wave reflection, which contributes to backward-travelling pressure, than AP or AIx (Townsend *et al.*, 2015). Accordingly, AP was interpreted as an index of systolic pressure augmentation only, which is determined primarily by aortic reservoir pressure (Davies *et al.*, 2010; Schultz *et al.*, 2015).

Finally, as a cross-sectional study design was utilised in this study, it is not possible to determine the effects of chronic habitual exercise within individuals. Future studies should complete longitudinal follow-up studies in endurance-trained, recreationally-active and sedentary men and women of different ethnicities to discern the effects of age, sex, race and habitual exercise on the aortic haemodynamic profile.

4.5 Conclusion

These data provide important new information on the effects of habitual exercise on aortic haemodynamics in middle-aged and young normotensive men. This study shows an age-dependent effect of habitual exercise on aortic haemodynamics with lower aortic blood pressure in young, but not middle-aged, runners compared to nonrunners. The reason(s) for this difference with age are unclear, but may be related to the age-dependent effects of habitual endurance exercise on ascending aortic stiffness. Future studies are required to investigate the interaction between age, habitual endurance exercise and potential differences in regional aortic stiffness (i.e. ascending vs arch vs descending). Furthermore, the data presented here show that there is no influence of habitual exercise on adjusted aortic stiffness or systolic pressure augmentation in either age group. These data highlight the importance of selecting recreationally-active, but not sedentary, control groups when determining the effects of habitual endurance exercise; as well as the importance of appropriately adjusting aPWV and AP.

4.6 Study Hypotheses

i) Adjusted aortic stiffness and aortic blood pressure would be lower in middle-aged runners compared to nonrunners, with no difference in systolic pressure augmentation between groups. **(REJECTED)**

This chapter reports no effect of habitual exercise on the aortic haemodynamic profile in middle-aged normotensive men. The next chapter will determine the effects of habitual endurance exercise on the primary factor which regulates peripheral blood pressure at rest, the vascular sympathetic baroreflex.

CHAPTER 5. EXPERIMENTAL STUDY 2

THE EFFECT OF CHRONIC HABITUAL ENDURANCE EXERCISE ON THE AUTONOMIC REGULATION OF RESTING BRACHIAL BLOOD PRESSURE IN NORMOTENSIVE MIDDLE-AGED MEN

A version of this thesis chapter has been published in the

American Journal of Physiology - Heart and Circulatory Physiology

Wakeham DJ, Lord RN, Talbot JS, Lodge FM, Curry BA, Dawkins TG, Simpson LL, Shave RE, Pugh CJA & Moore JP (2019). Upward resetting of the vascular sympathetic baroreflex in middle-aged male runners. *Am J Physiol Heart Circ Physiol* 317, H181-H189.

5.1 Introduction

Healthy human ageing exerts a marked influence on the determinants of arterial blood pressure. As reported in the previous chapter, habitual endurance exercise did not influence aortic stiffness, systolic pressure augmentation or aortic blood pressure in normotensive middle-aged men. A hallmark age-related change in the cardiovascular system, in relation to peripheral blood pressure, is the elevation of MSNA burst incidence and frequency (Iwase *et al.*, 1991; Ebert *et al.*, 1992; Matsukawa *et al.*, 1998b). Notably, this age-related increase in MSNA reflects a change in the autonomic regulation of peripheral (i.e. brachial) blood pressure. However, the increase in vascular sympathetic vasoconstrictor drive does not necessarily lead to arterial hypertension (Taylor and Tan, 2014). Whether habitual exercise influences the autonomic regulation of peripheral blood pressure in healthy ageing is the focus of this experimental chapter.

The interaction of chronic habitual exercise training and age-related changes to vascular sympathetic activity is unclear. This is despite arterial baroreflex control of MSNA (i.e. the vascular sympathetic limb of the arterial baroreflex) being the primary mechanism through which the autonomic nervous system regulates vascular resistance. Thus, the vascular sympathetic baroreflex plays a pivotal role in blood pressure homeostasis. To date, relatively little consensus exists among previous microneurographic studies, which have found that basal MSNA burst frequency for middle-aged and older endurance-trained men is either higher (Ng *et al.*, 1994a), not different (Notarius *et al.*, 2012) or lower (Studinger *et al.*, 2009), compared to sedentary peers. Furthermore, quantification of MSNA burst occurrence relative to the number of opportunities for a burst (i.e. burst incidence; the vascular sympathetic baroreflex operating point) does not provide further clarity. However, the method of burst quantification provides different neurophysiological insight into regulation of vascular sympathetic activity (Charkoudian and Wallin, 2014). Burst frequency indicates the amount of sympathetic activity (or local noradrenaline release) that the

vasculature is exposed to in a given time period (Wallin *et al.*, 1992). In contrast, burst incidence indicates the probability of a sympathetic burst occurring at a given arterial pressure (McAllen and Malpas, 1997). Baroreceptor signals influence both the timing and the probability of sympathetic bursts over a wide pressure range. Burst incidence, therefore, is an index of sympathetic burst 'gating', which couples a burst of MSNA with a cardiac cycle (Keller *et al.*, 2006), rather than vascular sympathetic activity *per se*. In the only study to consider the influence of chronic exercise training in healthy ageing on vascular sympathetic baroreflex function, the training status of healthy older males had no effect on resting diastolic blood pressure (DBP; operating pressure), MSNA burst incidence (operating point), or the MSNA responsiveness (gain) assessed during a modified Oxford baroreceptor test (Studinger *et al.*, 2009).

The cardiovagal baroreflex plays a primary role in the regulation of cardiac interval at rest (Dampney, 2017), and thus contributes to the support of arterial pressure via eliciting changes in cardiac output. Despite this, the vascular sympathetic limb of the arterial baroreflex contributes more to the support of arterial blood pressure at rest and during physiological stress (Ogoh *et al.*, 2003a; Dampney, 2017), by mediating reflex changes in vascular resistance. Cross-sectional evidence from middle-aged and older men indicates that chronic endurance training is associated with greater cardiovagal baroreflex responsiveness when compared to sedentary, but not moderately exercising, middle-aged men (Monahan *et al.*, 2000). The finding of greater cardiovagal baroreflex responsiveness in middle-aged and older endurance-trained compared sedentary individuals has been observed in several other studies to date (Davy *et al.*, 1996; Davy *et al.*, 1998a; Monahan *et al.*, 2000; Hunt *et al.*, 2001; Monahan *et al.*, 2001a; Pierce *et al.*, 2016).

Taking the various uncertainties into account, the primary aim of this chapter was to determine the effect of chronic habitual endurance exercise on the autonomic regulation of resting brachial blood pressure by the vascular sympathetic baroreflex in normotensive middle-aged men. Furthermore, to discern the effect of exercise training from age, a

secondary aim was to compare the sympathetic control of blood pressure between young runners and young nonrunners. Based upon limited data, it was hypothesised that vascular sympathetic baroreflex function would not be different between middle-aged runners and nonrunners.

5.2 Methodology

Study Overview

For this experimental chapter, participants underwent comprehensive haemodynamic and neurophysiological assessment (Figure 21).

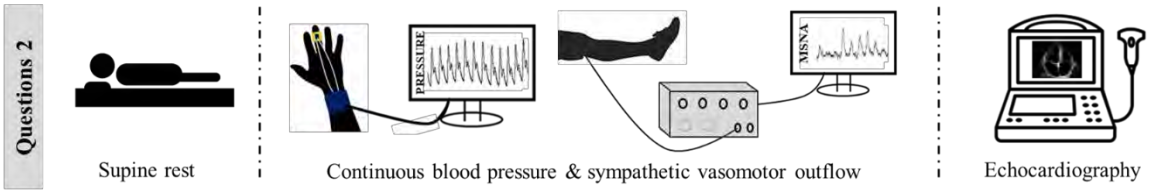


Figure 21 – Study 2 Schematic.

Experimental Measures

Heart rate and arterial blood pressure

Heart rate and brachial artery blood pressure were monitored continuously via three-lead electrocardiography and finger photoplethysmography, respectively (Finometer® Pro, FMS, Groningen, Netherlands). Participants were supine throughout testing. A blood pressure cuff was attached to the second or third digit of the right hand, which was height corrected for the distance from the heart to the finger cuff. The Finometer® Pro enabled continuous beat-by-beat arterial blood pressure monitoring via a photo-electric plethysmograph and a servo-controlled setpoint adjuster within the finger cuff. To assess arterial pressure, unloaded arterial volume is determined when intra- and extra-arterial pressure are matched by adjustments of external pressure on the artery. When pressures are matched, extra-arterial pressure is an indirect measure of intra-arterial pressure (Boehmer, 1987). This technique is termed the volume-clamp method, as first described by Peňáz (1973). The dynamic servo-

controlled setpoint adjuster modulates cuff pressure and allows for, via an infrared light-emitting diode in the cuff, automated assessment of arterial unloaded volume. This is used to re-calibrate pressure throughout recordings (Wesseling, 1995). Furthermore, a return to flow (RTF) calibration is used to determine brachial SBP, via automated sphygmomanometry, which finger SBP is subtracted from. This calibration corrects for brachial-radial PPA. Alongside a digital filter which adjusts for change in pulse wave morphology, the RTF calibration enables reconstruction of the brachial artery pressure waveform (Bos *et al.*, 1996). This reconstruction of the brachial artery wave form has been validated for use to estimate brachial artery blood pressure from finger photoplethysmography (Guelen *et al.*, 2008). These data were sampled at 1000Hz using a commercial data acquisition system and stored for offline data analysis (Chart Version 8, Lab Chart Pro, AD Instruments, UK).

Muscle sympathetic nerve activity

The microneurography technique involves the insertion of a tungsten microelectrode into a peripheral nerve (Figure 22A). As shown in Figure 22B, there are many axons within the nerve in which the recording electrode is placed. Accordingly, microneurography enables researchers to directly record either multi-unit (an average from multiple axons) or single-unit (from one axon) MSNA. The collection and analysis of multi-unit and single-unit nerve recordings differs markedly. Due to the greater understanding and more widespread use of the multi-unit signal in the relevant literature, this thesis focuses upon multi-unit MSNA as assessed from the integrated neurogram (Figure 22C). From the integrated neurogram, four indices of MSNA can be quantified (Figure 22D): i) burst frequency, ii) burst amplitude (or area), iii) total activity, and iv) burst incidence. Each index of MSNA provides different insight into the autonomic regulation and neural control of blood pressure (Charkoudian and Wallin, 2014). MSNA total activity, calculated as the sum of MSNA burst frequency and amplitude, reflects the “amount” of MSNA directed to blood vessels per unit time. These indices are used to quantify the resting level of MSNA and as an index of neural control of

blood pressure when assessing the transduction of changes in MSNA into changes in vascular tone. Importantly, the arterial baroreflex couples MSNA bursts with the cardiac cycle. Thus, as MSNA burst frequency/total activity are time-dependent, they are influenced by the number of "opportunities" (i.e. cardiac cycles) for a burst to occur per unit time. MSNA burst incidence, however, reflects the probability of burst occurrence, which is independent of the number of cardiac cycles. Accordingly, MSNA burst incidence is used as an index of the arterial baroreflex (autonomic) regulation of MSNA. Thus, reporting all indices of MSNA together provides more comprehensive insight into the sympathetic control of blood pressure.

Multi-unit muscle sympathetic nerve activity was obtained via microneurography using a recording system (Nerve Traffic Analyser, Model 663 C, University of Iowa, Iowa City, IA, USA) following a recognized technique (Sundlof and Wallin, 1978). These data were sampled at 1000Hz using a commercial data acquisition system and stored for offline data analysis (Chart Version 8, Lab Chart Pro, AD Instruments, UK). First, external mapping of the nerve was conducted to track the route of the nerve. This enables directed insertion of a unipolar tungsten microelectrode (FHC, Bowdoin, ME), with shaft diameter of 0.1 mm (impedance 1-5 MW), across the skin at the popliteal fossa and into the fibular nerve by an experienced microneurographer. A maximum search time of 60 minutes was stipulated to minimize the risk of mild paraesthesia, which occurs in < 5% of cases, when the recording microelectrode is in place. Importantly, this paraesthesia is not permanent and disappears with removal of the microelectrode (Meah *et al.*, 2019). A reference electrode was placed subcutaneously approximately 2-3 cm from the site of the recording electrode. The recorded raw neurogram was amplified (70 000 to 160 000 fold), band-pass filtered (700 to 2000 Hz), full-wave rectified and integrated with a resistance-capacitance circuit (time constant 0.1 sec).

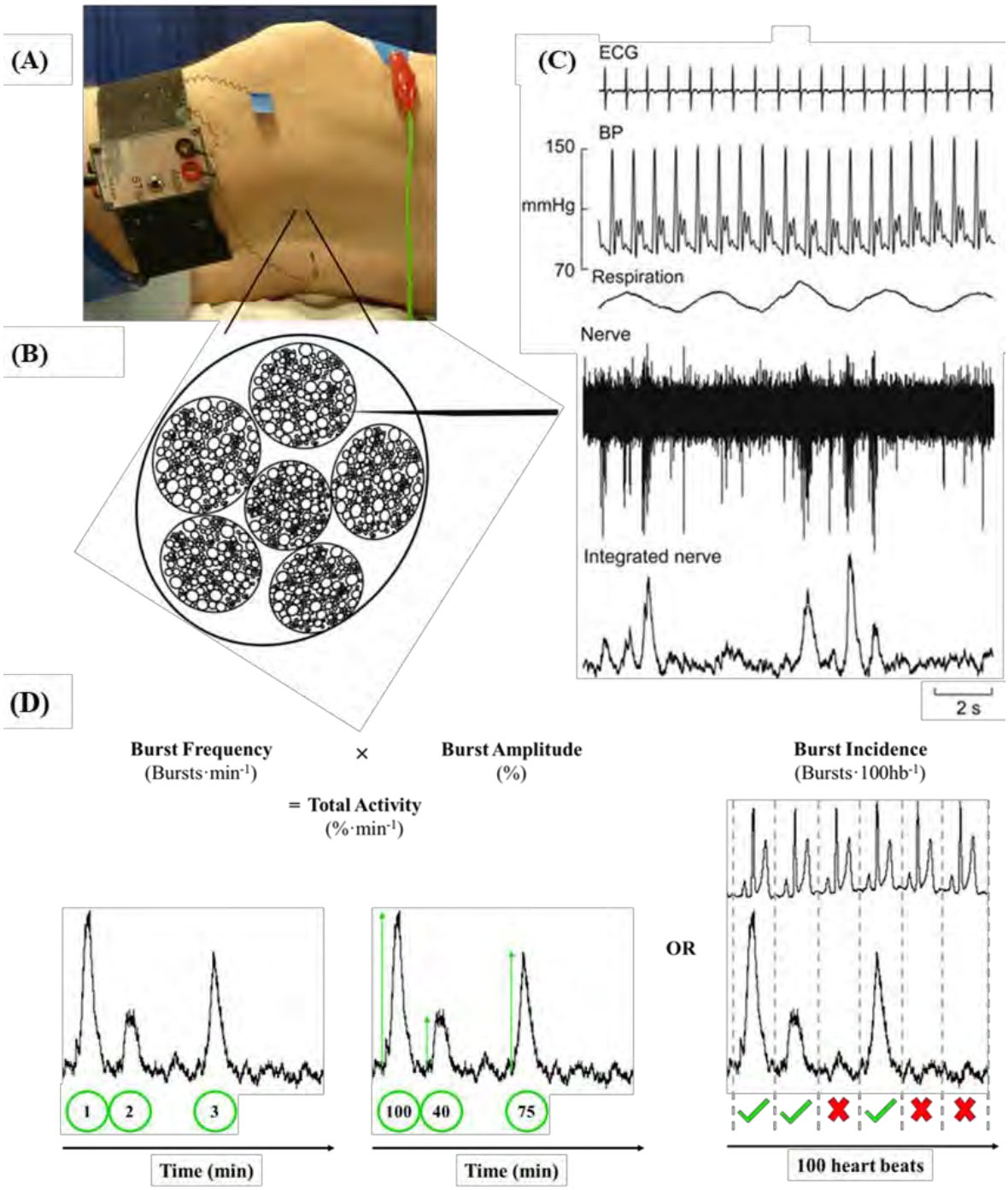


Figure 22 – Sympathetic microneurography and quantifying muscle sympathetic nerve activity. (A) The technique of microneurography requires the insertion of two tungsten electrodes subcutaneously. The first electrode (with a blue tag) is inserted superficially under the skin and acts as a reference electrode. **(B)** The second, recording, electrode is inserted into a nerve fascicle in the fibular nerve to record either single-unit (from one axon) or multi-unit (an average from multiple axons) sympathetic nerve activity. **(C)** In this example, muscle sympathetic nerve activity (MSNA) is being recorded. The raw nerve signal is amplified (in part by the preamplifier strapped to the participants leg) and then integrated. The positive peaks in the integrated nerve signal are termed bursts of MSNA which are identified and analysed post-hoc. **(D)** The four indices of MSNA are used to provide different insight into the neural control and autonomic regulation of blood pressure (see text). Burst amplitude (or area) is dependent on the proximity of the recording electrode to the nerve axon(s) it is recording from. To account for this limitation when conducting multi-unit analyses, the tallest MSNA burst in a recording period is assigned a value of 100 (%); all other bursts are expressed as a percentage of this largest normalised burst. *Panels A-C and D are adapted from Macefield (2013) and Busch (2018), respectively.*

Satisfactory recordings of MSNA were identified, dependent on the following criteria (White *et al.*, 2015), (i) pulse-synchronous bursts of activity, (ii) increased burst occurrence in response to voluntary apnoea, (iii) unaffected burst pattern during stroking of the skin and (iv) 3:1 signal to noise ratio. Respiratory rate was monitored via a nasal cannula attached to a capnograph (Capnocheck® Sleep Capnograph, Smiths Medical, UK) to confirm that participants avoided breath holding (Delius *et al.*, 1972b). No adverse events or complications occurred during or were reported following the microneurography procedure in any subject. At least 10 minutes after an acceptable MSNA recording site was found, echocardiograms were acquired. The reliability of MSNA has been determined previously, showing that despite large inter-individual variability there is strong intra-individual variability over the short, medium and long-term (Fagius and Wallin, 1993; Grassi *et al.*, 1997; Kimmerly *et al.*, 2004; Fonkoue and Carter, 2015; Notay *et al.*, 2016).

Assessment of arterial baroreflex function

Brachial blood pressure, R-R interval and MSNA recordings were acquired during six minutes of spontaneous breathing to characterize vascular sympathetic and cardiovagal baroreflex function; the analyses of these data are described later.

Cardiac Ultrasound (Echocardiography)

High-resolution ultrasonography allows for determination of internal bodily structures via ultrasonic waves (> 20,000 Hz; a frequency too high for the human to hear) which are sent and received by a transducer. Within each transducer piezoelectrical crystals generate and receive ultrasonic waves, which are reflected (or echo) back to the transducer from tissues at different depths, enabling simultaneous assessment of deep and superficial body structures (Galiuto, 2011). The reflected waves cause disturbances to the crystals within the transducer. The magnitude of this disturbance is converted to a grey-scale (B-mode) image which enables the assessment of the heart (termed echocardiography) and blood vessels (termed vascular ultrasonography, see chapter 6).

In this thesis transthoracic echocardiography was used to assess the morphology of the left ventricle to enable characterisation of ventricular volumes (Radjenovic and Roditi, 2019). Echocardiograms were acquired using a commercially available ultrasound system (Vivid E9, GE Medical, Norway) with a 1.5 to 4 MHz array probe to determine left ventricular stroke volume. Images were obtained from apical 4- and 2-chamber views by a single experienced sonographer (see Appendix III) and saved for offline analysis with commercially available software (EchoPAC, BT12, GE Medical, Norway).

Data Analyses

The reconstructed brachial arterial pressure waveform was calibrated at regular intervals to the average resting systolic and diastolic pressures measured, at that time point, via manual sphygmomanometry (Welch Allyn Durashock DS66, UK). This was conducted to adjust for any potential drift in the measurement which may occur during extended recording periods.

Left ventricular stroke volume was estimated using the Simpson's-biplane method (Lang *et al.*, 2015); thus permitting determination of cardiac output (heart rate x stroke volume) and total peripheral resistance (TPR; MAP/cardiac output). Stroke volume was calculated as the absolute difference between left ventricular end-diastolic and end-systolic volumes, via tracing around the endocardial borders at each time point within the cardiac cycle, in both the 4- and 2-chamber views. The coefficient of variation for left ventricular stroke volume in this study was 6.4% (see Table 2). The average of three cardiac cycles was used for analysis and calculation of related haemodynamics. Satisfactory images for the quantification of stroke volume were not recorded in one individual (one middle-aged runner). Allometric scaling of left ventricular stroke volume and cardiac output to body surface area, to account for between-group differences in body size (Dewey et al., 2008), did not change the findings from this study. Thus, only absolute stroke volume and cardiac output are reported.

Multi-unit bursts of MSNA were verified via visual inspection following adjustment for baroreflex latency (time between R wave and peak burst height; Table 4) (White *et al.*, 2015), which aligned each burst with the appropriate R wave of the ECG. MSNA was quantified as burst frequency (bursts per minute [bursts·min^{-1}]) and burst incidence (bursts per 100 heartbeats [bursts·100hb^{-1}]). Examples of the dynamic relationship between beat-by beat arterial pressure and bursts of MSNA at rest are shown in Figure 23.

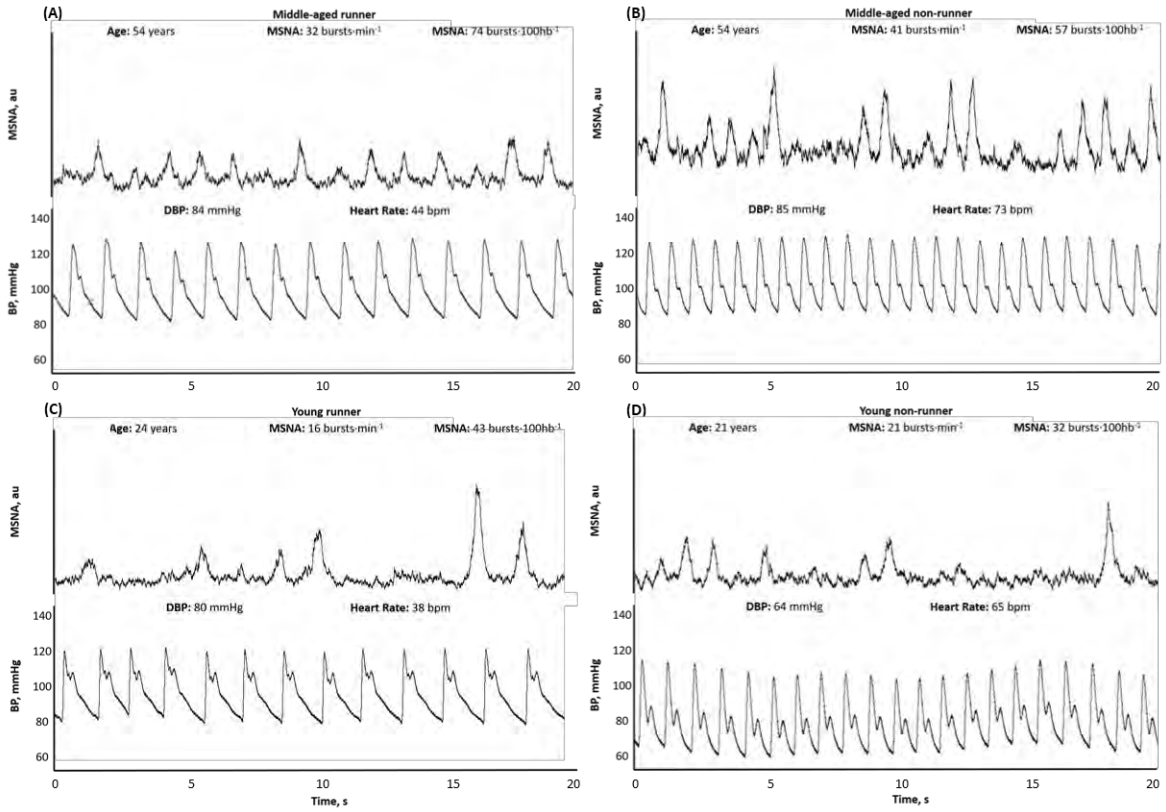


Figure 23 – Example recordings of muscle sympathetic nerve activity and brachial blood pressure during supine rest. 20 seconds of resting muscle sympathetic nerve activity (MSNA) and blood pressure (BP) data are shown from one representative participant per group: (A) Middle-aged runner; (B) Middle-aged non-runner; (C) Young runner; (D) Young non-runner.

The slope of the stimulus-response relationship between DBP and MSNA burst probability was calculated to represent vascular sympathetic baroreflex gain (Sundlof and Wallin, 1978; Kienbaum *et al.*, 2001). DBP was averaged into bins of 2 mmHg increments, to minimize the influence of respiration on MSNA and to maximize the number of data points for inclusion in the linear regression model (Figure 24). The percentage of cardiac

cycles associated with a burst of MSNA (ranging from zero to 100%), per bin of DBP, was used to calculate burst probability. Data were included for further analysis if, (i) at least five data points for each linear regression were available and (ii) a correlation coefficient of ≥ 0.5 was present (Hart *et al.*, 2011). Mean values and tests of statistical significance are presented for 20 middle-aged (11 runners) and 21 younger men (11 runners). Numerical weighting was adopted for this analyses to minimize the influence of differences in the number of cardiac cycles within each DBP bin (Kienbaum *et al.*, 2001). The operating pressure and point of the vascular sympathetic baroreflex was determined from mean diastolic pressure and corresponding average burst incidence, respectively.

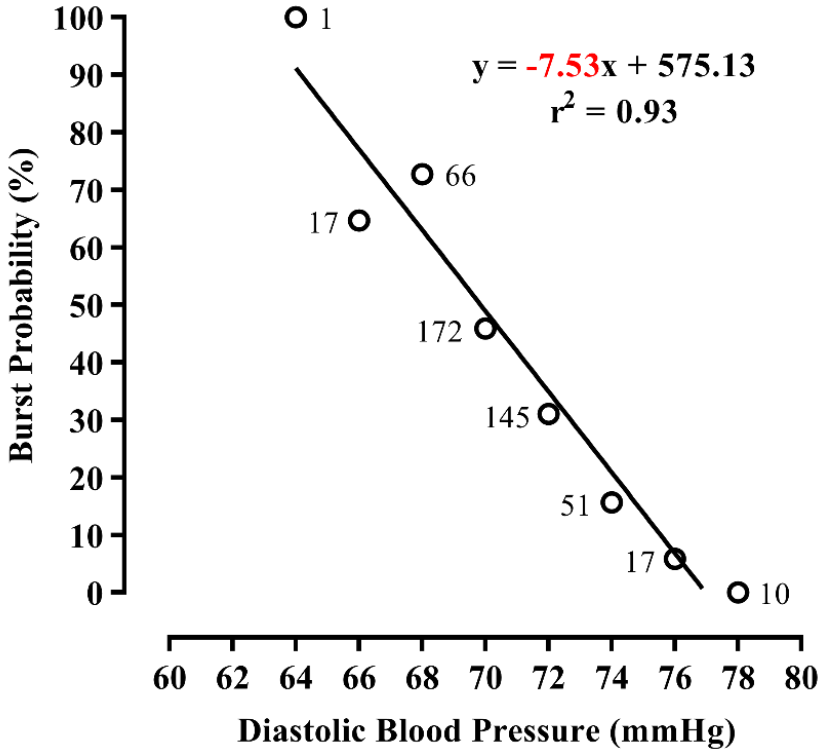


Figure 24 – Assessment of vascular sympathetic baroreflex gain. An example of the weighted linear regression analyses used to determine vascular sympathetic baroreflex gain from the slope (identified in red). The number by each data point represents the number of cardiac cycles per 2 mmHg bin.

Cardiovagal baroreflex gain was assessed by the sequence method using customized computer software (CardioSeries version 2.4, Ribeirao Preto, São Paulo, Brazil; Figure 25). Thresholds for a minimum change in SBP and R-R interval were set at 1 mmHg and 6 ms,

respectively (Parati *et al.*, 1988). If average R-R interval for an individual was ≥ 800 ms a delay of 1 beat was applied so that the SBP was regressed against the following R-R interval (Eckberg and Eckberg, 1982). Data were included for further analysis upon conditions of (i) a minimum of three data points for a linear regression were available and (ii) a correlation coefficient of ≥ 0.8 was present (Parati *et al.*, 2000). The operating point of the cardiovagal baroreflex was determined from mean prevailing SBP and corresponding average R-R interval. Data, including "up" and "down" sequence gain, and the number of baroreflex sequences, are presented for 20 middle-aged (11 runners) and 21 younger men (11 runners).

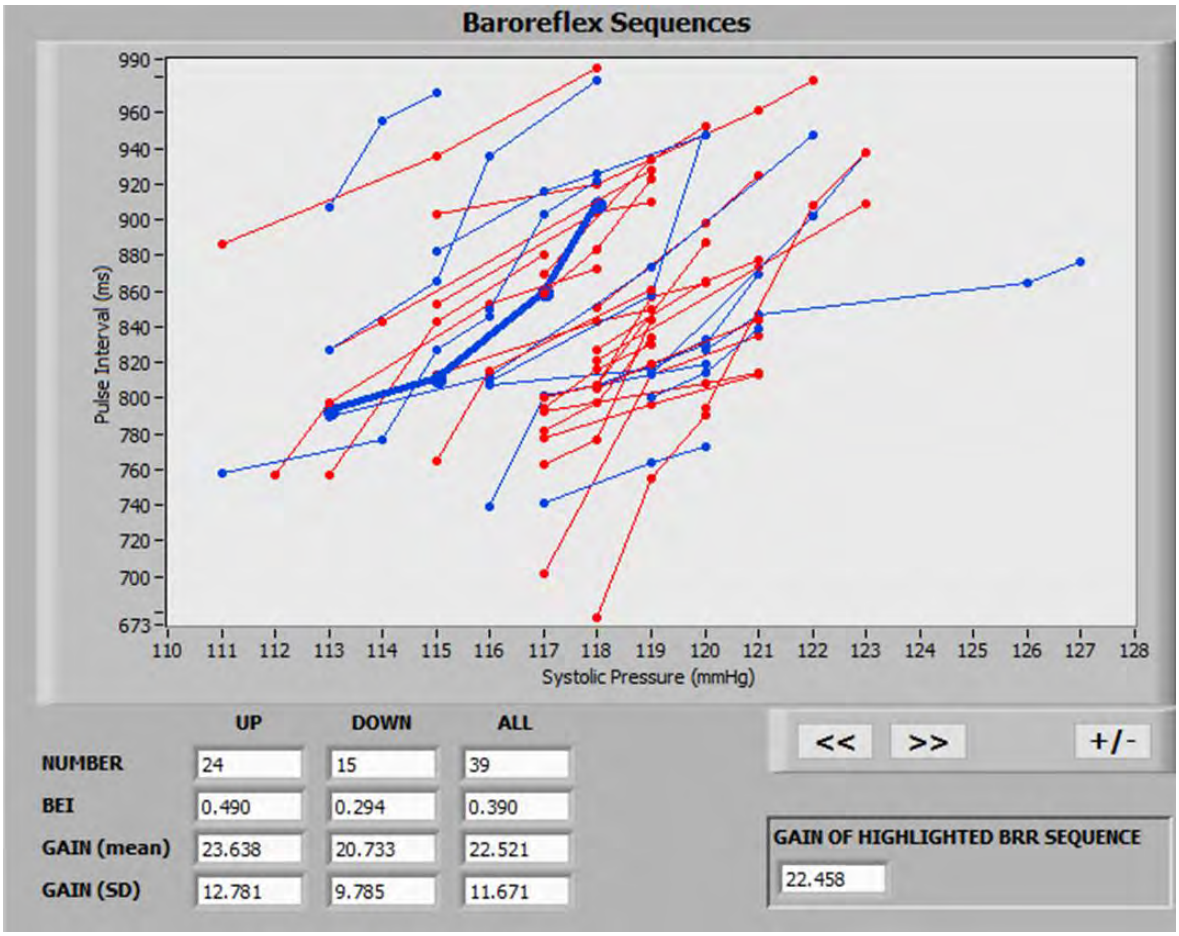


Figure 25 – Assessment of cardiovagal baroreflex gain. An example of the sequence method analysis, using the CardioSeries software (CardioSeries version 2.4, Ribeirao Preto, São Paulo, Brazil), to determine the average of both "up" (red slopes) and "down" (blue slopes) sequences.

Statistical Analyses

In line with the primary (MR vs MNR) and secondary (YR vs YNR) aims of this study, and after checking compliance with basic parametric assumptions, between-group differences

were assessed for each comparison via independent samples t-tests. Alpha was set *a priori* as $P < 0.05$. All statistical analyses were completed using SPSS for Windows (Version 24, Chicago, IL) and data are reported as mean ± standard deviation.

5.3 Results

Table 4 presents the between-group differences in haemodynamics, MSNA and cardiovagal baroreflex gain. Arterial baroreflex function is presented in Figure 26.

Resting haemodynamics, and vascular sympathetic neural activity

In middle-aged runners compared to nonrunners heart rate was lower and stroke volume was higher. There were no other differences in resting haemodynamics in middle-aged men and MSNA burst frequency was not different.

As for middle-aged men, stroke volume was higher and heart rate was lower in young runners compared to nonrunners. MSNA burst frequency and the other haemodynamic indices were not significantly different between young men.

Arterial baroreflex function

Among middle-aged men, there was no difference between runners and nonrunners for the diastolic operating pressure of the vascular sympathetic baroreflex (*hedges g* = 0.3, P = 0.525); however, the corresponding MSNA operating point was higher in the runners (*hedges g* = 1.1, P = 0.008; Figure 26A). The vascular sympathetic baroreflex gain was not influenced by habitual exercise in middle-aged men (-6.1 ± 3.3 vs. -7.3 ± 2.9 %·mmHg^{-1}, *hedges g* = 0.4, P = 0.582; Figure 26A). Vascular sympathetic baroreflex latency was shorter in middle-aged runners compared to nonrunners (Table 4). As for the cardiovagal baroreflex, the systolic operating pressure was not different between middle-aged runners and nonrunners (*hedges g* = 0.2, P = 0.622), but the corresponding operating R-R interval was higher for runners (*hedges g* = 1.6, P = 0.001; Figure 26B). The cardiovagal baroreflex gain (33.6 ± 13.7 vs 25.5 ± 11.9, P = 0.174; Figure 26B) and number of combined baroreflex sequences (25 ± 12 vs. 37 ± 17, P = 0.103) were not different in middle-aged men; data for

positive and negative pressure ramps and the number of sequences per ramp are presented in Table 4.

Among young men, there was no significant difference in the vascular sympathetic operating pressure (*hedges g* = 0.4, *P* = 0.281), corresponding MSNA operating point (*hedges g* = 0.5, *P* = 0.244) or gain (-5.8 ± 1.9 vs. -6.7 ± 3.9 %·mmHg^{-1}, *hedges g* = -0.3, *P* = 0.670) between young runners and nonrunners (Figure 26A). For the cardiovagal limb of the arterial baroreflex, the systolic operating pressure was lower (*hedges g* = -1.2, *P* = 0.012) and the corresponding R-R interval was higher (*hedges g* = 3.0, *P* < 0.001) for young runners when compared to young nonrunners. The cardiovagal baroreflex gain was not different (37.2 ± 13.8 vs 26.4 ± 10.3, *P* = 0.061; Figure 27B) but was assessed during fewer baroreflex sequences in young runners compared to nonrunners (11 ± 17 vs. 50 ± 23, *P* < 0.001).

