THE ACUTE AND CHRONIC EFFECTS OF ISOMETRIC EXERCISE ON HAEMODYNAMIC, AUTONOMIC AND CARDIAC FUNCTION IN A PRE-HYPERTENSIVE POPULATION

by
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Thesis submitted
for the Degree of Doctor of Philosophy

2017

ABSTRACT

Raised blood pressure (BP) remains the leading modifiable risk factor for cardiovascular disease morbidity and mortality globally. As such, primary prevention strategies are required to improve risk factors to prevent the development of hypertension (HTN). Isometric exercise training (IET) is becoming an established intervention for reducing resting BP. However, few studies have investigated the effects of IET in a population at increased risk of developing HTN. Therefore, this thesis examined the effects of IET, using a novel home-based wall squat intervention, in a pre-hypertensive male population. Specifically, the thesis aimed to explore the potential mechanism/s responsible for improved BP control using an acute isometric exercise (IE) stimuli and a four-week IET intervention. Firstly, acute IE was shown to elicit a step-wise increase in BP, heart rate and cardiac output and associated increase in sympathetic activity. In the immediate recovery period, there was a hypotensive response, which was associated with parasympathetic activation, increased baroreceptor reflex control and reduced peripheral vascular resistance. The hypotensive response was also associated with improved indices of cardiac function, including a reduced estimated filling pressure. Four weeks of IET was shown to significantly reduce resting and ambulatory BP. Improved autonomic cardiovascular control, with increased parasympathetic over sympathetic activity, greater baroreceptor reflex sensitivity and reduced peripheral vascular resistance potentially mediated the decreased BP. A reduction in plasma interleukin-6 and asymmetric dimethylarginine suggests an anti-inflammatory response and improved vascular function, respectively, following IET. Finally, improved myocardial diastolic function suggests positive cardiac adaptations in response to BP reductions. Collectively, the findings of this thesis highlight potential mechanistic pathways for improved BP control in a pre-hypertensive population and demonstrates wider cardiovascular benefits of IET beyond BP reductions, which are important observations for risk reduction in this population.

Work from this thesis has contributed to the following publications:

Taylor, K.A., Wiles, J.D., Coleman, D.A., Sharma, R., and O'Driscoll, JM. (2016) Acute cardiac autonomic responses to an incremental isometric exercise test in pre-hypertensive males. In: European College of Sport Science, 6-9th July, 2016, Vienna, Austria.

Wiles, J.D., Taylor, K.A., Coleman, D.A. and O'Driscoll, JM. (2017). Blood pressure responses to isometric exercise; safety considerations for exercise prescription. In: American College of Sports Medicine 64th Annual Meeting and Congress on Exercise is Medicine, 30th May-3rd June, 2017, Denver, Colorado, US. *Medicine and Science in Sports and Exercise*. 49:407.

Taylor, K.A., Wiles, J.D., Coleman, D.A. and O'Driscoll, JM. (2017) Ambulatory blood pressure responses to home-based isometric exercise training in pre-hypertensive males. In: American College of Sports Medicine 64th Annual Meeting and Congress on Exercise is Medicine, 30th May-3rd June, 2017, Denver, Colorado, US. *Medicine and Science in Sports and Exercise*. 49:409.

Taylor, K.A., Wiles, J.D., Coleman, D.A., Sharma, R., and O'Driscoll, JM. (2017) Continuous cardiac autonomic and haemodynamic responses to isometric exercise. *Medicine and Science in Sports and Exercise*. 49(8). pp 1511-1519.

O'Driscoll, J.M., Taylor, K.A., Wiles, J.D., Coleman, D.A. and Sharma, R. (2017) Acute cardiac functional and mechanical responses to isometric exercise in pre-hypertensive males. *Physiological Reports*. 5(7). e13236.

Acknowledgements

I would like to thank the Section of Sport and Exercise Sciences at Canterbury Christ Church University for providing me with the opportunity to conduct this research.

I would also like to take this opportunity to express my sincere gratitude to the following people, without whom this research would not have been possible:

To my supervisor Dr Jamie O'Driscoll, your passion for physiology is contagious and your mentorship and encouragement over the course of my studies have made my experience a thoroughly enjoyable one. I am indebted to you for the immeasurable time and effort you have invested, in order to train me and provide me with skills and research experience, which extend beyond the scope of this work. Your perfectionism and attention for detail will never be unappreciated, and I could not have asked for better supervision.

To my supervisor Dr Jonathan Wiles, for always being on hand to provide valuable feedback. Your experience and expertise in this field of isometric research has significantly aided the progression of this work.

To Dr Damian Coleman, thank you for your valued guidance with data analysis.

To my colleagues, who have become great friends through this process; my fellow PhD student James Scales, thank you for being on hand to lend your body to research practice, for modelling, and to escape for coffee or beer when needed. I have very much enjoyed the healthy competitive atmosphere, which has led to the timely submission of both of our theses. To Dr Samantha Saunders and Dr Lucinda Howland, for your encouragement and advice from a position of experience, and to the lab team, Dan Stretch, Dan Tolhurst and Sam Todd for always being on hand to provide fantastic technical support.

To my fiancé, James, thank you for your patience throughout this process and for making my priorities your own. You have been a constant source of support and encouragement. I owe you some weekends!

To my parents, Helen and Adrian, for your unwavering support in everything I do. You will both never cease to amaze me and are the inspiration for everything I have ever achieved.

Finally, to the participants of these studies; this research could not have happened without you. Thank you for your commitment and diligent participation in your training. My appreciation is limitless.

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ABBREVIATIONS

A	Peak late filling velocity
A’	Peak late diastolic mitral annular tissue velocity
ABP	Ambulatory blood pressure
ACE	Angiotensin converting enzyme
ADMA	Asymmetric dimethylarginine
ANOVA	Analysis of variance
ANP	Atrial naturietic peptide
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
BNP	Brain naturietic peptide
BP	Blood pressure
BPV	Blood pressure variability
BRS	Baroreceptor sensitivity
BSA	Body surface area
Ca2+	Calcium
CAD	Coronary artery disease
CHF	Congestive heart failure
CI	Confidence interval
CoV	Coefficient of variation
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
dBP	Diastolic blood pressure
E	Peak early filling velocity
E’	Peak early diastolic mitral annular tissue velocity
ECG	Electrocardiogram
EDHF	Endothelium derived hyperpolarising factor
EDV	End diastolic volume
EMG	Electromyography
eNOS	Endothelial nitric oxide synthase
ESV	End systolic volume
ET	Ejection time
H_1	Histamine 1 receptor
H_2	Histamine 2 receptor
HF	High frequency
HFnu	High frequency normalised units
HR	Heart rate
HRV	Heart rate variability
Hs-CRP	High sensitivity C-Reactive protein
HTN	Hypertension
ICAM	Intercellular adhesion molecule
ICG	Impedance cardiography
IE	Isometric exercise

IET	Isometric exercise training
IHD	Ischaemic heart disease
IHG	Isometric handgrip
IL-	Interleukin-
ILT	Isometric leg training
IVCT	Isovolumetric contraction time
IVRT	Isovolumetric relaxation time
IVSd	Interventricular septal diameter
LA	Left atrium
LF	Low frequency
LFnu	Low frequency normalised units
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVIDd	Left ventricular internal diameter diastole
LVIDs	Left ventricular internal diameter systole
LVMI	Left ventricular mass index
LVPWd	Left ventricular posterior wall thickness diastole
mBP	Mean blood pressure
MI	Myocardial infarction
MPI	Myocardial performance index
MSNA	Muscle sympathetic nerve activity
MV	Mitral valve
MVC	Maximal voluntary contraction
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
NTN	Normotension
O_2	Oxygen
PA	Physical activity
PI	Pulse interval
PO_2	Partial pressure oxygen
PP	Pulse pressure
PSD	Power spectral density
$\dot{Q}$	Cardiac output
$\dot{Q}I$	Cardiac index
RAAS	Renin angiotensin aldosterone system
RPD	Rate of perceived discomfort
RPP	Rate pressure product
RV	Right ventricle
RWT	Relative wall thickness
sBP	Systolic blood pressure
SD	Standard deviation
SI	Stroke index