Table 4 – Haemodynamics, muscle sympathetic nerve activity and cardiovagal baroreflex gain

Variable	**YNR**	**YR**	***P***	**MNR**	**MR**	***P***
n	10	13		10	13	
Haemodynamics						
Heart rate (bpm)	64 ± 9	45 ± 5	**< 0.001**	56 ± 9	43 ± 7	**< 0.001**
Stroke volume (ml)	61 ± 5	92 ± 8	**< 0.001**	62 ± 8	70 ± 11	**0.029**
Cardiac output ($L \cdot min^{-1}$)	3.8 ± 0.5	4.1 ± 0.5	0.204	3.4 ± 0.5	3.0 ± 0.6	0.099
TPR ($mmHg \cdot L \cdot min^{-1}$)	24.3 ± 4.3	21.3 ± 3.4	0.077	29.1 ± 3.1	31.6 ± 4.9	0.183
MAP (mmHg)	90 ± 9	86 ± 6	0.086	95 ± 8	92 ± 5	0.541
Respiration rate ($breaths \cdot min^{-1}$)	13 ± 3	15 ± 1	0.051	11 ± 3	12 ± 4	0.387
Muscle Sympathetic Nerve Activity						
Burst Frequency ($bursts \cdot min^{-1}$)	18 ± 8	16 ± 9	0.601	28 ± 13	30 ± 6	0.578
Burst Incidence ($bursts \cdot 100hb^{-1}$)	27 ± 12	36 ± 22	0.244	50 ± 23	72 ± 15	**0.008**
Baroreflex latency (seconds)	1.26 ± 0.05	1.23 ± 0.07	0.273	1.24 ± 0.07	1.14 ± 0.12	**0.023**
Cardiovagal Baroreflex Gain						
'Up' Gain ($ms \cdot mmHg^{-1}$)	31 ±12	41 ± 21	0.187	28 ± 13	34 ± 15	0.293
# of sequences (n)	20 ± 12	8 ± 4	**0.004**	17 ± 9	11 ± 6	0.147
'Down' Gain ($ms \cdot mmHg^{-1}$)	24 ± 10	33 ± 12	0.079	23 ± 12	33 ± 17	0.159
# of sequences (n)	30 ± 14	9 ± 5	**< 0.001**	20 ± 9	13 ± 8	0.107

Data are presented as mean ± standard deviation. P values were determined from independent samples t-tests. *Abbreviations: MAP, mean arterial pressure; MNR, Middle-aged nonrunner; MR, Middle-aged runner; n, number; TPR, total peripheral resistance. YNR, Young nonrunner; YR, Young runner.*

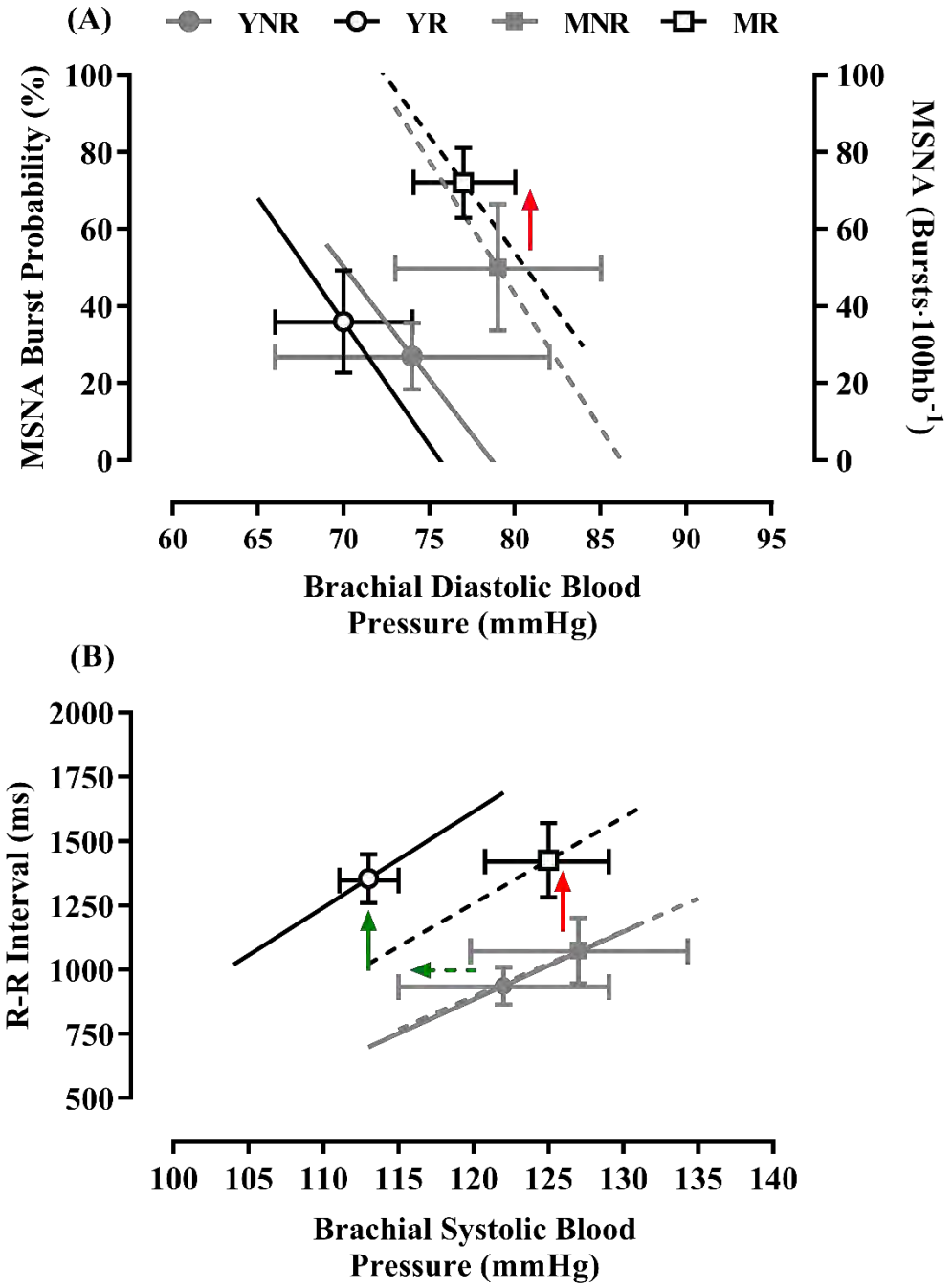


Figure 26 – Effects of habitual exercise on vascular sympathetic and cardiovagal baroreflex function. (A) Group mean regressions between diastolic blood pressure (DBP) and muscle sympathetic nerve activity (MSNA) burst probability/incidence are presented with the sympathetic operating points superimposed on the regression lines. Middle-age runners had similar operating DBP compared to middle-aged nonrunners but the corresponding level of MSNA was higher (by 22 bursts·100hb^{-1}; red arrow), with similar sympathetic baroreflex gain between groups. However, in young men training status had no influence on the operating DBP, corresponding level of MSNA or sympathetic baroreflex gain. **(B)** Group mean regressions between systolic blood pressure (SBP) and R-R interval (sequence method) are shown with the operating points of the cardiac baroreflex overlaid on the regression lines. Middle-aged runners had similar operating SBP and cardiovagal baroreflex gain compared to middle-aged nonrunners, but the corresponding R-R interval was longer (by 352 msec; red arrow). In contrast, when compared to young nonrunners, the operating SBP was set leftward (by 9 mmHg; green dashed arrow) in young runners with a longer corresponding R-R interval (by 418 msec; green solid arrow), with similar cardiac baroreflex gain between groups. *Abbreviations: MNR, Middle-aged nonrunner; MR, Middle-aged runner; YNR, Young nonrunner; YR, Young runner. NB: Baroreflex responsiveness data are presented from: 9 middle-aged nonrunners, 11 middle-aged runners, 10 young nonrunners, 11 young runners.*

5.4 Discussion

The principal findings from this chapter are as follows. First, for middle-aged men, in contrast to our hypothesis, chronic endurance exercise training sets the operating point of the vascular sympathetic baroreflex at a burst occurrence that is higher than for recreationally-active peers. Second, the higher vascular sympathetic operating point in middle-aged runners occurs despite no differences in the operating pressure, vascular sympathetic baroreflex gain or basal MSNA burst frequency. Third, for younger men, habitual endurance training has no effect on the vascular sympathetic operating pressure, point, gain or basal MSNA burst frequency compared with recreationally-active peers. Taken together, these findings indicate that chronic habitual endurance exercise training elicits some form of cardiovascular or neural remodelling occurs in middle-aged men, that plays a critical role in the baroreflex regulation of vascular sympathetic activity and therefore control of peripheral blood pressure.

The effect of habitual exercise on vascular sympathetic baroreflex function

In the middle-aged men studied here, basal MSNA was similar between endurance-trained runners and nonrunners during supine rest. An intriguing finding, however, is that the middle-aged runners exhibit a greater MSNA burst occurrence, by 40-50 %; this is despite no significant difference in the operating DBP. These data for MSNA burst frequency and occurrence suggest that chronic habitual exercise alters the gating of sympathetic bursts (i.e. baroreflex regulation) without influencing the frequency of sympathetic bursts *per* minute (i.e. amount of neurotransmitter release per unit time). Although this might seem contradictory, burst frequency and incidence provide slightly different neurophysiological information (Wallin *et al.*, 1992; McAllen and Malpas, 1997; Charkoudian and Wallin, 2014). Furthermore, reciprocal interplay between exercise bradycardia (i.e. fewer opportunities for a burst), and greater burst incidence (operating point) explain why the burst frequency for middle-aged runners and nonrunners is similar.

The upward setting of the MSNA operating point in middle-aged runners occurs without any change in the ability to increase or decrease vascular sympathetic activity during spontaneous fluctuations in resting arterial pressure. In other words, vascular sympathetic baroreflex overall gain is unaffected by habitual exercise. Stüdinger and colleagues (2009), using the modified Oxford baroreceptor test, also observed that overall gain was similar among older trained and sedentary men. Unlike the present study, however, these authors observed no difference in the vascular sympathetic baroreflex operating point but found resting burst frequency to be marginally lower for endurance-trained versus recreationally-active middle-aged males.

Other studies of endurance-trained and sedentary middle-aged and older individuals have recorded resting MSNA without specifically addressing the function of the vascular sympathetic baroreflex. Notarius and colleagues (2012) observed that MSNA burst incidence was higher, while basal MSNA burst frequency was similar, for moderately endurance-trained middle-aged men compared with sedentary peers (whom had high-normal blood pressure). In contrast, Ng and co-workers (1994a) reported higher sympathetic burst incidence and frequency for older-endurance trained athletes. However, these findings may reflect an older cohort, or inclusion of endurance-trained females, for whom sympathetic burst incidence and frequency were markedly higher compared with sedentary peers (Ng *et al.*, 1994a). Whilst it is difficult to explain this lack of consensus in the literature, it may reflect the differences in the endurance phenotype across the studies. Furthermore, factors that influence basal vascular sympathetic outflow with human ageing, such as abdominal adiposity (Jones *et al.*, 1997) and blood volume (Best *et al.*, 2014), all are influenced by the "dose" of endurance exercise training.

To isolate the effect of habitual endurance exercise training from that of age, younger males were also studied here. As for the older men, no difference was found for basal burst frequency between well-trained runners and nonrunners. The vascular sympathetic

baroreflex operating point was marginally higher for the runners (by ~33%), but not significantly different unlike in the middle-aged groups. These findings for young men are similar to those of previous cross-sectional studies (Svedenhag *et al.*, 1984; Seals, 1991; Christou *et al.*, 2003). Furthermore, vascular sympathetic baroreflex gain was similar for young runners and nonrunners. Thus, these data suggest that chronic habitual endurance exercise in young men does not "induce" a higher operating point of the vascular sympathetic baroreflex.

Differences in resting heart rate between young runners and nonrunners are comparable with those for the middle-aged men. However, one noteworthy distinction relates to the difference in resting stroke volume. For young well-trained men, stroke volume during supine rest was 50% greater than that of age-matched nonrunners. For middle-aged men, resting stroke volume was only 12% greater for runners compared with age-matched nonrunners. This lesser difference in stroke volume may explain why habitual endurance exercise only effects the operating point for the vascular sympathetic baroreflex for middle-aged runners. That is, compared to young runners, middle-aged runners are more reliant on MSNA support of arterial blood pressure rather than cardiac output. However, further investigation of the potential interaction of left ventricular stroke volume, the vascular sympathetic baroreflex and habitual exercise is required.

The interpretation for young men in this study is consistent with a previous report that endurance training does not influence autonomic support of blood pressure in the young (Jones *et al.*, 2002). However, these findings do contrast with those from another study by Alvarez and colleagues (2005). As in the present study, burst occurrence was marginally higher in trained men, while basal MSNA burst frequency was similar. However, when adiposity is taken into account, burst occurrence and burst frequency both were greater for endurance-trained versus sedentary men (Alvarez *et al.*, 2005). Furthermore, in contrast to the data presented in this chapter, sympathetic baroreflex gain (assessed during the modified

oxford baroreceptor test) was lower in endurance-trained compared with sedentary young men; an effect irrespective of percentage body fat (Alvarez *et al.*, 2005). This suggests that body composition may be important, at least in younger men; however, the lack of difference in the vascular sympathetic operating point between the young men in this chapter remained following ANCOVA with either BMI ($P = 0.265$) or body fat percentage ($P = 0.153$).

The effect of habitual exercise on cardiovagal baroreflex control

Although considerable debate exists surrounding mechanism(s) involved (Billman, 2017; Boyett *et al.*, 2017), it is well known that endurance athletes display exercise-induced bradycardia. In spite of this, arterial baroreflex regulation of blood pressure is mediated predominantly via sympathetic vascular control, rather than by reflex changes in cardiac interval (Dampney, 2017). Nevertheless, the effects of habitual endurance exercise on the responsiveness of the cardiovagal baroreflex in middle-age were studied here. For well-trained middle-aged men, as expected, the cardiovagal baroreflex operated around a longer R-R interval at rest; however, both operating pressure and gain were similar between middle-aged runners and nonrunners. Previous work has shown that middle-aged endurance trained men display greater cardiovagal baroreflex gain than sedentary controls, but not moderately-active, age-matched peers (Monahan *et al.*, 2000). In the case of the younger trained men, the cardiovagal baroreflex also operated around a longer heart period, with a lower operating pressure but no difference in baroreflex gain compared with age-matched nonrunners. This finding for cardiovagal baroreflex gain is in agreement with previous studies in younger men (Monahan *et al.*, 2000; Christou *et al.*, 2003; Alvarez *et al.*, 2005).

Remodelling of the vascular sympathetic baroreflex

Mechanosensory transduction, central processing, and efferent neurotransmission are integrated as the three primary components of baroreflex regulation of vascular tone and R-R interval, and therefore arterial pressure. It is proposed that human ageing may have opposing influences on the mechanical and neural components of the vascular sympathetic

baroreflex (Studinger *et al.*, 2009). However, it is only possible to speculate upon potential sites where additional remodelling in the vascular sympathetic baroreflex arc might have occurred in committed middle-aged runners, which may explain the findings presented here. Many years of training may influence the strength and/or timing of barosensory signals controlling vascular sympathetic burst occurrence. This could arise from altered mechanosensory transduction, in the more compliant carotid arteries (Tanaka *et al.*, 2000; Talbot *et al.*, 2020) but not aorta (Chapter 4) and/or a change to the threshold for baroreceptor activation. Specifically, during the longer diastole, in runners, more complete elastic recoil could lead to a longer period of 'silence' in the afferent baroreceptor signal (Kienbaum *et al.*, 2001). The apparent lack of a similar upward setting of the MSNA operating point for younger trained men, who also display bradycardia, does contradict this. However, endurance-training induced cardiovascular remodelling may only lead to upward setting of the vascular sympathetic baroreflex in middle-aged men due to increased autonomic support of blood pressure with age (Jones *et al.*, 2001).

Animal studies indicate that chronic exercise potentially influences baroreceptor control of sympathetic bursts at brain structures including, the nucleus tractus solitairius, the paraventricular nucleus of the hypothalamus, and the rostral ventrolateral medulla (Mueller *et al.*, 2017). Brain imaging studies have identified some of these sites as regions of baroreflex control in humans (Kimmerly *et al.*, 2005; Kramer *et al.*, 2014). Notably, however, short-term exercise training in animals leads to reduction in the level of vascular sympathetic activity; whether these same brain regions remodel in the opposite manner to facilitate increases in sympathetic outflow with chronic exercise, as shown in this chapter, is unclear. It is possible, therefore, that neural plasticity and exercise-induced changes in central processing underpin the higher sympathetic burst occurrence in middle-aged trained males.

Changes to efferent neurotransmission in the vasculature also may mediate the upward setting of the vascular sympathetic baroreflex in middle-aged runners. Vasoconstrictor responsiveness to noradrenaline declines with advancing age (Dinenno *et al.*, 2002; Smith *et al.*, 2007), which may counteract the effects of elevated MSNA burst frequency. Alpha-adrenergic vasoconstrictor responsiveness has been shown to be reduced following short-term (8 weeks) exercise training in middle-age (Mortensen *et al.*, 2014). This may contribute to the findings of Notarius and colleagues (2012), where they observed lower sympathetic vascular transduction, assessed during baroreflex-mediated sympathoexcitation, in endurance-trained compared to sedentary middle-aged men. Previously, a reduction of sympathetic vascular transduction has been proposed to contribute to orthostatic intolerance observed in some highly-trained individuals (van Lieshout, 2003). Another possibility is that this higher vascular sympathetic baroreflex operating point may be a compensatory mechanism to offset training-induced microvascular adaptation, for example exercise-induced angiogenesis (Laughlin *et al.*, 2012). Each of these possibilities require investigation. Notably, irrespective of the location(s), habitual endurance exercise-induced cardiovascular remodelling does not alter vascular sympathetic baroreflex gain, at least not the integrated gain.

Methodological Considerations

There are some limitations of this chapter which warrant consideration. First, vascular sympathetic baroreflex gain was calculated by associating spontaneous fluctuations in DBP to the probability of MSNA burst occurrence. Burst strength (i.e amplitude) was not taken into account because baroreceptor signals modulate burst occurrence, whereas less is known as to whether baroreflex mechanisms govern burst amplitude (McAllen and Malpas, 1997; Kienbaum *et al.*, 2001). In addition, vascular sympathetic baroreflex gain was not assessed separately during “ups” and “downs” in pressure; therefore this analysis does not take baroreflex hysteresis into account (Hart *et al.*, 2011).

Second, many external factors could influence the results presented here. It is reported that dietary salt and nitrate can influence vascular sympathetic activity (Matthews *et al.*, 2017; Notay *et al.*, 2017). Although diet was not controlled in this study and may have affected the outcomes, all participants did attend the laboratory following a 6 hour fast. Every effort was made to accurately record the number of years over which an individual had exercised at their current level. In addition, self-reported training history was recorded in runners and there was a clear difference in maximal aerobic capacity between the trained and recreationally-active groups. However, group allocation, determined by habitual endurance training, may limit the conclusions based on other components of exercise training. These components include mode, intensity, duration, all of which may have an impact on cardiac, vascular and neural remodelling. Furthermore, we did not collect any objective measures of physical activity levels. Because sex of the participants was controlled for in this study, future studies are required to further address potential sex differences in the effects of habitual exercise on vascular sympathetic baroreflex function. Although all participants were non-obese, adiposity was not specifically controlled for, which is known to influence vascular sympathetic activity. However, post-hoc analysis suggests that percentage body fat was not a significant covariate for any indices of MSNA in this study.

Finally, the *a priori* intention of this study was to investigate the effect that chronic habitual endurance exercise has on vascular sympathetic activity and vascular sympathetic baroreflex regulation of resting blood pressure in healthy middle-aged men. However, young men were also studied to discern the effect of habitual exercise independently of cardiovascular ageing. The use of independent samples t-tests reflects these *a priori* questions. To limit the chance of a type 1 error, the statistical comparisons to determine the effects of habitual exercise were not completed in one statistical model.

5.5 Conclusion

This study demonstrates upward setting of the vascular sympathetic baroreflex regulation of MSNA in habitually endurance-trained middle-aged men. Notably, upward setting of the vascular sympathetic baroreflex coupled with exercise-induced bradycardia, results in a similar basal MSNA burst frequency compared with recreationally-active peers. Furthermore, this study demonstrates that training status does not significantly influence the MSNA operating point for younger well-trained men, who also display similar exercise-induced bradycardia and basal MSNA burst frequency compared with recreationally-active peers. Notably, there was no effect of habitual exercise on vascular sympathetic or cardiovagal baroreflex gain studied here, which were assessed during supine rest only. The effects of habitual exercise on vascular sympathetic and cardiovagal baroreflex function during reflex sympathoexcitation are unclear but warrant future investigation. Remodelling within the vascular sympathetic baroreflex arc, culminating in a higher MSNA operating point, may also be an example of phenotypic adaptation to chronic (~30 years) habitual endurance exercise. This occurs, presumably, to maintain resting vascular tone and blood pressure and to complement cardiac and vascular adaptations to chronic endurance exercise training.

5.6 Study Hypothesis

i) Vascular sympathetic baroreflex function would not be different between middle-aged runners and nonrunners **(REJECTED)**.

This chapter shows that chronic habitual endurance exercise until middle-aged normotensive influences the operating point of the vascular sympathetic baroreflex, despite no influence on the operating pressure or gain. This occurs independently of differences in the aortic haemodynamic profile (Chapter 4). The influence of habitual exercise on the effects of increases in MSNA on vascular tone, and subsequently blood pressure, are

however unclear. The effects of habitual exercise on efferent neurotransmission in the vasculature (i.e. sympathetic vascular transduction) will be addressed in the final (next) experimental chapter.

CHAPTER 6. EXPERIMENTAL STUDY 3

THE EFFECT OF CHRONIC HABITUAL ENDURANCE EXERCISE ON SYMPATHETIC VASCULAR TRANSDUCTION DURING SYMPATHOEXCITATION IN NORMOTENSIVE MIDDLE-AGED MEN

6.1 Introduction

Chronic habitual endurance exercise in middle-aged men does not influence either aortic haemodynamics (Chapter 4) or resting vascular sympathetic activity despite the upward setting of the vascular sympathetic baroreflex (Chapter 5). The final research question addressed in this thesis is whether chronic habitual endurance exercise influences the third component of the vascular sympathetic baroreflex arc, which is efferent neurotransmission in the vasculature (as outlined in Chapter 2). This component can be assessed by relating MSNA and vascular and/or blood pressure responses during sympathoexcitation and is termed sympathetic vascular transduction. The influence of habitual endurance exercise on sympathetic vascular transduction is not well studied to date despite the important role it plays in the regulation of blood pressure.

This research question arises from the report that short-term (8 weeks) aerobic exercise training reduces vascular alpha-adrenergic sensitivity in normotensive middle-aged individuals (Mortensen *et al.*, 2014). This study reported blunted vascular responses to intra-arterial infusions of tyramine, which stimulates the endogenous release of noradrenaline, following exercise training. Notably, this could explain the previous finding by Notarius and colleagues (2012). In this study of moderately endurance-trained middle-aged men, there was no relationship between MSNA and forearm vascular resistance (FVR) during sympathoexcitation induced by lower body negative pressure (LBNP). In contrast, the sedentary middle-aged men displayed a positive association between MSNA and FVR across LBNP. These findings led the authors to suggest that there was an alteration in sympathetic vascular transduction in middle-aged endurance-trained men (Notarius *et al.*, 2012). However, resting blood pressure was different between these groups of middle-aged men, with the sedentary group having high-normal blood pressure. Thus, it is unclear as to whether the differences in the MSNA-FVR relationship reflects the effects of habitual exercise on sympathetic vascular transduction *per se* or between-group differences in arterial pressure. In other words, it is unclear whether sympathetic vascular transduction was exaggerated in

the sedentary individuals in this study. Furthermore, endurance-trained men were compared to sedentary and not recreationally-active men.

Given this uncertainty, the primary aim of this study was to determine the effect of chronic habitual endurance exercise on sympathetic vascular transduction in normotensive middle-aged men. To address this aim, participants underwent multiple conditions which elevate vascular sympathetic activity (termed sympathoexcitation): i) cold pressor test (CPT); ii) static hand grip exercise (SHG), where both central command (Williamson, 2010) and the muscle metaboreflex (Mitchell *et al.*, 1983) are engaged; and, iii) isolated muscle metaboreflex activation following SHG, with post-exercise muscle ischaemia (PEMI). To quantify sympathetic vascular transduction, relationships between MSNA and FVR and forearm vascular conductance (FVC) and systemic vasomotor tone (DBP) were constructed for CPT and PEMI. These linear slopes were compared between middle-aged runners and nonrunners. In line with the report of lower vascular alpha-adrenergic sensitivity following short term exercise training in middle-age (Mortensen *et al.*, 2014), the hypothesis for this study was that sympathetic vascular transduction would be lower in middle-aged male runners compared to age-matched recreationally-active nonrunners. Testing this hypothesis also enabled investigation of a potential explanation for the higher set point of the vascular sympathetic baroreflex in middle-aged male runners reported in this thesis. (Chapter 5). As a secondary aim, sympathetic vascular transduction was also compared for young runners and nonrunners to isolate the effect of habitual exercise from that of age.

6.2 Methodology

Study Overview

For this experimental chapter, participants underwent continuous recordings of blood pressure, vascular sympathetic activity (i.e. MSNA) and forearm blood flow (FBF) during CPT, SHG and PEMI (Figure 27). Echocardiography was also performed during baseline and at the end of each stimulus. SHG was utilised to elicit a period of isolated muscle

metaboreflex activation. However, sympathetic vascular transduction was only assessed during CPT and PEMI. During SHG cardiac output and contralateral forearm, but not leg, blood flow is increased (Eklund *et al.*, 1974; Jacobsen *et al.*, 1994); the increase in FBF appears to be mediated via β-mediated vasodilation (Eklund and Kaijser, 1976). Notably, alpha-adrenergic blockade significantly blunts the pressor response to CPT (Monahan *et al.*, 2013) and PEMI (Kiviniemi *et al.*, 2012) which highlights that the response to these stimuli is primarily mediated via the sympathetic vascular response. Therefore, the forearm vascular and blood pressure responses during CPT and PEMI primarily reflect the ability of MSNA to elicit changes in vascular tone, as there is no confounding influence of an increase in cardiac output and/or FBF on sympathetic vasoconstriction.

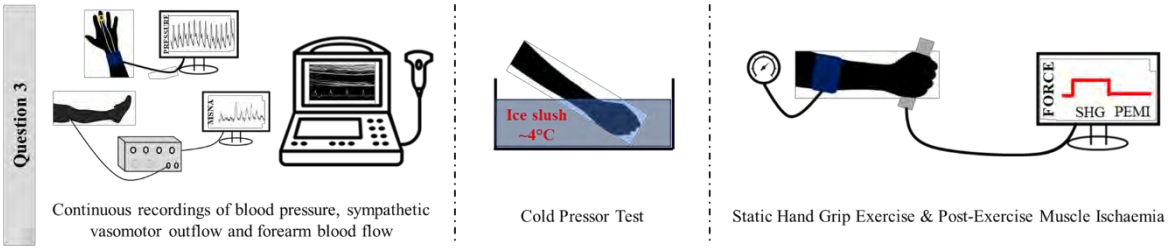


Figure 27 – Study 3 Schematic.

Cold pressor test, static hand grip exercise and post-exercise muscle ischemia

Following one minute of baseline, all participants submerged their left hand, up to the wrist, in an ice water bath (2-4°C) for two minutes. After at least 10 minutes of recovery from CPT, during which the hand was warmed, SHG exercise was performed at 35% of maximal voluntary contraction (MVC) until task failure using a commercially-available device (MLT003/D, ADInstruments, UK) gripped with the left hand (5 participants were left hand dominant). Task failure was determined when participants were unable to maintain 85% of the required force for > 3 seconds. The MVC was calculated as the highest value of three maximal efforts completed prior to the SHG test. During SHG, participants were given continuous visual and auditory feedback to help maintain the required force. At the point of task failure, PEMI was established which lasted for two minutes. This involved inflation, to

220 mmHg, of a cuff positioned around the forearm of the left arm, immediately distal to the olecranon process, and attached to a rapid inflation and deflation pneumatic device (E20 Rapid Cuff Inflation System, D.E. Hokanson, Bellevue, USA). Beat-by-beat R-R interval, blood pressure (contralateral hand), brachial artery diameter and blood velocity (contralateral arm) and MSNA (lower limb) were collected continuously throughout each of the tests. Each stimulus was performed with participants supine. Furthermore, stroke volume was assessed during the final 15 seconds of baseline, CPT, SHG and PEMI. Participants were instructed to avoid breath holding during each test, which was monitored throughout as all participants were instrumented with a nasal cannula attached to a capnograph (Capnocheck® Sleep Capnograph, Smiths Medical, UK).

Experimental Measurements

Haemodynamics

Beat-by-beat brachial artery blood pressure was monitored via finger photoplethysmography and R-R interval was recorded using a three-lead electrocardiogram (Finometer® Pro, FMS, Groningen, Netherlands), as for Chapter 5. Reconstructed brachial artery blood pressure waveforms were calibrated against average resting SBP and DBP measured via manual sphygmomanometry (Welch Allyn Durashock DS66, UK). Stroke volume was recorded via echocardiography (Vivid E9, GE Medical, Norway) as outlined in the previous chapter; however, only single-plane (4-chamber) images were used to calculate left ventricular stroke volume in this chapter, due to it not being possible to record reliable apical 2-chamber images during each stress.

Forearm Blood flow

B-mode images of the brachial artery, in the distal third of the upper right arm, were obtained to determine arterial diamater by one experienced vascular sonographer (see Appendix III) using a 12-MHz linear array transducer attached to a high-resolution ultrasound machine (Vivid Q, GE Medical, Norway). Doppler ultrasound was used to record blood velocity.

Doppler ultrasound

The Doppler effect enables determination of the velocity of motion of blood or tissue, which is important for both vascular and cardiac imaging (although only relevant for vascular assessments in this thesis). This Doppler effect is the change in the received frequency of vibrations due to the movement of the object (e.g. blood) which reflects the ultrasound vibrations back to the transducer. The magnitude of this shift in received frequency is directly related to the velocity of movement (McDicken and Anderson, 2011). Due to very high temporal resolution, Doppler ultrasound enables real-time recording of sharp peaks and troughs in velocity signals and when coupled with B-mode images, termed Duplex ultrasonography (Figure 28), it is possible to image both arterial diameter and blood (Doppler) velocity simultaneously. Accordingly, duplex ultrasonography was utilised to record brachial artery diameter and blood velocity throughout each stimulus.

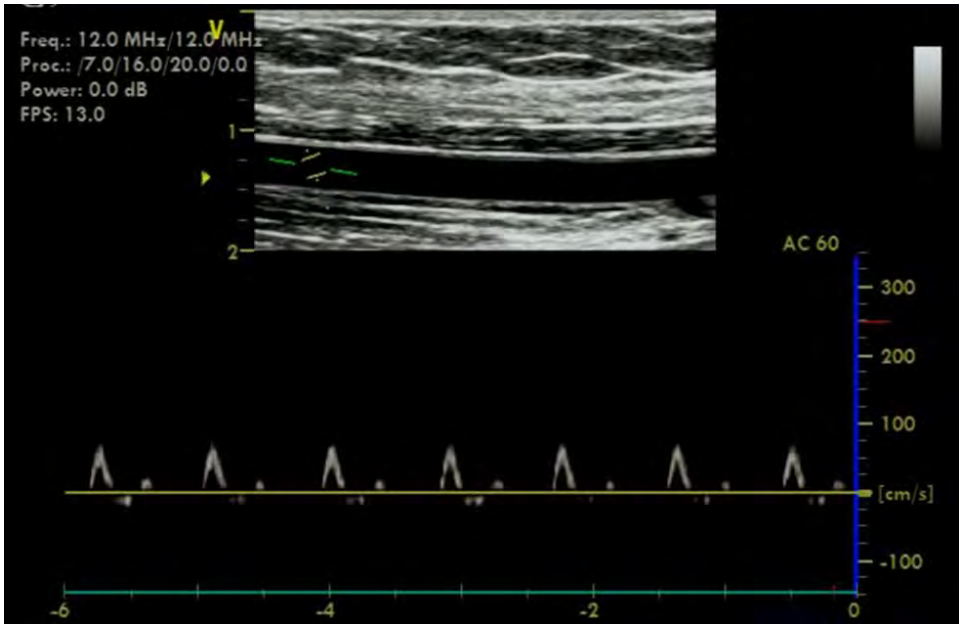


Figure 28 – Example of a duplex ultrasound image. The right brachial artery was imaged using duplex ultrasonography to determine simultaneous recordings of arterial diameter and blood (Doppler) velocity.

Muscle Sympathetic Nerve Activity

Multi-unit MSNA was recorded from the fibular nerve using microneurography, as described in Chapter 5. The recording of MSNA was maintained following the six minutes

of resting data which was analysed and reported on in Chapter 5. The microneurography electrode was not adjusted from recording MSNA throughout this study protocol unless the neural signal became diminished or was lost. In these instances, the microelectrode was repositioned to enable acquisition of an acceptable MSNA signal in line with the criteria set out in Chapter 5.

Data Analyses

The two minutes of CPT and PEMI were divided into quartiles, so that data bins were 30 seconds in duration. To account for inter-individual variability, SHG duration (i.e. time to failure) was divided into quintiles, that is 20, 40, 60, 80 and 100%. Haemodynamic, neural and vascular data were averaged across each quartile of CPT and PEMI and each quintile of SHG.

Blood pressure, R-R interval and MSNA were sampled at 1000 Hz on a personal computer using a commercially-available data acquisition system (Chart Version 8, LabChart Pro, ADInstruments, UK) and saved for offline analysis, as outlined for chapter 5.

Stroke volume was calculated as detailed in the previous chapter, but only in one plane as noted above. The assessment of stroke volume permitted the calculation of cardiac output (heart rate x stroke volume), TPR (MAP/cardiac output) and total vascular conductance (TVC; cardiac output/MAP). As for Chapter 5, allometrically scaling stroke volume and cardiac output to body surface area did not change the findings from this study; accordingly, only absolute stroke volume and cardiac output are reported. Stroke volume, and associated haemodynamics, are reported for 42 individuals during all stimuli (7 middle-aged nonrunners, 12 middle-aged runners, 10 young nonrunners and 13 young runners).

Post-hoc analysis of synchronised arterial diameter and blood flow velocity data were performed using custom semi-automated operator-independent arterial wall-tracking software (Woodman *et al.*, 2001), which is independent of investigator bias. The intra-observer coefficient of variation when analysing arterial diameter using this software is 7%

(Woodman *et al.*, 2001). The coefficients of variation for brachial artery diameter, blood velocity and forearm blood flow are 4.2%, 22.1% and 27.2%, respectively (see Table 2). Video files were imported into the software (BloodFlow Analysis, version 4.0) and each file underwent separate calibration to enable determination of artery diameter (cm) and blood (Doppler) velocity (time-averaged maximum velocity [TA_{MAX}], expressed in $cm \cdot s^{-1}$). Subsequently, region of interest boxes were placed over a segment of the arterial wall, which was stable throughout the recording period, as well as over the doppler signal to enable frame-by-frame recording (at 30 Hz, i.e. 30 frames per second) of arterial diameter and Doppler velocity. Arterial diameter was determined by a wall-tracking algorithm which identifies the top and bottom walls by a rake routine which scans up the region of interest and detects the most rapid change in the pixel intensity (i.e. the high contrast of the bright arterial walls compared to the dark vessel lumen). From the synchronised measures of arterial diameter and blood velocity, FBF was calculated as the product of lumen cross-sectional area (CSA) and average TA_{MAX} Doppler velocity (as below).

Calculation of Forearm flood blow:

$$\text{Forearm blood flow} = ((\text{Diameter}/2)^2 \times 3.14) \times \text{Doppler Velocity} \times 60)$$

Where: Brachial diameter is in cm; 3.14 represents π; Doppler velocity is TA_{MAX} in $cm \cdot s^{-1}$; 60 is used to convert $ml \cdot sec^{-1}$ to $ml \cdot min^{-1;}$ FBF is in $ml \cdot min^{-1}$ (Hearon et al., 2017).

To index forearm vascular responses during all stimuli FVR ([MAP/FBF]*100, $100mmHg^{-1} \cdot ml \cdot min^{-1}$) and FVC ([FBF/MAP]*100], $ml \cdot min^{-1} \cdot 100mmHg^{-1}$) were calculated. Brachial artery diameter did not change during any stimulus, for any group; therefore, the changes in blood velocity underpinned any observed changes in FBF. To compare the forearm sympathetic vasoconstrictor responses during CPT and PEMI, the percentage change (%Δ) in both FVR and FVC were calculated from baseline to the final quartile, for example: ([FVR final time point-FVR Baseline]/[FVR Baseline]) (Buckwalter and Clifford, 2001). FVR is the primary index of arterial vascular tone during CPT and PEMI, as blood

pressure changes more than flow (Lautt, 1989); nevertheless, both FVR and FVC are presented for completeness.

Following alignment of each MSNA burst with the appropriate R wave of the ECG, inter-recording differences in electrode position were accounted for via normalising burst amplitude, which is dependent on the size (Steinback *et al.*, 2010) and number (Ninomiya *et al.*, 1993) of neurons firing in close proximity to the recording electrode. The largest spontaneous burst was assigned a value of 100 and all other bursts were expressed relative to this value. MSNA was quantified as burst frequency (bursts·min^{-1}), mean burst amplitude (%), total activity (burst frequency x burst amplitude; %·min^{-1}) and burst incidence (bursts·100hb^{-1}). In this chapter, MSNA total activity was focused upon to compare between-group responses to CPT, SHG and PEMI and to assess sympathetic vascular transduction. However, analyses of between-group differences were also conducted using MSNA burst frequency alone, an index of vascular sympathetic activity which is less dependent on electrode position. The findings based upon MSNA burst frequency and total activity were similar, accordingly only data on MSNA total activity are reported. During SHG and PEMI it was not possible to obtain satisfactory recordings of MSNA from one middle-aged nonrunner; therefore, data are reported on 9 middle-aged nonrunners during these stimuli. Thirty seconds of representative MSNA and blood pressure data are presented from one young (Figure 29, A, B and C) and one middle-aged man (Figure 29, D, E and F) during baseline, at the end of SHG and at the end of PEMI.

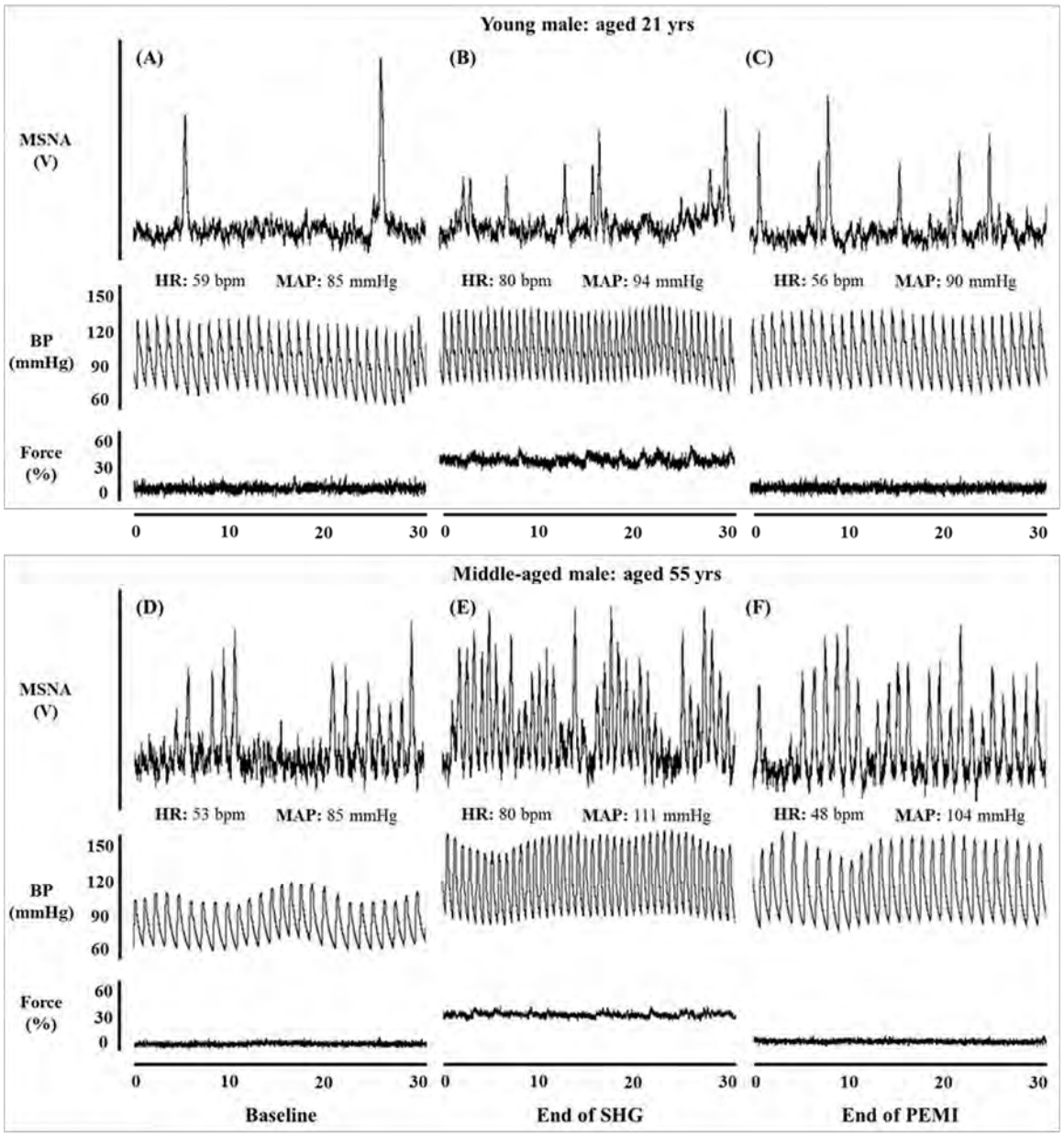


Figure 29 – Representative neural and blood pressure data from one young and one middle-aged man. Thirty seconds of raw muscle sympathetic nerve activity (MSNA) and brachial blood pressure (BP) data are shown from one young **(panels A, B and C)** and one middle-aged male participant **(panels D, E and F)** at baseline, the end of static hand grip (SHG) and the end of post-exercise muscle ischemia (PEMI), respectively.

To determine sympathetic vascular transduction, stimulus-response relationships between indices of MSNA (burst frequency & total activity) and indices of vasomotor tone (FVR, FVC and DBP) were constructed, and slopes were calculated from linear regression analysis, as performed previously (Halliwill *et al.*, 1996). DBP was used as it is a reproducible, easy to measure, target variable of vascular sympathetic activity which

indicates systemic vascular responses (Briant *et al.*, 2016). To generate sympathetic vascular transduction slopes during CPT and PEMI, the relationship between MSNA burst frequency and total activity was plotted against FVR/FVC/DBP from each quartile during CPT and PEMI. The baseline value for each variable was included in the regression analyses. Due to similar between-group differences when sympathetic vascular transduction was determined using MSNA burst frequency and total activity, only data using total activity are reported.

Statistical Analyses

As the resting data have been reported and compared in Chapter 5, only data collected during CPT, SHG and PEMI are presented and analysed here; that is from the one-minute baseline until the end of each test. In line with the primary (MR vs MNR) and secondary (YR vs YNR) aims of this study, and after checking data compliance with parametric assumptions, the haemodynamic, neural and vascular responses to each stimulus were compared between the middle-aged and the young groups. To do this, linear mixed-effects models (including subjects as a random factor) were used to determine the main effects of group and time (baseline and end of stimulus), as well as for the presence of a significant group*time interactions for each between-group comparison during each stimulus. In the presence of a significant group*time interaction, post-hoc multiple comparisons would be conducted using Fisher's LSD, as this study conducted pre-planned statistical analyses in line with the primary and secondary aims (Perneger, 1998; Cramer *et al.*, 2016). Data collected during the intermediate phases of CPT and PEMI are presented in Figures 30 and 31, respectively, without error bars, to illustrate the response of each variable as these intermediate data points were used to calculate the sympathetic vascular transduction slopes. However, no statistical analyses were undertaken on these intermediate data points. Following the linear mixed model analyses, the primary research question was addressed by comparing sympathetic vascular transduction slopes between the two middle-aged and between the two young groups via independent samples Mann-Whitney U tests. Independent samples t-tests were used to compare the sympathetic vasoconstrictor responses between groups. All statistical

analyses were performed using SPSS (Version 24, Chicago, IL) and all data are reported as mean ± standard deviation. Alpha was set *a priori* at $P < 0.05$.

6.3 Results

Middle-aged men

The haemodynamic, neural and vascular responses to CPT and SHG/PEMI are reported in Tables 5 and 6, respectively. Significant main effects or group*time interactions are described in text. The responses of FVR, DBP and MSNA total activity to CPT and PEMI are presented separately in Figure 30, as these data were utilised to assess sympathetic vascular transduction. Sympathetic vascular transduction slopes, with DBP as the vascular outcome, are also shown in Figure 30. The forearm vascular transduction slopes are presented alongside forearm sympathetic vasoconstrictor responses during CPT and PEMI in Table 7.

Cold pressor test

Throughout CPT, heart rate and MAP were lower and stroke volume, MSNA burst frequency and incidence were higher in middle-aged runners compared to middle-aged nonrunners. In response to the CPT, TPR and all indices of blood pressure and MSNA increased, whereas stroke volume and TVC decreased, with no between-group differences in the responses (i.e. no significant time*group interactions). Sympathetic vascular transduction, using either FVR, FVC or DBP as the vascular outcome, was not significantly different between middle-aged runners and nonrunners during CPT. Forearm sympathetic vasoconstriction was also similar between groups during CPT.

Static Hand Grip Exercise and Post-Exercise Muscle Ischaemia

Time to fatigue during SHG was similar in middle-aged runners and nonrunners (255 ± 87 vs 242 ± 129 seconds, $P = 0.798$). Throughout SHG, heart rate was significantly lower in runners and stroke volume and MSNA burst incidence were higher. Heart rate, cardiac

output, MAP, MSNA burst frequency, incidence and amplitude and FBF increased; whereas stroke volume decreased during SHG. There were no significant group*time interactions.

During PEMI, heart rate and MSNA burst amplitude were lower in middle-aged runners compared to age-matched nonrunners; whereas stroke volume and MSNA burst incidence were higher in runners. TPR, MAP and MSNA burst frequency, incidence and amplitude all increased during PEMI; whereas, stroke volume, cardiac output, and TVC decreased. There were no significant group*time interactions during PEMI. Sympathetic vascular transduction and forearm sympathetic vasoconstrictor responses were not significantly different between groups during PEMI.

Table 5 – Haemodynamic, neural and vascular responses to CPT in middle-aged runners and nonrunners

Variable	Group	n	Baseline	End	Group *P*	Time *P*	Int. *P*
Haemodynamic							
Heart Rate (bpm)	NR	10	58 ± 9	62 ± 14	**< 0.001**	0.474	0.743
	R	13	46 ± 10	47 ± 10			
Stroke Volume (ml)	NR	7	73 ± 8	64 ± 9	**< 0.001**	**0.005**	0.591
	R	12	84 ± 8	78 ± 7			
Cardiac Output ($l \cdot min^{-1}$)	NR	7	4.5 ± 0.8	4.1 ± 1.0	0.132	0.318	0.924
	R	12	4.0 ± 1.0	3.7 ± 0.8			
TPR ($mmHg \cdot l \cdot min^{-1}$)	NR	7	22.0 ± 3.4	27.6 ± 5.5	0.250	**0.016**	0.831
	R	12	24.8 ± 5.8	29.5 ± 7.5			
TVC ($l \cdot min^{-1} \cdot mmHg$)	NR	7	0.046 ± 0.006	0.038 ± 0.007	0.401	**0.019**	0.699
	R	12	0.043 ± 0.011	0.036 ± 0.008			
MAP (mmHg)	NR	10	96 ± 8	108 ± 8	**0.031**	**0.001**	0.626
	R	12	92 ± 6	100 ± 12			
Neural							
MSNA BF ($bursts \cdot min^{-1}$)	NR	10	19 ± 11	36 ± 11	**0.012**	**< 0.001**	0.183
	R	13	31 ± 8	40 ± 11			
MSNA BI ($bursts \cdot 100hb^{-1}$)	NR	10	32 ± 16	59 ± 18	**< 0.001**	**< 0.001**	0.208
	R	13	69 ± 20	83 ± 17			
MSNA BA (%)	NR	10	64 ± 11	88 ± 42	0.269	**0.001**	0.651
	R	13	52 ± 10	83 ± 27			
Vascular							
FBF ($ml \cdot min^{-1}$)	NR	6	93 ± 16	102 ± 15	0.769	0.588	0.997
	R	9	98 ± 48	107 ± 55			
FVC ($ml \cdot min^{1} \cdot 100mmHg^{-1}$)	NR	6	95 ± 15	89 ± 16	0.413	0.725	0.937
	R	9	104 ± 47	103 ± 52			

Data are presented as mean ± standard deviation. Significant main effects or interactions are highlighted in bold, determined via a linear mixed model. *Abbreviations: BA, burst amplitude; BF, burst frequency; BI, burst incidence; CPT, cold pressor test; DBP, diastolic blood pressure; FBF, forearm blood flow; FVC, forearm vascular conductance; Int., Interaction; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; n, sample size; NR, nonrunner; R, runner; TPR, total peripheral resistance; TVC, total vascular conductance.*

Table 6 – Haemodynamic, neural and vascular responses to SHG and PEMI in middle-aged runners and nonrunners

				SHG				PEMI			
Variable	**Group**	**n**	**Baseline**	**End SHG**	**Group *P***	**Time *P***	**Int. *P***	**End PEMI**	**Group *P***	**Time *P***	**Int. *P***
Haemodynamic											
Heart Rate	NR	9	59 ± 8	81 ± 11	**0.002**	**< 0.001**	0.706	56 ± 10	**< 0.001**	0.654	0.713
(bpm)	R	13	47 ± 9	72 ± 13				46 ± 11			
Stroke Volume	NR	6	73 ± 7	61 ± 8	**0.007**	**0.001**	0.990	62 ± 8	**0.021**	**0.001**	0.591
(ml)	R	12	82 ± 10	70 ± 9				68 ± 9			
Cardiac Output	NR	6	4.4 ± 0.8	5.2 ± 0.9	0.363	**0.014**	0.749	3.6 ± 0.8	0.149	**0.015**	0.979
($l \cdot min^{-1}$)	R	12	3.9 ± 1.1	5.0 ± 1.2				3.1 ± 0.8			
TPR	NR	6	22.7 ± 3.1	23.2 ± 3.6	0.380	0.901	0.731	32.5 ± 6.2	0.319	**0.003**	0.907
($mmHg \cdot l \cdot min^{-1}$)	R	12	25.5 ± 6.8	24.4 ± 7.9				36.0 ± 12.6			
TVC	NR	6	0.045 ± 0.006	0.044 ± 0.007	0.841	0.793	0.615	0.032 ± 0.007	0.500	**0.001**	0.828
($l \cdot min^{-1} \cdot mmHg$)	R	12	0.042 ± 0.011	0.045 ± 0.015				0.030 ± 0.009			
MAP	NR	9	97 ± 7	118 ± 8	0.267	**< 0.001**	0.985	111 ± 7	0.080	**< 0.001**	0.6538
(mmHg)	R*	12	94 ± 8	115 ± 12				104 ± 11			
Neural											
MSNA BF	NR	9	23 ± 12	47 ± 10	0.414	**< 0.001**	0.450	36 ± 9	0.298	**0.005**	0.450
($bursts \cdot min^{-1}$)	R	13	29 ± 10	47 ± 16				37 ± 12			
MSNA BI	NR	9	39 ± 19	63 ± 14	**0.008**	**0.011**	0.208	64 ± 17	**0.003**	**0.004**	0.208
($bursts \cdot 100hb^{-1}$)	R	13	64 ± 22	70 ± 19				74 ± 22			
MSNA BA	NR	9	64 ± 14	107 ± 68	0.163	**0.001**	0.507	101 ± 50	**0.034**	**0.002**	0.219
(%)	R	13	56 ± 7	85 ± 22				73 ± 22			
Vascular											
FBF	NR	7	101 ± 23	151 ± 58	0.608	**0.018**	0.758	102 ± 15	0.701	0.935	0.995
($ml \cdot min^{-1}$)	R**	6	106 ± 73	169 ± 63				107 ± 55			
FVC	NR	7	103 ± 20	108 ± 40	0.522	0.583	0.744	78 ± 26	0.960	0.179	0.850
($ml \cdot min^{1} \cdot 100mmHg^{-1}$)	R**	6	97 ± 59	128 ± 52				82 ± 45			

Data are presented as mean ± standard deviation. Significant main effects or interactions are highlighted in bold, determined via separate linear mixed models for SHG and PEMI. * represents n = 13 during PEMI. ** represents n = 6 NRs and n = 7 Rs, during PEMI. *Abbreviations: BA, burst amplitude; BF, burst frequency; BI, burst incidence; FBF, forearm blood flow; FVC, forearm vascular conductance; Int., interaction; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; n, sample size; NR, nonrunner; PEMI, post-exercise muscle ischaemia; R, runner; SHG, static hand grip exercise; TPR, total peripheral resistance; TVC, total vascular conductance.*

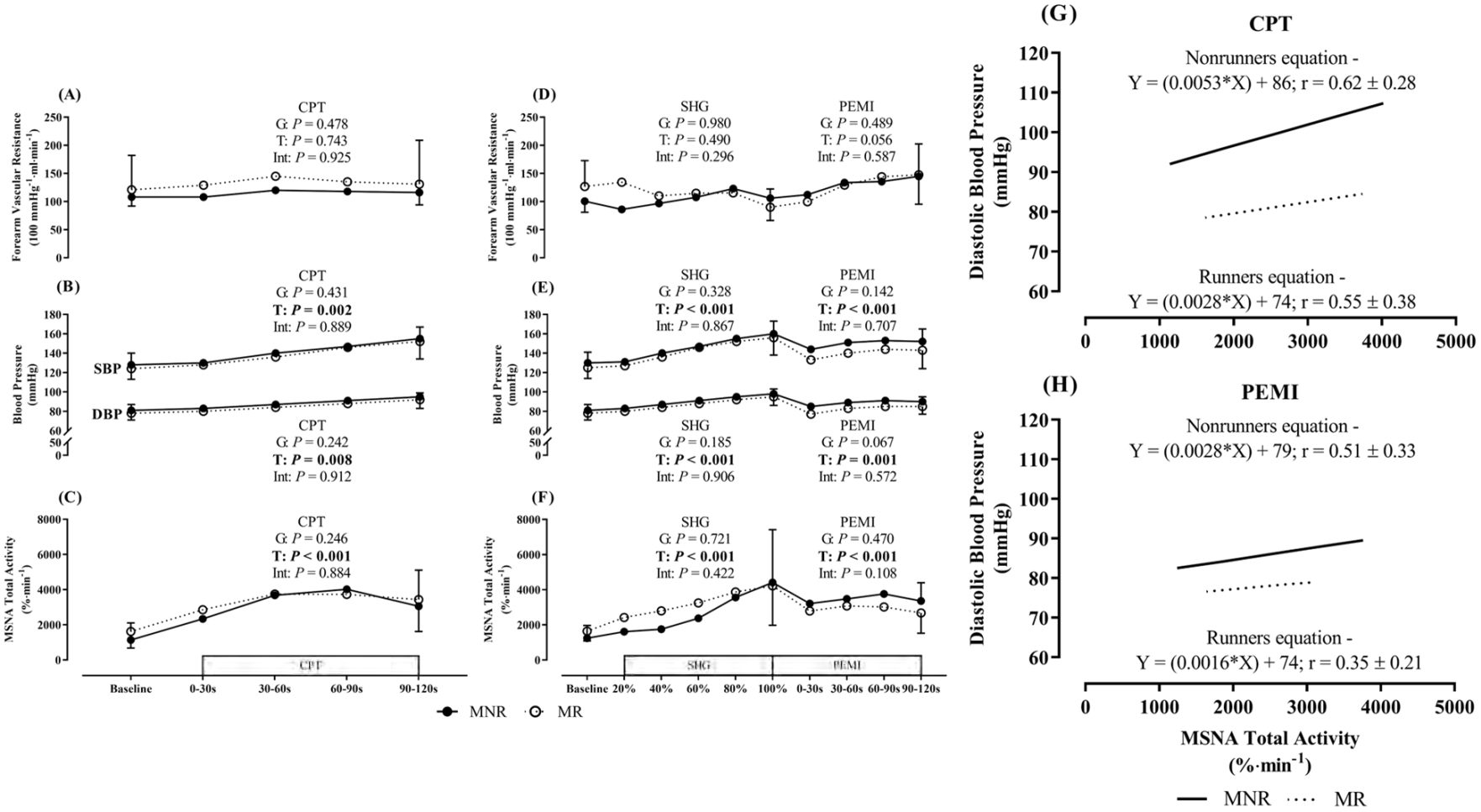


Figure 30 – The forearm vascular, blood pressure and neural responses to CPT and SHG & PEMI in middle-aged runners and nonrunners. (A-F) Data are presented as mean with standard deviation error bars. The data points without error bars represent continuously collected data which are shown for completeness only; no statistical analyses were conducted on these data but are presented as they were used to construct the transduction slopes. Main effects of group and time and group*time interactions were determined from separate linear mixed models comparing baseline to the end of CPT **(Panels A-C)**, SHG and PEMI **(Panels D-F)**; significant ($P < 0.05$) main effects or group*time interactions are reported in bold. **(G-H)** Data are presented as mean sympathetic vascular transduction slopes from linear regression analyses of MSNA total activity and diastolic blood pressure in 10 middle-aged nonrunners and 13 middle-aged runners for the CPT (G) and PEMI (H). Effect sizes and *P* values are presented within Table 7. *Abbreviations: CPT, cold pressor test; G, main effect of group; Int, group*time interaction; M, middle-aged nonrunner; MR, middle-aged runner; PEMI, post-exercise muscle ischaemia; s, seconds; SHG, static hand grip exercise; T, main effect of time.*

Table 7 – Sympathetic vascular transduction and forearm sympathetic vasoconstriction during CPT and PEMI in middle-aged runners and nonrunners

Variable		Cold Pressor Test				Post-Exercise Muscle Ischaemia			
		nonrunners	**runners**	***Effect Size***	***P***	**nonrunners**	**runners**	***Effect Size***	***P***
Slopes									
TA-FVR ($100mmHg^{-1}\cdot ml\cdot min^{-1}\cdot\%\cdot min^{-1}$)	*n*	6	8			5	7		
	Slope	0.0241 ± 0.0517	0.0047 ± 0.0172	0.5	0.282	-0.0005 ± 0.0085	0.0098 ± 0.0145	-0.8	0.432
	r	*0.38 ± 0.32*	*0.27 ± 0.29*	*0.3*	*0.852*	*0.33 ± 0.17*	*0.25 ± 0.26*	*0.3*	*0.627*
TA-FVC ($ml\cdot min^{-1}\cdot 100mmHg^{-1}\cdot\%\cdot min^{-1}$)	*n*	6	9			5	7		
	Slope	-0.0282 ± 0.0610	-0.0017 ± 0.0107	0.6	0.456	0.0027 ± 0.0086	-0.0115 ± 0.0107	1.3	0.106
	r	*0.43 ± 0.31*	*0.34 ± 0.32*	*0.3*	*0.615*	*0.33 ± 0.19*	*0.20 ± 0.27*	*0.5*	*0.688*
TA-DBP ($mmHg\cdot\%\cdot min^{-1}$)	*n*	10	13			9	13		
	Slope	0.0053 ± 0.0077	0.0028 ± 0.0031	0.4	0.563	0.0028 ± 0.0022	0.0016 ± 0.0089	0.2	0.794
	r	*0.62 ± 0.28*	*0.55 ± 0.38*	*0.2*	*0.563*	*0.51 ± 0.33*	*0.35 ± 0.21*	*0.6*	*0.393*
Sympathetic Vasoconstriction	*n*	6	9			7	9		
FVR (%Δ)		7 ± 12	6 ± 19	0.1	0.889	23 ± 46	6 ± 26	0.5	0.351
FVC (%Δ)		-6 ± 11	-3 ± 16	-0.2	0.748	-10 ± 26	-2 ± 21	-0.3	0.468

Data are presented as mean ± standard deviation. Significant differences are highlighted in bold, determined via ANOVA. * represents data reported for 10 middle-aged nonrunners and 13 middle-aged runners. *Abbreviations: CPT, cold pressor test; DBP, diastolic blood pressure; FVC, forearm vascular conductance; FVR, forearm vascular conductance; PEMI, post-exercise muscle ischaemia; TA, MSNA total activity.*

Young men

The haemodynamic, neural and vascular responses to CPT and SHG and PEMI are reported in Tables 8 and 9, respectively. Significant main effects or group*time interactions are described in text. FVR, DBP and MSNA total activity are presented in Figure 31. Sympathetic vascular transduction slopes, with DBP as the vascular outcome, are also shown in Figure 31. The sympathetic vascular transduction slopes are presented alongside forearm sympathetic vasoconstrictor responses during CPT and PEMI in Table 10.

Cold pressor test

Throughout CPT, heart rate, cardiac output, TVC, SBP and FVC were lower in young runners compared to nonrunners; whereas, stroke index, TPR, FVR, and MSNA burst incidence were higher in runners. During CPT, TPR, blood pressure (all indices) and MSNA (all indices) increased; stroke volume and TVC decreased. There were no significant differences in the response to CPT between groups. Sympathetic vascular transduction was not significantly different between young runners and nonrunners when assessed using any vascular outcome during CPT. There was also no significant effect of habitual exercise in young men on forearm sympathetic vasoconstriction during CPT.

Static Hand Grip Exercise and Post-Exercise Muscle Ischaemia

Time to fatigue was not different during SHG between young runners and nonrunners (216 ± 161 vs 154 ± 63 seconds, $P = 0.282$). Heart rate, cardiac output, SBP and MAP were lower in young runners compared to nonrunners throughout SHG; whereas, stroke volume, FVR, MSNA burst frequency, incidence and total activity were higher in runners. During SHG, heart rate, cardiac output, TVC, blood pressure (all indices), MSNA burst frequency, amplitude and total activity, and FBF increased; whilst stroke volume and TPR decreased. There were no significant group*time interactions during SHG.

During PEMI, heart rate, cardiac output and SBP were lower in young runners compared to nonrunners; stroke volume, TPR and MSNA burst incidence were higher in

runners. In response to PEMI, TPR, blood pressure (all indices) and MSNA (all indices) increased and stroke volume, cardiac output and TVC decreased. There were no significant group*time interactions during PEMI. Neither sympathetic vascular transduction assessed during nor the forearm sympathetic vasoconstrictor responses to PEMI were significantly different between young runners and nonrunners.

Table 8 – Haemodynamic, neural and vascular responses to CPT in young runners and nonrunners

Variable	Group	n	Baseline	End	Group *P*	Time *P*	Int. *P*
Haemodynamic							
Heart Rate (bpm)	NR	10	67 ± 10	72 ± 14	**< 0.001**	0.310	0.516
	R	13	48 ± 9	50 ± 10			
Stroke Volume (ml)	NR	10	86 ± 16	76 ± 16	0.057	**0.036**	0.828
	R	13	93 ± 11	85 ± 14			
Cardiac Output ($l \cdot min^{-1}$)	NR	10	5.7 ± 0.9	5.3 ± 0.7	**< 0.001**	0.281	0.912
	R	13	4.5 ± 0.9	4.2 ± 1.1			
TPR ($mmHg \cdot l \cdot min^{-1}$)	NR	10	16.7 ± 3.4	19.4 ± 2.9	**0.002**	**0.022**	0.737
	R	13	20.7 ± 4.2	24.2 ± 5.9			
TVC ($l \cdot min^{-1} \cdot mmHg$)	NR	10	0.062 ± 0.012	0.053 ± 0.008	**0.003**	**0.018**	0.680
	R	13	0.051 ± 0.011	0.044 ± 0.012			
MAP (mmHg)	NR	10	93 ± 9	102 ± 8	0.065	**0.001**	0.589
	R	13	90 ± 5	96 ± 7			
Neural							
MSNA BF ($bursts \cdot min^{-1}$)	NR	10	14 ± 8	26 ± 15	0.820	**< 0.001**	0.888
	R	13	15 ± 7	27 ± 9			
MSNA BI ($bursts \cdot 100hb^{-1}$)	NR	10	21 ± 13	35 ± 16	**0.004**	**0.001**	0.423
	R	13	32 ± 17	55 ± 19			
MSNA BA (%)	NR	10	63 ± 9	80 ± 23	0.441	**0.001**	0.502
	R	13	56 ± 10	79 ± 22			
Vascular							
FBF ($ml \cdot min^{-1}$)	NR	9	122 ± 45	120 ± 42	0.050	0.757	0.872
	R	10	95 ± 45	89 ± 43			
FVC ($ml \cdot min^{1} \cdot 100mmHg^{-1}$)	NR	9	135 ± 50	121 ± 44	**0.016**	0.874	0.931
	R	10	107 ± 49	92 ± 44			

Data are presented as mean ± standard deviation. Significant main effects or interactions are highlighted in bold, determined via a linear mixed model. *Abbreviations: BA, burst amplitude; BF, burst frequency; BI, burst incidence; CPT, cold pressor test; FBF, forearm blood flow; FVC, forearm vascular conductance; Int., interaction; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; n, sample size; NR, nonrunner; R, runner; TPR, total peripheral resistance; TVC, total vascular conductance.*

Table 9 – Haemodynamic, neural and vascular responses to SHG and PEMI in young runners and nonrunners

				SHG				PEMI			
Variable	**Group**	**n**	**Baseline**	**End SHG**	**Group *P***	**Time *P***	**Int. *P***	**End PEMI**	**Group *P***	**Time *P***	**Int. *P***
Haemodynamic											
Heart Rate	NR	10	70 ± 13	99 ± 9	**< 0.001**	**< 0.001**	0.981	66 ± 11	**< 0.001**	0.393	0.521
(bpm)	R	13	48 ± 7	77 ± 17				47 ± 7			
Stroke Volume	NR	10	83 ± 13	75 ± 11	**0.001**	**0.004**	0.346	72 ± 14	**0.001**	**0.003**	0.617
(ml)	R	13	99 ± 14	84 ± 11				85 ± 12			
Cardiac Output	NR	10	5.8 ± 1.5	7.4 ± 0.9	**0.016**	**< 0.001**	0.823	4.7 ± 1.1	**0.015**	**0.009**	0.514
($l \cdot min^{-1}$)	R	13	4.7 ± 0.8	6.5 ± 1.7				4.0 ± 1.1			
TPR	NR	10	17.4 ± 3.8	14.4 ± 2.1	**0.049**	**0.009**	0.950	23.0 ± 4.4	**0.049**	**< 0.001**	0.589
($mmHg \cdot l \cdot min^{-1}$)	R	13	19.6 ± 3.8	16.6 ± 4.29				27.0 ± 7.4			
TVC	NR	10	0.061 ± 0.016	0.071 ± 0.009	0.074	**0.009**	0.826	0.045 ± 0.009	0.052	**< 0.001**	0.692
($l \cdot min^{-1} \cdot mmHg$)	R	13	0.052 ± 0.011	0.064 ± 0.017				0.040 ± 0.010			
MAP	NR	10	97 ± 9	121 ± 11	**0.028**	**< 0.001**	0.844	122 ± 7	0.100	**< 0.001**	0.483
(mmHg)	R	13	91 ± 6	116 ± 7				120 ± 11			
Neural											
MSNA BF	NR	10	11 ± 7	19 ± 12	**0.017**	**< 0.001**	0.214	22 ± 11	0.050	**< 0.001**	0.611
($bursts \cdot min^{-1}$)	R	13	15 ± 9	32 ± 15				29 ± 9			
MSNA BI	NR	10	17 ± 10	21 ± 12	**< 0.001**	0.074	0.328	34 ± 17	**0.003**	**< 0.001**	0.246
($bursts \cdot 100hb^{-1}$)	R	13	33 ± 19	47 ± 21				62 ± 19			
MSNA BA	NR	10	62 ± 15	85 ± 32	0.235	**0.001**	0.843	76 ± 23	0.619	**0.001**	0.477
(%)	R	13	56 ± 7	77 ± 23				77 ± 21			
Vascular											
FBF	NR	9	121 ± 50	211 ± 70	0.086	**0.001**	0.620	113 ± 50	0.179	0.909	0.707
($ml \cdot min^{-1}$)	R*	12	93 ± 48	161 ± 96				97 ± 54			
FVC	NR	9	128 ± 50	175 ± 51	0.148	0.050	0.756	92 ± 35	0.281	0.071	0.679
($ml \cdot min^{1} \cdot 100mmHg^{-1}$)	R*	12	104 ± 55	159 ± 92				96 ± 52			

Data are presented as mean ± standard deviation. Significant main effects or interactions are highlighted in bold, determined via separate linear mixed models. * represents n = 13 during PEMI. *Abbreviations: BA, burst amplitude; BF, burst frequency; BI, burst incidence; FBF, forearm blood flow; FVC, forearm vascular conductance; Int., interaction; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; n, sample size; NR, nonrunner; PEMI, post-exercise muscle ischaemia; R, runner; SHG, static hand grip exercise; TPR, total peripheral resistance; TVC, total vascular conductance.*

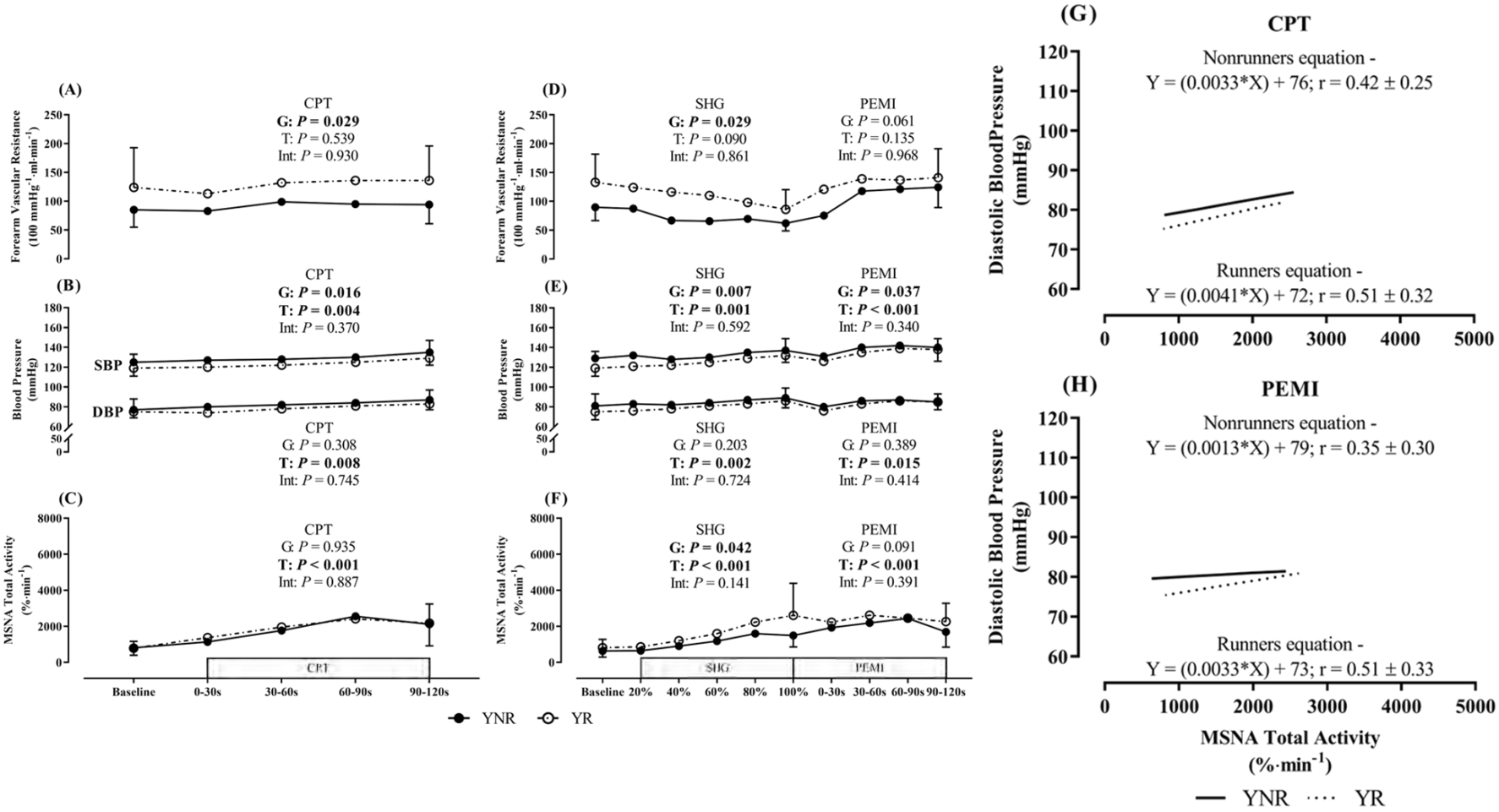


Figure 31 – The forearm vascular, blood pressure and neural responses to CPT and SHG & PEMI in young runners and nonrunners. (A-F) Data are presented as mean with standard deviation error bars. The data points without error bars represent continuously collected data which are shown for completeness only; no statistical analyses were conducted on these data. Main effects of group and time and group*time interactions were determined from separate linear mixed models comparing baseline to the end of CPT **(Panels A-C)**, SHG and PEMI **(Panels D-F)**; significant ($P < 0.05$) main effects or group*time interactions are reported in bold. **(G-H)** Data are presented as mean slopes from linear regression analyses in 10 young nonrunners and 13 young runners. Effect sizes and *P* values are presented within Table 10. *Abbreviations: CPT, cold pressor test; DBP, diastolic blood pressure; G, main effect of group; Int, group*time interaction; M, middle-aged nonrunner; MR, middle-aged runner; PEMI, post-exercise muscle ischaemia; s, seconds; SBP, systolic blood pressure; SHG, static hand grip exercise; T, main effect of time.*

Table 10 – Sympathetic vascular transduction and forearm sympathetic vasoconstriction during CPT and PEMI in young runners and nonrunners

Variable		CPT				PEMI			
		nonrunners	**runners**	***Effect Size***	***P***	**nonrunners**	**runners**	***Effect Size***	***P***
Slopes									
TA-FVR ($100mmHg^{-1}\cdot ml\cdot min^{-1}\cdot\%\cdot min^{-1}$)	*n*	9	9			8	11		
	Slope	0.0049 ± 0.0061	0.0042 ± 0.0147	0.0	0.489	0.0048 ± 0.0100	0.0114 ± 0.0384	-0.2	0.657
	r	*0.35 ± 0.33*	*0.32 ± 0.27*	*0.0*	*1.000*	*0.15 ± 0.12*	*0.24 ± 0.23*	*-0.5*	*0.840*
TA-FVC ($ml\cdot min^{-1}\cdot 100mmHg^{-1}\cdot\%\cdot min^{-1}$)	*n*	9	8			8	12		
	Slope	-0.0061 ± 0.0107	-0.0048 ± 0.0092	0.1	0.606	-0.0068 ± 0.0168	-0.0013 ± 0.0157	0.3	0.678
	r	*0.36 ± 0.32*	*0.32 ± 0.27*	*0.1*	*0.888*	*0.17 ± 0.13*	*0.28 ± 0.23*	*-0.5*	*0.592*
TA-DBP ($mmHg\cdot\%\cdot min^{-1}$)	*n*	9	13			10	13		
	Slope	0.0033 ± 0.0021	0.0041 ± 0.0026	-0.3	0.744	0.0013 ± 0.0053	0.0033 ± 0.0023	0.5	0.563
	r	*0.42 ± 0.25*	*0.51 ± 0.32*	*-0.3*	*0.512*	*0.35 ± 0.30*	*0.51 ± 0.33*	*-0.5*	*0.693*
Sympathetic Vasoconstriction	*n*	9	10			9	13		
FVR (%Δ)		12 ± 14	18 ± 30	-0.3	0.598	22 ± 34	11 ± 26	0.4	0.441
FVC (%Δ)		-10 ± 12	-10 ± 22	0.0	0.927	-11 ± 29	-5 ± 20	-0.2	0.678

Data are presented as mean ± standard deviation. *Abbreviations: CPT, cold pressor test; DBP, diastolic blood pressure; FVC, forearm vascular conductance; FVR, forearm vascular conductance; PEMI, post-exercise muscle ischaemia; TA, MSNA total activity.*

6.4 Discussion

The main novel findings from this study are as follows. First, in contrast to the primary hypothesis, sympathetic vascular transduction during sympathoexcitation, associated with increases in arterial pressure, was not significantly different between middle-aged endurance-trained runners and age-matched recreationally-active men. Second, sympathetic vascular transduction was also not different between young endurance-trained runners and age-matched recreationally-active men. Together, this finding for middle-aged men suggests that sympathetic vascular transduction is not an explanation for the upward setting of the vascular sympathetic baroreflex in chronic habitually endurance-trained male runners, as presented in the previous chapter of this thesis.