SV	Stroke volume
TFM	Task Force® Monitor
TNF-a	Tumor necrosis factor alpha
TPR	Total peripheral resistance
TPRI	Total peripheral resistance index
TTE	Transthoracic echocardiography
VCAM	Vascular cell adhesion molecule
VLF	Very low frequency

CHAPTER 1:

Introduction

Arterial hypertension (HTN) remains the leading risk factor for cardiovascular disease morbidity and mortality, which in 2013 accounted for 10.4 million premature deaths and 208.1 million disability adjusted life years globally (Forouzanfar *et al.*, 2016). Despite this knowledge, HTN remains highly prevalent, affecting more than 25% of the population, with projections that by 2025, one in three adults will be classified as hypertensive (Kearney *et al.*, 2005). The projections are largely based on HTN being under-recognised and under-controlled (Joffres *et al.*, 2013). Recent evidence has suggested a reduced risk of mortality from treating HTN to a target blood pressure (BP) level towards the normotensive range (Wright *et al.*, 2015; Xie *et al.*, 2016). Furthermore, a reform in clinical practice towards prevention and primary care, has resulted in a greater emphasis on identifying those at increased risk of developing HTN (Naylor *et al.*, 2015). Pre-hypertension (pre-HTN) is considered to precede the development of HTN and is associated with a considerably increased risk of cardiovascular disease (CVD) compared to populations with optimal BP (Vasan *et al.*, 2001a; Liszka *et al.*, 2005; Leitschuh *et al.*, 1991; Guo *et al.*, 2013; Huang *et al.*, 2013). At present, approximately 30% of the population are considered to be pre-hypertensive (Gupta *et al.*, 2010; Wang and Wang, 2004). This population has therefore been recognised as a target to reduce BP in order to reduce the incidence of HTN and associated CVD morbidity and mortality. As such, non-pharmacological interventions are frequently recommended with the aim of reducing BP towards optimal levels.

Growing evidence supports isometric exercise training (IET) as a non-pharmacological intervention to reduce resting BP. The short time commitment, minimal equipment requirements and the ability to perform IET in the home, provides a sound alternative exercise modality to traditional modes of physical activity (PA) for the management of BP. Importantly, IET may reduce perceived barriers to PA, such as lack of time, which has been regularly reported (Salmon, 2001). Historically, research by Kiveloff and Huber (1971) noted the benefits of short duration isometric contractions of various muscle groups on reducing resting systolic BP (sBP) and diastolic BP (dBP) over a period of 5-8 weeks, and Buck and Donner (1985) observed that regular exposure to moderate or heavy isometric stimuli within the work place was related to a reduced incidence of HTN. Since these early observations, a number of studies have been performed to assess the effects of different modes of IET with resting BP the primary

outcome variable (see Chapter 2, table 2.2). A protocol of 4 x 2-minute isometric exercise (IE) bouts to form one session of IE, proposed by Wiley *et al.* (1992a) has been adopted across this field of research and a number of studies exist, which appear to advocate the use of IE as a method of reducing BP, despite undefined mechanistic pathways for the observed physiological adaptations.

Short-term IET interventions, prescribed using either handgrip training or leg training protocols, have demonstrated BP reductions similar to those induced by aerobic exercise training (Borjesson *et al.*, 2016), which is already widely recommended as a non-pharmacological intervention strategy to reduce resting BP (Pescatello *et al.*, 2004a). Findings from meta-analyses of IET studies have reported mean BP reductions ranging from 5.2-10.9 mmHg in sBP and 3.9-6.2 mmHg in dBP (Inder *et al.*, 2016; Cornelissen and Smart, 2013), while individual studies have been associated with reductions of 13 mmHg and 15 mmHg in sBP and dBP, respectively (Wiley *et al.*, 1992a). It has been suggested that BP reductions may be due to changes in autonomic regulation (Millar *et al.*, 2013; Taylor *et al.*, 2003), and improvements in vascular function (McGowan *et al.*, 2006b; Badrov *et al.*, 2016), however findings are limited and inconclusive.

Investigating the acute cardiac autonomic and haemodynamic responses continuously during IE, as well as selected parameters pre and post multiple isometric contractions, comprising a session of IE, may help to elucidate potential mechanisms for reductions in resting BP observed following IET. Furthermore, despite the efficacy of IET to reduce resting BP, few studies have explored wider cardiovascular adaptations that may be associated with reductions in resting BP.

1.2 Thesis overview

The purpose of this thesis was to investigate the acute and chronic physiological effects of isometric wall squat exercise, in order to ascertain potential mechanistic pathways for BP reductions observed through IET. All participants in this thesis were pre-hypertensive males, with no known co-morbidities. Participants were not taking

any medication, were aged between 30 and 65 years and were considered physically inactive based on weekly participation in less than 150-minutes of physical activity. This thesis is divided into Chapters, which are linked by the common theme of isometric wall squat exercise. Initially, in Chapter 2 of this thesis, a full review of literature explores the key physiological (acute and chronic) concepts of IE and the existing evidence of BP reduction following isometric muscle contraction. In particular, the role of IE and mechanistic pathways for reduced BP is discussed. All methodology common to experimental Chapters is described in Chapter 3: General methods, while specific details are provided in individual study Chapters. Although the same isometric wall squat exercise was used throughout this thesis, the acute responses are investigated in Chapters 4 and 5, and use a single isometric session as the physiological stimulus, which is referred to as isometric exercise (IE). The chronic responses to an intervention programme of isometric exercise training are investigated in Chapters 6, 7 and 8, and is referred to as IET. Chapter 9 presents an overall integrated discussion of the experimental findings. Future research directions and limitations for all experimental Chapters are collectively addressed in Chapter 9.

CHAPTER 2:

Review of Literature

2.1 Introduction

Cardiovascular disease (CVD) is the leading preventable cause of morbidity and mortality worldwide. It is estimated to cause over 31% of all deaths (WHO, 2013) and is predicted to remain the leading cause of death through to 2020 (Murray and Lopez, 1997). Coronary artery disease (CAD), ischaemic heart disease (IHD), myocardial infarction (MI), congestive heart failure (CHF), peripheral artery disease and cerebrovascular accidents (CVA), are all diseases of the cardiovascular system and are associated with accelerated CVD mortality. Epidemiological research has evolved our understanding of the aetiology of CVD through consistency of observed associations and biological probability in experimental studies. However, CVD causation is complex and still not completely understood.

Susceptibility to CVD increases through various non-modifiable risk factors including genetics, family history, ethnicity, sex and age (Sharman and Stowasser, 2009). There are also a number of modifiable risk factors that predict the development of CVD; including smoking, excessive alcohol consumption, hypercholesterolaemia, obesity, other preventable diseases such as diabetes and renal disease and increasingly physical inactivity (Wong *et al.*, 2001). Hypertension, which is characterised by a sustained elevation in arterial BP ≥140 mmHg and/or ≥90 mmHg in systolic and diastolic BP respectively, is the leading attributable risk factor for increased risk of CVD mortality (Mancia *et al.*, 2013).