The primary aim of this study was to investigate the effect of chronic habitual endurance exercise on sympathetic vascular transduction in middle-aged males. A previous study suggested that sympathetic vascular transduction is lower in habitually endurance-trained middle-aged men, possibly mitigating against an age-related increase in vascular sympathetic activity (Notarius *et al.*, 2012). In that study, there was no significant relationship between MSNA-FVR in moderately endurance-trained middle-aged men during LBNP, unlike the significant correlation observed in age-matched sedentary men. However, the sedentary group had a higher resting SBP, which remained higher throughout LBNP, and the endurance-trained group had a mean $\dot{V}O_2$ Peak of 42.7 $mL \cdot kg^{-1} \cdot min^{-1}$ which was 129% of predicted (Notarius *et al.*, 2012). Given the interest in the chronic effects of habitual endurance exercise in this thesis, sympathetic vascular transduction was assessed in this study using a cohort of normotensive habitually endurance-trained runners and age-matched recreationally-active nonrunners. In comparison to the participants studied by Notarius and colleagues (2012), the runners studied here presented with a $\dot{V}O_2$ Peak of 50.7 $mL \cdot kg^{-1} \cdot min^{-1}$ which, using the same equation, was 143% of predicted, suggesting the runners studied in this thesis were more well-trained than those studied previously (Notarius *et al.*, 2012).

However, in contrast to findings of Notarius and colleagues, this study found that sympathetic vascular transduction was not different between trained and untrained middle-aged males. Furthermore, in the current study, habitual exercise had no influence on sympathetic vascular transduction in younger males. This finding also contradicts a previous report which found smaller vasomotor responses despite a greater increase in MSNA in young endurance-trained compared to untrained men (Fadel *et al.*, 2001). Notably, the vasoconstrictor response during orthostatic stress also is attenuated for young endurance-trained men (Raven *et al.*, 1984; Smith *et al.*, 1988; Stevens *et al.*, 1992; Ogoh *et al.*, 2003b). Thus, it has been suggested that endurance training may decrease sympathetic vascular control (van Lieshout, 2003). Lower sympathetic vascular transduction could contribute to orthostatic hypotension and intolerance in some endurance-trained athletes (Levine *et al.*, 1991a; Levine *et al.*, 1991b), and why "trained men can run, but they cannot stand" (Greenleaf *et al.*, 1981). However, future studies are warranted to further explore whether habitual exercise does influence sympathetic vascular transduction.

Compared with these previous studies, a different methodological approach was utilised to characterise sympathetic vascular transduction in this chapter. Here, sympathoexcitation was associated with elevated arterial pressure, which contrasts sympathoexcitation induced by LBNP (Notarius *et al.*, 2012) and thigh cuff release (Fadel *et al.*, 2001). This differential effect on blood pressure may explain the contrasting findings for sympathetic vascular transduction in this study. Notably, however, non-alpha-adrenergic effects on total vascular conductance appear to be greater during baroreflex stress (Kiviniemi *et al.*, 2012), unlike the primarily adrenergic mediated responses to CPT (Monahan *et al.*, 2013) and PEMI (Kiviniemi *et al.*, 2012). Collectively, the findings of this study may reflect that, as part of an integrated pressor response dependent primarily on alpha-adrenergic vasoconstriction, habitual exercise does not influence sympathetic vascular transduction. Indeed, any difference in sympathetic vascular transduction between endurance-trained and untrained men could be apparent only during a hypotensive stimulus; whereby reflex

sympathoexcitation is fundamental to "defend" arterial pressure. Whether blunted sympathetic vascular transduction during hypotension is mediated by neural mechanisms, such as lower rate coding, or recruitment, of previously silent sympathetic axons (Shoemaker *et al.*, 2018), or vascular mechanisms, such as reduced alpha-adrenergic responsiveness (Mortensen *et al.*, 2014), requires further study. Nevertheless, in the present study, utilising previously employed hypertensive physiological stressors (Halliwill *et al.*, 1996; Jarvis *et al.*, 2011; Tan *et al.*, 2013; Usselman *et al.*, 2015; Hissen *et al.*, 2019; Engelland *et al.*, 2020), sympathetic vascular transduction was not influenced by habitual exercise in middle-aged or young men. Future studies should provide further evidence as to the effects of habitual exercise on sympathetic vascular transduction throughout healthy human ageing utilising hypo- and hypertensive stimuli and recording vascular responses in both upper and lower limbs, as the effects may be stimulus and/or limb (i.e. trained vs untrained) dependent.

The hypothesis tested in the current study was formulated based upon a report of lower vascular alpha-adrenergic sensitivity following short term endurance-training in middle-age (Mortensen *et al.*, 2014). Importantly, to the best of the authors knowledge, no study has specifically investigated the effect of chronic habitual endurance exercise on vascular adrenergic sensitivity. Therefore, differential effects of acute and chronic endurance training may explain the disparity between the findings from Mortensen and colleagues (2014) and those presented here. Furthermore, differences in the methodological approaches, that is assessment of vascular alpha-adrenergic sensitivity via intra-arterial tyramine infusion in the lower limb versus vascular transduction assessed in the upper limb, may also contribute. Adrenergic sensitivity of vessels appears greater in the lower compared to the upper limbs (Pawelczyk and Levine, 2002); whether this difference is accentuated by exercise training is unknown (i.e. trained lower vs untrained upper limbs). Future studies should determine the effects of chronic habitual exercise of vascular adrenergic sensitivity, alongside assessments of sympathetic vascular transduction, in both trained and untrained limbs.

Another finding of note in this study relates to the changes in DBP and MSNA burst incidence (i.e. vascular sympathetic baroreflex resetting) in response to CPT, SHG and PEMI. It is well known that the vascular sympathetic baroreflex resets during these stimuli (Cui *et al.*, 2002; Ichinose *et al.*, 2004; Ichinose *et al.*, 2006), to mediate the increase in arterial pressure. Although not tested specifically in the present study, the findings of similar increases in DBP and MSNA burst incidence in response to all stimuli suggest that the resetting of the vascular sympathetic baroreflex operating point occurs independently of habitual exercise in middle-aged and young men. Notably, baroreflex gain was not assessed during any stimulus in this study. This observation is of interest in middle-aged men especially, since the resting vascular sympathetic baroreflex operating point is higher in middle-aged runners (Chapter 5). Thus, runners can only increase burst incidence from rest by around one third before they hit the "ceiling" of MSNA burst incidence (i.e. 100 bursts·100hb^{-1}). Whereas, middle-aged nonrunners have the capacity to double the MSNA operating point before reaching this ceiling. Hypothetically, operating closer to the "ceiling" may make it more difficult for middle-aged runners to increase MSNA burst incidence, requiring a greater change in MSNA burst amplitude. Nevertheless, during moderate sympathetic stress at utilised in this study, vascular sympathetic baroreflex resetting is proportionally similar for middle-aged runners and nonrunners. Furthermore, the response of MSNA burst amplitude was similar between middle-aged groups. This suggests that the higher resting operating point is not a barrier to elevating MSNA in middle-aged runners. Whether this is the case during more severe sympathetic stress, for example combined head-up tilt and LBNP to presyncope (el-Bedawi and Hainsworth, 1994), is unknown but warrants future investigation.

Methodological considerations

There are some limitations of this chapter which warrant consideration. First, in the present study MSNA was recorded from the fibular nerve and local blood flow was recorded in the brachial artery. During SHG the increases in MSNA are similar between upper and lower

limbs, but are lesser in the lower limb during PEMI (Wallin *et al.*, 1989). Importantly, however, there were no differences in sympathetic vascular transduction when calculated using a systemic vascular outcome (DBP). Future studies should record both upper and lower limb arterial and venous tone, with simultaneous fibular and radial MSNA, during each stimulus to further characterise sympathetic vascular control.

Second, there are multiple methods being employed within the literature to assess sympathetic vascular transduction, with no consensus as to which is the most appropriate. In this study sympathetic vascular transduction was assessed from the slope of the relationship between vascular sympathetic activity and indices of vascular tone calculated using an established method (Halliwill *et al.*, 1996). This analysis is used widely in the literature, but it presumes a linear relationship between vascular sympathetic activity and vasomotor responses and does not assess the temporal relationship between variables. In addition, forearm sympathetic vasoconstriction (%Δ in FVR and FVC) was assessed, a measure mathematically-independent of MSNA; these findings support the conclusion of no effects of habitual exercise on sympathetic vascular control. Whether habitual exercise related differences in sympathetic vascular transduction exist at rest (Fairfax *et al.*, 2013), but not during reflex sympathoexcitation, is unknown but warrants further investigation.

Finally, in the current study only males were included due to sex-based differences in sympathetic vascular control (Briant *et al.*, 2016), to isolate the effects of habitual exercise. Future studies should assess whether there are sex-dependent effects of habitual exercise on sympathetic vascular transduction, as there are sex-related differences in the level of resting MSNA in older age (Ng *et al.*, 1994a).

6.5 Conclusion

This chapter provides important new insight into the interaction of habitual exercise and efferent neurotransmission in the vasculature, studied here as sympathetic vascular transduction. Chronic habitual endurance exercise does not influence sympathetic vascular

transduction in normotensive, middle-aged men. Furthermore, this effect does not appear to age-dependent, as sympathetic vascular transduction was similar for young groups. When considered alongside the key novel finding from Chapter 5, it appears that the upward setting of the vascular sympathetic baroreflex in middle-aged men is not explained by lower efferent neurotransmission in the vasculature.

6.6 Hypotheses

i) Middle-aged runners would have lower sympathetic vascular transduction compared to age-matched nonrunners **(REJECTED)**.

CHAPTER 7. GENERAL DISCUSSION

7.1 Introduction

The primary aim of this thesis was to characterise the effects of chronic habitual endurance exercise on central haemodynamics and peripheral blood pressure regulation in normotensive middle-aged men. Three discrete research questions were posed and addressed in one large overarching study. Young habitually endurance-trained and recreationally-active men were also recruited to discern the effects of habitual exercise independently of age. The findings from these three related thesis studies provide a comprehensive insight into the determinants of blood pressure in the central and peripheral arteries, as data for all three studies were collected in the same individuals.

7.2 Summary of key findings

The main findings from this thesis are outlined within Figure 32, which describe the effects of habitual exercise on aortic haemodynamics and brachial blood pressure regulation in normotensive middle-aged men.

The first main finding from this thesis was that, in opposition to the working hypothesis, the aortic haemodynamic profile (stiffness, systolic pressure augmentation and pressure) was not different between middle-aged habitually endurance-trained and recreationally-active men. Notably, the data presented in chapter 4 contend the well-accepted notion that habitual exercise lowers aortic stiffness; aPWV was not different between middle-aged runners and nonrunners following the appropriate statistical adjustment for aMAP and heart rate.

The second key finding, again in contrast to the hypothesis, was that middle-aged habitually endurance-trained men were found to have a higher operating point (MSNA burst incidence) of the vascular sympathetic baroreflex. The higher MSNA operating point in middle-aged runners occurred despite similar operating diastolic pressure and vascular sympathetic baroreflex gain. The higher likelihood of MSNA burst occurrence in middle-

aged runners may represent a fundamental physiological adaptation to chronic endurance exercise training that supports resting peripheral arterial blood pressure.

Lastly, the third key finding from this thesis was that habitual endurance exercise did not influence sympathetic vascular transduction in middle-aged men. This finding contrasts the *a-priori* hypothesis. Thus, at least when compared to age-matched recreationally-active men, middle-aged runners do not present with lower efferent neurotransmission in the vasculature.

7.2.1 Habitual exercise, central haemodynamics and peripheral blood pressure regulation

The main novel finding from this thesis is that the vascular sympathetic baroreflex operating point is higher in middle-aged male endurance-trained runners compared to age and blood pressure matched recreationally-active nonrunners (Chapter 5). This occurs despite no significant between-group differences in aortic haemodynamics and sympathetic vascular transduction. All hypotheses tested within this thesis were rejected, and all findings appear to contradict the previous literature. The reason(s) for the disparity between the findings from this thesis and from the previous literature could be related to differences in study design. Specifically, the studies in this thesis were developed to investigate the effects of habitual endurance exercise on central and peripheral blood pressure in the same sample of middle-aged men. This study design provides important new insight which may not have been revealed previously, as these assessments have been conducted in different samples of individuals across different laboratories. Furthermore, unlike most previous studies, endurance-trained runners were compared to age matched recreationally-active, but not sedentary, nonrunners; all of whom were normotensive. Thus, these data highlight the effects of habitual endurance exercise without the confounding effects of sedentarism and elevated blood pressure.

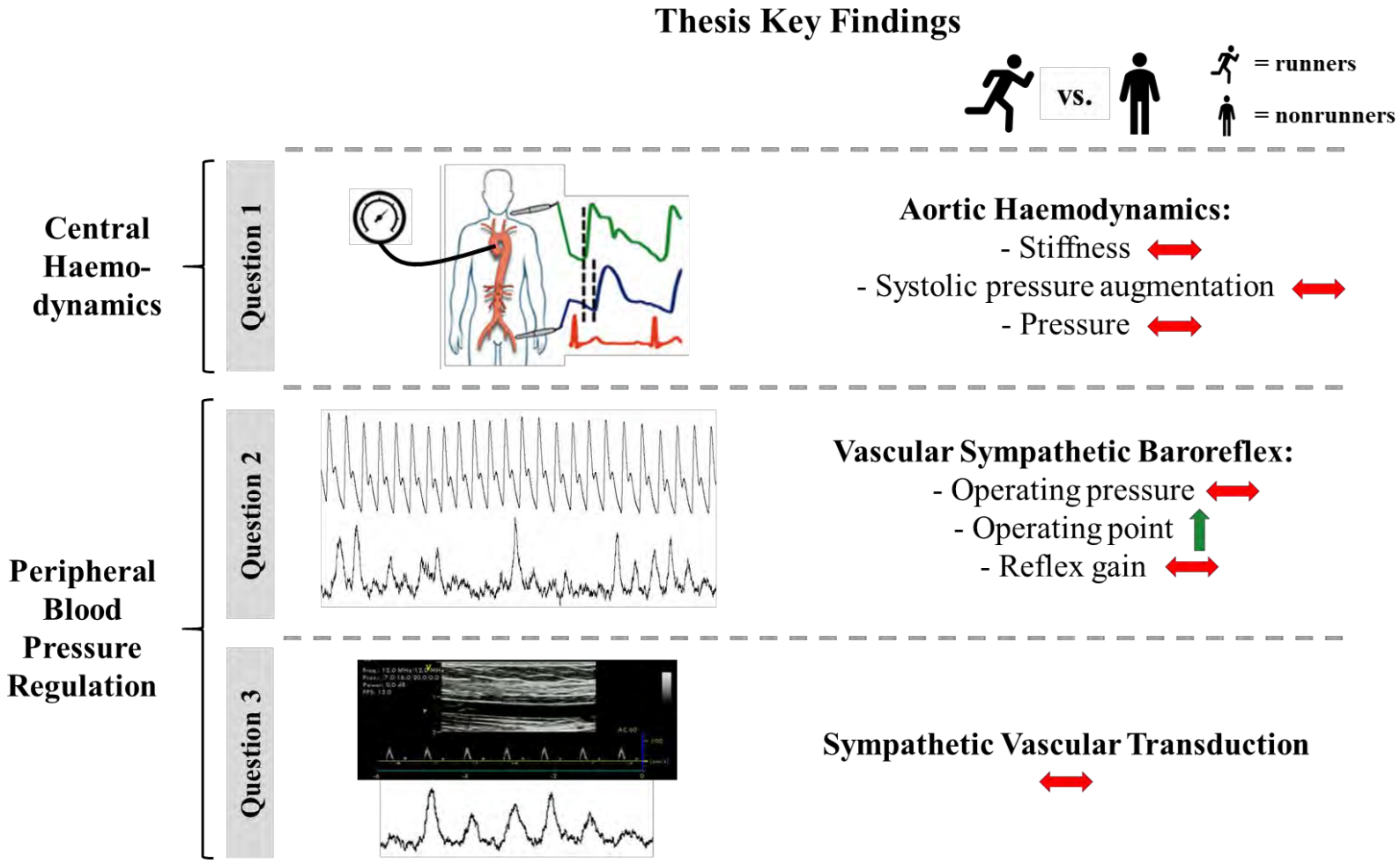


Figure 32 – Effects of habitual endurance exercise on central haemodynamics and peripheral blood pressure regulation in middle-aged men. The findings from study 1 (chapter 4; top), 2 (chapter 5; middle) and 3 (chapter 6; bottom). Significant differences between groups are shown by the green directional arrows; no significant difference between groups is shown by the red bi-directional arrows.

It is important to consider the primary components of the arterial baroreflex arc when attempting to understand the contributing mechanism(s) to this upward setting of the vascular sympathetic baroreflex in middle-aged runners. The three primary components are: (1) mechanosensory transduction, which is influenced by barosensory vessel pressure and stiffness (as assessed in the aorta in Chapter 4); (2) central processing; and (3) efferent neurotransmission of nerve signals into an end-organ response (i.e. sympathetic vascular transduction as assessed in Chapter 6). Based upon the data reported in this thesis, it is possible to rule out some potential mechanism(s) which may contribute to the upward setting of the vascular sympathetic baroreflex. First, aortic stiffness and pressure were not different between middle-aged groups (Chapter 4). Along with the same parameters within the carotid arteries, aortic stiffness and pressure play a key role in the arterial baroreceptor sensing of mechanical deformation and subsequent afferent signalling to the brainstem. The study of mechanical deformation and the associated changes in afferent nerve traffic are difficult to study in humans. Thus, these data provide some insight into the central pressure which distends the aortic baroreceptors. However, aortic pressure and stiffness are not indices of mechanical transduction *per se*. Second, left ventricular stroke volume was greater in middle-aged runners compared to nonrunners (Chapter 5). Hypothetically, this would lead to greater likelihood of baroreflex engagement (i.e. gate closing) with each cardiac systole, due to the interaction of a greater stroke volume in an aorta of similar stiffness (Chapter 4). Whether the baroreceptors themselves reset (Chapleau *et al.*, 1988) following exercise training is unknown; however, it is not currently possible to test this in humans. Third, there was no difference in the vascular sympathetic baroreflex operating pressure or gain between middle-aged men (Chapter 5). Thus, independently of differences in the stimulus to, and the responsiveness of, the vascular sympathetic baroreflex the level of MSNA was set higher in middle-aged runners. Fourth, sympathetic vascular transduction was not different between middle-aged groups (Chapter 6), which, if lower, could lead to increases in MSNA burst incidence due to reduced efferent neurotransmission in the vasculature. Together, these four

factors which could influence the vascular sympathetic operating point are not effected by chronic habitual endurance exercise until middle-age; accordingly, other mechanism(s), independently of differences in aspects of mechanical transduction or efferent neurotransmission, likely contribute to this difference between groups.

One possible mechanism is the interaction between exercise-induced bradycardia and left ventricular stroke volume in middle-aged runners. Stroke volume was only moderately (12%) higher in middle-aged runners when compared to age-matched nonrunners. In young men, however, stroke volume was much greater (50% higher) in runners compared to nonrunners, with a similar magnitude of exercise-induced bradycardia. Notably, there was no difference in the vascular sympathetic operating point in young men. Thus, the smaller magnitude of difference in stroke volume between middle-aged runners and nonrunners may contribute to the chronic endurance exercise related upward setting of the vascular sympathetic baroreflex. The difference in stroke volume between middle-aged groups may not be able to offset the increased microvascular density following endurance exercise training (Hoier *et al.*, 2012; Laughlin *et al.*, 2012; Baum *et al.*, 2015; Haas and Nwadozi, 2015). Accordingly, in an ageing system which is already more reliant on the autonomic support of blood pressure (Jones *et al.*, 2001), likely mediated by lower blood volume (Best *et al.*, 2014), the moderately higher stroke volume in middle-aged endurance runners may not be able to maintain arterial pressure during a longer diastolic period. This, in turn, may contribute to why middle-aged runners operate at DBP which is further leftward of the midpoint (termed T50) of their baroreflex operating pressure range (Figure 33B), compared to nonrunners, quantified as the DBP offset (T50-DBP, mmHg; Figure 33A). Operating leftward of T50 (i.e. a negative DBP offset) is associated with higher MSNA burst probability (Wehrwein *et al.*, 2010). Importantly, operating DBP and T50 were similar between middle-aged groups. As the operating DBP for middle-aged nonrunners was the same as their T50 value (DBP offset = 0 mmHg) MSNA burst incidence was 50

bursts·100hb^{-1}; whereas, the operating DBP for runners was lower (DBP offset = 6 mmHg) than T50, equating to an average MSNA burst incidence of 72 bursts·100hb^{-1} (Figure 33B).

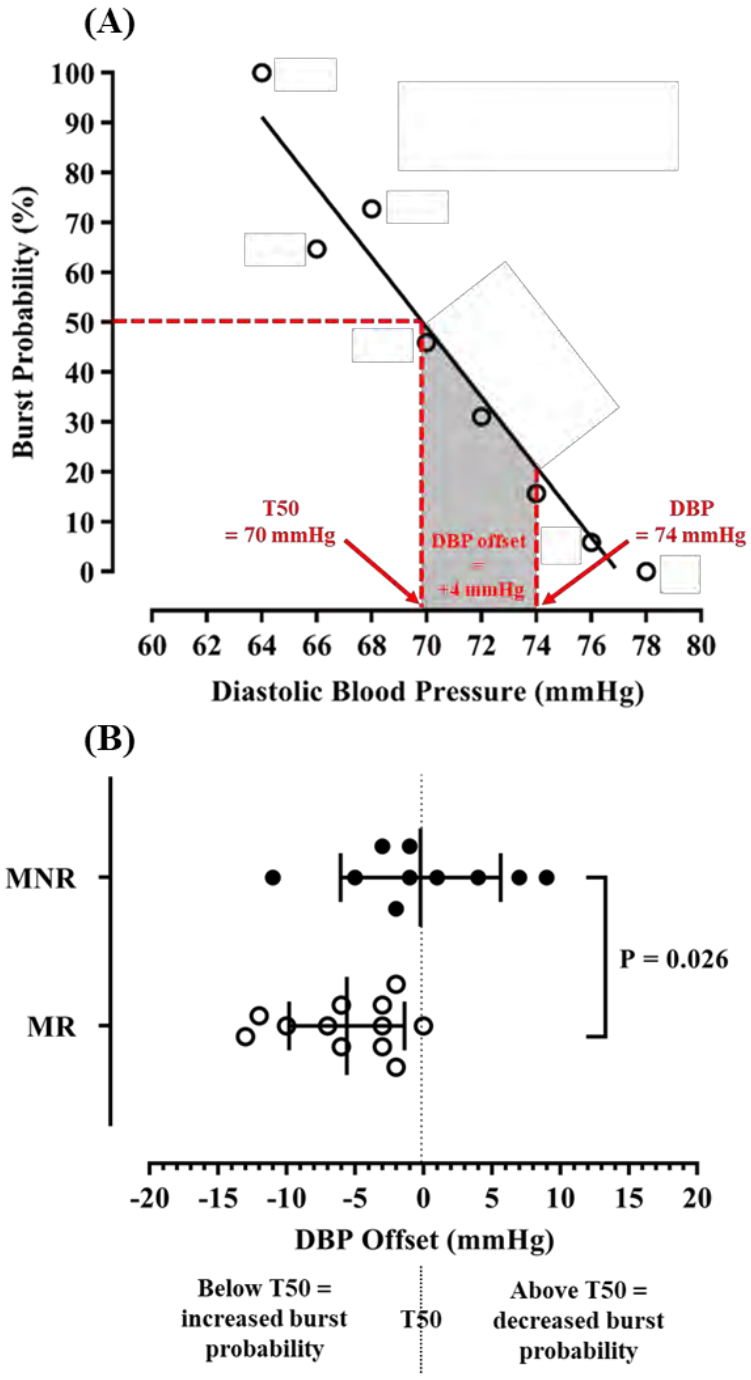


Figure 33 – Diastolic blood pressure offset from the midpoint (T50) of the operating pressure range. (A) T50 is determined from the linear regression equation determined by the analysis of vascular sympathetic baroreflex gain. T50 is calculated as the diastolic blood pressure (DBP) associated with a 50% likelihood of MSNA burst occurrence, as shown here from a young individual. The DBP offset represents the difference between the T50 and the operating DBP. **(B)** T50 is represented by 0. The more positive the DBP offset the lower the MSNA burst probability and vice versa. The average DBP offset was more negative in the middle-aged runners compared to the nonrunners; accordingly, the average MSNA burst probability was higher in middle-aged runners, despite the midpoint of the baroreflex operating range (i.e. T50) being similar between groups (P = 0.304).

The reason(s) for the age-dependent effect of habitual endurance exercise on the magnitude of difference in left ventricular stroke volume are unclear. In previous studies of middle-aged and older individuals, using both transthoracic echocardiography and cardiac MRI, habitual exercise has been shown to be associated with a greater left ventricular stroke volume by 17-40% (Arbab-Zadeh *et al.*, 2004; Wilson *et al.*, 2011; Bohm *et al.*, 2016; Maessen *et al.*, 2017). In these studies, however, endurance trained individuals were compared to age-matched sedentary counterparts. Thus, the finding of a smaller magnitude of difference between middle-aged groups here, of 12%, may be mediated by the greater level of physical activity in the middle-aged nonrunners studied in this thesis. In young individuals, using both transthoracic echocardiography and cardiac MRI, left ventricular stroke has been shown to be between 17-55% higher in endurance athletes (Levine *et al.*, 1991b; Scharhag *et al.*, 2002; D'Andrea *et al.*, 2011; Cooke *et al.*, 2018). Thus, in support of the data presented within this thesis, previous studies also report a smaller range of effects of habitual endurance exercise on the magnitude of difference in left ventricular stroke volume in middle-aged/older compared to younger individuals. Again, the reasons for this difference are unclear but may be related to age-associated changes in left ventricular diastolic function (Park *et al.*, 2007) or blood volume (Best *et al.*, 2014). However, an effect of age was not apparent in the nonrunners studied here as stroke volume was marginally higher with age (+2%), unlike the effects of age in the runners (-24%). The aforementioned studies (Levine *et al.*, 1991b; Scharhag *et al.*, 2002; Arbab-Zadeh *et al.*, 2004; D'Andrea *et al.*, 2011; Wilson *et al.*, 2011; Bohm *et al.*, 2016; Maessen *et al.*, 2017; Cooke *et al.*, 2018) used either transthoracic echocardiography or cardiac MRI to determine ventricular volumes. Although echocardiography underestimates absolute ventricular volumes compared to cardiac MRI (Gardner *et al.*, 2009; Greupner *et al.*, 2012; Marwick *et al.*, 2013), the magnitude of difference between-groups is likely to be similar between techniques. Together, when the data presented within this thesis are compared to data from previous studies, it appears that the magnitude of difference in left ventricular stroke volume is smaller

in middle-aged runners compared to nonrunners, but similar in young runners compares to nonrunners.

Physical-activity dependent neuroplasticity could also contribute to the higher operating point of the vascular sympathetic baroreflex in middle-aged runners. The phenomenon of physical-activity dependent neuroplasticity describes alterations within the NTS and RVLM, amongst other brain regions, which adapt following sustained physical (in)activity (Mueller *et al.*, 2017). In the animal studies which report this neuroplasticity within the central circuitry, the level of peripheral vascular sympathetic activity reduces following short-term exercise training (Mueller *et al.*, 2017). Whether these same brain regions can also remodel to facilitate increases in vascular sympathetic activity with chronic exercise in humans is unclear. It is possible, therefore, that neuroplasticity and exercise-induced changes in central processing underpin the higher MSNA burst incidence in middle-aged trained males. However, central processing is difficult to study in humans. Recently, recordings of MSNA have been conducted during functional MRI to assess the activation of different brain regions, including the NTS and RVLM, and relate this brain activity to the firing of MSNA bursts (Macefield and Henderson, 2019). More studies like these will further the understanding of the autonomic control of the circulation in humans. Despite suggesting this as a mechanism contributing to the upward baroreflex setting, the signal which leads to this is not clear.

In summary, the mechanism(s) which underpin this upward setting of the vascular sympathetic baroreflex are unclear but are unlikely to be related to differences in mechanosensory transduction or efferent neurotransmission in the vasculature. Thus, the most likely mechanism is related to a change in central processing within the brainstem following chronic habitual endurance exercise training. These data provide novel insight into central haemodynamics and the autonomic regulation and neural control of the circulation following chronic habitual endurance exercise. Furthermore, this thesis challenges the

conventional wisdom regarding the central haemodynamic and neuro-circulatory adaptations with habitual endurance exercise.

7.3 Significance

Exercise training is known to induce significant cardiac and vascular remodelling in healthy, sedentary and patient populations (Hellsten and Nyberg, 2015). The effects of short-term exercise training leads to beneficial cardiovascular adaptations; however, the responses to short-term (i.e. weeks) exercise training, independent of the population, are not likely to reflect the adaptations to chronic (i.e. years) exercise training. Furthermore, both the number and magnitude of the physiological adaptations that occur are likely dependent on training intensity and duration. The overarching aim of this thesis was to determine the effects of chronic habitual endurance exercise on central haemodynamics and peripheral blood pressure regulation. The principal finding from this thesis is related to the effects of habitual exercise on the vascular sympathetic baroreflex regulation of peripheral blood pressure.

The effects of exercise training on the autonomic nervous system, specifically vascular sympathetic activity (i.e. MSNA), have been shown to be minimal in healthy individuals. However, significant reductions in MSNA are often observed following exercise training in patient populations (Carter and Ray, 2015). To date, the effects of exercise training on MSNA are often extrapolated from the studies reporting effects in patients to healthy individuals, despite no clear evidence base for this conclusion. Furthermore, when endurance-trained individuals are studied they are often compared to sedentary individuals, despite the negative cardiovascular consequences of sedentarism (Lavie *et al.*, 2019). Therefore, the findings regarding the effects of habitual exercise may differ when healthy, normotensive, recreationally-active individuals are used as a control group compared to studies recruiting sedentary controls. In line with this, the operating point of the vascular sympathetic baroreflex was higher in normotensive middle-aged runners compared to age-matched nonrunners and was not different between young runners and nonrunners.

Therefore, these data clearly show that, in middle-aged and young normotensive men, chronic endurance exercise was not associated with lower MSNA and provide evidence that exercise training can lead to increases in the MSNA operating point, in the context of the arterial baroreflex, to help support peripheral blood pressure in middle-aged men.

There are other examples of physiological elevations in MSNA in response to cardiovascular remodelling. For example, MSNA increases by ~100% during normotensive healthy pregnancy (Reyes *et al.*, 2018). This occurs due to many factors, including the significant decrease in total vascular resistance which is not offset by an appropriate increase in cardiac output (Meah *et al.*, 2016). Accordingly, this physiological increase in the MSNA operating point is a necessary adaptation to aid in the support of arterial blood pressure. Although orders of magnitude smaller compared to the vascular adaptations to pregnancy, the changes in microvascular density that occur with chronic exercise, mediated by exercise-induced angiogenesis within the skeletal muscle vasculature (Hoier *et al.*, 2012; Laughlin *et al.*, 2012; Baum *et al.*, 2015; Haas and Nwadozi, 2015), may lead to a higher vascular sympathetic baroreflex operating point. Furthermore, in this thesis the training-related effect on stroke volume was only moderate in middle-age; thus, the magnitude of difference in stroke volume may not be large enough to offset the increased microvascular density associated with chronic endurance exercise training. Therefore, heightened levels of MSNA alongside normal blood pressure may represent an important and necessary autonomic adaptation to aid in the regulation of peripheral blood pressure. However, chronically high levels of vascular sympathetic activity are suggested to have negative consequences for the cardiovascular system. Yet, this conclusion is not binary. This is because the level of sympathetic outflow is dependent on the integration of systemic physiology (Charkoudian and Wallin, 2014). The cause(s) for the high levels of sympathetic outflow, and to which end-organ (i.e. skeletal muscle vasculature versus heart) need to be considered before determining whether, or not, the level of sympathetic outflow is of negative consequence

(Fisher and Paton, 2012). High levels of sympathetic outflow to other organs or vascular beds, as opposed to the skeletal muscle vasculature, may have important clinical consequences, as reviewed previously (Fisher and Paton, 2012).

A key theme that has been referred to throughout this thesis is the appropriate selection of a control group when determining the effects of habitual endurance exercise on physiological function. The effects of differences in physical activity levels should be determined by comparing habitually trained or sedentary individuals to recreationally-active individuals. Recreationally-active individuals, who meet the current exercise recommendations of at least 150 minutes of moderate or 75 minutes of vigorous physical activity per week, as set out by the UK Chief Medical Officers (2019), may more likely reflect the "normal" healthy ageing process. This is supported by the data presented in this thesis as only heart rate, stroke volume and the MSNA operating point were significantly different between middle-aged groups. This suggests that chronic endurance exercise does elicit marked adaptations within the cardiovascular system, when compared to recreationally-active individuals.

7.4 Future Directions

Although there are many research questions regarding the effects of chronic endurance exercise training on central and peripheral blood pressure that have come from this thesis, future studies should focus on three main themes based upon the data presented here:

1. The effects of chronic exercise "dose" (i.e. sedentary vs recreationally-active vs endurance-trained) on the aortic haemodynamic profile and vascular sympathetic activity. These data should be collected across the lifespan (i.e. young vs middle-aged vs older adults); studies should also determine the effects of sex and race. This will have important implications for the understanding of the integrative cardiovascular control of blood pressure with healthy human ageing.

2. The mechanism(s) underpinning the upward setting of the vascular sympathetic limb of the arterial baroreflex following chronic habitual exercise. The use of functional MRI could be coupled with measurements of MSNA, as conducted previously (Macefield and Henderson, 2019), to characterise whether changes in the central neural circuitry occur with habitual exercise in humans.
3. To comprehensively determine the effects of habitual exercise on efferent neurotransmission in the vasculature. Future studies should assess vascular adrenergic sensitivity as well as sympathetic vascular transduction at rest and during hypo- and hypertensive stimuli. Vascular responses should be assessed in both upper and lower limbs, as the effects may be stimulus and/or limb (i.e. trained vs untrained) dependent. The effects of habitual exercise are not well documented across the lifespan and may potentially represent an important component of the cardiovascular adaptation to habitual exercise that, to date, remains relatively unknown.

7.5 Thesis Limitations

There are some general limitations relating to the findings from this thesis that warrant consideration. First, the sample size of groups studied in this thesis is small and therefore these data need to be collected in a larger population to confirm/refute the findings presented here. However, this study was designed to comprehensively assess the integrative regulation of arterial blood pressure in four groups, which required simultaneous data collection from multiple investigators. This study, albeit in a small sample size, does provide novel insight into the effects of habitual endurance exercise on central haemodynamics and peripheral blood pressure regulation. Second, only males were studied in thesis due to the sex-based differences in central haemodynamics and sympathetic vascular regulation (Hart *et al.*, 2009a; Casey *et al.*, 2011; Hart *et al.*, 2012a; Hart *et al.*, 2012b; Hart *et al.*, 2013). Future investigations should also determine the effects of sex and race on the interaction between age and habitual endurance exercise. Third, in conscious humans it is only possible to record

continuous sympathetic vasomotor activity directed to the skeletal muscle vasculature (i.e. MSNA); whether the findings presented here would be similar in other vascular regions is unknown. Notably, however, the muscle circulation is the largest within the body, thus is of prime importance in the regulation of arterial blood pressure (Dornhorst, 1963). Fourth, baroreflex function was only assessed at rest in this thesis; the effects of habitual endurance exercise on baroreflex function during physiological stress are unclear but warrant further investigation. Finally, sympathetic vascular transduction was assessed during two stressors associated with increases in arterial blood pressure. Whether the findings presented here would be similar when assessed during supine rest or other stressors associated with a fall or no significant change in arterial pressure is unknown.

7.6 Conclusion

The data in the thesis challenges conventional wisdom which calls for further studies to (re)evaluate the effects of chronic habitual endurance exercise on aortic stiffness and the autonomic regulation of vascular sympathetic activity. These two parameters contribute to central and peripheral blood pressure, respectively. Currently, lower aortic stiffness and vascular sympathetic activity are thought to be "hallmark" adaptations to habitual endurance exercise; however, data included in this thesis suggest that this may not be the case. This thesis shows that chronic (~30 years) habitual endurance exercise has no effect on the aortic haemodynamic profile (stiffness, systolic pressure augmentation and pressure), peripheral blood pressure, vascular sympathetic and cardiovagal operating pressure and gain, and sympathetic vascular transduction. However, habitual endurance exercise in middle-age does lead to an increased likelihood of MSNA burst occurrence at rest. This higher likelihood of MSNA burst occurrence in middle-aged male runners may represent a fundamental physiological adaptation to chronic habitual endurance exercise. This adaptation may be necessary to support resting peripheral blood pressure in an expanded cardiovascular system with greater reserve.

References

2019. *UK Chief Medical Officers' Physical Activity Guidelines* [Online]. Available: https://www.gov.uk/government/publications/physical-activity-guidelines-uk-chief-medical-officers-report [Accessed 07/05/2020].

Alvarez, G. E., Halliwill, J. R., Ballard, T. P., Beske, S. D. & Davy, K. P. 2005. Sympathetic neural regulation in endurance-trained humans: fitness vs. fatness. *J Appl Physiol (1985),* 98**,** 498-502.

Arbab-Zadeh, A., Dijk, E., Prasad, A., Fu, Q., Torres, P., Zhang, R., Thomas, J. D., Palmer, D. & Levine, B. D. 2004. Effect of aging and physical activity on left ventricular compliance. *Circulation,* 110**,** 1799-805.

Atkinson, G. & Batterham, A. M. 2013. The percentage flow-mediated dilation index: a large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. *Vasc Med,* 18**,** 354-65.

Babcock, M. C., Robinson, A. T., Migdal, K. U., Watso, J. C., Wenner, M. M., Stocker, S. D. & Farquhar, W. B. 2019. Reducing Dietary Sodium to 1000 mg per Day Reduces Neurovascular Transduction Without Stimulating Sympathetic Outflow. *Hypertension,* 73**,** 587-593.

Baksi, A. J., Treibel, T. A., Davies, J. E., Hadjiloizou, N., Foale, R. A., Parker, K. H., Francis, D. P., Mayet, J. & Hughes, A. D. 2009. A meta-analysis of the mechanism of blood pressure change with aging. *J Am Coll Cardiol,* 54**,** 2087-92.

Baum, O., Gubeli, J., Frese, S., Torchetti, E., Malik, C., Odriozola, A., Graber, F., Hoppeler, H. & Tschanz, S. A. 2015. Angiogenesis-related ultrastructural changes to capillaries in human skeletal muscle in response to endurance exercise. *J Appl Physiol (1985),* 119**,** 1118-26.

Beard, J. R., Officer, A., De Carvalho, I. A., Sadana, R., Pot, A. M., Michel, J. P., Lloyd-Sherlock, P., Epping-Jordan, J. E., Peeters, G., Mahanani, W. R., Thiyagarajan, J. A. & Chatterji, S. 2016. The World report on ageing and health: a policy framework for healthy ageing. *Lancet,* 387**,** 2145-2154.

Ben-Shlomo, Y., Spears, M., Boustred, C., May, M., Anderson, S. G., Benjamin, E. J., Boutouyrie, P., Cameron, J., Chen, C. H., Cruickshank, J. K., Hwang, S. J., Lakatta, E. G., Laurent, S., Maldonado, J., Mitchell, G. F., Najjar, S. S., Newman, A. B., Ohishi, M., Pannier, B., Pereira, T., Vasan, R. S., Shokawa, T., Sutton-Tyrell, K., Verbeke, F., Wang, K. L., Webb, D. J., Willum Hansen, T., Zoungas, S., Mceniery, C. M., Cockcroft, J. R. & Wilkinson, I. B. 2014. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol,* 63**,** 636-646.

Benarroch, E. E. 2008. The arterial baroreflex: functional organization and involvement in neurologic disease. *Neurology,* 71**,** 1733-8.

Benetos, A., Thomas, F., Joly, L., Blacher, J., Pannier, B., Labat, C., Salvi, P., Smulyan, H. & Safar, M. E. 2010. Pulse pressure amplification a mechanical biomarker of cardiovascular risk. *J Am Coll Cardiol,* 55**,** 1032-7.

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M., Delling, F. N., Deo, R., De Ferranti, S. D., Ferguson, J. F., Fornage, M., Gillespie, C., Isasi, C. R., Jimenez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., Lichtman, J. H., Lisabeth, L., Liu, S., Longenecker, C. T., Lutsey, P. L., Mackey, J. S., Matchar, D. B., Matsushita, K., Mussolino, M. E., Nasir, K., O'flaherty, M., Palaniappan, L. P., Pandey, A., Pandey, D. K., Reeves, M. J., Ritchey, M. D., Rodriguez, C. J., Roth, G. A., Rosamond, W. D., Sampson, U. K. A., Satou, G. M., Shah, S. H., Spartano, N. L., Tirschwell, D. L., Tsao, C. W., Voeks, J. H., Willey, J. Z., Wilkins, J. T., Wu, J. H., Alger, H. M., Wong, S. S., Muntner, P.,

American Heart Association Council On, E., Prevention Statistics, C. & Stroke Statistics, S. 2018. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation,* 137**,** e67-e492.

Best, S. A., Okada, Y., Galbreath, M. M., Jarvis, S. S., Bivens, T. B., Adams-Huet, B. & Fu, Q. 2014. Age and sex differences in muscle sympathetic nerve activity in relation to haemodynamics, blood volume and left ventricular size. *Exp Physiol,* 99**,** 839-48.

Bhella, P. S., Hastings, J. L., Fujimoto, N., Shibata, S., Carrick-Ranson, G., Palmer, M. D., Boyd, K. N., Adams-Huet, B. & Levine, B. D. 2014. Impact of lifelong exercise "dose" on left ventricular compliance and distensibility. *J Am Coll Cardiol,* 64**,** 1257-66.

Bhuva, A. N., D'silva, A., Torlasco, C., Jones, S., Nadarajan, N., Van Zalen, J., Chaturvedi, N., Lloyd, G., Sharma, S., Moon, J. C., Hughes, A. D. & Manisty, C. H. 2020. Training for a First-Time Marathon Reverses Age-Related Aortic Stiffening. *J Am Coll Cardiol,* 75**,** 60-71.

Billman, G. E. 2017. Counterpoint: Exercise training-induced bradycardia: the case for enhanced parasympathetic regulation. *J Appl Physiol (1985),* 123**,** 686-688.

Bjarnegard, N., Lanne, T., Cinthio, M., Ekstrand, J., Hedman, K., Nylander, E. & Henriksson, J. 2018. Vascular characteristics in young women-Effect of extensive endurance training or a sedentary lifestyle. *Acta Physiol (Oxf),* 223**,** e13041.

Boehmer, R. D. 1987. Continuous, real-time, noninvasive monitor of blood pressure: Penaz methodology applied to the finger. *J Clin Monit,* 3**,** 282-7.

Bohm, P., Schneider, G., Linneweber, L., Rentzsch, A., Kramer, N., Abdul-Khaliq, H., Kindermann, W., Meyer, T. & Scharhag, J. 2016. Right and Left Ventricular Function and Mass in Male Elite Master Athletes: A Controlled Contrast-Enhanced Cardiovascular Magnetic Resonance Study. *Circulation,* 133**,** 1927-35.

Bonyhay, I., Jokkel, G., Karlocai, K., Reneman, R. & Kollai, M. 1997. Effect of vasoactive drugs on carotid diameter in humans. *Am J Physiol,* 273**,** H1629-36.

Booth, J. 1977. A short history of blood pressure measurement. *Proc R Soc Med,* 70**,** 793-9.

Bos, W. J., Van Goudoever, J., Van Montfrans, G. A., Van Den Meiracker, A. H. & Wesseling, K. H. 1996. Reconstruction of brachial artery pressure from noninvasive finger pressure measurements. *Circulation,* 94**,** 1870-5.

Boyett, M. R., Wang, Y., Nakao, S., Ariyaratnam, J., Hart, G., Monfredi, O. & D'souza, A. 2017. Point: Exercise training-induced bradycardia is caused by changes in intrinsic sinus node function. *J Appl Physiol (1985),* 123**,** 684-685.

Briant, L. J., Burchell, A. E., Ratcliffe, L. E., Charkoudian, N., Nightingale, A. K., Paton, J. F., Joyner, M. J. & Hart, E. C. 2016. Quantifying sympathetic neuro-haemodynamic transduction at rest in humans: insights into sex, ageing and blood pressure control. *J Physiol,* 594**,** 4753-68.

Bristow, J. D., Gribbin, B., Honour, A. J., Pickering, T. G. & Sleight, P. 1969. Diminished baroreflex sensitivity in high blood pressure and ageing man. *J Physiol,* 202**,** 45P-46P.

Buckwalter, J. B. & Clifford, P. S. 2001. The paradox of sympathetic vasoconstriction in exercising skeletal muscle. *Exerc Sport Sci Rev,* 29**,** 159-63.

Bunsawat, K., Grigoriadis, G., Schroeder, E. C., Rosenberg, A. J., Rader, M. M., Fadel, P. J., Clifford, P. S., Fernhall, B. & Baynard, T. 2019. Preserved ability to blunt sympathetically-mediated vasoconstriction in exercising skeletal muscle of young obese humans. *Physiol Rep,* 7**,** e14068.

Burnstock, G. 2013. Chapter 3 - Cotransmission in the autonomic nervous system. *In:* BUIJS, R. M. & SWAAB, D. F. (eds.) *Handbook of Clinical Neurology.* Elsevier.

Busch, S. A. 2018. *Sympathetic Function in Sherpa and Lowlanders at High Altitude.* Master of Science, University of Alberta.

Carter, J. R. 2019. Microneurography and sympathetic nerve activity: a decade-by-decade journey across 50 years. *J Neurophysiol,* 121**,** 1183-1194.

Carter, J. R. & Ray, C. A. 2015. Sympathetic neural adaptations to exercise training in humans. *Auton Neurosci,* 188**,** 36-43.

Casey, D. P., Curry, T. B., Joyner, M. J., Charkoudian, N. & Hart, E. C. 2011. Relationship between muscle sympathetic nerve activity and aortic wave reflection characteristics in young men and women. *Hypertension,* 57**,** 421-7.

Chapleau, M. W. 2012. Chapter 33 - Baroreceptor Reflexes. *In:* ROBERTSON, D., BIAGGIONI, I., BURNSTOCK, G., LOW, P. A. & PATON, J. F. R. (eds.) *Primer on the Autonomic Nervous System (Third Edition).* San Diego: Academic Press.

Chapleau, M. W., Hajduczok, G. & Abboud, F. M. 1988. Mechanisms of resetting of arterial baroreceptors: an overview. *Am J Med Sci,* 295**,** 327-34.

Chapleau, M. W. & Sabharwal, R. 2011. Methods of assessing vagus nerve activity and reflexes. *Heart Fail Rev,* 16**,** 109-27.

Charkoudian, N. & Wallin, B. G. 2014. Sympathetic neural activity to the cardiovascular system: integrator of systemic physiology and interindividual characteristics. *Compr Physiol,* 4**,** 825-50.

Chen, C. H., Nevo, E., Fetics, B., Pak, P. H., Yin, F. C., Maughan, W. L. & Kass, D. A. 1997. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation,* 95**,** 1827-36.

Chirinos, J. A., Segers, P., Hughes, T. & Townsend, R. 2019. Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review. *J Am Coll Cardiol,* 74**,** 1237-1263.

Christou, D. D., Jones, P. P., Jordan, J., Diedrich, A., Robertson, D. & Seals, D. R. 2005. Women have lower tonic autonomic support of arterial blood pressure and less effective baroreflex buffering than men. *Circulation,* 111**,** 494-8.

Christou, D. D., Jones, P. P. & Seals, D. R. 2003. Baroreflex buffering in sedentary and endurance exercise-trained healthy men. *Hypertension,* 41**,** 1219-22.

Cohen, J. 1992. A power primer. *Psychol Bull,* 112**,** 155-9.

Cooke, S., Samuel, T. J., Cooper, S. M. & Stohr, E. J. 2018. Adaptation of myocardial twist in the remodelled athlete's heart is not related to cardiac output. *Exp Physiol,* 103**,** 1456-1468.

Craig, J. C., Broxterman, R. M., La Salle, D. T., Cerbie, J., Ratchford, S. M., Gifford, J. R., Bunsawat, K., Nelson, A. D., Bledsoe, A. D., Morgan, D. E., Wray, D. W., Richardson, R. S. & Trinity, J. D. 2020. The role of endothelin A receptors in peripheral vascular control at rest and during exercise in patients with hypertension. *J Physiol,* 598**,** 71-84.

Cramer, A. O., Van Ravenzwaaij, D., Matzke, D., Steingroever, H., Wetzels, R., Grasman, R. P., Waldorp, L. J. & Wagenmakers, E. J. 2016. Hidden multiplicity in exploratory multiway ANOVA: Prevalence and remedies. *Psychon Bull Rev,* 23**,** 640-7.

Crecelius, A. R., Kirby, B. S., Hearon, C. M., Jr., Luckasen, G. J., Larson, D. G. & Dinenno, F. A. 2015. Contracting human skeletal muscle maintains the ability to blunt alpha1 -adrenergic vasoconstriction during KIR channel and Na(+) /K(+) -ATPase inhibition. *J Physiol,* 593**,** 2735-51.

Cui, J., Wilson, T. E. & Crandall, C. G. 2002. Baroreflex modulation of muscle sympathetic nerve activity during cold pressor test in humans. *Am J Physiol Heart Circ Physiol,* 282**,** H1717-23.

D'andrea, A., Naeije, R., D'alto, M., Argiento, P., Golia, E., Cocchia, R., Riegler, L., Scarafile, R., Limongelli, G., Di Salvo, G., Citro, R., Caso, P., Russo, M. G., Calabro, R. & Bossone, E. 2011. Range in pulmonary artery systolic pressure among highly trained athletes. *Chest,* 139**,** 788-794.

Dampney, R. a. L. 2017. Resetting of the Baroreflex Control of Sympathetic Vasomotor Activity during Natural Behaviors: Description and Conceptual Model of Central Mechanisms. *Front Neurosci,* 11**,** 461.

Davies, J. E., Baksi, J., Francis, D. P., Hadjiloizou, N., Whinnett, Z. I., Manisty, C. H., Aguado-Sierra, J., Foale, R. A., Malik, I. S., Tyberg, J. V., Parker, K. H., Mayet, J. & Hughes, A. D. 2010. The arterial reservoir pressure increases with aging and is the major determinant of the aortic augmentation index. *Am J Physiol Heart Circ Physiol,* 298**,** H580-6.

Davy, K. P., Desouza, C. A., Jones, P. P. & Seals, D. R. 1998a. Elevated heart rate variability in physically active young and older adult women. *Clin Sci (Lond),* 94**,** 579-84.

Davy, K. P., Miniclier, N. L., Taylor, J. A., Stevenson, E. T. & Seals, D. R. 1996. Elevated heart rate variability in physically active postmenopausal women: a cardioprotective effect? *Am J Physiol,* 271**,** H455-60.

Davy, K. P., Seals, D. R. & Tanaka, H. 1998b. Augmented cardiopulmonary and integrative sympathetic baroreflexes but attenuated peripheral vasoconstriction with age. *Hypertension,* 32**,** 298-304.

Davy, K. P., Tanaka, H., Andros, E. A., Gerber, J. G. & Seals, D. R. 1998c. Influence of age on arterial baroreflex inhibition of sympathetic nerve activity in healthy adult humans. *Am J Physiol,* 275**,** H1768-72.

Delius, W., Hagbarth, K. E., Hongell, A. & Wallin, B. G. 1972a. General characteristics of sympathetic activity in human muscle nerves. *Acta Physiol Scand,* 84**,** 65-81.

Delius, W., Hagbarth, K. E., Hongell, A. & Wallin, B. G. 1972b. Manoeuvres affecting sympathetic outflow in human muscle nerves. *Acta Physiol Scand,* 84**,** 82-94.

Dempsey, P. C., Strain, T., Khaw, K. T., Wareham, N. J., Brage, S. & Wijndaele, K. 2020. Prospective Associations of Accelerometer-Measured Physical Activity and Sedentary Time With Incident Cardiovascular Disease, Cancer, and All-Cause Mortality. *Circulation,* 141**,** 1113-1115.

Denham, J., Brown, N. J., Tomaszewski, M., Williams, B., O'brien, B. J. & Charchar, F. J. 2016. Aortic augmentation index in endurance athletes: a role for cardiorespiratory fitness. *Eur J Appl Physiol,* 116**,** 1537-44.

Difrancesco, D. 2010. The role of the funny current in pacemaker activity. *Circ Res,* 106**,** 434-46.

Dinenno, F. A., Dietz, N. M. & Joyner, M. J. 2002. Aging and forearm postjunctional alpha-adrenergic vasoconstriction in healthy men. *Circulation,* 106**,** 1349-54.

Dornhorst, A. C. 1963. Clinical implications of recent peripheral vascular studies. *British Medical Bulletin,* 19**,** 161-162.

Draghici, A. E. & Taylor, J. A. 2016. The physiological basis and measurement of heart rate variability in humans. *J Physiol Anthropol,* 35**,** 22.

Ebert, T. J. & Cowley, A. W., Jr. 1992. Baroreflex modulation of sympathetic outflow during physiological increases of vasopressin in humans. *Am J Physiol,* 262**,** H1372-8.

Ebert, T. J., Morgan, B. J., Barney, J. A., Denahan, T. & Smith, J. J. 1992. Effects of aging on baroreflex regulation of sympathetic activity in humans. *Am J Physiol,* 263**,** H798-803.

Eckberg, D. L. & Eckberg, M. J. 1982. Human sinus node responses to repetitive, ramped carotid baroreceptor stimuli. *Am J Physiol,* 242**,** H638-44.

Eckel, R. H., Jakicic, J. M., Ard, J. D., De Jesus, J. M., Houston Miller, N., Hubbard, V. S., Lee, I. M., Lichtenstein, A. H., Loria, C. M., Millen, B. E., Nonas, C. A., Sacks, F. M., Smith, S. C., Jr., Svetkey, L. P., Wadden, T. A., Yanovski, S. Z., Kendall, K. A., Morgan, L. C., Trisolini, M. G., Velasco, G., Wnek, J., Anderson, J. L., Halperin, J. L., Albert, N. M., Bozkurt, B., Brindis, R. G., Curtis, L. H., Demets, D., Hochman, J. S., Kovacs, R. J., Ohman, E. M., Pressler, S. J., Sellke, F. W., Shen, W. K., Smith, S. C., Jr., Tomaselli, G. F. & American College of Cardiology/American Heart Association Task Force on Practice, G. 2014. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation,* 129**,** S76-99.

Edwards, D. G. & Lang, J. T. 2005. Augmentation index and systolic load are lower in competitive endurance athletes. *Am J Hypertens,* 18**,** 679-83.

Eklund, B. & Kaijser, L. 1976. Effect of regional alpha- and beta-adrenergic blockade on blood flow in the resting forearm during contralateral isometric handgrip. *J Physiol,* 262**,** 39-50.

Eklund, B., Kaijser, L. & Knutsson, E. 1974. Blood flow in resting (contralateral) arm and leg during isometric contraction. *J Physiol,* 240**,** 111-24.

El-Bedawi, K. M. & Hainsworth, R. 1994. Combined head-up tilt and lower body suction: a test of orthostatic tolerance. *Clin Auton Res,* 4**,** 41-7.

Engelland, R. E., Hemingway, H. W., Tomasco, O. G., Olivencia-Yurvati, A. H. & Romero, S. A. 2020. Neural Control of Blood Pressure is Altered Following Isolated Leg Heating in Aged Humans. *Am J Physiol Heart Circ Physiol*.

Esler, M., Hastings, J., Lambert, G., Kaye, D., Jennings, G. & Seals, D. R. 2002. The influence of aging on the human sympathetic nervous system and brain norepinephrine turnover. *Am J Physiol Regul Integr Comp Physiol,* 282**,** R909-16.

Esler, M. D., Turner, A. G., Kaye, D. M., Thompson, J. M., Kingwell, B. A., Morris, M., Lambert, G. W., Jennings, G. L., Cox, H. S. & Seals, D. R. 1995. Aging effects on human sympathetic neuronal function. *Am J Physiol,* 268**,** R278-85.

Fadel, P. J., Stromstad, M., Hansen, J., Sander, M., Horn, K., Ogoh, S., Smith, M. L., Secher, N. H. & Raven, P. B. 2001. Arterial baroreflex control of sympathetic nerve activity during acute hypotension: effect of fitness. *Am J Physiol Heart Circ Physiol,* 280**,** H2524-32.

Fagius, J. & Wallin, B. G. 1993. Long-term variability and reproducibility of resting human muscle nerve sympathetic activity at rest, as reassessed after a decade. *Clin Auton Res,* 3**,** 201-5.

Fairfax, S. T., Holwerda, S. W., Credeur, D. P., Zuidema, M. Y., Medley, J. H., Dyke, P. C., 2nd, Wray, D. W., Davis, M. J. & Fadel, P. J. 2013. The role of alpha-adrenergic receptors in mediating beat-by-beat sympathetic vascular transduction in the forearm of resting man. *J Physiol,* 591**,** 3637-49.

Fisher, J. P. & Paton, J. F. 2012. The sympathetic nervous system and blood pressure in humans: implications for hypertension. *J Hum Hypertens,* 26**,** 463-75.

Fonkoue, I. T. & Carter, J. R. 2015. Sympathetic neural reactivity to mental stress in humans: test-retest reproducibility. *Am J Physiol Regul Integr Comp Physiol,* 309**,** R1380-6.

Fortier, C. & Agharazii, M. 2016. Arterial Stiffness Gradient. *Pulse (Basel),* 3**,** 159-66.

Galiuto, L. 2011. *The EAE textbook of echocardiography,* Oxford, Oxford University Press.

Gardner, B. I., Bingham, S. E., Allen, M. R., Blatter, D. D. & Anderson, J. L. 2009. Cardiac magnetic resonance versus transthoracic echocardiography for the assessment of cardiac volumes and regional function after myocardial infarction: an intrasubject comparison using simultaneous intrasubject recordings. *Cardiovasc Ultrasound,* 7**,** 38.

Gates, P. E., Tanaka, H., Graves, J. & Seals, D. R. 2003. Left ventricular structure and diastolic function with human ageing. Relation to habitual exercise and arterial stiffness. *Eur Heart J,* 24**,** 2213-20.

Gourine, A. V. & Ackland, G. L. 2019. Cardiac Vagus and Exercise. *Physiology (Bethesda),* 34**,** 71-80.

Grassi, G., Bolla, G., Seravalle, G., Turri, C., Lanfranchi, A. & Mancia, G. 1997. Comparison between reproducibility and sensitivity of muscle sympathetic nerve traffic and plasma noradrenaline in man. *Clin Sci (Lond),* 92**,** 285-9.

Greaney, J. L. & Kenney, W. L. 2017. Measuring and quantifying skin sympathetic nervous system activity in humans. *J Neurophysiol,* 118**,** 2181-2193.

Greaney, J. L., Schwartz, C. E., Edwards, D. G., Fadel, P. J. & Farquhar, W. B. 2013. The neural interaction between the arterial baroreflex and muscle metaboreflex is preserved in older men. *Exp Physiol,* 98**,** 1422-31.

Greaney, J. L., Stanhewicz, A. E., Kenney, W. L. & Alexander, L. M. 2014. Muscle sympathetic nerve activity during cold stress and isometric exercise in healthy older adults. *J Appl Physiol (1985),* 117**,** 648-57.

Greenleaf, J. E., Sciaraffa, D., Shvartz, E., Keil, L. C. & Brock, P. J. 1981. Exercise training hypotension: implications for plasma volume, renin, and vasopressin. *J Appl Physiol Respir Environ Exerc Physiol,* 51**,** 298-305.

Greupner, J., Zimmermann, E., Grohmann, A., Dubel, H. P., Althoff, T. F., Borges, A. C., Rutsch, W., Schlattmann, P., Hamm, B. & Dewey, M. 2012. Head-to-head comparison of left ventricular function assessment with 64-row computed tomography, biplane left cineventriculography, and both 2- and 3-dimensional transthoracic echocardiography: comparison with magnetic resonance imaging as the reference standard. *J Am Coll Cardiol,* 59**,** 1897-907.

Gribbin, B., Pickering, T. G., Sleight, P. & Peto, R. 1971. Effect of age and high blood pressure on baroreflex sensitivity in man. *Circ Res,* 29**,** 424-31.

Guelen, I., Westerhof, B. E., Van Der Sar, G. L., Van Montfrans, G. A., Kiemeneij, F., Wesseling, K. H. & Bos, W. J. 2008. Validation of brachial artery pressure reconstruction from finger arterial pressure. *J Hypertens,* 26**,** 1321-7.

Haas, T. L. & Nwadozi, E. 2015. Regulation of skeletal muscle capillary growth in exercise and disease. *Appl Physiol Nutr Metab,* 40**,** 1221-32.

Hagbarth, K. E. & Macefield, V. G. 1995. The fusimotor system. Its role in fatigue. *Adv Exp Med Biol,* 384**,** 259-70.

Hagbarth, K. E. & Vallbo, A. B. 1968. Pulse and respiratory grouping of sympathetic impulses in human muscle-nerves. *Acta Physiol Scand,* 74**,** 96-108.

Hainsworth, R. 2014. Cardiovascular control from cardiac and pulmonary vascular receptors. *Exp Physiol,* 99**,** 312-9.

Halliwill, J. R., Taylor, J. A. & Eckberg, D. L. 1996. Impaired sympathetic vascular regulation in humans after acute dynamic exercise. *J Physiol,* 495 (Pt 1)**,** 279-88.

Harris, R. A., Padilla, J., Hanlon, K. P., Rink, L. D. & Wallace, J. P. 2007. Reproducibility of the flow-mediated dilation response to acute exercise in overweight men. *Ultrasound Med Biol,* 33**,** 1579-85.

Hart, E. C., Charkoudian, N. & Joyner, M. J. 2012a. Chapter 54 - Sex Differences in Autonomic Function. *In:* ROBERTSON, D., BIAGGIONI, I., BURNSTOCK, G., LOW, P. A. & PATON, J. F. R. (eds.) *Primer on the Autonomic Nervous System (Third Edition).* San Diego: Academic Press.

Hart, E. C., Charkoudian, N., Joyner, M. J., Barnes, J. N., Curry, T. B. & Casey, D. P. 2013. Relationship between sympathetic nerve activity and aortic wave reflection characteristics in postmenopausal women. *Menopause,* 20**,** 967-72.

Hart, E. C., Charkoudian, N., Wallin, B. G., Curry, T. B., Eisenach, J. H. & Joyner, M. J. 2009a. Sex differences in sympathetic neural-hemodynamic balance: implications for human blood pressure regulation. *Hypertension,* 53**,** 571-6.

Hart, E. C., Joyner, M. J., Wallin, B. G. & Charkoudian, N. 2012b. Sex, ageing and resting blood pressure: gaining insights from the integrated balance of neural and haemodynamic factors. *J Physiol,* 590**,** 2069-79.

Hart, E. C., Joyner, M. J., Wallin, B. G., Johnson, C. P., Curry, T. B., Eisenach, J. H. & Charkoudian, N. 2009b. Age-related differences in the sympathetic-hemodynamic balance in men. *Hypertension,* 54**,** 127-33.

Hart, E. C., Joyner, M. J., Wallin, B. G., Karlsson, T., Curry, T. B. & Charkoudian, N. 2010. Baroreflex control of muscle sympathetic nerve activity: a nonpharmacological measure of baroreflex sensitivity. *Am J Physiol Heart Circ Physiol,* 298**,** H816-22.

Hart, E. C., Wallin, B. G., Barnes, J. N., Joyner, M. J. & Charkoudian, N. 2014. Sympathetic nerve activity and peripheral vasodilator capacity in young and older men. *Am J Physiol Heart Circ Physiol,* 306**,** H904-9.

Hart, E. C., Wallin, B. G., Curry, T. B., Joyner, M. J., Karlsson, T. & Charkoudian, N. 2011. Hysteresis in the sympathetic baroreflex: role of baseline nerve activity. *J Physiol,* 589**,** 3395-404.

Hearon, C. M., Jr., Richards, J. C., Racine, M. L., Luckasen, G. J., Larson, D. G. & Dinenno, F. A. 2019. Amplification of endothelium-dependent vasodilatation in contracting human skeletal muscle: role of KIR channels. *J Physiol,* 597**,** 1321-1335.

Hearon, C. M., Jr., Richards, J. C., Racine, M. L., Luckasen, G. J., Larson, D. G. & Dinenno, F. A. 2020. Augmentation of endothelium-dependent vasodilatory signalling improves functional sympatholysis in contracting muscle of older adults. *J Physiol,* 598**,** 2323-2336.

Hearon, C. M., Jr., Richards, J. C., Racine, M. L., Luckasen, G. J., Larson, D. G., Joyner, M. J. & Dinenno, F. A. 2017. Sympatholytic effect of intravascular ATP is independent of nitric oxide, prostaglandins, Na(+) /K(+) -ATPase and KIR channels in humans. *J Physiol,* 595**,** 5175-5190.

Hellsten, Y. & Nyberg, M. 2015. Cardiovascular Adaptations to Exercise Training. *Compr Physiol,* 6**,** 1-32.

Herbert, A., Cruickshank, J. K., Laurent, S., Boutouyrie, P. & Reference Values for Arterial Measurements, C. 2014. Establishing reference values for central blood pressure and its amplification in a general healthy population and according to cardiovascular risk factors. *Eur Heart J,* 35**,** 3122-33.

Herring, N. & Paterson, D. J. 2018. *Levick's Introduction to Cardiovascular Physiology, Sixth Edition,* Florida, Taylor & Francis Group, LLC.

Hickson, S. S., Nichols, W. W., Yasmin, Mcdonnell, B. J., Cockcroft, J. R., Wilkinson, I. B. & Mceniery, C. M. 2016. Influence of the central-to-peripheral arterial stiffness gradient on the timing and amplitude of wave reflections. *Hypertens Res,* 39**,** 723-729.

Hissen, S. L. 2019. *Interactions between sympathetic baroreflex sensitivity and vascular transduction in males and females.* Doctor of Philosophy, Western Sydney University.

Hissen, S. L., Macefield, V. G., Brown, R. & Taylor, C. E. 2019. Sympathetic baroreflex sensitivity is inversely related to vascular transduction in men but not women. *Am J Physiol Heart Circ Physiol,* 317**,** H1203-H1209.

Hogan, N., Kardos, A., Paterson, D. J. & Casadei, B. 1999. Effect of exogenous nitric oxide on baroreflex function in humans. *Am J Physiol,* 277**,** H221-7.

Hoier, B., Nordsborg, N., Andersen, S., Jensen, L., Nybo, L., Bangsbo, J. & Hellsten, Y. 2012. Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. *J Physiol,* 590**,** 595-606.

Hughes, A. D., Park, C., Davies, J., Francis, D., Mc, G. T. S. A., Mayet, J. & Parker, K. H. 2013. Limitations of augmentation index in the assessment of wave reflection in normotensive healthy individuals. *PLoS One,* 8**,** e59371.

Hunt, B. E., Farquhar, W. B. & Taylor, J. A. 2001. Does reduced vascular stiffening fully explain preserved cardiovagal baroreflex function in older, physically active men? *Circulation,* 103**,** 2424-7.

Huybrechts, S. A., Devos, D. G., Vermeersch, S. J., Mahieu, D., Achten, E., De Backer, T. L., Segers, P. & Van Bortel, L. M. 2011. Carotid to femoral pulse wave velocity: a comparison of real travelled aortic path lengths determined by MRI and superficial measurements. *J Hypertens,* 29**,** 1577-82.

Ichinose, M., Saito, M., Kondo, N. & Nishiyasu, T. 2006. Time-dependent modulation of arterial baroreflex control of muscle sympathetic nerve activity during isometric exercise in humans. *Am J Physiol Heart Circ Physiol,* 290**,** H1419-26.

Ichinose, M., Saito, M., Wada, H., Kitano, A., Kondo, N. & Nishiyasu, T. 2004. Modulation of arterial baroreflex control of muscle sympathetic nerve activity by muscle metaboreflex in humans. *Am J Physiol Heart Circ Physiol,* 286**,** H701-7.

Iwase, S., Mano, T., Watanabe, T., Saito, M. & Kobayashi, F. 1991. Age-related changes of sympathetic outflow to muscles in humans. *J Gerontol,* 46**,** M1-5.

Jacobsen, T. N., Hansen, J., Nielsen, H. V., Wildschiodtz, G., Kassis, E., Larsen, B. & Amtorp, O. 1994. Skeletal muscle vascular responses in human limbs to isometric handgrip. *Eur J Appl Physiol Occup Physiol,* 69**,** 147-53.

Jarvis, S. S., Vangundy, T. B., Galbreath, M. M., Shibata, S., Okazaki, K., Reelick, M. F., Levine, B. D. & Fu, Q. 2011. Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans. *Am J Physiol Regul Integr Comp Physiol,* 301**,** R193-200.

Jones, P. P., Davy, K. P., Alexander, S. & Seals, D. R. 1997. Age-related increase in muscle sympathetic nerve activity is associated with abdominal adiposity. *Am J Physiol,* 272**,** E976-80.

Jones, P. P., Shapiro, L. F., Keisling, G. A., Jordan, J., Shannon, J. R., Quaife, R. A. & Seals, D. R. 2001. Altered autonomic support of arterial blood pressure with age in healthy men. *Circulation,* 104**,** 2424-9.

Jones, P. P., Shapiro, L. F., Keisling, G. A., Quaife, R. A. & Seals, D. R. 2002. Is autonomic support of arterial blood pressure related to habitual exercise status in healthy men? *J Physiol,* 540**,** 701-6.

Jose, A. D. & Collison, D. 1970. The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc Res,* 4**,** 160-7.

Joyce, W., White, D. W., Raven, P. B. & Wang, T. 2019. Weighing the evidence for using vascular conductance, not resistance, in comparative cardiovascular physiology. *J Exp Biol,* 222.

Joyner, M. J., Barnes, J. N., Hart, E. C., Wallin, B. G. & Charkoudian, N. 2015. Neural control of the circulation: how sex and age differences interact in humans. *Compr Physiol,* 5**,** 193-215.

Karamanoglu, M., O'rourke, M. F., Avolio, A. P. & Kelly, R. P. 1993. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J,* 14**,** 160-7.

Katona, P. G., Mclean, M., Dighton, D. H. & Guz, A. 1982. Sympathetic and parasympathetic cardiac control in athletes and nonathletes at rest. *J Appl Physiol Respir Environ Exerc Physiol,* 52**,** 1652-7.

Kaufmann, H., Norcliffe-Kaufmann, L. & Palma, J. A. 2020. Baroreflex Dysfunction. *N Engl J Med,* 382**,** 163-178.

Keller, D. M., Cui, J., Davis, S. L., Low, D. A. & Crandall, C. G. 2006. Heat stress enhances arterial baroreflex control of muscle sympathetic nerve activity via increased sensitivity of burst gating, not burst area, in humans. *J Physiol,* 573**,** 445-51.

Kienbaum, P., Karlssonn, T., Sverrisdottir, Y. B., Elam, M. & Wallin, B. G. 2001. Two sites for modulation of human sympathetic activity by arterial baroreceptors? *J Physiol,* 531**,** 861-9.

Kienbaum, P. & Peters, J. 2004. Muscle sympathetic baroreflex sensitivity is different at rest and during evoked hypotension. *Basic Res Cardiol,* 99**,** 152-8.

Kimmerly, D. S., O'leary, D. D., Menon, R. S., Gati, J. S. & Shoemaker, J. K. 2005. Cortical regions associated with autonomic cardiovascular regulation during lower body negative pressure in humans. *J Physiol,* 569**,** 331-45.

Kimmerly, D. S., O'leary, D. D. & Shoemaker, J. K. 2004. Test-retest repeatability of muscle sympathetic nerve activity: influence of data analysis and head-up tilt. *Auton Neurosci,* 114**,** 61-71.

Kiviniemi, A. M., Frances, M. F., Rachinsky, M., Craen, R., Petrella, R. J., Huikuri, H. V., Tulppo, M. P. & Shoemaker, J. K. 2012. Non-alpha-adrenergic effects on systemic vascular conductance during lower-body negative pressure, static exercise and muscle metaboreflex activation. *Acta Physiol (Oxf),* 206**,** 51-61.

Knez, W. L., Sharman, J. E., Jenkins, D. G. & Coombes, J. S. 2008. Central hemodynamics in ultra-endurance athletes. *J Sci Med Sport,* 11**,** 390-5.

Kollias, A., Ntineri, A., Stambolliu, E., Papadatos, S., Vazeou, A. & Stergiou, G. S. 2018. 24-hour ambulatory central blood pressure variability and target-organ damage in adolescents and young adults. *Journal of Hypertension,* 36**,** e155-e156.

Kramer, H. H., Ament, S. J., Breimhorst, M., Klega, A., Schmieg, K., Endres, C., Buchholz, H. G., Elam, M., Schreckenberger, M. & Birklein, F. 2014. Central correlation of muscle sympathetic nerve activation during baroreflex unloading - a microneurography-positron emission tomography study. *Eur J Neurosci,* 39**,** 623-9.

La Rovere, M. T., Pinna, G. D., Maestri, R. & Sleight, P. 2013. Clinical value of baroreflex sensitivity. *Neth Heart J,* 21**,** 61-3.