Physiologically, BP is the hydrostatic pressure that blood exerts on the blood vessels as it is transported through the systemic and pulmonary circulation. Systolic blood pressure (sBP) describes the pressure during myocardial contraction, whilst diastolic blood pressure (dBP) refers to the arterial pressure during myocardial relaxation and the refilling phase of the cardiac cycle.

Internationally recognised guidelines classify optimal sBP as <120 mmHg and optimal dBP as <80 mmHg (WHO, 2007; Mancia *et al.,* 2013; Public Health England 2014). A sBP of ≥140 mmHg and/or dBP of ≥90 mmHg remains the most commonly used criteria for the diagnosis of HTN. Internationally recognised BP classifications are shown in Table 2.1.

Table 2.1: Blood pressure classifications (Chobanian *et al.*, 2003).

Classification	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)
Optimal	<120	<80
Pre-Hypertension	120-139	80-89
Stage 1 Hypertension	140-159	90-99
Stage 2 Hypertension	>160	>100

New guidelines for the 'Prevention, Detection, Evaluation and Management of High Blood Pressure in Adults' (Whelton *et al,* 2017) have been recently published. The new recommendations are yet to be universally adopted in clinical practice, however these have removed the former classification of pre-hypertension, re-defining BP from 120-129 mmHg (sBP) and <80 mmHg as 'elevated' and lowering the classification of 'Stage 1 HTN' to 130-139 mmHg (sBP) and 80-89 mmHg (dBP). The purpose of these changes is to encourage the earlier prescription of anti-hypertensive treatment interventions in order to reduce the subsequent and progressive risk of CVD, as well as the cumulative physiological decline that occurs from living with raised BP. Indeed it has long been understood that BP above the optimal values of 115mmHg is associated with an increased risk of CVD compared with optimal BP (Lewington *et al,* 2002). However, for the purpose of this thesis, and given that the new recommendations are yet to be adopted by health treatment guidelines, and published research to date does not refer to these new classifications, the terminology 'pre-hypertension' and associated BP values (Chobanian *et al,* 2003; Table 2.1) will be used throughout this thesis.

If the recently recommended guidelines are adopted, then the prevalence of diagnosed hypertension is set to drastically increase, however at present hypertension affects approximately 35-40% of the population of Europe (Mancia *et al.*, 2013) and >50% of the UK population >60 years old (NICE, 2011), demonstrating that the prevalence of HTN is common and strongly influenced by age. Indeed, it is common for sBP to

increase progressively with age (Franklin *et al.*, 1997a), often due to atherosclerosis within the arteries, resulting in decreased luminal diameter, and reduced arterial elasticity (Mancia *et al.*, 2013; Wilson *et al.*, 1998).

Elevation of sBP is strongly associated with an increase in cardiovascular events (Weber *et al.*, 2013). Indeed, the risk of CVD doubles for each 20 mmHg and 10 mmHg increase in sBP and dBP respectively above optimum values (WHO, 2013) with a clear link between sBP >140 mmHg and increased cardiovascular morbidity (Franklin *et al.*, 1997a). Conversely, an isolated reduction in sBP by 5 mmHg has been demonstrated to reduce the risk of CAD mortality by 9%, the risk of CVA mortality by 14% and the risk of all-cause mortality by 7% (Stamler *et al.*, 1989). In addition, a 5 mmHg reduction in dBP could reduce the risk of CAD by 29% and CVA by 46% (Macmahon *et al.*, 1990). However, up to 60% of individuals with HTN are unaware of their condition and are therefore failing to manage their BP to within optimal levels (Public Health England, 2014). Despite the condition being widely under diagnosed, HTN remains the most frequently encountered condition by general practitioners (Sharman and Stowasser, 2009), with varying degrees of success, as HTN as a whole accounts for 57 million disability associated life years (WHO, 2010a).

Various effective pharmacological treatment options have been shown to successfully reduce BP and to combat HTN; however in addition to side effects, these usually involve lifelong adherence and have a high socio-economic cost (Heidenreich *et al.*, 2011). Greater than optimum BP accounts for over 10% of the worlds healthcare expenditure and up to 25% of European healthcare costs (Gaziano *et al.*, 2009). In the UK in 2006, approximately £1 billion was spent on anti-hypertensive drug therapies (NICE, 2011) while in the United States in 2010 this cost reached $46.4 billion (Go *et al.*, 2014). Widespread reductions in BP into optimal ranges across the UK, could save £850m over 10 years in health and social care costs associated with HTN (Public Health England, 2014). It is accepted that lifestyle factors directly contribute to incidences of HTN; as such, there is significant justification to explore new and effective lifestyle interventions.

As previously indicated, physical inactivity has been recognised as a predictor of CVD and a precursor to the development of HTN (Prasad and Das, 2009). Current guidelines

recommend participation in moderate intensity physical activity for at least 30 minutes, 5 days per week (WHO, 2010a). Physical inactivity, the persistent inability to achieve this recommendation, is a major risk factor contributing to the widespread development of HTN (Pescatello *et al.*, 2004a). A review by Public Health England (2014) further supports the well-established relationship between habitual physical inactivity and higher than optimal BP. The risk of developing HTN is reduced by up to 52% in people who are able to maintain cardiovascular fitness through regular physical activity (Blair *et al.*, 1984). Furthermore, good physical fitness has been associated with lower risk of all-cause mortality and cardiovascular events (Kodama *et al.*, 2009).

2.2 Pre-hypertension

Given the worldwide prevalence of HTN, recent research has focused attention on preventing the pathogenesis of HTN in those at risk of developing high BP and those with higher than optimal BP. Readings in-between optimum values and a diagnosis of Stage 1 HTN (120-139 mmHg systolic and 80-89 mmHg diastolic) are very common and are still currently classified as 'normal'. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) (Chobanian *et al.*, 2003) termed this level of BP 'pre-hypertension' (pre-HTN) and highlighted it as 'an area of interest for further research and innovation' (Public Health England, 2014, p16). Further epidemiological data shows that 93% of those with pre-hypertensive BP present with at least one additional CVD risk factor (Liszka *et al.*, 2005). The same data concluded that pre-HTN was a predictor of 9.1% of all deaths, the greatest attributable number of which occurred in males aged 45-64, or persons aged 65-74 (Russell *et al.*, 2004).

Research suggests that individuals classified as pre-hypertensive are more likely to become hypertensive than those with optimal BP (Vasan, 2001). Furthermore, patients with BP in the upper range of pre-HTN (130- 139 mmHg sBP and 85-89 mmHg dBP) are twice as likely as those in the lower range of pre-HTN (120-129 mmHg sBP and 80-84 mmHg dBP) to develop HTN (Vasan *et al.*, 2001b) suggesting a linear increase in overall risk as BP increases. In support, Liszka *et al.* (2005) also reported the upper

range of systolic pre-HTN (130-139 mmHg) to be a greater predictor of mortality than lower range systolic pre-HTN.

As well as being a precursor for the development of HTN, pre-HTN is also associated with increased rates of MI, CAD and increased risk of CVD (Qureshi *et al.*, 2005b). Blood pressure reductions in pre-hypertensive populations may therefore induce considerable reductions in cardiovascular risk. A meta-analysis, comprising results from 61 observational studies, including over 1 million participants and 12.7 million person years, suggested that a 10 mmHg reduction in sBP and a 5 mmHg reduction in dBP is associated with a 40% reduced risk of CVA mortality and a 30% reduced risk of IHD mortality down to BP >115 mmHg (sBP) and >75 mmHg (dBP). Moreover, a sBP reduction of just 2 mmHg could reduce the risk of CVA mortality by 10% and the risk of IHD by 7% in a middle aged population without CVD (Lewington *et al.*, 2002). Targeting pre-HTN through lifestyle modification has the potential to reverse or prevent pathological BP increases with age and reduce overall cardiovascular risk.