Lacolley, P., Regnault, V., Segers, P. & Laurent, S. 2017. Vascular Smooth Muscle Cells and Arterial Stiffening: Relevance in Development, Aging, and Disease. *Physiol Rev,* 97**,** 1555-1617.

Laitinen, T., Hartikainen, J., Vanninen, E., Niskanen, L., Geelen, G. & Lansimies, E. 1998. Age and gender dependency of baroreflex sensitivity in healthy subjects. *J Appl Physiol (1985),* 84**,** 576-83.

Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W. & Voigt, J. U. 2015. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr,* 28**,** 1-39 e14.

Laughlin, M. H., Davis, M. J., Secher, N. H., Van Lieshout, J. J., Arce-Esquivel, A. A., Simmons, G. H., Bender, S. B., Padilla, J., Bache, R. J., Merkus, D. & Duncker, D. J. 2012. Peripheral circulation. *Compr Physiol,* 2**,** 321-447.

Lautt, W. W. 1989. Resistance or conductance for expression of arterial vascular tone. *Microvasc Res,* 37**,** 230-6.

Lavie, C. J., Ozemek, C., Carbone, S., Katzmarzyk, P. T. & Blair, S. N. 2019. Sedentary Behavior, Exercise, and Cardiovascular Health. *Circ Res,* 124**,** 799-815.

Lazarus, N. R. & Harridge, S. D. 2010. Exercise, physiological function, and the selection of participants for aging research. *J Gerontol A Biol Sci Med Sci,* 65**,** 854-7.

Levine, B. D., Buckey, J. C., Fritsch, J. M., Yancy, C. W., Jr., Watenpaugh, D. E., Snell, P. G., Lane, L. D., Eckberg, D. L. & Blomqvist, C. G. 1991a. Physical fitness and cardiovascular regulation: mechanisms of orthostatic intolerance. *J Appl Physiol (1985),* 70**,** 112-22.

Levine, B. D., Lane, L. D., Buckey, J. C., Friedman, D. B. & Blomqvist, C. G. 1991b. Left ventricular pressure-volume and Frank-Starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance. *Circulation,* 84**,** 1016-23.

Limberg, J. K., Casey, D. P., Trinity, J. D., Nicholson, W. T., Wray, D. W., Tschakovsky, M. E., Green, D. J., Hellsten, Y., Fadel, P. J., Joyner, M. J. & Padilla, J. 2020. Assessment of resistance vessel function in human skeletal muscle: guidelines for experimental design, Doppler ultrasound, and pharmacology. *Am J Physiol Heart Circ Physiol,* 318**,** H301-H325.

Macefield, V. G. 2013. Chapter 28 - Sympathetic microneurography. *In:* BUIJS, R. M. & SWAAB, D. F. (eds.) *Handbook of Clinical Neurology.* Elsevier.

Macefield, V. G. & Henderson, L. A. 2019. Identifying Increases in Activity of the Human RVLM Through MSNA-Coupled fMRI. *Front Neurosci,* 13**,** 1369.

Maessen, M. F., Eijsvogels, T. M., Stevens, G., Van Dijk, A. P. & Hopman, M. T. 2017. Benefits of lifelong exercise training on left ventricular function after myocardial infarction. *Eur J Prev Cardiol,* 24**,** 1856-1866.

Marwick, T. H., Neubauer, S. & Petersen, S. E. 2013. Use of cardiac magnetic resonance and echocardiography in population-based studies: why, where, and when? *Circ Cardiovasc Imaging,* 6**,** 590-6.

Matsukawa, T., Sugiyama, Y., Iwase, S. & Mano, T. 1994. Effects of aging on the arterial baroreflex control of muscle sympathetic nerve activity in healthy subjects. *Environ Med,* 38**,** 81-4.

Matsukawa, T., Sugiyama, Y. & Mano, T. 1996. Age-related changes in baroreflex control of heart rate and sympathetic nerve activity in healthy humans. *J Auton Nerv Syst,* 60**,** 209-12.

Matsukawa, T., Sugiyama, Y., Watanabe, T., Kobayashi, F. & Mano, T. 1998a. Baroreflex control of muscle sympathetic nerve activity is attenuated in the elderly. *J Auton Nerv Syst,* 73**,** 182-5.

Matsukawa, T., Sugiyama, Y., Watanabe, T., Kobayashi, F. & Mano, T. 1998b. Gender difference in age-related changes in muscle sympathetic nerve activity in healthy subjects. *Am J Physiol,* 275**,** R1600-4.

Matthews, E. L., Brian, M. S., Edwards, D. G., Stocker, S. D., Wenner, M. M. & Farquhar, W. B. 2017. Blood pressure responses to dietary sodium: Association with autonomic cardiovascular function in normotensive adults. *Auton Neurosci,* 208**,** 51-56.

Mcallen, R. M. & Malpas, S. C. 1997. Sympathetic burst activity: characteristics and significance. *Clin Exp Pharmacol Physiol,* 24**,** 791-9.

Mcdicken, W. N. & Anderson, T. 2011. CHAPTER 1 - Basic physics of medical ultrasound. *In:* ALLAN, P. L., BAXTER, G. M. & WESTON, M. J. (eds.) *Clinical Ultrasound (Third Edition).* Edinburgh: Churchill Livingstone.

Mcdonnell, B. J., Maki-Petaja, K. M., Munnery, M., Yasmin, Wilkinson, I. B., Cockcroft, J. R. & Mceniery, C. M. 2013. Habitual exercise and blood pressure: age dependency and underlying mechanisms. *Am J Hypertens,* 26**,** 334-41.

Mceniery, C. M., Yasmin, Hall, I. R., Qasem, A., Wilkinson, I. B., Cockcroft, J. R. & Investigators, A. 2005. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol,* 46**,** 1753-60.

Mceniery, C. M., Yasmin, Mcdonnell, B., Munnery, M., Wallace, S. M., Rowe, C. V., Cockcroft, J. R., Wilkinson, I. B. & Anglo-Cardiff Collaborative Trial, I. 2008. Central pressure: variability and impact of cardiovascular risk factors: the Anglo-Cardiff Collaborative Trial II. *Hypertension,* 51**,** 1476-82.

Meah, V. L., Busch, S. A., Jones, K. E., Davenport, M. H. & Steinback, C. D. 2019. A review of acute responses, after-effects and chronic complications related to microneurography. *Clinical Neurophysiology*.

Meah, V. L., Cockcroft, J. R., Backx, K., Shave, R. & Stohr, E. J. 2016. Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. *Heart,* 102**,** 518-26.

Mitchell, G. F., Parise, H., Benjamin, E. J., Larson, M. G., Keyes, M. J., Vita, J. A., Vasan, R. S. & Levy, D. 2004. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension,* 43**,** 1239-45.

Mitchell, J. H., Kaufman, M. P. & Iwamoto, G. A. 1983. The exercise pressor reflex: its cardiovascular effects, afferent mechanisms, and central pathways. *Annu Rev Physiol,* 45**,** 229-42.

Monahan, K. D., Dinenno, F. A., Seals, D. R., Clevenger, C. M., Desouza, C. A. & Tanaka, H. 2001a. Age-associated changes in cardiovagal baroreflex sensitivity are related to central arterial compliance. *Am J Physiol Heart Circ Physiol,* 281**,** H284-9.

Monahan, K. D., Dinenno, F. A., Seals, D. R. & Halliwill, J. R. 2001b. Smaller age-associated reductions in leg venous compliance in endurance exercise-trained men. *Am J Physiol Heart Circ Physiol,* 281**,** H1267-73.

Monahan, K. D., Dinenno, F. A., Tanaka, H., Clevenger, C. M., Desouza, C. A. & Seals, D. R. 2000. Regular aerobic exercise modulates age-associated declines in cardiovagal baroreflex sensitivity in healthy men. *J Physiol,* 529 Pt 1**,** 263-71.

Monahan, K. D., Eskurza, I. & Seals, D. R. 2004. Ascorbic acid increases cardiovagal baroreflex sensitivity in healthy older men. *Am J Physiol Heart Circ Physiol,* 286**,** H2113-7.

Monahan, K. D., Feehan, R. P., Sinoway, L. I. & Gao, Z. 2013. Contribution of sympathetic activation to coronary vasodilatation during the cold pressor test in healthy men: effect of ageing. *J Physiol,* 591**,** 2937-47.

Monahan, K. D., Tanaka, H., Dinenno, F. A. & Seals, D. R. 2001c. Central arterial compliance is associated with age- and habitual exercise-related differences in cardiovagal baroreflex sensitivity. *Circulation,* 104**,** 1627-32.

Mortensen, S. P., Nyberg, M., Gliemann, L., Thaning, P., Saltin, B. & Hellsten, Y. 2014. Exercise training modulates functional sympatholysis and alpha-adrenergic vasoconstrictor responsiveness in hypertensive and normotensive individuals. *J Physiol,* 592**,** 3063-73.

Mueller, P. J., Clifford, P. S., Crandall, C. G., Smith, S. A. & Fadel, P. J. 2017. Integration of Central and Peripheral Regulation of the Circulation during Exercise: Acute and Chronic Adaptations. *Compr Physiol,* 8**,** 103-151.

Namasivayam, M., Adji, A. & O'rourke, M. F. 2010. Aortic augmentation index and aging: mathematical resolution of a physiological dilemma? *Hypertension,* 56**,** e9-10.

Narkiewicz, K., Phillips, B. G., Kato, M., Hering, D., Bieniaszewski, L. & Somers, V. K. 2005. Gender-selective interaction between aging, blood pressure, and sympathetic nerve activity. *Hypertension,* 45**,** 522-5.

Ng, A. V., Callister, R., Johnson, D. G. & Seals, D. R. 1993. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. *Hypertension,* 21**,** 498-503.

Ng, A. V., Callister, R., Johnson, D. G. & Seals, D. R. 1994a. Endurance exercise training is associated with elevated basal sympathetic nerve activity in healthy older humans. *J Appl Physiol (1985),* 77**,** 1366-74.

Ng, A. V., Callister, R., Johnson, D. G. & Seals, D. R. 1994b. Sympathetic neural reactivity to stress does not increase with age in healthy humans. *Am J Physiol,* 267**,** H344-53.

Nichols, W. W., Denardo, S. J., Wilkinson, I. B., Mceniery, C. M., Cockcroft, J. & O'rourke, M. F. 2008. Effects of arterial stiffness, pulse wave velocity, and wave reflections on the central aortic pressure waveform. *J Clin Hypertens (Greenwich),* 10**,** 295-303.

Nichols, W. W., Nichols, W. W. M. S. B. F. I. A. & Mcdonald, D. a. B. F. I. A. 2011. *McDonald's blood flow in arteries : theoretical, experimental and clinical principles,* London, Hodder Arnold.

Ninomiya, I., Malpas, S. C., Matsukawa, K., Shindo, T. & Akiyama, T. 1993. The amplitude of synchronized cardiac sympathetic nerve activity reflects the number of activated pre- and postganglionic fibers in anesthetized cats. *J Auton Nerv Syst,* 45**,** 139-47.

Notarius, C. F., Murai, H., Morris, B. L. & Floras, J. S. 2012. Effect of fitness on reflex sympathetic neurovascular transduction in middle-age men. *Med Sci Sports Exerc,* 44**,** 232-7.

Notay, K., Incognito, A. V. & Millar, P. J. 2017. Acute beetroot juice supplementation on sympathetic nerve activity: a randomized, double-blind, placebo-controlled proof-of-concept study. *Am J Physiol Heart Circ Physiol,* 313**,** H59-H65.

Notay, K., Seed, J. D., Incognito, A. V., Doherty, C. J., Nardone, M., Burns, M. J. & Millar, P. J. 2016. Validity and reliability of measuring resting muscle sympathetic nerve activity using short sampling durations in healthy humans. *J Appl Physiol (1985),* 121**,** 1065-1073.

O'leary, D. S. 1991. Regional vascular resistance vs. conductance: which index for baroreflex responses? *Am J Physiol,* 260**,** H632-7.

O'leary, D. S. 1996. Heart rate control during exercise by baroreceptors and skeletal muscle afferents. *Med Sci Sports Exerc,* 28**,** 210-7.

Ogoh, S., Fadel, P. J., Nissen, P., Jans, O., Selmer, C., Secher, N. H. & Raven, P. B. 2003a. Baroreflex-mediated changes in cardiac output and vascular conductance in response to alterations in carotid sinus pressure during exercise in humans. *J Physiol,* 550**,** 317-24.

Ogoh, S., Volianitis, S., Nissen, P., Wray, D. W., Secher, N. H. & Raven, P. B. 2003b. Carotid baroreflex responsiveness to head-up tilt-induced central hypovolaemia: effect of aerobic fitness. *J Physiol,* 551**,** 601-8.

Opthof, T. 2000. The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc Res,* 45**,** 177-84.

Otsuki, T., Maeda, S., Iemitsu, M., Saito, Y., Tanimura, Y., Ajisaka, R. & Miyauchi, T. 2007a. Relationship between arterial stiffness and athletic training programs in young adult men. *Am J Hypertens,* 20**,** 967-73.

Otsuki, T., Maeda, S., Iemitsu, M., Saito, Y., Tanimura, Y., Ajisaka, R. & Miyauchi, T. 2007b. Vascular endothelium-derived factors and arterial stiffness in strength- and endurance-trained men. *Am J Physiol Heart Circ Physiol,* 292**,** H786-91.

Ottaviani, M. M., Wright, L., Dawood, T. & Macefield, V. G. 2020. In-vivo recordings from the human vagus nerve using ultrasound-guided microneurography. *J Physiol*.

Parati, G., Di Rienzo, M., Bertinieri, G., Pomidossi, G., Casadei, R., Groppelli, A., Pedotti, A., Zanchetti, A. & Mancia, G. 1988. Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension,* 12**,** 214-22.

Parati, G., Di Rienzo, M. & Mancia, G. 2000. How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life. *J Hypertens,* 18**,** 7-19.

Parati, G., Frattola, A., Di Rienzo, M., Castiglioni, P., Pedotti, A. & Mancia, G. 1995. Effects of aging on 24-h dynamic baroreceptor control of heart rate in ambulant subjects. *Am J Physiol,* 268**,** H1606-12.

Park, H. S., Naik, S. D., Aronow, W. S., Ahn, C. W., Mcclung, J. A. & Belkin, R. N. 2007. Age- and sex-related differences in the tissue Doppler imaging parameters of left ventricular diastolic dysfunction. *Echocardiography,* 24**,** 567-71.

Pauca, A. L., O'rourke, M. F. & Kon, N. D. 2001. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension,* 38**,** 932-7.

Pawelczyk, J. A. & Levine, B. D. 2002. Heterogeneous responses of human limbs to infused adrenergic agonists: a gravitational effect? *J Appl Physiol (1985),* 92**,** 2105-13.

Penaz, J. Photoelectric measurement of blood pressure, volume and flow in the finger. Digest of the 10th Internation Conference on Medical and Biological Engineering, 1973 Dresden. 104.

Peng, X., Schultz, M. G., Picone, D. S., Black, J. A., Dwyer, N., Roberts-Thomson, P., Davies, J. E. & Sharman, J. E. 2017. Arterial reservoir characteristics and central-to-peripheral blood pressure amplification in the human upper limb. *J Hypertens,* 35**,** 1825-1831.

Perneger, T. V. 1998. What's wrong with Bonferroni adjustments. *BMJ,* 316**,** 1236-8.

Pierce, G. L., Harris, S. A., Seals, D. R., Casey, D. P., Barlow, P. B. & Stauss, H. M. 2016. Estimated aortic stiffness is independently associated with cardiac baroreflex sensitivity in humans: role of ageing and habitual endurance exercise. *J Hum Hypertens,* 30**,** 513-20.

Pinna, G. D., Maestri, R. & La Rovere, M. T. 2015. Assessment of baroreflex sensitivity from spontaneous oscillations of blood pressure and heart rate: proven clinical value? *Physiol Meas,* 36**,** 741-53.

Radjenovic, A. & Roditi, G. 2019. Chapter 7 - Clinical Cardiovascular Imaging. *In:* TOUYZ, R. M. & DELLES, C. (eds.) *Textbook of vascular medicine.* Springer Nature Switzerland AG.

Raven, P. B., Rohm-Young, D. & Blomqvist, C. G. 1984. Physical fitness and cardiovascular response to lower body negative pressure. *J Appl Physiol Respir Environ Exerc Physiol,* 56**,** 138-44.

Ray, C. A. & Monahan, K. D. 2002. Sympathetic vascular transduction is augmented in young normotensive blacks. *J Appl Physiol (1985),* 92**,** 651-6.

Reyes, L. M., Usselman, C. W., Skow, R. J., Charkoudian, N., Staab, J. S., Davenport, M. H. & Steinback, C. D. 2018. Sympathetic neurovascular regulation during pregnancy: A longitudinal case series study. *Exp Physiol,* 103**,** 318-323.

Robinson, A. T., Babcock, M. C., Watso, J. C., Brian, M. S., Migdal, K. U., Wenner, M. M. & Farquhar, W. B. 2019. Relation between resting sympathetic outflow and vasoconstrictor responses to sympathetic nerve bursts: sex differences in healthy young adults. *Am J Physiol Regul Integr Comp Physiol,* 316**,** R463-R471.

Roman, M. J., Devereux, R. B., Kizer, J. R., Lee, E. T., Galloway, J. M., Ali, T., Umans, J. G. & Howard, B. V. 2007. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. *Hypertension,* 50**,** 197-203.

Roman, M. J., Devereux, R. B., Kizer, J. R., Okin, P. M., Lee, E. T., Wang, W., Umans, J. G., Calhoun, D. & Howard, B. V. 2009. High central pulse pressure is independently associated with adverse cardiovascular outcome the strong heart study. *J Am Coll Cardiol,* 54**,** 1730-4.

Rudas, L., Crossman, A. A., Morillo, C. A., Halliwill, J. R., Tahvanainen, K. U., Kuusela, T. A. & Eckberg, D. L. 1999. Human sympathetic and vagal baroreflex responses to sequential nitroprusside and phenylephrine. *Am J Physiol,* 276**,** H1691-8.

Sagawa, K. 1983. Baroreflex Control of Systemic Arterial Pressure and Vascular Bed. *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow.* Bethesda, MD: American Physiological Society.

Scharhag, J., Schneider, G., Urhausen, A., Rochette, V., Kramann, B. & Kindermann, W. 2002. Athlete's heart: right and left ventricular mass and function in male endurance athletes and untrained individuals determined by magnetic resonance imaging. *J Am Coll Cardiol,* 40**,** 1856-63.

Schultz, M. G., Davies, J. E., Hardikar, A., Pitt, S., Moraldo, M., Dhutia, N., Hughes, A. D. & Sharman, J. E. 2014. Aortic reservoir pressure corresponds to cyclic changes in aortic volume: physiological validation in humans. *Arterioscler Thromb Vasc Biol,* 34**,** 1597-603.

Schultz, M. G., Hughes, A. D., Davies, J. E. & Sharman, J. E. 2015. Associations and clinical relevance of aortic-brachial artery stiffness mismatch, aortic reservoir function, and central pressure augmentation. *Am J Physiol Heart Circ Physiol,* 309**,** H1225-33.

Seals, D. R. 1991. Sympathetic neural adjustments to stress in physically trained and untrained humans. *Hypertension,* 17**,** 36-43.

Seals, D. R., Brunt, V. E. & Rossman, M. J. 2018. Keynote lecture: strategies for optimal cardiovascular aging. *Am J Physiol Heart Circ Physiol,* 315**,** H183-H188.

Seals, D. R., Nagy, E. E. & Moreau, K. L. 2019. Aerobic exercise training and vascular function with ageing in healthy men and women. *J Physiol,* 597**,** 4901-4914.

Shantsila, A., Mcintyre, D. B., Lip, G. Y., Fadel, P. J., Paton, J. F., Pickering, A. E. & Fisher, J. P. 2015. Influence of age on respiratory modulation of muscle sympathetic nerve activity, blood pressure and baroreflex function in humans. *Exp Physiol,* 100**,** 1039-51.

Sharman, J. E. & Laurent, S. 2013. Central blood pressure in the management of hypertension: soon reaching the goal? *J Hum Hypertens,* 27**,** 405-11.

Shibata, S., Fujimoto, N., Hastings, J. L., Carrick-Ranson, G., Bhella, P. S., Hearon, C. M., Jr. & Levine, B. D. 2018. The effect of lifelong exercise frequency on arterial stiffness. *J Physiol,* 596**,** 2783-2795.

Shoemaker, J. K., Klassen, S. A., Badrov, M. B. & Fadel, P. J. 2018. Fifty years of microneurography: learning the language of the peripheral sympathetic nervous system in humans. *J Neurophysiol,* 119**,** 1731-1744.

Smith, E. G., Voyles, W. F., Kirby, B. S., Markwald, R. R. & Dinenno, F. A. 2007. Ageing and leg postjunctional alpha-adrenergic vasoconstrictor responsiveness in healthy men. *J Physiol,* 582**,** 63-71.

Smith, M. L., Hudson, D. L., Graitzer, H. M. & Raven, P. B. 1988. Blood pressure regulation during cardiac autonomic blockade: effect of fitness. *J Appl Physiol (1985),* 65**,** 1789-95.

Smulyan, H., Marchais, S. J., Pannier, B., Guerin, A. P., Safar, M. E. & London, G. M. 1998. Influence of body height on pulsatile arterial hemodynamic data. *J Am Coll Cardiol,* 31**,** 1103-9.

Smyth, H. S., Sleight, P. & Pickering, G. W. 1969. Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. *Circ Res,* 24**,** 109-21.

Steinback, C. D., Fraser, G. M., Usselman, C. W., Reyes, L. M., Julian, C. G., Stickland, M. K., Chari, R. S., Khurana, R., Davidge, S. T. & Davenport, M. H. 2019. Blunted sympathetic neurovascular transduction during normotensive pregnancy. *J Physiol,* 597**,** 3687-3696.

Steinback, C. D., Salmanpour, A., Breskovic, T., Dujic, Z. & Shoemaker, J. K. 2010. Sympathetic neural activation: an ordered affair. *J Physiol,* 588**,** 4825-36.

Stevens, G. H., Foresman, B. H., Shi, X., Stern, S. A. & Raven, P. B. 1992. Reduction in LBNP tolerance following prolonged endurance exercise training. *Med Sci Sports Exerc,* 24**,** 1235-44.

Stoner, L., Faulkner, J., Lowe, A., D, M. L., J, M. Y., Love, R. & D, S. R. 2014. Should the augmentation index be normalized to heart rate? *J Atheroscler Thromb,* 21**,** 11-6.

Stuckless, T. J. R., Vermeulen, T. D., Brown, C. V., Boulet, L. M., Shafer, B. M., Wakeham, D. J., Steinback, C. D., Ayas, N. T., Floras, J. S. & Foster, G. E. 2020. Acute intermittent hypercapnic hypoxia and sympathetic neurovascular transduction in men. *J Physiol,* 598**,** 473-487.

Studinger, P., Goldstein, R. & Taylor, J. A. 2009. Age- and fitness-related alterations in vascular sympathetic control. *J Physiol,* 587**,** 2049-57.

Sundlof, G. & Wallin, B. G. 1978. Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J Physiol,* 274**,** 621-37.

Svedenhag, J., Wallin, B. G., Sundlof, G. & Henriksson, J. 1984. Skeletal muscle sympathetic activity at rest in trained and untrained subjects. *Acta Physiol Scand,* 120**,** 499-504.

Talbot, J. S., Lord, R. N., Wakeham, D. J., Dawkins, T. G., Curry, B. A., Brown, M., Lodge, F. M. & Pugh, C. J. A. 2020. The influence of habitual endurance exercise on carotid artery strain and strain rate in young and middle-aged men. *Exp Physiol*.

Tan, A. Y. & Verrier, R. L. 2013. Chapter 12 - The role of the autonomic nervous system in cardiac arrhythmias. *In:* BUIJS, R. M. & SWAAB, D. F. (eds.) *Handbook of Clinical Neurology.* Elsevier.

Tan, C. O., Tamisier, R., Hamner, J. W. & Taylor, J. A. 2013. Characterizing sympathetic neurovascular transduction in humans. *PLoS One,* 8**,** e53769.

Tan, I., Kiat, H., Barin, E., Butlin, M. & Avolio, A. P. 2016a. Effects of pacing modality on noninvasive assessment of heart rate dependency of indices of large artery function. *J Appl Physiol (1985),* 121**,** 771-780.

Tan, I., Spronck, B., Kiat, H., Barin, E., Reesink, K. D., Delhaas, T., Avolio, A. P. & Butlin, M. 2016b. Heart Rate Dependency of Large Artery Stiffness. *Hypertension,* 68**,** 236-42.

Tanaka, H., Desouza, C. A. & Seals, D. R. 1998. Absence of age-related increase in central arterial stiffness in physically active women. *Arterioscler Thromb Vasc Biol,* 18**,** 127-32.

Tanaka, H., Dinenno, F. A., Monahan, K. D., Clevenger, C. M., Desouza, C. A. & Seals, D. R. 2000. Aging, habitual exercise, and dynamic arterial compliance. *Circulation,* 102**,** 1270-5.

Tanaka, H., Tarumi, T. & Rittweger, J. 2019. Aging and Physiological Lessons from Master Athletes. *Compr Physiol,* 10**,** 261-296.

Tarumi, T., Gonzales, M. M., Fallow, B., Nualnim, N., Pyron, M., Tanaka, H. & Haley, A. P. 2013. Central artery stiffness, neuropsychological function, and cerebral perfusion in sedentary and endurance-trained middle-aged adults. *J Hypertens,* 31**,** 2400-9.

Taylor, C. E., Willie, C. K., Ainslie, P. N. & Tzeng, Y. C. 2014. Assessment of human baroreflex function using carotid ultrasonography: what have we learnt? *Acta Physiol (Oxf),* 211**,** 297-313.

Taylor, J. A. & Tan, C. O. 2014. BP regulation VI: elevated sympathetic outflow with human aging: hypertensive or homeostatic? *Eur J Appl Physiol,* 114**,** 511-9.

Terwoord, J. D., Hearon, C. M., Jr., Racine, M. L., Ketelhut, N. B., Luckasen, G. J., Richards, J. C. & Dinenno, F. A. 2020. KIR channel activation links local vasodilatation with muscle fibre recruitment during exercise in humans. *J Physiol*.

Thrasher, T. N. 2004. Baroreceptors and the long-term control of blood pressure. *Exp Physiol,* 89**,** 331-5.

Thrasher, T. N. 2005. Baroreceptors, baroreceptor unloading, and the long-term control of blood pressure. *Am J Physiol Regul Integr Comp Physiol,* 288**,** R819-27.

Torebjork, H. E. & Hallin, R. G. 1974. Identification of afferent C units in intact human skin nerves. *Brain Res,* 67**,** 387-403.

Townsend, R. R., Wilkinson, I. B., Schiffrin, E. L., Avolio, A. P., Chirinos, J. A., Cockcroft, J. R., Heffernan, K. S., Lakatta, E. G., Mceniery, C. M., Mitchell, G. F., Najjar, S. S., Nichols, W. W., Urbina, E. M., Weber, T. & American Heart Association Council On, H. 2015. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. *Hypertension,* 66**,** 698-722.

Usselman, C. W., Wakefield, P. K., Skow, R. J., Stickland, M. K., Chari, R. S., Julian, C. G., Steinback, C. D. & Davenport, M. H. 2015. Regulation of sympathetic nerve activity during the cold pressor test in normotensive pregnant and nonpregnant women. *Hypertension,* 66**,** 858-64.

Vaitkevicius, P. V., Fleg, J. L., Engel, J. H., O'connor, F. C., Wright, J. G., Lakatta, L. E., Yin, F. C. & Lakatta, E. G. 1993. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation,* 88**,** 1456-62.

Vallbo, A. B. & Hagbarth, K. E. 1967. Impulses recorded with micro-electrodes in human muscle nerves during stimulation of mechanoreceptors and voluntary contractions. *Electroencephalogr Clin Neurophysiol,* 23**,** 392.

Vallbo, A. B., Hagbarth, K. E. & Wallin, B. G. 2004. Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol (1985),* 96**,** 1262-9.

Van Bortel, L. M., Laurent, S., Boutouyrie, P., Chowienczyk, P., Cruickshank, J. K., De Backer, T., Filipovsky, J., Huybrechts, S., Mattace-Raso, F. U., Protogerou, A. D., Schillaci, G., Segers, P., Vermeersch, S., Weber, T., Artery, S., European Society of Hypertension Working Group on Vascular, S., Function & European Network for Noninvasive Investigation of Large, A. 2012. Expert consensus document on the

measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens,* 30**,** 445-8.

Van Bortel, L. M., Segers, P. & De Backer, T. 2020. Misconceptions About Arterial Stiffness May Lead to Erroneous Conclusions. *Am J Hypertens,* 33**,** 402-404.

Van Lieshout, J. J. 2003. Exercise training and orthostatic intolerance: a paradox? *J Physiol,* 551**,** 401.

Vianna, L. C., Hart, E. C., Fairfax, S. T., Charkoudian, N., Joyner, M. J. & Fadel, P. J. 2012. Influence of age and sex on the pressor response following a spontaneous burst of muscle sympathetic nerve activity. *Am J Physiol Heart Circ Physiol,* 302**,** H2419-27.

Vlachopoulos, C., Aznaouridis, K., O'rourke, M. F., Safar, M. E., Baou, K. & Stefanadis, C. 2010. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J,* 31**,** 1865-71.

Vranish, J. R., Holwerda, S. W., Young, B. E., Credeur, D. P., Patik, J. C., Barbosa, T. C., Keller, D. M. & Fadel, P. J. 2018. Exaggerated Vasoconstriction to Spontaneous Bursts of Muscle Sympathetic Nerve Activity in Healthy Young Black Men. *Hypertension,* 71**,** 192-198.

Vyas, M., Izzo, J. L., Jr., Lacourciere, Y., Arnold, J. M., Dunlap, M. E., Amato, J. L., Pfeffer, M. A. & Mitchell, G. F. 2007. Augmentation index and central aortic stiffness in middle-aged to elderly individuals. *Am J Hypertens,* 20**,** 642-7.

Wallin, B. G., Esler, M., Dorward, P., Eisenhofer, G., Ferrier, C., Westerman, R. & Jennings, G. 1992. Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J Physiol,* 453**,** 45-58.

Wallin, B. G. & Nerhed, C. 1982. Relationship between spontaneous variations of muscle sympathetic activity and succeeding changes of blood pressure in man. *J Auton Nerv Syst,* 6**,** 293-302.

Wallin, B. G., Victor, R. G. & Mark, A. L. 1989. Sympathetic outflow to resting muscles during static handgrip and postcontraction muscle ischemia. *Am J Physiol,* 256**,** H105-10.

Wang, J. J., O'brien, A. B., Shrive, N. G., Parker, K. H. & Tyberg, J. V. 2003. Time-domain representation of ventricular-arterial coupling as a windkessel and wave system. *Am J Physiol Heart Circ Physiol,* 284**,** H1358-68.

Wang, K. L., Cheng, H. M., Sung, S. H., Chuang, S. Y., Li, C. H., Spurgeon, H. A., Ting, C. T., Najjar, S. S., Lakatta, E. G., Yin, F. C., Chou, P. & Chen, C. H. 2010. Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities: a community-based study. *Hypertension,* 55**,** 799-805.

Wehrwein, E. A. & Joyner, M. J. 2013. Chapter 8 - Regulation of blood pressure by the arterial baroreflex and autonomic nervous system. *In:* BUIJS, R. M. & SWAAB, D. F. (eds.) *Handbook of Clinical Neurology.* Elsevier.

Wehrwein, E. A., Joyner, M. J., Hart, E. C., Wallin, B. G., Karlsson, T. & Charkoudian, N. 2010. Blood pressure regulation in humans: calculation of an "error signal" in control of sympathetic nerve activity. *Hypertension,* 55**,** 264-9.

Wesseling, K. H., Wit, B.D., Hoeven, G.M., Goudoever, J.V., & Settels, J.J. 1995. Physiocal, calibrating finger vascular physiology for finapres. *Homeostasis,* 36**,** 67-82.

White, D. W. & Raven, P. B. 2014. Autonomic neural control of heart rate during dynamic exercise: revisited. *J Physiol,* 592**,** 2491-500.

White, D. W., Shoemaker, J. K. & Raven, P. B. 2015. Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. *Auton Neurosci,* 193**,** 12-21.

Wilkinson, I. B., Maccallum, H., Flint, L., Cockcroft, J. R., Newby, D. E. & Webb, D. J. 2000. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol,* 525 Pt 1**,** 263-70.

Wilkinson, I. B., Maccallum, H., Hupperetz, P. C., Van Thoor, C. J., Cockcroft, J. R. & Webb, D. J. 2001. Changes in the derived central pressure waveform and pulse pressure in response to angiotensin II and noradrenaline in man. *J Physiol,* 530**,** 541-50.

Wilkinson, I. B., Mohammad, N. H., Tyrrell, S., Hall, I. R., Webb, D. J., Paul, V. E., Levy, T. & Cockcroft, J. R. 2002. Heart rate dependency of pulse pressure amplification and arterial stiffness. *Am J Hypertens,* 15**,** 24-30.

Williamson, J. W. 2010. The relevance of central command for the neural cardiovascular control of exercise. *Exp Physiol,* 95**,** 1043-8.

Wilson, M., O'hanlon, R., Prasad, S., Deighan, A., Macmillan, P., Oxborough, D., Godfrey, R., Smith, G., Maceira, A., Sharma, S., George, K. & Whyte, G. 2011. Diverse patterns of myocardial fibrosis in lifelong, veteran endurance athletes. *J Appl Physiol (1985),* 110**,** 1622-6.

Woodman, R. J., Playford, D. A., Watts, G. F., Cheetham, C., Reed, C., Taylor, R. R., Puddey, I. B., Beilin, L. J., Burke, V., Mori, T. A. & Green, D. 2001. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol (1985),* 91**,** 929-37.

Zeng, W. Z., Marshall, K. L., Min, S., Daou, I., Chapleau, M. W., Abboud, F. M., Liberles, S. D. & Patapoutian, A. 2018. PIEZOs mediate neuronal sensing of blood pressure and the baroreceptor reflex. *Science,* 362**,** 464-467.

Appendices

Appendix I – Ethical Approval

CARDIFF METROPOLITAN UNIVERSITY APPLICATION FOR ETHICS APPROVAL

When undertaking a research or enterprise project, Cardiff Met staff and students are obliged to complete this form in order that the ethics implications of that project may be considered.

If the project requires ethics approval from an external agency (e,g., NHS), you will not need to seek additional ethics approval from Cardiff Met. You should however complete Part One of this form and attach a copy of your ethics letter(s) of approval in order that your School has a record of the project.

The document ***Guidelines for obtaining ethics approval*** will help you complete this form. It is available from the Cardiff Met website. The School or Unit in which you are based may also have produced some guidance documents, please consult your supervisor or School Ethics Coordinator.

Once you have completed the form, sign the declaration and forward to the appropriate person(s) in your School or Unit.

PLEASE NOTE:
Participant recruitment or data collection MUST NOT commence until ethics approval has been obtained.

PART ONE

Name of applicant:	Denis Wakeham
Supervisor (if student project):	Dr Chris Pugh
School / Unit:	Cardiff School of Sport
Student number (if applicable):	St20093328
Programme enrolled on (if applicable):	MPhil/PhD in Cardiovascular Physiology and Health
Project Title:	The effect of age and fitness status on sympathetic regulation of vascular function
Expected start date of data collection:	27/06/2016
Approximate duration of data collection:	18 months
Funding Body (if applicable):	N/A
Other researcher(s) working on the project:	Dr. Rachel Lord[1], Dr. Jonathan Moore[2], Prof. Rob Shave[1], Dr. Chris Pugh[1]. [1]Cardiff School of Sport, Cardiff Metropolitan University. [2]School of Sport, Health & Exercise Sciences, Bangor University.
Will the study involve NHS patients or staff?	No
Will the study involve taking samples of human origin from participants?	Yes

Does your project fall entirely within one of the following categories:	
Paper based, involving only documents in the public domain	No

Laboratory based, not involving human participants or human tissue samples	No
Practice based not involving human participants (eg curatorial, practice audit)	No
Compulsory projects in professional practice (eg Initial Teacher Education)	No
A project for which external approval has been obtained (e.g., NHS)	No
If you have answered YES to any of these questions, expand on your answer in the non-technical summary. No further information regarding your project is required. If you have answered NO to all of these questions, you must complete Part 2 of this form	

In no more than 150 words, give a non-technical summary of the project
The autonomic nervous system (ANS) is responsible for regulating the body's involuntary actions and plays an essential role in the regulation of the cardiovascular system (the heart and blood vessels). An important pathway within the ANS is the sympathetic nervous system. Generally, when sympathetic nervous activity (SNA) increases, blood pressure and heart rate increase. As we get older, SNA increases and the function of the cardiovascular system deteriorates. It has been suggested that these changes contribute to the age-related increase in blood pressure. Exercise training is known to reduce the effects of ageing on the cardiovascular system and some evidence suggests that exercise training can reduce SNA and BP. However, it is currently unknown whether fitness status can influence the age-related changes to the cardiovascular system and SNA. Therefore, we aim to investigate the influence of fitness status on the cardiovascular system and SNA in young and old individuals.