2.3 Cardiovascular System – Physiological dysfunction associated with pre-hypertension

Many lifestyle risk factors and genetic factors are understood to be associated with essential HTN (Poulter *et al.*, 2015), however no single physiological mechanism for raised BP has been defined. Increases in BP are directly associated with the dysregulation of cardiovascular control pathways. Such pathways are independently associated with an increased risk of CVD and premature mortality, and will be discussed in this section. However, it remains unclear as to whether any, or all, of such mechanisms are the cause or effect of raised BP. Furthermore, essential HTN, which makes up 95% of all HTN, is considered a heterogeneous disorder and different patients have different causal factors that lead to high BP (Carretero and Oparil, 2000).

The cardiovascular system is a continuous closed-loop organ system made up of the myocardium, the pulmonary and systemic circulatory systems and blood as a transport medium. The purpose of this highly responsive system is to maintain homeostasis within the body, however when BP increases from normotension (NTN) to pre-HTN and HTN, progressive dysregulation of the cardiovascular system occurs. There is no

specific level of BP where cardiovascular and end organ complications start to occur; thus the definition of HTN is arbitrary but needed for practical reasons in patient assessment and treatment (Carretero and Oparil, 2000). There is a well-reported positive and continuous correlation between BP and the risk of CVD, even in NTN (Lewington *et al.*, 2002), suggesting a continuum between raised BP and increased cardiovascular risk, and the potential dysregulation of cardiovascular control systems, as demonstrated in Figure 2.1. Such systems may contribute to the pathogenesis of HTN, or dysregulation may occur as a result of increases in BP, and as such may be important mechanistic pathways when studying possible reductions in BP. It is well understood that those with pre-HTN have a heightened susceptibility to the development of established HTN (Vasan *et al.*, 2001b) as well as a markedly increased risk of the development of CVD (Vasan *et al.*, 2001a), yet the physiological mechanisms for raised BP in pre-HTN are unclear.

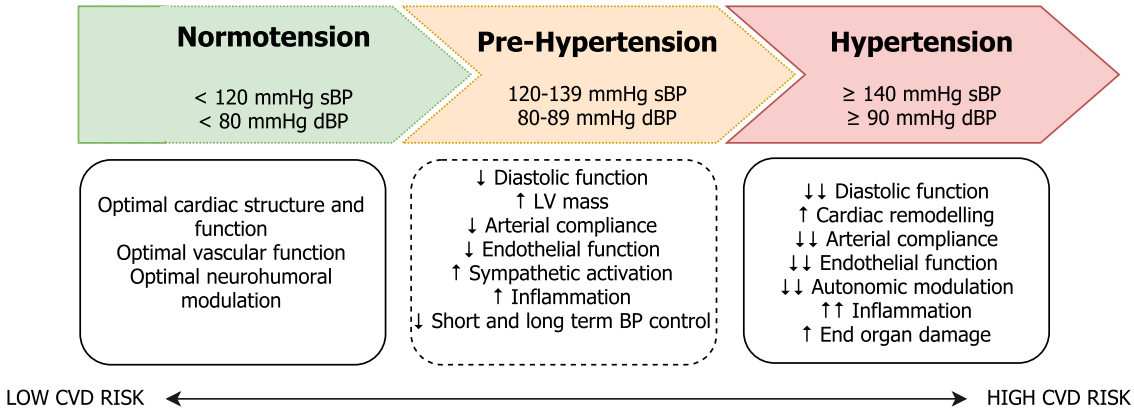

Figure 2.1. Cardiovascular dysregulation associated with increases in BP and the directional increase in CVD risk. Note: ↓ represents the potential deterioration of the physiological parameter; ↓↓ represents the potential worsening of the physiological parameter; ↑ represents the potential increase in the physiological parameter; ↑↑ represents the potential worsened increase in the physiological parameter.

2.3.1 Adverse cardiac function and performance

The average human cardiac cycle lasts 0.9 s, at a heart rate (HR) of 67 b·min^{-1}, two thirds of which is passive filling during ventricular diastole after which point atrial contraction actively increases ventricular filling by up to 20%. The active atrial contribution increases with age and HR, such as during exercise, as there is less time for passive filling between beats (Strait and Lakatta, 2012; Dai *et al.*, 2012). When HR is raised, all stages of the cardiac cycle are shortened as the whole cycle can last for as little as 0.3 s (at a HR of 180 b·min^{-1}). Ventricular systole shortens moderately to ~0.2 s, therefore diastolic filling shortens substantially. Hypertension and pre-HTN are associated with increases in HR (King et al, 2006), as well as prolonged isovolumetric contraction time (IVCT) (Drukteinis *et al.*, 2007) when compared with NTN. Indeed the Bowditch staircase effect (Opie, 2004) highlights that an increase in HR also increases myocardial oxygen demand, and enhances the force of ventricular contraction and lengthens filling intervals. This can lead to increased myocardial tension and contribute to heart failure. Cardiac time intervals have been shown to independently predict future cardiovascular events (Biering-Sorensen *et al.*, 2015). In addition, myocardial performance index (MPI) combines cardiac time intervals to provide a sensitive measure to account for both systolic and diastolic function (Masugata *et al,* 2009), which may be of value in healthy patients who do not display abnormalities of traditional structural and echocardiographic measures.

The LV exerts a mean pressure of 120 mmHg while the mean pressure of the RV is only 25 mmHg (Levick, 2003). However an increase in BP is associated with pathological remodelling of the myocardium and can effect cardiac performance in HTN (Mihl *et al.*, 2008). Adverse remodelling, such as left ventricular hypertrophy (LVH) refers to thickening of the walls of the LV, which can lead to an overall increase in LV mass (Vakili *et al.*, 2001). The addition of sarcomeres in parallel causes the internal volume of the chamber to be reduced, and the thickening of the walls allows for a compensatory increase in contractile force to occur (Levick, 2003). This occurs in response to chronic pressure overload on the heart, which will affect cardiac performance through reductions in stretch and contractility. Stroke volume (SV) is dependent on the interplay of diastolic stretch (pre-load), contractility and arterial pressure (after-load), therefore reduced stretch, contractility and volume will cause a

reduction in SV. In order to maintain sufficient cardiac output (Q̇), a compensatory increase in HR and increase in inotropy occurs via sympathetic activation of the ANS. The ANS is capable of modulating the rate of contraction (chronotropy), strength of contraction (inotropy) rate of conduction (dromotropy) and rate of relaxation (lusitropy) through central and peripheral feedback mechanisms. According to Starlings Law of the heart, SV will be greater with greater end diastolic volume (EDV). Increased myocardial contractility, preload and afterload (EDV-ESV) cause an increase in SV and subsequently Q̇ (Davis et al, 2012). Indeed, in addition to raised BP, SV is raised in HTN (McEniery *et al.*, 2005) causing a greater stroke work.

Eccentric LV remodelling occurs when the LV mass increases overall while the wall thickness remains the same (Vakili *et al.*, 2001). The causes of differing geometric patterns of remodelling are poorly understood; however, it is thought that eccentric adaptations may be caused by an increase in blood volume (volume overload) while concentric hypertrophy is caused by a combination of pressure overload and volume overload (Opie, 2004). Concentric hypertrophy carries the worst prognosis, as the thickened ventricle carries a greater risk of myocardial ischaemia.