DECLARATION: **I confirm that this project conforms with the Cardiff Met Research Governance Framework**	
Signature of the applicant: Denis Wakeham	Date: 11.05.16
FOR STUDENT PROJECTS ONLY	
Name of supervisor: Dr. Chris Pugh	Date: 11.05.16
Signature of supervisor: 	

CARDIFF METROPOLITAN UNIVERSITY APPLICATION FOR ETHICS APPROVAL

Research Ethics Committee use only	
Decision reached:	Project approved ☐ Project approved in principle ☑ Decision deferred ☐ Project not approved ☐ Project rejected ☐
Project reference number: 16/7/02R	
Name: Dr. Brendan Cropley	Date: 06/07/2016
Signature: 	
Details of any conditions upon which approval is dependant: 1. The project is approved pending confirmation of compliance with the HTA framework.	

Appendix II – Participant Information Sheet and Consent Form

Cardiff Metropolitan University | Prifysgol Metropolitan Caerdydd

The effect of age and fitness status on sympathetic regulation of vascular function

Participant Information Sheet

Principal investigator: **Mr Denis Wakeham**
Co-investigators: Dr Rachel Lord, Dr Jonathan Moore, Professor Rob Shave & Dr Chris Pugh

This document provides information on:

1. The background and aim of the research project
2. Your role as a participant
3. The role of the researchers
4. Benefits of taking part
5. What does the study involve and how will data be collected?
6. Risks
7. How the results will be used
8. Your rights

IMPORTANT: The purpose of this document is to assist you in making an informed decision about whether you wish to volunteer for this research project. Your right as a voluntary participant is that you are **free to enter or withdraw from the study at any time**. After you have read this document thoroughly, you are encouraged to ask any questions you may have.

1. Background and aims of the research project

- The autonomic nervous system is responsible for regulating the body's involuntary actions and plays an essential role in the regulation of the cardiovascular system (the heart and blood vessels).
- An important pathway within the autonomic nervous system is the sympathetic nervous system. Generally, when sympathetic nerve activity increases, blood pressure and heart rate increase.
- As we get older, resting sympathetic nerve activity increases and the function of the cardiovascular system deteriorates because our heart and blood vessels become stiffer.
- Exercise training is known to reduce the effects of ageing on the heart and blood vessels and some evidence suggests that exercise training can reduce sympathetic nerve activity.
- It is currently unknown whether fitness status (high fit vs. low fit) can influence the age-related changes to the cardiovascular system and sympathetic nerve activity.
- **Therefore, the aim of this study is to investigate** the influence of fitness status on the cardiovascular system and sympathetic nerve activity in different age groups.

Cardiff School of Sport
Cyncoed Campus, Cyncoed
Road, Cardiff, CF23 6XD UK
Tel:
Fax:
Email:
www.cardiffmet.ac.uk

PARTICIPANT CONSENT FORM

Reference Number:

Participant name or Study ID Number:

Title of Project: The effect of age and fitness status on sympathetic regulation of vascular function

Name of Researchers: Mr Denis Wakeham, Dr Rachel Lord, Dr Jonathan Moore, Professor Rob Shave and Dr Chris Pugh

---

Participant to complete this section: **Please initial each box**

1. I confirm that I have read the information sheet and that I understand everything the study entails. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason. ☐

3. I am happy for my data to be used for publication in scientific journals. ☐

4. I am happy to be contacted again in the future for subsequent studies. ☐

5. I agree to take part in the above study. ☐

_____
Name of Participant

_____ _____
Signature of Participant Date

_____
Name of person taking consent

_____ _____
Signature of person taking consent Date

Appendix III – Contributions to data collection.

Table 11 – Experimenter contribution to collection of primary outcomes.

Experimenter	Techniques
Visit 1	
Denis J. Wakeham	Manual Sphygmomanometry to determine arterial blood pressure; Indirect calorimetry to determine VO2 Max.
Tony G. Dawkins	Manual Sphygmomanometry to determine arterial blood pressure; Applanation tonometry to determine aortic blood pressure and aortic and brachial pulse wave velocity (i.e. stiffness).
Visit 2	
Denis J. Wakeham	Manual Sphygmomanometry to determine arterial blood pressure; Finger Photoplethysmography to record continuous arterial blood pressure; Capnography to record respiratory rate.
Jonathan P. Moore	Microneurography to record muscle sympathetic nerve activity.
Rachel N. Lord	Echocardiography to determine left ventricular stroke volume.
Christopher J. A. Pugh	Vascular Ultrasonography to determine brachial artery diameter, blood flow velocity and forearm blood flow.

Appendix IV – Published manuscripts from this thesis

Chapter 5 (Study 2) was published in the May issue of the American Journal of Physiology – Heart and Circulatory Physiology in 2019:

Wakeham, D. J., Lord, R. N., Talbot, J. S., Lodge, F. M., Curry, B.A., Dawkins, T. G., Simpson, L. L. S., Shave, R., Pugh, C. J. A. & Moore, J. P. (2019). Upward resetting of the vascular sympathetic baroreflex in middle-aged runners. *American Journal of Physiology – Heart & Circulatory Physiology* 317, H181-H189. PMID: 31050557

Am J Physiol Heart Circ Physiol 317: H181–H189, 2019.
First published May 3, 2019; doi:10.1152/ajpheart.00106.2019.

RESEARCH ARTICLE | *Integrative Cardiovascular Physiology and Pathophysiology*

Upward resetting of the vascular sympathetic baroreflex in middle-aged male runners

Denis J. Wakeham,[1] Rachel N. Lord,[1] Jack S. Talbot,[1] Freya M. Lodge,[2] Bryony A. Curry,[1] Tony G. Dawkins,[1] Lydia L. Simpson,[3] Rob E. Shave,[1,4] Christopher J. A. Pugh,[1]* and Jonathan P. Moore[3]*

[1]*Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, United Kingdom;* [2]*Cardiff and Vale University Health Board, University Hospital of Wales, Cardiff, United Kingdom;* [3]*Physical Activity for Health and Well-Being Centre, School of Sport, Health and Exercise Sciences, Bangor University, Bangor, United Kingdom; and* [4]*Centre for Heart, Lung, and Vascular Health, University of British Columbia Okanagan, Kelowna, BC, Canada*

Submitted 19 February 2019; accepted in final form 1 May 2019

Wakeham DJ, Lord RN, Talbot JS, Lodge FM, Curry BA, Dawkins TG, Simpson LL, Shave RE, Pugh CJ, Moore JP. Upward resetting of the vascular sympathetic baroreflex in middle-aged male runners. *Am J Physiol Heart Circ Physiol* 317: H181–H189, 2019. First published May 3, 2019; doi:10.1152/ajpheart.00106.2019.—This study focused on the influence of habitual endurance exercise training (i.e., committed runner or nonrunner) on the regulation of muscle sympathetic nerve activity (MSNA) and arterial pressure in middle-aged (50 to 63 yr, $n = 23$) and younger (19 to 30 yr; $n = 23$) normotensive men. Hemodynamic and neurophysiological assessments were performed at rest. Indices of vascular sympathetic baroreflex function were determined from the relationship between spontaneous changes in diastolic blood pressure (DBP) and MSNA. Large vessel arterial stiffness and left ventricular stroke volume also were measured. Paired comparisons were performed within each age category. Mean arterial pressure and basal MSNA bursts/min were not different between age-matched runners and nonrunners. However, MSNA bursts/100 heartbeats, an index of baroreflex regulation of MSNA (vascular sympathetic baroreflex operating point), was higher for middle-aged runners ($P = 0.006$), whereas this was not different between young runners and nonrunners. The slope of the DBP-MSNA relationship (vascular sympathetic baroreflex gain) was not different between groups in either age category. Aortic pulse wave velocity was lower for runners of both age categories ($P < 0.03$), although carotid β-stiffness was lower only for middle-aged runners ($P = 0.04$). For runners of both age categories, stroke volume was larger, whereas heart rate was lower (both $P < 0.01$). In conclusion, we suggest that neural remodeling and upward setting of the vascular sympathetic baroreflex compensates for cardiovascular adaptations after many years committed to endurance exercise training, presumably to maintain arterial blood pressure stability.

NEW & NOTEWORTHY Exercise training reduces muscle sympathetic burst activity in disease; this is often extrapolated to infer a similar effect in health. We demonstrate that burst frequency of middle-aged and younger men committed to endurance training is not different compared with age-matched casual exercisers. Notably, well-trained, middle-aged runners display similar arterial pressure but higher sympathetic burst occurrence than untrained peers. We suggest that homeostatic plasticity and upward setting of the vascular sympathetic baroreflex maintains arterial pressure stability following years of training.

aging; baroreflex; blood pressure; exercise physiology; sympathetic nervous system

INTRODUCTION

Human aging exerts a marked influence on blood pressure, which is the primary regulated variable of the cardiovascular system. Two hallmarks of cardiovascular aging are large-vessel arterial stiffening (30) and chronic elevation of muscle sympathetic nerve activity (MSNA) (27). The conventional wisdom is that these factors, among others, contribute to the age-related increase in arterial blood pressure observed in Western society beyond 50 years of age (13).

Arterial baroreflex control of MSNA (i.e., vascular sympathetic baroreflex) is the primary mechanism through which the autonomic nervous system regulates vasomotor tone and thus plays a pivotal role in blood pressure homeostasis. The age-related increase in MSNA is underpinned by resetting of the vascular sympathetic baroreflex (31), whereby the "operating point" [i.e., mean resting diastolic blood pressure (DBP) and corresponding MSNA bursts per 100 heartbeats, a measure of the probability of a burst occurrence] resets upward and rightward. Vascular sympathetic baroreflex "resetting" with age occurs in the absence of a change of reflex "gain" (i.e., responsiveness to acute changes in blood pressure) (11, 25, 26). Notably, however, it appears that a rise in arterial pressure does not necessarily follow progressive elevation in resting vascular sympathetic activity with advancing age (49). In contrast, baroreflex-mediated cardiac parasympathetic control (i.e., cardiovagal baroreflex gain) is progressively impaired with advancing age (11, 32). Alterations to mechanosensory transduction and neural control (44) may explain these changes to the vascular sympathetic and cardiovagal limbs of the arterial baroreflex with human aging.

Long-term aerobic exercise training mitigates against some of the hallmarks of cardiovascular aging. For example, lifelong endurance exercise training offsets age-related stiffening of the aorta (51) and carotid artery (47). However, the interaction of committed exercise training and age-related changes to vascu-

* C. J. A. Pugh and J. P. Moore contributed equally to this work as co-senior authors.

Address for reprint requests and other correspondence: J. P. Moore, Physical Activity for Health and Well-Being Centre, School of Sport, Health and Exercise Sciences, Bangor Univ., LL57 2PZ, UK (e-mail: j.p.moore@bangor.ac.uk).

lar sympathetic activity is unclear. To date, relatively little consensus exists among previous microneurographic studies, which have found basal MSNA burst frequency for middle-aged and older endurance-trained men is either higher (37), not different (38), or lower (44) compared with untrained peers. Furthermore, quantification of the number of burst occurrences relative to the number of opportunities for a burst (i.e., burst incidence) does not provide clarity. However, the method of burst quantification provides different neurophysiological insight into regulation of vascular sympathetic activity (5). Burst frequency is reflective of the amount of sympathetic activity (or neurotransmitter release) that the vasculature is exposed to in a given time period (53). In contrast, burst incidence indicates the probability of a sympathetic burst occurring at a given arterial pressure (29). Furthermore, baroreceptor signals over a wide pressure range influence both the timing and the probability of sympathetic bursts. We contend, therefore, that burst incidence is an index of the baroreflex "gating" sympathetic bursts (20, 21) rather than sympathetic outflow per se. In the only study to consider the influence of aging and chronic exercise training on vascular sympathetic baroreflex control, the training status of healthy older men had no effect on MSNA burst incidence (vascular sympathetic baroreflex operating point) or the MSNA responsiveness (gain) to a modified Oxford baroreceptor test (44). In contrast, cross-sectional evidence from middle-aged and older men indicates that vigorous long-term endurance training attenuates the aging-related decline in cardiovagal baroreflex responsiveness (34).

Taking the various aforementioned uncertainties into account, the primary aim of this cross-sectional study was to investigate the effect that habitual endurance exercise training has on regulation of vascular sympathetic burst activity and resting blood pressure in healthy middle age. Because of marked sex differences in sympathetic regulation (18) and autonomic support of blood pressure (6), only men were studied to experimentally isolate the influence of long-term endurance training as much as possible. Furthermore, to examine the effect of exercise training independently of aging, a secondary aim was to compare the sympathetic control of blood pressure between young runners and young nonrunners. To address these aims, we performed comprehensive hemodynamic and neurophysiological assessments and measured central artery stiffness and left ventricular stroke volume in four groups of healthy normotensive men: middle-aged committed runners, middle-aged nonrunners, younger runners, and younger nonrunners. Based upon limited data, we hypothesized that the vascular sympathetic baroreflex control would not be different between well-trained runners and nonrunners.

METHODS

Ethical approval. This study conformed to the most recent Declaration of Helsinki, except for registration in a database. The Research Ethics Committee at the Cardiff School of Sport and Health approved all study procedures (16/7/02R), and participants provided written, informed consent before entering the study.

Participants. Between August 2016 and August 2017, the eligibility to participate was assessed for 70 men. Forty-six participants completed the study. Each participant was categorized according to his age (i.e., middle aged or young) and training status (i.e., committed runner or nonrunner) (Table 1). Among middle-aged men, runners performed ≥25 mi of moderate to intense training per week for ≥10 yr (n = 13), whereas nonrunners were casually recreationally active, i.e., ≤3 h of structured physical activity per week for ≥10 yr (n = 10). In the case of the young men, runners performed ≥50 mi of training per week (n = 13), and nonrunners performed ≤3 h of structured physical activity per week for ≥2 yr (n = 10). All participants were free of known cardiovascular, metabolic, or other chronic diseases; normotensive (<140/90 mmHg when supine); nonsmokers; and nonobese (BMI, <30 kg/m^2) as assessed by medical history, manual sphygmomanometry (Welch Allyn, UK), and measurement of height and body mass. Middle-aged men were further evaluated by resting and maximal exercise electrocardiogram.

Experimental overview. Participants completed one screening visit and 2 days of physiological testing, with a minimum of 1 wk between the tests. All screening and physiological tests were performed at the Cardiff School of Sport and Health Sciences in a quiet, temperature-controlled (22–24°C) environment. We requested that participants abstain from caffeine, alcohol, and strenuous exercise for 24 h before arrival at the laboratory on each visit; no participants took medication at the time of testing. On one testing day, assessment of body composition (bioelectrical impedance analysis; Bodystat 1500, Bodystat, Douglas, Isle of Man) and measurement of arterial stiffness were followed by a maximal incremental exercise test. On the other testing

Table 1. *Participant characteristics*

	Young Nonrunners	Young Runners	Middle-Aged Nonrunners	Middle-Aged Runners
Subjects, n	10	13	10	13
Demographics				
Age, yr	23 (21–25)	22 (21–24)	53 (52–55)	57 (54–59)
Stature, cm	178.1 (174.0–182.3)	179.9 (176.9–183.0)	175.6 (170.5–180.6)	174.7 (170.9–178.5)
Body mass, kg	80.4 (68.8–92.0)	67.0 (63.9–70.0)*	80.9 (73.8–88.0)	66.1 (61.3–70.9)†
BMI, kg/m^2	25.4 (22.1–28.8)	20.8 (19.9–21.6)*	26.2 (24.0–28.5)	21.6 (20.7–22.6)†
Body fat, %	19.7 (15.2–24.1)	10.7 (7.8–13.6)*	26.8 (20.4–33.3)	17.5 (15.6–19.3)†
Blood pressure, mmHg				
SBP	119 (109–128)	111 (108–114)*	119 (113–124)	118 (113–123)
DBP	71 (67–76)	66 (62–69)*	76 (73–80)	74 (70–78)
Cardiorespiratory fitness				
$\dot{V}O_{2peak}$, ml·kg^{-1}·min^{-1}	36.5 (31.9–41.0)	60.6 (55.0–66.2)*	32.6 (26.6–38.6)	50.7 (47.0–54.4)†
$\dot{V}O_{2peak}$, %predicted	86 (82–103)	116 (116–141)*	106 (87–129)	143 (129–155)†
Training history				
Exercise per wk, mi		65 (56–73)		34 (28–39)
Training history, yr		8 (5–11)		29 (28–40)

Values are means (95% confidence interval). BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; $\dot{V}O_{2peak}$, peak oxygen consumption. *P < 0.05 young runner vs. young nonrunner; †P < 0.05 middle-aged runner vs. middle-aged nonrunner.

day, having fasted for 6 h, participants underwent cardiovascular and sympathetic neural assessments.

Assessment of arterial stiffness. Sequential ECG-gated arterial pressure waveforms were recorded in accordance with current guidelines (50) from the carotid and femoral arteries at the site of maximal arterial pulsation, enabling the calculation of aortic pulse wave velocity (SphygmoCor, AtCor Medical, Sydney, Australia). Furthermore, the β-stiffness index of the right common carotid artery was determined via high-resolution ultrasonography, using a 12-MHz linear array transducer (Vivid Q, GE Medical, Norway) as previously described (19). Central blood pressure was estimated to calculate β-stiffness index by applying a generalized transfer function (41) to radial arterial waveforms, collected via a high-fidelity micromanometer-tipped probe (SphygmoCor, AtCor Medical). Carotid artery β-stiffness index is reported in 44 individuals (9 young nonrunners, 12 young runners, 10 middle-aged nonrunners, and 13 middle-aged runners).

Cardiopulmonary exercise test. All participants completed an incremental exercise test to exhaustion on a cycle ergometer (Lode Corival, Groningen, The Netherlands) to assess peak oxygen consumption. Cycling was chosen for reasons of safety and assessment of the exercise electrocardiogram. Each increment corresponded to an increase of 20 W/min (middle-aged runners started at 90 W and young runners started at 120 W; middle-aged and young nonrunners started at 30 W and 50 W, respectively). During the maximal exercise test, oxygen consumption was measured continuously via a breath-by-breath analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). Heart rate was measured throughout the exercise test via either a chest strap in the young groups (Polar Electro, RS400, Finland) or 12-lead electrocardiography in middle-aged men (Oxycon Pro, Jaeger).

Hemodynamics and sympathetic neural activity. Heart rate and blood pressure were monitored continuously via three-lead electrocardiography and finger photoplethysmography (FinometerPro, FMS, Groningen, The Netherlands) with participants supine. The arterial pressure waveform was calibrated at regular intervals to the average resting systolic and diastolic pressures measured via manual sphygmomanometry. Echocardiograms were acquired using a commercially available ultrasound system (Vivid E9, GE Medical) with a 1.5- to 4-MHz array probe. Images were obtained from apical four- and two-chamber views by a single experienced sonographer (RNL) and saved for offline analysis with commercially available software (EchoPAC, BT12, GE Medical).

Multiunit MSNA was obtained by microneurography using a recording system (Nerve Traffic Analyzer, Model 663 C, University of Iowa, Iowa City, IA) and following a recognized technique (45). In brief, a unipolar tungsten microelectrode (FHC, Bowdoin, ME) with shaft diameter of 0.1 mm (impedance, 1–5 MW) was placed across the skin at the popliteal fossa and inserted into the peroneal nerve by an experienced microneurographer (JPM). A reference electrode was placed subcutaneously ~2–3 cm above from the site of the recording electrode. The recorded neurogram was amplified (70,000- to 160,000-fold), band-pass filtered (700 to 2,000 Hz), full-wave rectified, and integrated with a resistance-capacitance circuit (time constant 0.1 s). Satisfactory recordings of MSNA were identified, dependent on the following criteria (54): *1*) pulse-synchronous "bursts" of activity, *2*) increased burst occurrence in response to voluntary apnea, *3*) unaffected burst pattern during stroking of the skin, and *4*) 3:1 signal-to-noise ratio. At least 10 min after an acceptable MSNA recording site was found, echocardiograms and other baseline data were acquired. Hemodynamic and neural data were sampled at 1,000 Hz using a commercial data acquisition system and stored for offline data analysis (Chart Version 8, Laboratory Chart Pro, AD Instruments, UK).

Assessment of arterial baroreflex function. Hemodynamic and neural recordings were acquired for 6 min to characterize the arterial baroreflex regulation of MSNA and interbeat RR interval. Respiratory rate was monitored via a nasal cannula (Capnocheck Sleep Capnograph, Smiths Medical, UK) to ensure that the participants had a regular breathing pattern because of the influence of breath hold on MSNA (9). Examples of the dynamic relationship between beat-by-beat arterial pressure and bursts of MSNA are shown in Fig. 1.

Data analyses. Stroke volume was estimated using the Simpson's biplane method (24), thus permitting determination of cardiac output (heart rate × stroke volume) and the total peripheral resistance (cardiac output/mean arterial pressure). Satisfactory images for the quantification of stroke volume were not recorded in 1 individual (1 middle-aged runner); accordingly, stroke volume, cardiac output, and total peripheral resistance data are reported for 45 individuals.

Multiunit bursts of MSNA were verified by two investigators (DJW and JPM) via visual inspection following adjustment for baroreflex latency (54) (time between R wave and peak burst height), which aligned each burst with the appropriate R wave of the ECG. MSNA was quantified as burst frequency (bursts/min) and burst incidence [bursts/100 heartbeats (hb)].

The slope of the stimulus-response relationship between DBP and MSNA burst probability was calculated to represent vascular sympathetic baroreflex gain (21, 45). Briefly, DBP was averaged into 2-mmHg bins to minimize the influence of respiration on MSNA and to maximize the number of data points for inclusion in the linear regression model. The percentage of cardiac cycles associated with a burst of MSNA (ranging from 0 to 100%) per bin of DBP was used to calculate burst probability. Data were included for further analysis if *1*) at least five data points for each linear regression were available and *2*) a correlation coefficient of ≥ -0.5 was present (14). Mean values and tests of statistical significance are presented for 20 middle-aged (11 runners) and 20 younger (11 runners) men. Statistical weighting was adopted for this analysis to minimize the influence of differences in the number of cardiac cycles within each DBP bin (21). The operating point of the vascular sympathetic baroreflex was determined from mean diastolic pressure and corresponding average burst incidence.

Cardiovagal baroreflex gain was assessed by the sequence method using customized computer software (Cardioseries version 2.4, Ribeirao Preto, São Paulo, Brazil). If RR interval was ≥800 ms, a delay of 1 beat was applied so that the systolic blood pressure was regressed against the following RR interval (12). Data were included for further analysis upon condition of *1*) a minimum of three data points for a linear regression were available and *2*) a correlation coefficient of ≥0.8 was present (40). The operating point of the cardiovagal baroreflex was determined from mean prevailing systolic blood pressure and corresponding average RR interval. Data, including positive and negative ramp gains and the number of sequences, are presented for 20 middle-aged (11 runners) and 21 younger (11 runners) men.

Statistical analyses. In line with our primary (i.e., middle-aged runner vs. age-matched nonrunner) and secondary (i.e., younger runner vs. age-matched nonrunner) aims, and after checking compliance with basic parametric assumptions, we assessed between-group differences for middle-aged runners and nonrunners and for young runners and nonrunners via independent *t*-tests. α was set a priori as $P < 0.05$. All statistical analyses were completed using Statistics Package for Social Sciences for Windows (version 23, Chicago, IL), and data are reported as means (95% confidence interval).

RESULTS

Participant demographics. By design, training and cardiorespiratory fitness (peak oxygen consumption) were greater for runners compared with age-matched nonrunners (middle aged and young, $P < 0.001$; Table 1). Runners had lower body mass (middle aged, $P = 0.001$; young, $P = 0.003$), body mass index (middle aged and young, $P < 0.001$), and body fat percentage (middle aged and young, $P < 0.001$) than age-matched nonrunners. Systolic blood pressure ($P = 0.041$) and DBP ($P = 0.027$) were

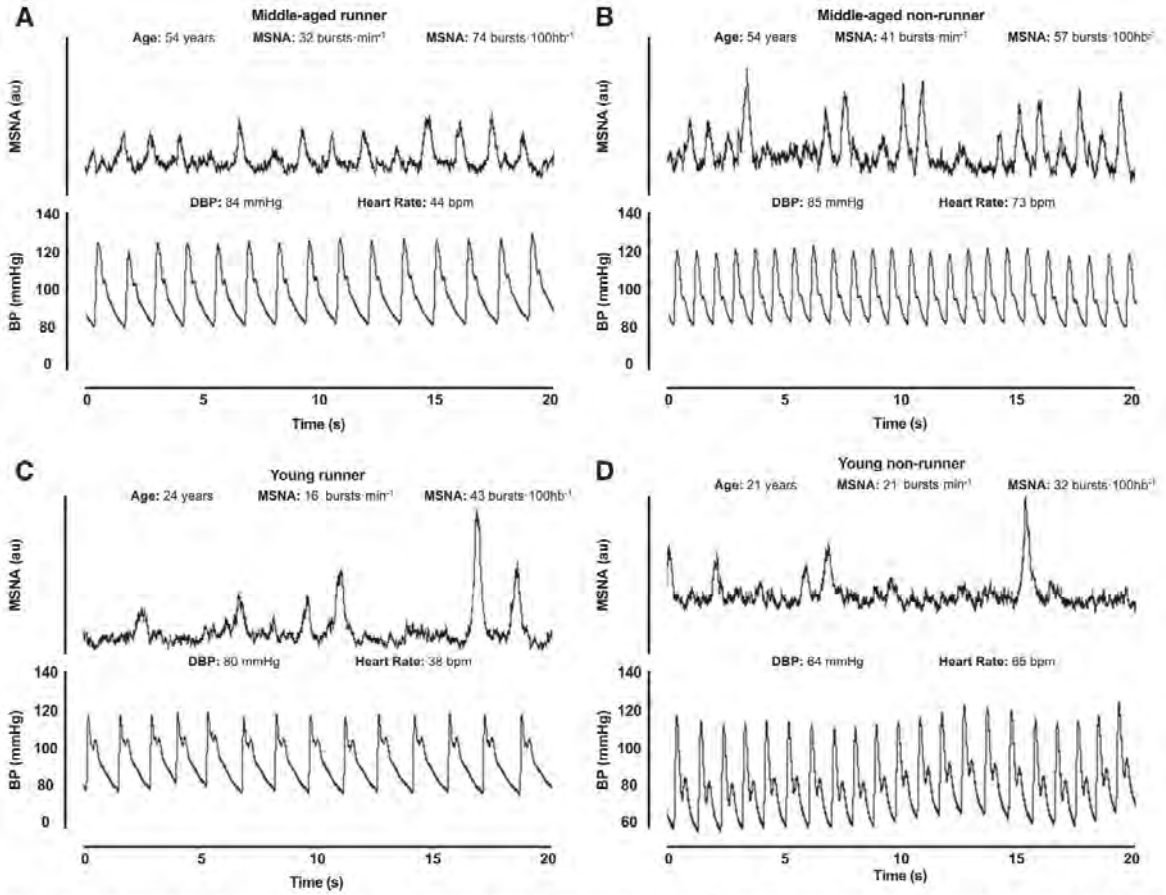


Fig. 1. Example recordings of muscle sympathetic nerve activity (MSNA) and blood pressure (BP) during supine rest. Twenty seconds of resting MSNA and BP data are shown from one representative participant per group: middle-aged runner (*A*), middle-aged nonrunner (*B*), young runner (*C*), and young nonrunner (*D*). au, arbitrary unit; DBP, diastolic blood pressure.

lower for young runners compared with age-matched nonrunners. Screening blood pressures were not different among middle-aged runners and untrained peers.

Resting hemodynamics and vascular sympathetic neural activity. Stroke volume was higher (middle aged, $P = 0.03$; young, $P < 0.01$) and heart rate was lower (middle aged, $P < 0.001$; young, $P < 0.001$) between both groups of runners compared with age-matched nonrunners (Table 2). There were no other differences in resting hemodynamic parameters between runners and nonrunners for either age category. Resting MSNA burst frequencies were not different among middle-aged runners and nonrunners or among young runners and age-matched nonrunners. Burst incidence data are considered in the following section.

Arterial baroreflex function. Among middle-aged men, there was no difference between runners and nonrunners for the diastolic operating pressure of the vascular sympathetic baroreflex ($P = 0.57$); however, the corresponding operating MSNA (i.e., bursts/100 hb) was higher in the runners ($P < 0.01$; Fig. 2*A*). Among young men, there was no significant difference in vascular sympathetic operating point between runners and nonrunners (DBP, $P = 0.23$; corresponding MSNA bursts/100 hb, $P = 0.24$). The vascular sympathetic baroreflex gain (i.e., slope of the DBP-MSNA relationship) was not influenced by the training status of either middle-aged [−6.07 (−8.80 to −3.55) vs. −7.30 (−10.49 to −4.12) %·mmHg, $P = 0.55$] or younger [−6.68 (−13.1 to −2.33) vs. −5.82 (−7.15 to −4.49) %·mmHg, $P = 0.58$] men.

Among middle-aged runners and nonrunners, there was no difference in the prevailing systolic pressure for the cardiovagal baroreflex ($P = 0.58$), but the corresponding RR interval was higher for runners ($P < 0.01$; Fig. 2*B*). Among young men, the prevailing systolic pressure was lower ($P = 0.02$) and the corresponding RR interval was higher for runners ($P < 0.01$). The cardiovagal baroreflex gain was not different between runners and nonrunners of both age groups; data for positive and negative pressure ramps and the number of sequences per ramp are presented in Table 3.

Arterial stiffness. Runners had lower aortic pulse wave velocity (middle aged, $P = 0.026$; young, $P = 0.027$) compared

Table 2. *Resting hemodynamics and basal sympathetic nervous system activity*

	Young		Middle Aged	
	Nonrunners	Runners	Nonrunners	Runners
Subjects, *n*	10	13	10	13
Central artery stiffness				
aPWV, m/s	5.8 (5.2–6.3)	5.1 (4.8–5.3)*	7.5 (6.9–8.1)	6.8 (6.2–7.3)†
β-stiffness index	2.96 (2.52–3.40)	2.38 (2.03–2.74)	5.06 (3.94–6.19)	4.06 (3.25–4.88)†
Hemodynamics				
Heart rate, beats/min	64 (57–70)	45 (41–48)*	56 (49–62)	43 (38–47)†
Stroke volume, ml	61 (57–64)	92 (87–97)*	62 (56–68)	70 (63–77)†
Cardiac output, l/min	3.8 (3.5–4.2)	4.1 (3.8–4.4)	3.4 (3.0–3.8)	3.0 (2.6–3.3)
TPR, mmHg·l^{-1}·min^{-1}	24.3 (21.2–27.4)	21.3 (19.2–23.3)	29.1 (26.8–31.3)	31.6 (28.4–34.7)
MAP, mmHg	90 (83–97)	84 (81–88)	95 (89–101)	93 (90–96)
Respiration rate, breaths/min	13 (10–15)	15 (14–16)	11 (9–13)	12 (10–14)
Muscle sympathetic nerve activity				
Burst frequency, bursts/min	18 (12–23)	16 (10–21)	28 (19–38)	31 (27–34)
Burst incidence, bursts/100 heartbeats	27 (19–36)	36 (23–50)	50 (33–66)	72 (63–81)†

Value are means (95% confidence interval). Note: We were unable to quantify β-stiffness index in 1 young nonrunner and 1 young runner; accordingly, data are reported for 44 individuals. Furthermore, stroke volume was unobtainable for one middle-aged runner. Accordingly, stroke volume, cardiac output, and total peripheral resistance (TPR) data are reported in 45 individuals. aPWV, aortic pulse wave velocity; MAP, mean arterial pressure. *$P < 0.05$ young runner vs. young nonrunner; †$P < 0.05$ middle-aged runner vs. middle-aged nonrunner.

with age-matched nonrunners (Table 2). In contrast, the β-stiffness index of the carotid artery was lower only for the middle-aged runners compared with age-matched nonrunners ($P = 0.041$).

DISCUSSION

The principal findings are as follows: *1*) for middle-aged men, many years of moderate to vigorous endurance exercise training sets the operating point of the vascular sympathetic baroreflex at a burst occurrence that is higher than for peers who have not trained; *2*) higher burst occurrence does not influence overall reflex gain, basal burst frequency, or resting arterial pressure; and *3*) for younger men, endurance training has a limited effect on the operating point, and there are no differences in vascular sympathetic baroreflex reflex gain or basal burst frequency compared with untrained peers. Taken together, these findings indicate that some form of remodeling in middle-aged men following many years of committed endurance exercise training plays a critical role in the baroreflex control of vascular sympathetic bursts and resting blood pressure.

The effect of training on vascular sympathetic baroreflex control. Regardless of the training status, we observed similar frequencies of sympathetic bursts in microneurographic recordings taken from middle-aged men during supine rest. An intriguing finding, however, is that the well-trained men exhibit

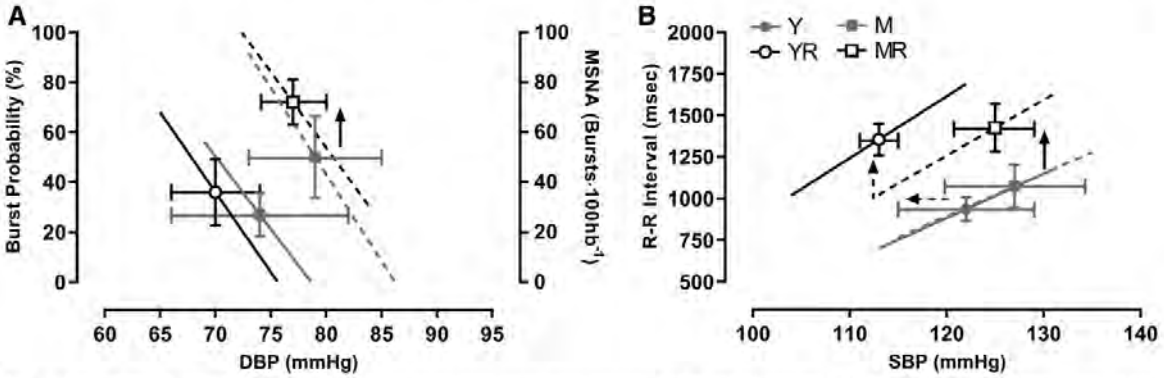


Fig. 2. Sympathetic and cardiac baroreflex function. *A*: group mean regressions between diastolic blood pressure (DBP) and muscle sympathetic nerve activity (MSNA) are presented with the sympathetic operating points superimposed on the regression lines. Middle-aged runners had similar operating DBP compared with middle-aged nonrunners, but the corresponding level of MSNA was higher (by 22 bursts/100 heartbeats; solid black arrow), despite similar sympathetic baroreflex gain. However, in young men, training status had no influence on the operating DBP, corresponding level of MSNA, or sympathetic baroreflex gain. *B*: group mean regressions between systolic blood pressure (SBP) and RR interval (sequence method) are shown with the operating points of the cardiac baroreflex overlaid on the regression lines. Middle-aged runners had similar operating SBP and cardiovagal baroreflex gain [33.6 (24.5–42.8) vs. 25.5 (16.2–34.7) ms/mmHg, $P = 0.16$] compared with middle-aged nonrunners, but the corresponding RR interval was longer (by 352 ms; solid black arrow). In contrast, when compared with young nonrunners, the operating SBP was set leftward (by 9 mmHg; black dashed arrow) in young runners with a longer corresponding RR interval (by 418 ms; black dashed arrow), despite similar cardiac baroreflex gain [37.2 (28.1–46.3) vs. 26.4 (19.1–33.8) ms/mmHg, $P = 0.06$]. Baroreflex responsiveness data are presented from 10 young nonrunners, 11 young runners, 9 middle-aged nonrunners, and 11 middle-aged runners. M, middle-aged nonrunners; MR, middle-aged runner; Y, young nonrunner; YR, young runner.

Table 3. *Cardiovagal baroreflex gain and the number of sequences for positive and negative pressure ramps*

	Young		Middle Aged	
	Nonrunners	Runners	Nonrunners	Runners
Subjects, *n*	10	11	9	13
"Up" gain, ms/mmHg	31 (23–39)	41 (27–55)	28 (18–37)	34 (25–44)
Sequences, *n*	20 (12–29)	8 (5–11)	17 (10–24)	11 (8–16)
"Down" gain, ms/mmHg	24 (17–32)	33 (22–45)	23 (14–32)	33 (22–45)
Sequences, *n*	30 (20–39)	9 (6–12)	20 (13–26)	13 (8–19)

Values are means (95% confidence interval).

a greater MSNA burst occurrence, and by some margin (approximately 40 to 50%); this occurs without any obvious difference in the corresponding diastolic pressure stimulus. Together, we interpret these data for MSNA burst frequency and occurrence as evidence that many years of training alters the gating of sympathetic bursts (i.e., baroreflex control) without influencing the frequency of sympathetic bursts per minute (i.e., rate of neurotransmitter release). Although this might seem contradictory, burst frequency and occurrence provide slightly different neurophysiological information (5, 29, 53). Furthermore, reciprocal interplay between exercise bradycardia (i.e., fewer opportunities for a burst per minute) and the higher MSNA operating point (i.e., greater burst occurrence) explains why the burst frequency for trained runners and nonrunners is similar.

Our data indicate that an exercise training-induced upward setting for the MSNA operating point in middle age occurs without any change in the ability to increase or decrease vasoconstrictor outflow during fluctuations of resting arterial pressure. In other words, vascular sympathetic baroreflex overall gain is unaffected by training. Stüdinger and colleagues (44), using the modified Oxford baroreceptor test, also observed that overall gain was similar among older-trained and untrained men. Unlike the present study, however, no difference was observed for sympathetic burst occurrence, and resting burst frequency was marginally lower for endurance-trained versus untrained middle-aged men.