Cardiac remodelling is strongly associated with incidences of HTN. Hammond *et al.* (1986) found that 20% of patients presenting with mild HTN and 50% of patients presenting with severe HTN displayed LV hypertrophy assessed by echocardiography. Left ventricular hypertrophy has been associated repeatedly with an increased risk of CVAs, cardiovascular death, and all-cause mortality (Levy *et al.*, 1990b; Kahn *et al.*, 1996; Koren *et al.*, 1991). A meta-analysis of 20 studies investigating the prognostic implications of LV hypertrophy (n=48,545) found that baseline diagnosis using echocadiography revealed more cases of LV hypertrophy than studies that used electrocardiogram (ECG). This indicates a greater sensitivity of the echocardiogram for measuring LV hypertrophy. It has been suggested that LV mass and geometry measured via transthoracic echocardiography is able to stratify risk more strongly than measures of BP and other risk factors (Koren *et al.*, 1991).

Although lesser than in hypertensive participants, epidemiological research (The Strong Heart Study) of participants aged 14-39 reported that pre-hypertensive individuals displayed a greater LV mass, LV mass index (LVMI), and an increased

relative wall thickness than normotensive counterparts (Drukteinis *et al.*, 2007). Further population based studies have also concluded strong associations between greater LV mass and higher BP within the pre-hypertensive range (Gardin *et al.*, 2002; Lorber *et al.*, 2003).

The consequences of cardiac remodelling include a reduction in ventricular compliance. When the ventricle is no longer able to compensate for pressure overload with hypertrophy, its radius and wall stress increase excessively and systolic dysfunction can occur (Federman and Hess, 1994). This causes a reduction in left ventricular ejection fraction (LVEF) and the ventricular contraction may no longer be strong enough to eject an adequate SV to maintain $\dot{Q}$. Therefore the heart no longer functions as a pump and ventricular failure occurs. In early cardiac remodelling it is likely that there will be no observable symptoms, highlighting the importance of treating elevated BP in its early stages prior to the onset of myocardial remodelling and overt CVD (Lalande and Johnson, 2008).

Studies in athletes have demonstrated considerable adaptations in cardiac structure and function (Baggish and Wood, 2011a) and exercise interventions in diseased populations have demonstrated attenuated pathogenesis and reduced remodelling (Libonati, 2013) suggesting that exercise training interventions may reduce cardiac structural deterioration and improve cardiac performance.

2.3.2 Peripheral vascular activity

The contracting myocardium provides the necessary pressures for the blood to be distributed into the systemic and pulmonary circulation. Resistance can be described as the difference in pressure needed to drive one unit of blood flow in steady state ($mmHg \cdot mL \cdot min^{-1}$) and arterioles constitute the major resistance that the ventricles must pump against and collectively constitute peripheral or systemic vascular resistance (Levick, 2003).

Changes in vascular resistance are regulated by the vasoconstriction and vasodilation of the narrow terminal branches of the arterial system. Total peripheral resistance (TPR), sometimes referred to as systemic vascular resistance, is a measure of

resistance to blood flow by the systemic vasculature, which plays a crucial role in the control of circulation. Total peripheral resistance is interrelated with $\dot{Q}$ and mean arterial BP (TPR x $\dot{Q}$ = mBP).

Total peripheral resistance can be affected by the degradation of blood vessels and ineffective autonomic control of vasomotor centres. Pre-HTN is associated with a higher TPR, indicating potential arterial stiffness (Drukteinis *et al.*, 2007; Tomiyama and Yamashina, 2012) or impaired vasomotor regulation. Although best measured through the use of invasive catheterisation, non-invasive techniques have also been widely used to measure TPR and provide a good insight into resistance to blood flow (Gerhard-Herman *et al.*, 2006). Pathogenesis of HTN is associated with increased arterial stiffness, and reduced lumen diameter (Mayet and Hughes, 2003).

Vascular regulation is subject to a hierarchy of local and extrinsic control processes. Intrinsic mechanisms include the Bayliss myogenic response to arterial pressure change, detection of temperature change, and the secretion and generation of endothelial agents and vasoactive metabolites. Extrinsic mechanisms include sympathetic vasoconstrictor nerves, parasympathetic vasodilator nerves and circulating hormones (Young *et al.*, 1987). Indeed, parasympathetic activation stimulates the endothelium to secrete NO.

Afferent vascular activity and efferent vascular control occur through impulses transmitted via one of the agonistic branches of the autonomic nervous system (ANS). Sympathetic innervation of the cardiovascular system is controlled from the medulla in the brain stem. Most arteries and arterioles are innervated; however, some of the smallest arterioles rely solely on local tissue metabolites for vasomotor regulation. Tissue are independently regulated by a specific region within the ventrolateral brainstem, which means that sympathetic vasoconstrictor activity is adjusted regionally according to circumstances (Levick, 2003).

Shear stress occurs when friction between the sliding laminae cause the molecules to tug against one another. The magnitude of shear stress is dependent on the rate of shear, and the viscosity of the blood. This shear stress tugs on the lining of the endothelium and stimulates the secretion of nitric oxide (NO). Endothelium derived

relaxing factor NO inhibits vascular myocyte contraction, causing vasodilation by diffusing into neighbouring vascular smooth muscle where it reacts with a group of enzymes to catalyse vascular relaxation (Levick, 2003).

During physical activity or heightened cardiovascular activation, when HR, BP and SV are increased, shear rate is also higher leading to a greater secretion of NO, stimulating localised vasodilation. Improvements in vascular function have been reported following exercise training (Pal *et al.*, 2013), which may mediate associated BP reductions following physical activity interventions. The augmented release of NO during exercise is thought to be a result of low tissue Oxygen (O_2) tension, or a mechanical reaction on the endothelium caused by increased shear rate (Opie, 2004). Although an increase in shear rate is favourable, excessive shear stress due to enduring high pressures within the arteries, such as with raised resting BP, can reduce arterial compliance, impede local vasodilator control mechanisms and damage the arterial wall, which has been shown to accelerate atherosclerosis and CVD mortality (Opie, 2004). Indeed pre-HTN has been associated with impaired endothelial function (Giannotti *et al.*, 2010) and endothelial dysfunction is understood to be a determinant of the development of atherosclerosis in HTN (Puddu *et al.*, 2000). Therefore any improvements in vascular regulation or vascular structure and function may be able to induce reductions in TPR and BP, thus attenuating the pathogenesis of HTN.

2.3.3 Autonomic nervous system

The autonomic nervous system is an efferent neural pathway transporting information from the brain to central and peripheral tissues. As such, the ANS control mechanisms are fundamental to the regulation of the cardiovascular system and maintenance of homeostasis. Impaired autonomic function is associated with an increased risk of all-cause mortality (Gerritsen *et al.*, 2001) and is implicated in the development of HTN (Schroeder *et al.*, 2003). Sympathetic nervous system over-activity in a resting state has been demonstrated even in the early stages of HTN, prior to the development of symptoms (Carthy, 2014), through measurement of HRV and adrenergic overdrive (Julius *et al.*, 1991).

The specific causes of elevated BP as a result of sympathetic over activity remain inconclusively defined. However, it is known that mean BP is determined by $\dot{Q}$ and TPR, therefore sympathetic regulation of these factors plays a key role in elevated BP. Sympathetic activation of the sinoatrial and atrioventricular nodes will affect LV function, having an impact on chronotropic, inotropic, dromotropic and lucitropic activity of the myocardium, subsequently changing $\dot{Q}$. Sympathetic over activity places excessive demand on the cardiovascular systems, causing premature degradation, which can lead to cardiac and vascular remodelling, as discussed in sections 2.3.1 and 2.3.2.

Although invasive measures of ANS activity are preferable, HRV is a commonly used measured advocated by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Malik *et al.*, 1996). Heart rate variability is calculated by analysing the oscillations and interval times between consecutive R-R intervals on the ECG and is considered as a measure of neurocardiac function, reflecting heart-brain interactions and ANS dynamics (Shaffer *et al.*, 2014). Non-invasive measures of HRV include frequency domain analysis, time domain analysis, rhythm pattern analysis and non-linear methods.

Spectral frequencies of ECG data can be separated into their component parts and an indication of the dominant nervous activity can be established. The frequency domain method of establishing HRV, measures overall power spectral density (PSD) to provide information of how power variance distributes as a function of frequency. Frequency parameters are classified as very low frequency (VLF) low frequency (LF) and high frequency (HF) power, which change in relation to autonomic modulations of the heart period. LF has been associated with sympathetic activity while HF is associated with parasympathetic activity (Pomeranz *et al.*, 1985). The ratio of low and high frequencies (LF:HF ratio) is considered a representation of sympathovagal balance (Malliani, 1999), while expression of LF and HF in normalised units (LFnu and HFnu, respectively), represents the relative value of each power component. Sympathetic predominance (increased LF and reduced PSD) was observed in a third of pre-hypertensive young adults from the longitudinal Hypertension and Ambulatory Recording Venetia Study (Palatini *et al.*, 2006). Furthermore, a 6-year follow up study revealed greater progression to sustained HTN in participants with greater LF activity

and higher LFnu at baseline, highlighting the use of HRV as a further early predictor of CVD risk. A high prevalence of LF readings has also been shown to be a strong predictor of HTN in males (Singh *et al.*, 1998). In addition it has been shown that adrenergic overdrive precedes the elevation of BP and that adults who display a higher resting HR are more likely to develop HTN (Gudmundsdottir *et al.*, 2008).

The ANS is acutely controlled by the release of catecholamines following stimulation of the sympathetic cardioaccelerator nerves. Catecholamines contribute to the regulation of blood vessels and blood flow distribution. Adrenaline reacts with adrenoreceptors (α- and β-) to cause vasoconstriction and vasodilation. Higher levels of adrenaline result in vasoconstriction whereas lower levels of adrenaline result in the predominance of β-andrenoreceptor stimulation causing vasodilation and reduced TPR. In the heart, catecholamine induced β-stimulation causes positive inotropy, luscitropy, and chronotropy. High levels of catecholamines in a resting state indicates sympathetic overactivity; however, circulating plasma noradrenaline levels constitute only a fraction of the amount actually secreted from sympathetic nerve terminals (Esler *et al.*, 1990). Sympathetic overactivity is considered a predictor of the development of HTN and sympathetic activation is elevated in pre-hypertensive individuals compared with NTN (Duprez, 2008). Autonomic adjustments in parasympathetic and sympathetic activity have been widely reported following exercise training and it is thought that such neural control may be responsible for mediating vascular and cardiac responses to exercise training (Fisher *et al.*, 2015).

2.3.4 Neurohumoral control

In response to any infection or injury, the body initiates an autoimmune response to digest and destroy any invading bacteria. Blood circulation around the affected area increases, in addition to a supply of proteins, or cytokines, which fuel the immune response. Cytokines are released at the site of inflammation by localised inflammatory cells and are signalling molecules that mediate and regulate immunity and inflammation. This local response is accompanied by an acute-phase response, which involves the production of a distinct cascade of cytokines and cytokine inhibitors by the liver (Moshage, 1997; Baumann and Gauldie, 1994).

Systemic inflammation is the term used to describe this chronic state of inflammation, and low-grade systemic inflammation typically describes a two to threefold increase in systemic concentrations of tumor necrosis factor alpha (TNF- α), interleukins 1, 6 and 1ra (Il-1, Il-6, Il-1ra) and C-reactive protein (CRP) (Pedersen et al, 2005), all of which represent measureable markers of the degree of systemic inflammation. Low-grade inflammation is likely to be asymptomatic; however any raised systemic inflammation may be linked to the development of CVD.

When BP is raised, the blood vessels experience a chronic state of vascular stress. This may cause the endothelial cells to express cytokines, thus stimulating an inflammatory response (Chae *et al.*, 2001). Inflammation accelerates the artheroschlerotic process, which is associated with the pathogenesis of CVD (Ross, 1999). As such increased BP may be a stimulus for increases in inflammation which may accelerate atherosclerosis and CVD (Chae *et al.*, 2001).

A diseased state, even in its earliest stages is accompanied by chronic activation of the immune system, and the constant release of pro-inflammatory cytokines by immune related cells. Raised BP has been associated with elevated levels of TNF- α, high blood pressure variability (BPV) has been linked to raised IL-6 concentrations, raised ambulatory blood pressure (ABP) during sleep is associated with high sensitivity CRP (hs-CRP) (Kim *et al.*, 2008) and soluble intercellular adhesion molecule- 1 is a predictor of cardiovascular death (Chae *et al.*, 2001). Levels of inflammation and arterial BP increase concurrently (Chae *et al.*, 2001) and raised inflammation may act as a mediator between BPV and end organ damage (Kim *et al.*, 2008). Pre-hypertension is associated with raised neurohumoral activation, and higher concentrations of inflammatory markers, in comparison to optimal BP (Chae *et al.*, 2001).

In addition to inflammatory cytokines, some cytokines have anti-inflammatory properties. Interleukin-10 is also known as human cytokine inhibitory factor, and has been shown to inhibit the activation and effector function of T cells, such as TNF- α, to terminate inflammatory responses. Physical exercise has been linked to increases in the levels of circulating IL-10 (Ostrowski *et al.*, 1999), suggesting that physical activity may be able to elicit an anti-inflammatory response.

2.3.5 Baroreceptors

Baroreceptors are pressure sensors, located on the arterial side of the circulation in the carotid sinus and aortic arch. In order to maintain adequate organ perfusion, arterial BP is regulated within narrow limits, a process achieved via negative feedback. In response to distension or stretching of the artery walls, indicating acute changes in arterial pressure, baroreceptor action potential firing frequency increases. Conversely, a decrease in stretch, indicating a reduction in arterial BP, decreases receptor firing. In response to a rise or fall in arterial BP, adjustments in parasympathetic and sympathetic outflow mediate alterations in cardiac chronotropic, dromotropic and inotropic function and vasoconstriction or vasodilation of blood vessels to stabilise arterial pressure (Opie, 2004).

Baroreceptors form the first line of defence against transient increases or decreases in arterial BP and initiate reflex responses to return BP to its normal value when deviations are detected. Baroreceptors provide BP control within seconds and are therefore a short-term control mechanism to defend resting BP. Longer-term BP control, from a number of minutes to days, involves adjustments in total blood volume, salt and water balance under the influence of the renin-angiotensin-aldosterone system (RAAS) discussed in 2.3.6.

Any acute drop in BP causes a decrease in the distending pressure of baroreceptors resulting in a decreased frequency of afferent impulses to the nucleus solitarius. As a result there is an increase in sympathetic predominance over parasympathetic activity, prompting a reflex mediated increase in HR, contractility and $\dot{Q}$, and an increase in TPR. Higher baroreceptor reflex sensitivity (BRS) is considered to demonstrate greater parasympathetic control, which is associated with improved autonomic modulation and protection from CVD (La Rovere *et al.*, 2008a).

Baroreceptor reflex sensitivity is known to decline with age, as the distensibility of the arterial wall declines and central arterial compliance is reduced (Monahan *et al.*, 2001b). However reduced BRS is also prevalent in CVD (La Rovere *et al.*, 2008a), and is associated with an increased risk of morbidity and mortality (Ormezzano *et al.*,

2008; La Rovere *et al.*, 2008a). Baroreceptor reflex sensitivity is lower in hypertensive than in normotensive individuals, demonstrated by an impaired ability to control HR in response to higher arterial BP (Bristow *et al.*, 1969). An effective baroreflex would trigger a bradycardic HR response to elevated BP; however HTN is associated with a normal, or high resting HR. Reduced BRS provides a further marker of autonomic dysfunction in pre-HTN (Takeshita *et al.*, 1975).