Other studies of trained and untrained middle-aged and older people have recorded resting MSNA without specifically addressing vascular sympathetic baroreflex function. Notarius and colleagues (38) observed that burst occurrence was higher, whereas basal sympathetic burst frequency was similar, for endurance trained middle-aged men compared with sedentary peers. In contrast, Ng and coworkers (37) reported higher sympathetic burst occurrence and burst frequency for older endurance-trained athletes; however, these findings may reflect an older cohort or inclusion of endurance-trained women, for whom burst frequency was markedly higher compared with untrained peers. Although we cannot explain this lack of consensus, it may reflect the differences in the endurance phenotype across the studies. Factors that influence basal vascular sympathetic outflow with human aging, such as abdominal adiposity (15), distensibility of the barosensory vessel walls (48), and blood volume (2), all are influenced by the dose of endurance exercise training.

To isolate the effect of endurance exercise training from human aging, we also studied younger men. As with older men, we found no difference for basal burst frequency between well-trained runners and nonrunners. The burst occurrence was marginally higher for the runners, but this difference was modest in comparison with that between the older groups. These findings in young men are similar to previous cross-sectional studies (7, 43, 46). Furthermore, vascular sympathetic baroreflex gain is similar for trained runners and nonrunners. Thus, our data suggest that the endurance phenotype traits of young men do not include a higher operating point for the vascular sympathetic baroreflex.

Differences for aortic stiffness and resting heart rate between young runners and nonrunners are comparable with those for the middle-aged men. However, one noteworthy distinction relates to the difference in resting stroke volume. For young, well-trained men, stroke volume during supine rest was 50% greater than that of age-matched nonrunners. For older men, resting stroke volume was only 12% greater for runners compared with age-matched nonrunners. This lesser difference in stroke volume may explain why endurance training affects the operating point for the vascular sympathetic baroreflex only for committed middle-aged runners. That is, older runners rely more on vascular sympathetic neural activity than cardiac output to support arterial pressure. However, further investigation of potential interaction of left ventricular stroke volume and the vascular sympathetic baroreflex is required.

Our interpretation for young men in this study is consistent with a previous report that endurance training does not influence autonomic support of blood pressure in the young (17). However, our findings do contrast with those of a study by Alvarez and colleagues (1). As in the present study, burst occurrence was marginally higher in trained men, whereas basal MSNA burst frequency was similar. However, when adiposity is taken into account, burst occurrence and burst frequency both were greater for endurance-trained versus untrained men (1). Furthermore, in contrast to the present study, sympathetic baroreflex gain was lower for endurance-trained compared with untrained young men, an effect regardless of percentage body fat. This suggests that body composition may be important, at least in younger men.

The effect of training on cardiovagal baroreflex control. It is well known that endurance athletes display exercise-induced bradycardia, although considerable debate exists surrounding the mechanisms involved (3, 4). Furthermore, arterial baroreceptor control of blood pressure is mediated predominantly via sympathetic vascular regulation rather than by reflex changes in heart period (8). Nonetheless, we determined how habitual endurance exercise influenced the responsiveness of the cardiovagal baroreflex in middle age. For well-trained middle-aged men, as expected, the cardiovagal baroreflex operated around a considerably longer RR interval at rest; however, the baroreflex gain was similar among runners and nonrunners. Previous work has shown that middle-aged, endurance-trained men display greater cardiovagal baroreflex gain than sedentary controls, but not moderately active, age-matched peers (34). In the case of the younger trained men in this study, the cardiovagal baroreflex also operated around a longer heart period, without any difference in baroreflex gain compared with age-matched nonrunners; this finding for gain is in agreement with previous studies in younger men (1, 7, 34).

Remodeling of the vascular sympathetic baroreflex. Mechanosensory transduction, central mediation, and efferent neurotransmission are integrated into the baroreflex regulation of

vasomotor tone and arterial pressure. Furthermore, it is proposed that human aging may have opposing influences on mechanical and neural events (44). However, we can only speculate upon potential sites where additional remodeling might have occurred in committed middle-aged runners to explain our findings. Many years of training may influence the strength and/or timing of mechanosensory signals controlling efferent sympathetic burst occurrence; this could arise from altered vascular mechanics and/or a change to the threshold for baroreceptor activation. Specifically, well-trained middle-aged men have less stiff barosensory regions; furthermore, more complete elastic recoil during a longer diastolic period could lead to a longer interval of "silence" in the afferent baroreceptor signal (21). However, the apparent lack of a similar upward setting of the MSNA operating point for younger trained men, who also possess lesser vascular stiffness and display bradycardia, argues against this. However, endurance training-induced cardiovascular remodeling may only lead to upward vascular sympathetic baroreflex resetting in middle-aged men because of increased autonomic support of blood pressure with age (16).

Animal studies indicate that chronic exercise training potentially influences baroreceptor control of sympathetic bursts at brain structures including the nucleus tractus solitairius, the paraventricular nucleus of the hypothalamus, and the rostral ventrolateral medulla (36). Brain imaging studies have identified some of the same sites as regions of baroreflex control in humans (22, 23). It is possible, therefore, that neural plasticity and exercise-induced central remodeling previously observed in animals underpins the higher sympathetic burst occurrence in middle-aged, trained males.

Changes to efferent neurotransmission may also mediate upward vascular sympathetic baroreflex setting. Short-term exercise training reduces α-adrenergic vasoconstrictor responsiveness (35), and a reduction of sympathetic vascular transduction has been proposed to contribute to orthostatic intolerance observed in some highly trained individuals (52). Vasoconstrictor responsiveness to noradrenaline declines with advancing age (10), which may counteract the effects of elevated MSNA burst frequency (16). Furthermore, Notarius and colleagues (38) observed that sympathetic vascular transduction during baroreflex-mediated sympathoexcitation may be altered further in trained middle-aged men. Another possibility is that vascular sympathetic baroreflex resetting may be a compensatory mechanism to offset training-induced vascular changes (33, 42). All of these aforementioned possibilities require investigation. Notably, irrespective of the locations, exercise-induced remodeling does not alter vascular sympathetic baroreflex gain, at least not the integrated gain.

Experimental considerations. Vascular sympathetic baroreflex gain was calculated by associating spontaneous fluctuations in DBP to the occurrence of bursts of MSNA. We did not take strength (amplitude) of sympathetic bursts into account because baroreceptor signals modulate burst occurrence, whereas less is known of the mechanisms that govern amplitude (21, 29). Furthermore, we did not assess vascular sympathetic baroreflex gain to rising and falling pressures independently, and we acknowledge that this does not take baroreflex hysteresis into account (14).

It is reported that dietary salt and nitrate can influence sympathetic burst activity (28, 39). However, we did not control for diet in our study; therefore, we cannot exclude some influence on our data. Every effort was made to accurately record the number of years over which an individual had exercised at their current level. In addition, we recorded lifetime physical activity and exercise and observed a clear difference in maximal aerobic capacity between the trained and untrained groups. However, group allocation, determined by habitual endurance training, may limit the conclusions based on other components of exercise training. These components include mode, intensity, and duration, all of which may have an impact on cardiac, vascular, and neural remodeling. Because sex of the participants was controlled for in this study, future studies are required to properly address potential sex differences. Although our participants were nonobese, we did not specifically control for adiposity, which is known to influence sympathetic burst activity. However, post hoc analysis suggests that percentage body fat was not a significant covariate for any indices of sympathetic activity in this study. Finally, the a priori intention of our study was to investigate the effect that committed endurance exercise training has on elevated sympathetic neural activity and vascular sympathetic baroreflex control of resting blood pressure in healthy middle-aged men. However, we also studied young men to investigate the effect of endurance training independently of cardiovascular aging. The use of independent samples *t*-tests reflects these a priori questions. To limit the chance of a type 1 error, we did not perform statistical comparisons between middle-aged runners and young runners or middle-aged nonrunners and young nonrunners.

Conclusion. This study demonstrates upward setting of arterial baroreflex regulation of vascular sympathetic bursts following committed endurance training in middle-aged men. Importantly, vascular sympathetic baroreflex resetting coupled to exercise-induced bradycardia results in a similar basal burst frequency compared with untrained peers. Furthermore, the study demonstrates that training status does not influence the MSNA operating point for younger well–trained men, who also display similar sympathetic burst frequency compared with untrained peers. In our view, remodeling within the vascular sympathetic baroreflex arc, culminating in a higher MSNA operating point, is another example of phenotypic adaptation to lifelong (>25 yr) training. This occurs, presumably, to maintain resting vasomotor tone and blood pressure stability and to complement cardiac and vascular adaptations to many years of endurance exercise training.

ACKNOWLEDGMENTS

We thank all participants for taking part in this study.

GRANTS

D. J. Wakeham was supported by a PhD studentship from the School of Sport and Health, Cardiff Metropolitan University. L. L. Simpson was supported by a PhD studentship from the School of Sport Health and Exercise Sciences, Bangor University.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

D.J.W., R.N.L., R.E.S., C.J.A.P., and J.P.M. conceived and designed research; D.J.W., R.N.L., J.S.T., F.M.L., B.A.C., T.G.D., L.L.S., C.J.A.P., and J.P.M. performed experiments; D.J.W., R.N.L., J.S.T., F.M.L., and J.P.M.

analyzed data; D.J.W. and J.P.M. interpreted results of experiments; D.J.W. prepared figures; D.J.W. and J.P.M. drafted manuscript; D.J.W., R.N.L., R.E.S., C.J.A.P., and J.P.M. edited and revised manuscript; D.J.W., R.N.L., J.S.T., F.M.L., B.A.C., T.G.D., L.L.S., R.E.S., C.J.A.P., and J.P.M. approved final version of manuscript.

REFERENCES

1. **Alvarez GE, Halliwill JR, Ballard TP, Beske SD, Davy KP.** Sympathetic neural regulation in endurance-trained humans: fitness vs. fatness. *J Appl Physiol (1985)* 98: 498–502, 2005. doi:10.1152/japplphysiol.01020.2004.
2. **Best SA, Okada Y, Galbreath MM, Jarvis SS, Bivens TB, Adams-Huet B, Fu Q.** Age and sex differences in muscle sympathetic nerve activity in relation to haemodynamics, blood volume and left ventricular size. *Exp Physiol* 99: 839–848, 2014. doi:10.1113/expphysiol.2013.077248.
3. **Billman GE.** Counterpoint: exercise training-induced bradycardia: the case for enhanced parasympathetic regulation. *J Appl Physiol (1985)* 123: 686–688, 2017. doi:10.1152/japplphysiol.00605.2017.
4. **Boyett MR, Wang Y, Nakao S, Ariyaratnam J, Hart G, Monfredi O, D'Souza A.** Point: Exercise training-induced bradycardia is caused by changes in intrinsic sinus node function. *J Appl Physiol (1985)* 123: 684–685, 2017. doi:10.1152/japplphysiol.00604.2017.
5. **Charkoudian N, Wallin BG.** Sympathetic neural activity to the cardiovascular system: integrator of systemic physiology and interindividual characteristics. *Compr Physiol* 4: 825–850, 2014. doi:10.1002/cphy.c130038.
6. **Christou DD, Jones PP, Jordan J, Diedrich A, Robertson D, Seals DR.** Women have lower tonic autonomic support of arterial blood pressure and less effective baroreflex buffering than men. *Circulation* 111: 494–498, 2005. doi:10.1161/01.CIR.0000153864.24034.A6.
7. **Christou DD, Jones PP, Seals DR.** Baroreflex buffering in sedentary and endurance exercise-trained healthy men. *Hypertension* 41: 1219–1222, 2003. doi:10.1161/01.HYP.0000072011.17095.AE.
8. **Dampney RA.** Resetting of the baroreflex control of sympathetic vasomotor activity during natural behaviors: description and conceptual model of central mechanisms. *Front Neurosci* 11: 461, 2017. doi:10.3389/fnins.2017.00461.
9. **Delius W, Hagbarth KE, Hongell A, Wallin BG.** Manoeuvres affecting sympathetic outflow in human muscle nerves. *Acta Physiol Scand* 84: 82–94, 1972. doi:10.1111/j.1748-1716.1972.tb05158.x.
10. **Dinenno FA, Dietz NM, Joyner MJ.** Aging and forearm postjunctional alpha-adrenergic vasoconstriction in healthy men. *Circulation* 106: 1349–1354, 2002. doi:10.1161/01.CIR.0000028819.64790.BE.
11. **Ebert TJ, Morgan BJ, Barney JA, Denahan T, Smith JJ.** Effects of aging on baroreflex regulation of sympathetic activity in humans. *Am J Physiol* 263: H798–H803, 1992. doi:10.1152/ajpheart.1992.263.3.H798.
12. **Eckberg DL, Eckberg MJ.** Human sinus node responses to repetitive, ramped carotid baroreceptor stimuli. *Am J Physiol* 242: H638–H644, 1982. doi:10.1152/ajpheart.1982.242.4.H638.
13. **Franklin SS, Gustin W IV, Wong ND, Larson MG, Weber MA, Kannel WB, Levy D.** Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation* 96: 308–315, 1997. doi:10.1161/01.CIR.96.1.308.
14. **Hart EC, Wallin BG, Curry TB, Joyner MJ, Karlsson T, Charkoudian N.** Hysteresis in the sympathetic baroreflex: role of baseline nerve activity. *J Physiol* 589: 3395–3404, 2011. doi:10.1113/jphysiol.2011.208538.
15. **Jones PP, Davy KP, Alexander S, Seals DR.** Age-related increase in muscle sympathetic nerve activity is associated with abdominal adiposity. *Am J Physiol* 272: E976–E980, 1997. doi:10.1152/ajpendo.1997.272.6.E976.
16. **Jones PP, Shapiro LF, Keisling GA, Jordan J, Shannon JR, Quaife RA, Seals DR.** Altered autonomic support of arterial blood pressure with age in healthy men. *Circulation* 104: 2424–2429, 2001. doi:10.1161/hc4501.099308.
17. **Jones PP, Shapiro LF, Keisling GA, Quaife RA, Seals DR.** Is autonomic support of arterial blood pressure related to habitual exercise status in healthy men? *J Physiol* 540: 701–706, 2002. doi:10.1113/jphysiol.2001.013664.
18. **Joyner MJ, Barnes JN, Hart EC, Wallin BG, Charkoudian N.** Neural control of the circulation: how sex and age differences interact in humans. *Compr Physiol* 5: 193–215, 2015. doi:10.1002/cphy.c140005.
19. **Kawasaki T, Sasayama S, Yagi S, Asakawa T, Hirai T.** Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovasc Res* 21: 678–687, 1987. doi:10.1093/cvr/21.9.678.
20. **Keller DM, Cui J, Davis SL, Low DA, Crandall CG.** Heat stress enhances arterial baroreflex control of muscle sympathetic nerve activity via increased sensitivity of burst gating, not burst area, in humans. *J Physiol* 573: 445–451, 2006. doi:10.1113/jphysiol.2006.108662.
21. **Kienbaum P, Karlsson T, Sverrisdottir YB, Elam M, Wallin BG.** Two sites for modulation of human sympathetic activity by arterial baroreceptors? *J Physiol* 531: 861–869, 2001. doi:10.1111/j.1469-7793.2001.0861h.x.
22. **Kimmerly DS, O'Leary DD, Menon RS, Gati JS, Shoemaker JK.** Cortical regions associated with autonomic cardiovascular regulation during lower body negative pressure in humans. *J Physiol* 569: 331–345, 2005. doi:10.1113/jphysiol.2005.091637.
23. **Krämer HH, Ament SJ, Breimhorst M, Klega A, Schmieg K, Endres C, Buchholz HG, Elam M, Schreckenberger M, Birklein F.** Central correlation of muscle sympathetic nerve activation during baroreflex unloading - a microneurography-positron emission tomography study. *Eur J Neurosci* 39: 623–629, 2014. doi:10.1111/ejn.12437.
24. **Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU.** Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 28: 1–39.e14, 2015. doi:10.1016/j.echo.2014.10.003.
25. **Matsukawa T, Sugiyama Y, Iwase S, Mano T.** Effects of aging on the arterial baroreflex control of muscle sympathetic nerve activity in healthy subjects. *Environ Med* 38: 81–84, 1994.
26. **Matsukawa T, Sugiyama Y, Mano T.** Age-related changes in baroreflex control of heart rate and sympathetic nerve activity in healthy humans. *J Auton Nerv Syst* 60: 209–212, 1996. doi:10.1016/0165-1838(96)00057-4.
27. **Matsukawa T, Sugiyama Y, Watanabe T, Kobayashi F, Mano T.** Gender difference in age-related changes in muscle sympathetic nerve activity in healthy subjects. *Am J Physiol* 275: R1600–R1604, 1998. doi:10.1152/ajpregu.1998.275.5.R1600.
28. **Matthews EL, Brian MS, Edwards DG, Stocker SD, Wenner MM, Farquhar WB.** Blood pressure responses to dietary sodium: association with autonomic cardiovascular function in normotensive adults. *Auton Neurosci* 208: 51–56, 2017. doi:10.1016/j.autneu.2017.08.001.
29. **McAllen RM, Malpas SC.** Sympathetic burst activity: characteristics and significance. *Clin Exp Pharmacol Physiol* 24: 791–799, 1997. doi:10.1111/j.1440-1681.1997.tb02693.x.
30. **McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR; ACCT Investigators.** Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol* 46: 1753–1760, 2005. doi:10.1016/j.jacc.2005.07.037.
31. **Monahan KD.** Effect of aging on baroreflex function in humans. *Am J Physiol Regul Integr Comp Physiol* 293: R3–R12, 2007. doi:10.1152/ajpregu.00031.2007.
32. **Monahan KD, Dinenno FA, Seals DR, Clevenger CM, Desouza CA, Tanaka H.** Age-associated changes in cardiovagal baroreflex sensitivity are related to central arterial compliance. *Am J Physiol Heart Circ Physiol* 281: H284–H289, 2001. doi:10.1152/ajpheart.2001.281.1.H284.
33. **Monahan KD, Dinenno FA, Seals DR, Halliwill JR.** Smaller age-associated reductions in leg venous compliance in endurance exercise-trained men. *Am J Physiol Heart Circ Physiol* 281: H1267–H1273, 2001. doi:10.1152/ajpheart.2001.281.3.H1267.
34. **Monahan KD, Dinenno FA, Tanaka H, Clevenger CM, DeSouza CA, Seals DR.** Regular aerobic exercise modulates age-associated declines in cardiovagal baroreflex sensitivity in healthy men. *J Physiol* 529: 263–271, 2000. doi:10.1111/j.1469-7793.2000.00263.x.
35. **Mortensen SP, Nyberg M, Gliemann L, Thaning P, Saltin B, Hellsten Y.** Exercise training modulates functional sympatholysis and α-adrenergic vasoconstrictor responsiveness in hypertensive and normotensive individuals. *J Physiol* 592: 3063–3073, 2014. doi:10.1113/jphysiol.2014.273722.
36. **Mueller PJ, Clifford PS, Crandall CG, Smith SA, Fadel PJ.** Integration of central and peripheral regulation of the circulation during exercise: acute and chronic adaptations. *Compr Physiol* 8: 103–151, 2017. doi:10.1002/cphy.c160040.
37. **Ng AV, Callister R, Johnson DG, Seals DR.** Endurance exercise training is associated with elevated basal sympathetic nerve activity in

healthy older humans. *J Appl Physiol (1985)* 77: 1366–1374, 1994. doi:10.1152/jappl.1994.77.3.1366.

38. **Notarius CF, Murai H, Morris BL, Floras JS.** Effect of fitness on reflex sympathetic neurovascular transduction in middle-age men. *Med Sci Sports Exerc* 44: 232–237, 2012. doi:10.1249/MSS.0b013e31822a68a5.
39. **Notay K, Incognito AV, Millar PJ.** Acute beetroot juice supplementation on sympathetic nerve activity: a randomized, double-blind, placebo-controlled proof-of-concept study. *Am J Physiol Heart Circ Physiol* 313: H59–H65, 2017. doi:10.1152/ajpheart.00163.2017.
40. **Parati G, Di Rienzo M, Mancia G.** How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life. *J Hypertens* 18: 7–19, 2000. doi:10.1097/00004872-200018010-00003.
41. **Pauca AL, O'Rourke MF, Kon ND.** Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* 38: 932–937, 2001. doi:10.1161/hy1001.096106.
42. **Prior BM, Lloyd PG, Yang HT, Terjung RL.** Exercise-induced vascular remodeling. *Exerc Sport Sci Rev* 31: 26–33, 2003. doi:10.1097/00003677-200301000-00006.
43. **Seals DR.** Sympathetic neural adjustments to stress in physically trained and untrained humans. *Hypertension* 17: 36–43, 1991. doi:10.1161/01.HYP.17.1.36.
44. **Studinger P, Goldstein R, Taylor JA.** Age- and fitness-related alterations in vascular sympathetic control. *J Physiol* 587: 2049–2057, 2009. doi:10.1113/jphysiol.2009.170134.
45. **Sundlöf G, Wallin BG.** Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J Physiol* 274: 621–637, 1978. doi:10.1113/jphysiol.1978.sp012170.
46. **Svedenhag J, Wallin BG, Sundlöf G, Henriksson J.** Skeletal muscle sympathetic activity at rest in trained and untrained subjects. *Acta Physiol Scand* 120: 499–504, 1984. doi:10.1111/j.1748-1716.1984.tb07413.x.
47. **Tanaka H, Dinenno FA, Monahan KD, Clevenger CM, DeSouza CA, Seals DR.** Aging, habitual exercise, and dynamic arterial compliance. *Circulation* 102: 1270–1275, 2000. doi:10.1161/01.CIR.102.11.1270.
48. **Tanaka H, Dinenno FA, Seals DR.** Reductions in central arterial compliance with age are related to sympathetic vasoconstrictor nerve activity in healthy men. *Hypertens Res* 40: 493–495, 2017. doi:10.1038/hr.2016.182.
49. **Taylor JA, Tan CO.** BP regulation VI: elevated sympathetic outflow with human aging: hypertensive or homeostatic? *Eur J Appl Physiol* 114: 511–519, 2014. doi:10.1007/s00421-013-2731-7.
50. **Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM, Mitchell GF, Najjar SS, Nichols WW, Urbina EM, Weber T; American Heart Association Council on Hypertension.** Recommendations for improving and standardizing vascular research on arterial stiffness: a scientific statement from the American Heart Association. *Hypertension* 66: 698–722, 2015. doi:10.1161/HYP.0000000000000033.
51. **Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE, Yin FC, Lakatta EG.** Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation* 88: 1456–1462, 1993. doi:10.1161/01.CIR.88.4.1456.
52. **van Lieshout JJ.** Exercise training and orthostatic intolerance: a paradox? *J Physiol* 551: 401, 2003. doi:10.1113/jphysiol.2003.049205.
53. **Wallin BG, Esler M, Dorward P, Eisenhofer G, Ferrier C, Westerman R, Jennings G.** Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J Physiol* 453: 45–58, 1992. doi:10.1113/jphysiol.1992.sp019217.
54. **White DW, Shoemaker JK, Raven PB.** Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. *Auton Neurosci* 193: 12–21, 2015. doi:10.1016/j.autneu.2015.08.004.

Appendix IV – Other publications during PhD candidature

Articles in peer-reviewed journals

1. Pugh, C. J. A., Stone, K. J., Stohr, E. J., Mcdonnell, B. J., Thompson, J. E. S., Talbot, J. S., **Wakeham, D. J.**, Cockcroft, J. R. & Shave, R. (2018). Carotid artery wall mechanics in young males with high cardiorespiratory fitness. *Exp Physiol,* 103, 1277-1286. PMID: 29959801
2. Stuckless, T. J. R., Vermeulen, T. D., Brown, C. V., Boulet, L. B., Shafer, B. M., **Wakeham, D. J.,** Steinback, C. D., Ayas, N. T., Floras, J. S. & Foster, G. E. (2019). Acute intermittent hypercapnic hypoxia and sympathetic neurovascular transduction in men. *J Physiol.* PMID: 31805605
3. Talbot, J. S., Lord, R. N., **Wakeham, D. J.**, Dawkins, T. G., Curry, B. A., Brown, M., Lodge, F. M. & Pugh, C. J. A. 2020. The influence of habitual endurance exercise on carotid artery strain and strain rate in young and middle-aged men. *Exp Physiol*. PMID: 32578897
4. Lord, R. N., **Wakeham, D. J.,** Talbot, J. S., Simpson, L. L. S., Curry, B.A., Pugh, C. J. A., Shave, R., & Moore, J. P. The influence of barosensory vessel mechanics on the vascular sympathetic baroreflex: insights into ageing and blood pressure homeostasis Am J Physiol Heart Circ Physiol 319(2): H370-H376. PMID: 32648822
5. Coombs, G. B., Tremblay, J. C., Shkredova, D. A., Carr, J. M. J. R., **Wakeham, D. J.,** Patrician, A., & Ainslie, P. N. 2020. Distinct contributions of skin and core temperatures to flow-mediated dilation of the brachial artery following passive heating. *J Appl Physiol (1985).* PMID: 33119469

Articles under-review or in preparation

1. **Wakeham, D. J.,** Lord, R. N., Talbot, J. S., Lodge, F. M., Curry, B.A., Dawkins, T. G., Simpson, L. L. S., Pugh, C. J. A., Shave, R., & Moore, J. P. Chronic endurance exercise training does not modulate vascular neuroeffector transduction during sympathetic activation in male runners (*in preparation)*
2. **Wakeham, D. J.,** Lord, R. N., Talbot, J. S., Lodge, F. M., Curry, B.A., Dawkins, T. G., Simpson, L. L. S., Pugh, C. J. A., Shave, R., & Moore, J. P. The influence of habitual exercise on resting aortic haemodynamics in middle-aged and young men (*in preparation)*
3. **Wakeham, D. J.,** Lord, R. N., Talbot, J. S., Lodge, F. M., Curry, B.A., Dawkins, T. G., Simpson, L. L. S., Pugh, C. J. A., Shave, R., & Moore, J. P. No relationship between sympathetic vasomotor outflow and aortic systolic pressure augmentation in young or middle-aged men (*in preparation)*
4. **Wakeham, D. J.,** Pugh, C. J. A., Shave, R., & Moore, J. P. Insights into the effects of chronic habitual endurance exercise on cardiovascular regulation: a report on young identical twins and a father and son *(in preparation)*
5. Lord, R. N., **Wakeham, D. J.,** Talbot, J. S., Simpson, L. L. S., Curry, B.A., Pugh, C. J. A., Shave, R., & Moore, J. P. The influence of age and habitual exercise on left ventricular mechanics at rest and during whole body exercise (*in preparation)*

Appendix VII – Conference abstracts presented from data within this thesis

1. **Wakeham, D. J.,** Lord, R. N., Talbot, J. S., Curry, B.A., Brown, M., Dawkins, T. G., Shave, R., Moore, J. P. & Pugh, C. J. A. (2017) The effect of age and fitness on the sympathetic regulation of vascular function: Study protocol. Cardiff Metropolitan University Annual Academic Associate Poster Symposium 2017 held on May 4th, 2017. – Poster Communication and 3-minute thesis competition

Introduction Ageing is associated with increased sympathetic nerve activity and arterial stiffness, as well as decreased endothelial function. These maladaptations may, in part, explain the age-related increase in blood pressure observed in western society. Exercise training is known to reduce the effects of ageing on the cardiovascular system. However, it is currently unknown whether fitness status can influence the age-related changes to sympathetic nerve activity and the cardiovascular system. The aim of this study is to investigate the influence of fitness status on sympathetic regulation of vascular structure and function in young and middle-aged males.

Methods Forty-eight healthy males (12 per group) will be recruited into the following four groups: young untrained (aged 18-30yrs, <3hrs of exercise per week); young endurance trained runners (aged 18-30yrs, >60miles per week for >2yrs); middle-age untrained (aged 50-64yrs, <3hrs of exercise per week) and middle-age endurance trained runners (aged 50-64yrs, >30 miles per week for >10 yrs). All participants will be free of cardiovascular, metabolic, neurological and renal disease with no exercise contraindications. Participants will attend the laboratory on two separate occasions. Visit one involves an assessment of resting blood pressure before completing an incremental exercise test to exhaustion on a cycle ergometer. The second visit involves the assessment of sympathetic nerve activity (recorded from the peroneal nerve, via microneurography), as well as cardiac and vascular function (via high-resolution ultrasonography) at rest. The heart and brachial artery will then be imaged simultaneously, as well as continuous acquisition of beat-by-beat blood pressure (finger photoplethysmography) and sympathetic nerve activity, in response to the cold pressor test and static hand grip exercise.

Results The primary outcome of this study is to characterise the effect of age and fitness on sympathetic nerve activity and cardiovascular function at rest and during sympathoexcitatory stimuli. This study will provide further insight into the integrative mechanisms that underpin blood pressure control and lead to a better understanding of healthy cardiovascular ageing.

NB: Winner of the 3-minute thesis competition

2. **Wakeham, D. J.,** Lord, R. N., Talbot, J. S., Curry, B.A., Dawkins, T. G., Simpson, L. L. S., Shave, R., Moore, J. P. & Pugh, C. J. A. (2018) Elevated sympathetic vasoconstrictor drive to skeletal muscle in middle-aged male runners. Okanagan Cardiovascular and Respiratory Symposium held at Silver Start Mountain Resort, Canada, held between 15th-17th March, 2018. – Oral communication

Regular endurance exercise offsets age-related arterial stiffening, however, it is unclear whether endurance training influences age-related increases in sympathetic vasoconstrictor drive to skeletal muscle (MSNA). Therefore, we investigated the effect of aging and endurance training on MSNA in healthy, normotensive males. Mean arterial pressure (MAP; photoplethysmography), arterial stiffness (carotid-femoral pulse wave velocity; PWV) and MSNA (microneurography) were assessed at rest in four groups; 10 young (23±3 years) and 10 middle-aged (53±2 years) nonrunners and 13 young (22±3 years) and 13 middle-aged (57±5 years) runners. MAP was higher in middle-aged runners in comparison to young runners (93±6 *vs* 84±5 mmHg, P=0.004) but was not different between middle-aged and young nonrunners (95±9 *vs* 90±9 mmHg, P=0.12). MAP was lower in young runners compared to young nonrunners (P=0.07) with no difference between the middle-aged groups (P=0.12). PWV was higher in middle-aged compared to young men irrespective of training status (runners: 7.8±0.8 *vs* 6.2±0.5 m.s^{-1}, P<0.001: nonrunners: 8.6±0.9 *vs* 6.8±0.7 m.s^{-1}, P<0.001) and lower in runners compared to age-matched nonrunners (young, P=0.07; middle-aged, P<0.001). MSNA was higher in middle-aged compared to young men, again irrespective of training status (nonrunners: 50±23 *vs* 27±12 bursts·100 heartbeats^{-1}, P=0.009; runners: 72±15 *vs* 36±22 bursts·100 heartbeats^{-1}, P<0.001). MSNA was not different between young runners and nonrunners (P=0.239), whereas, MSNA was higher in middle-aged runners compared to nonrunners (P=0.006). High MSNA is related to increased cardiovascular risk, however, we propose that in middle-aged runners, with lower arterial stiffness, high MSNA is a necessary physiological adaptation required to maintain blood pressure.

NB: Runner up in the 10-minute oral presentation competition in the Vascular and Autonomic Physiology session

3. **Wakeham, D. J.,** Lord, R. N., Talbot, J. S., Curry, B.A., Dawkins, T. G., Simpson, L. L. S., Shave, R., Moore, J. P. & Pugh, C. J. A. (2018) Lifelong endurance training modifies sympathetic baroreflex control of neural vasoconstrictor tone at rest. European Early Career Physiologists Symposium held at the Queen Elizabeth II Conference Centre, London, held 13th September 2018. – Poster Communication

Changes in blood pressure during middle age are associated with elevated muscle sympathetic nerve activity (MSNA), alterations in the sympathetic baroreflex, and stiffening of the barosensitive aortic and carotid arteries. It is well known that lifelong endurance-training attenuates the age-related increase in arterial stiffness; however, the impact on sympathetic baroreflex function (i.e. operating pressure, set point and gain) is less clear. We examined sympathetic baroreflex control of MSNA in age-matched recreationally active middle-aged men (n = 10: age, 53 [52-55] years), and determined whether this differed from that of lifelong (29 [28-40] years of training) endurance-trained runners (n = 13: age, 57 [54-59] years). Indices of spontaneous sympathetic baroreflex function were determined from beat-by-beat changes in diastolic blood pressure (DBP, photoplethysmography) and the corresponding MSNA (microneurography) during six minutes of supine rest. Aortic (Carotid-Femoral Pulse Wave Velocity via applanation tonometry) and carotid (β Stiffness Index via B-mode ultrasound) stiffness were also measured. Data presented are means [95% confidence intervals] and were compared using independent t-tests. Resting heart rate (electrocardiogram) was lower in runners (43 [38-47] vs 56 [49-62] bpm, $P < 0.01$) but mean brachial artery pressure was not different (93 [90-96] vs 95 [89-101] mmHg, $P = 0.54$). Aortic (6.8 [6.2-7.3] vs 7.5 [6.9-8.1] m/s^{-1}, $P = 0.02$) and carotid artery stiffness (4.06 [3.25-4.88] vs 5.06 [3.94-6.19] AU, $P = 0.04$) were also lower in runners. Spontaneous sympathetic baroreflex operating DBP was similar (77 [74-81] vs 79 [73-85] mmHg, $P = 0.57$), as was MSNA burst frequency (31 [27-34] vs 28 [19-38] $bursts/min^{-1}$, $P = 0.55$) and sympathetic baroreflex gain (i.e. responsiveness) (-6.1 [-8.0 - -4.1] vs -6.9 [-9.8 - -4.0] $\%/mmHg^{-1}$, $P = 0.55$). However, the corresponding MSNA burst incidence (i.e.' set point') was higher in runners (72 [63-81] vs 50 [33-66] $bursts/100hb^{-1}$, $P < 0.01$). In conclusion, lifelong endurance runners had lower stiffness in barosensitive arteries, and an upward setting of the sympathetic baroreflex; meaning there was a higher likelihood of a burst of MSNA for a similar operating DBP. This was evident despite no difference in the responsiveness of MSNA to spontaneous fluctuations in blood pressure. We suggest that the greater MSNA burst incidence in lifelong runners is necessary to maintain resting blood pressure in the context of the training-induced bradycardia, skeletal muscle angiogenesis and possible reductions in α-adrenergic sensitivity. Investigation is required to explore the mechanisms that underpin modified sympathetic baroreflex function following lifelong endurance training.

NB: Winner of the 2018 European Early Career Physiologists Symposium Poster Competition

4. **Wakeham, D. J.,** Lord, R. N., Talbot, J. S., Curry, B.A., Dawkins, T. G., Simpson, L. L. S., Shave, R., Moore, J. P. & Pugh, C. J. A. (2018) Lifelong endurance training modifies sympathetic baroreflex control of neural vasoconstrictor tone at rest. Europhysiology held at the Queen Elizabeth II Conference Centre, London, held 14th-16th September 2018 – Poster Communication

The same abstract was submitted for the above early career meeting as this, the main Europhysiology meeting.

NB: Winner of the Early Career Researcher Poster competition for Human & Exercise Physiology at Europhysiology 2018

5. **Wakeham, D. J.,** Lord, R. N., Talbot, J. S., Lodge., F. M., Curry, B.A., Dawkins, T. G., Simpson, L. L. S., Shave, R., Pugh, C. J. A. & Moore, J. P. (2019) Sympathetic neurovascular transduction during static handgrip exercise and post-exercise muscle ischemia: No influence of habitual exercise or healthy ageing. Canadian Society of Exercise Physiology Conference, Delta Grand Resort in Kelowna, Canada between 6th-9th November 2019 – Oral communication

It is unclear whether chronic habitual endurance exercise training influences the response of the arterial vasculature to increases in muscle sympathetic nerve activity (MSNA). Therefore, exercise, a known activator of MSNA, was used to assess sympathetic neurovascular transduction (SNVT) in 22 middle-aged (50-63 years, n=13 runners) and 23 young (19-30 years, n=13 runners) normotensive male runners and nonrunners. Continuous blood pressure (photoplethysmography), MSNA (peroneal microneurography) and contralateral limb forearm blood flow (Doppler ultrasonography) responses were recorded during fatiguing static handgrip exercise (SHG; 35% of maximum) and isolated muscle metaboreflex activation (via post-exercise muscle ischaemia; PEMI). The slopes of the relationships between MSNA total activity and forearm vascular conductance and resistance were used to index SNVT during each stimulus. Furthermore, baseline arterial stiffness was characterised by aortic (aPWV) and brachial pulse wave velocity (bPWV). Habitual exercise did not affect SNVT in either age category. Therefore, the runner and non-runner data were pooled to assess the effects of ageing. The increase in blood pressure (P<0.001) and MSNA total activity (P=0.018) were greater in middle-aged men during SHG, but not PEMI. SNVT was not different. Notably, the change in systolic blood pressure was significantly related to aPWV (r=0.643, P<0.001) and bPWV (r=0.342, P=0.023) during SHG only. In conclusion, neither habitual endurance exercise nor healthy ageing influence the ability of the arterial vasculature to respond to increases in MSNA during exercise. However, arterial stiffening and a greater increase in MSNA likely contribute to an ageing-related increase in the blood pressure response to fatiguing SHG.