Autonomic dysfunction, defined as sympathetic overactivity and reduced BRS may be responsible for HTN. Sympathetic over activity in normal resting conditions will result in the baroreflex obtaining a higher set point (Chapleau *et al.*, 1989). A lack of parasympathetic tone, caused by baroreceptor denervation means that BP cannot be adequately reduced and the new, higher set point is then maintained causing sustained HTN (Joyner and Limberg, 2014). Humans with impaired baroreceptor function experience a larger than normal rise in arterial pressure in response to exercise (Smit, 2002; Prabhakar and Peng, 2004) due to an impaired effectiveness of the reflex pathway.

Chronic denervation of baroreceptors results in the inability to suppress sympathetic activity, resulting in a predominance of adrenergic drive. The baroreflex no longer acts as a buffer against acute changes in BP (Joyner and Limberg, 2014). Denervation of baroreceptors gives the illusion that blood volume has become too low, initiating mechanisms to retain fluid causing chronic elevation in BP.

As an acute BP control mechanism, the arterial baroreflex is involved in the acute BP response to exercise (Joyner, 2006). Furthermore, exercise training has been shown to improve BRS (Laterza *et al.*, 2007), a pathway which may be linked with improved BP following exercise training.

2.3.6 Renin angiotensin aldosterone system (RAAS)

The RAAS is a pathway also stimulated by an increase in sympathetic activity as a result of physical stressors and is considered to provide long term BP control. An increase in noradrenaline constricts blood flow to the kidneys, stimulating the release of renin. Renin activates the production of the hormones aldosterone and angiotensin.

Aldosterone causes arterial constriction and cause the kidneys to retain sodium, potassium and water, increasing plasma volume, and thus BP (Levick, 2003).

Angiotensin II is also a powerful vasoconstrictor hormone and stimulates further catecholamine release and inhibits noradrenaline re-uptake, therefore sustaining the sympathetic andrenergic effect. Patients with CVD, HTN and pre-HTN show markedly elevated levels of angiotensin II suggesting that the vasoconstrictor action of angiotensin II contributes to BP elevation above optimum levels. Angiotensin II also increases cardiac contractility and can lead to adverse cardiac and vascular remodelling (Duprez, 2008; Yamamoto *et al.*, 2006). Of interest, the prescription of an angiotensin receptor blocker in pre-hypertensives supressed the onset of HTN during a 2-year treatment period (Julius *et al.*, 2006).

The RAAS pathway is also modulated by hormone secretion in the heart. The release of atrial naturietic peptide (ANP) and brain naturietic peptide (BNP) form an important counter regulatory system for the maintenance of homeostatic BP. Both ANP and BNP are released in the heart in response to atrial and ventricular distension, and neurohumoral stimuli (Opie, 2004). Atrial naturietic peptide is secreted by specialised myocytes in the atria in response to high cardiac filling pressure and hypovolemic states. This hormone acts against aldosterone by reducing plasma volume and filling pressure by enhancing renal salt and water excretion, as well as causing moderate dilation of resistance vessels. Brain naturietic peptide is secreted by the ventricles of the heart in response to excessive stretching of cardiomyocytes and acts to reduce BP by decreasing blood volume and systemic vascular resistance.

The RAAS has been identified as a target for HTN management, as RAAS over-activity will lead to raised BP. Anti-hypertensive medications, such as angiotensin converting enzyme (ACE) inhibitors or diuretics, are tasked with disrupting elements of this system and to prevent sodium and water retention. Inhibition of angiotensin II production through pharmacological blockade intervention has proven beneficial in modifying the pathology of CVD (Heran *et al.*, 2008). In addition, exercise training has been demonstrated to reduce plasma renin activity (Goessler *et al.*, 2016), which

may lead to longer-term BP control, therefore physical activity may offer an alternative therapy for down regulation of the RAAS.

2.3.7 Summary

The mechanisms causing sustained elevation in arterial BP remain unknown. The integrative roles of Q̇ and TPR in mediating arterial pressure are understood; however cardiovascular control mechanisms are complex. Altered cardiac regulation, vascular modulation, ANS dysfunction and impaired short and long-term BP control systems may collectively and/or independently contribute to chronic elevations in arterial BP and progression to established HTN and increased CVD risk. It has also been widely demonstrated that those with pre-HTN also possess increased risk (Franklin *et al.*, 1997a; Vasan, 2001; Qureshi *et al.*, 2005b; Licitra *et al.*, 2012) and display signs of dysregulation of cardiovascular control mechanisms (Palatini *et al.*, 2006; Duprez, 2008; Gardin *et al.*, 2002; Lorber *et al.*, 2003; Drukteinis *et al.*, 2007; Carthy, 2014). In spite of an inconclusive understanding of BP control mechanisms, the importance of BP in relation to cardiovascular outcome is well established. The use of lifestyle interventions, such as physical activity, in a pre-hypertensive population may have the potential to reduce mortality, induce health benefits, and limit the pathogenesis of HTN via modulation of the cardiovascular regulatory pathways discussed.

2.4 Physical activity and blood pressure.

2.4.1 General overview

Physical inactivity, is currently the fourth leading cause of mortality worldwide (WHO, 2010a). It is widely accepted that those who remain physically active throughout their life are less likely to have raised BP, and that levels of physical activity and cardiorespiratory fitness are inversely associated with the development of HTN (Carnethon *et al.*, 2010; Parker *et al.*, 2007; Chase *et al.*, 2009). Physical activity accounts for any bodily movement by skeletal muscles that requires energy

expenditure (WHO, 2010b) and encompasses routine daily tasks such as household activities and commuting.

Recent research has focused on the role of exercise as a form of physical activity, which may be beneficial for cardiovascular health. Exercise training interventions have been shown to offer significant BP reductions in hypertensive, pre-hypertensive and normotensive individuals (Cornelissen and Smart, 2013). Guidelines presented by the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (Chobanian *et al.*, 2003), recommend dynamic, aerobic, endurance exercise training as a successful and viable primary treatment option for HTN. In patients who have been prescribed pharmacological treatment for HTN, 150 minutes of weekly physical activity has been deemed an effective exercise target to complement the use of anti-hypertensive medication (Mosca *et al.*, 2011).

Exercise interventions prescribing walking (Murphy *et al.*, 2006), jogging (Tsuda *et al.*, 2003), swimming (Tanaka *et al.*, 1997), cycling (Finucane *et al.*, 2010), upper limb cycling (Westhoff *et al.*, 2008) rowing (Santa-Clara *et al.*, 2003) and stair climbing (Jessup and Brozena, 2003), have all elicited clinically relevant BP reductions, and as such an increase in physical activity should be recommended by health practitioners to compliment any anti-hypertensive therapy (Semlitsch *et al.*, 2013).

In addition to aerobic training recommendations, the American Heart Association and other bodies have also endorsed resistance training as a physical activity option to improve cardiovascular health (Cornelissen *et al.*, 2011; Pescatello *et al.*, 2004a). Kelley and Kelley (2000) reported reductions of ~2% and 4% in resting sBP and dBP respectively in a meta-analysis of resistance training programmes. Furthermore Cornelissen *et al.* (2011) concluded mean BP reductions of 3.9 mmHg following dynamic resistance training programmes, complemented by improvements in O_2 consumption ($VO_{2\ peak}$) (10.6%), body fat and plasma triglycerides (0.6%).
Despite the well known benefits of physical activity for health and wellbeing, worldwide participation in regular physical activity is low. It is estimated that 50% of adults embarking on a new exercise programme drop out within the first 6 months, and that only 20% will continue to exercise after 24 months (Dishman, 1988). According to the Transtheoretical model of behaviour change (Prochaska and DiClemente, 1982)

even individuals contemplating behaviour change can take 2 years to implement any related lifestyle modification, and may never actually implement such changes. The most common barrier reported for low activity uptake and high drop out rate is a perceived lack of time (Zunft *et al.*, 1999). Physical activity guidelines equate to 3 hours of activity per week, and involve a target of participation on 5 or more days a week (Chobanian *et al.*, 2003). This time commitment may be perceived to be inaccessible to many adults, who elect instead to abstain from all physical activity. Indeed these guidelines have been criticised for a lack of evidence of their effectiveness for both health and accessibility (Weed, 2016). Further barriers that account for a large proportion of physical inactivity include cost (expensive equipment, gym memberships, and travel expenses) and self-esteem issues, as individuals wish to avoid the judgement of others in a fitness setting (Salmon, 2001). Prescription of any physical activity training must minimise barriers and provide clear outcome benefits to participants.

2.4.2 Isometric exercise and isometric exercise training

Isometric exercise (IE) is a mode of resistance exercise that involves maintaining a static muscle contraction against a fixed resistance. Muscle length stays constant and tension increases while a contraction is maintained against an immovable force (Fadel *et al.*, 2004; Lind, 2011). Isometric exercise has been previously employed in clinical settings, for the measurement of LV performance and overall cardiac function (Kivowitz *et al.*, 1971; Flessas *et al.*, 1976; Crawford *et al.*, 1979; Eshani *et al.*, 1981; Floras *et al.*, 1989; Fisher *et al.*, 1973; Mitchell *et al.*, 1980), but has more recently been researched in relation to BP management. Isometric exercise training (IET) involves the systematic use of IE in an evidence-based training programme to achieve physiological adaptations, and IET has been shown to induce significant reductions in resting BP (Inder *et al.*, 2016).

The prospect of IET as an exercise intervention has the potential to combat time constraints against physical activity. The most common IET session protocol requires just four 2-minute working intervals, completed 4 times per session on three days of the week (Millar *et al.*, 2008; Millar *et al.*, 2013; Wiley *et al.*, 1992a) (see Table 2.2), whereas aerobic exercise training sessions are typically longer in duration. In addition

to time, barriers preventing the participation in traditional aerobic exercise such as cost, equipment and self-confidence are diminished with IET, as it can be performed in a small space within the home (Goldring *et al.*, 2014).

2.4.2.1 Isometric exercise and the pressor response

The exaggerated BP responses associated with IE, mean that individuals with a higher resting BP may experience higher BPs during IE than those with normal resting BP. There are concerns that reaching high arterial pressures could induce an unnecessary pressure load on the heart (Millar *et al.*, 2014). This is of particular importance when the participant already has a greater risk of cardiovascular events, as extreme pressure loads have the potential to cause a cerebrovascular haemorrhage or LV failure (Mitchell and Wildenthal, 1974).

Safety considerations can reduce associated risks of excessive BP elevation. The maintenance of regular breathing and avoidance of the Valsalva manoeuvre is of high importance in order to avoid rapid increases in intrathoracic pressure. In addition, prescribing exercise of the appropriate intensity, by monitoring HR, BP and training dose to ensure individuals remain within BP safety guideline values will support the prescription of IE as an accessible non-pharmacological alternative for BP management. Advice from the American Heart Association Council (Brook *et al.*, 2013) concludes that low-moderate intensity resistance training produces safe and minimal haemodynamic responses.

Despite apparent increases in BP, it has been reported that a single isometric contraction produces equivalent or lower sBP pressure responses than dynamic aerobic exercise (Daniels, 2000; Chaney and Arndt, 1983). Furthermore, measures of rate pressure product (RPP), an index of myocardial oxygen consumption, have been reported to be lower during resistance training (Mota *et al.*, 2009) and isometric training (Fisher *et al.*, 1973; Carlson *et al.*, 2017) compared with aerobic exercise alternatives.

2.4.3 Anti-hypertensive effects of isometric exercise training

There is a growing body of evidence affirming that a programme of IET can elicit statistically significant reductions in sBP and dBP in normotensive, pre-hypertensive and hypertensive participants (Carlson *et al.*, 2014; Wiley *et al.*, 1992b; Wiles *et al.*, 2008; Badrov *et al.*, 2013a; Ray and Carrasco, 2000; Howden *et al.*, 2002; Millar *et al.*, 2009b), as evidenced in Table 2.2. Effect sizes have been established from a number of meta-analyses, highlighting the effectiveness of IET as an intervention to reduce resting BP. Cornelissen and Smart (2013) concluded that IET protocols elicited average BP reductions of 10.9 mmHg (sBP) and 6.4 mmHg (dBP), compared to reductions of just 3.4mmHg (sBP) and 2.5 mmHg (dBP) through endurance training protocols. Carlson *et al.* (2014) observed mean reductions of 6.77 mmHg (sBP) and 3.96 mmHg (dBP) in hypertensive and normotensive participants following ≥4 weeks of IET. Inder *et al.* (2016) recently reported mean reductions of 5.2 mmHg (sBP) and 3.91 mmHg (dBP), and observed that hypertensive and male participants experienced greater reductions in BP than normotensive and female participants. These reductions in resting BP following IET are in line with, and in some cases greater than, reductions elicited by aerobic training (Borjesson *et al.*, 2016). Despite the reported positive results, the absence of large-scale randomised control trials should not be ignored (Kelley and Kelley, 2010).

Interventions have employed the use of a variety of isometric training protocols, varying in length, training duration, and training intensity. Training intensity is most commonly expressed as a percentage of maximal voluntary contraction (MVC). Despite notable differences in successful training protocols and IE types (See Table 2.2), a moderate to high level of between-study heterogeneity has been reported in this field (Carlson *et al.*, 2014).

Table 2.2. Isometric exercise training protocols previously used and associated BP reductions

Reference	Study Design	Intervention Participants	BP Category	Mode	Training Intensity	Training Time.	Intervention Frequency /Duration	BP change mmHg	Other Measures
Wiley et al, (1992)	RCT	8	Pre-HTN	Unilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 3 min	3 x/week 8 weeks (Total: 192min)	sBP: ↓ 13 dBP: ↓ 15	
		10	Pre-HTN	Bilateral IHG	50% MVC	Exercise: 4 x 45 sec Rest: 1 min	5 x/week 5 weeks (Total: 75 min)	sBP: ↓ 10 dBP: ↓ 9	
Ray and Carrasco, (2000)	Cohort Controlled	9	NTN	Unilateral IHG	30% MVC	Exercise: 4 x 3 min Rest: 5 min	4 x/week 5 weeks (Total: 240min)	sBP: not significant dBP: ↓ 5 mBP: ↓4	
Howden et al, (2002)	Cohort Controlled	8	NTN	Bilateral Arm Flexion	30% MVC	Exercise: 4 x 2 min Rest: 3 min	3 x/week 5 weeks	sBP: ↓ 10 dBP: not significant	
		9	NTN	Bilateral Leg Extension	20% MVC	Exercise: 4 x 2 min Rest: 3 min	3 x/week 5 weeks (Total: 120 min)	sBP: ↓ 12 dBP: not significant	
Taylor et al, (2003)	RCT	9	HTN – medicated	Bilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 1 min	3 x/week 10 weeks (Total: 240 min)	sBP: ↓ 19 dBP: not significant mBP: ↓ 11	HRV (PSA) BPV – resting beat-to-beat
Peters et al, (2006)	Cohort	10	HTN – un-medicated	Bilateral IHG	50% MVC	Exercise: 4 x 45 sec Rest: 1 min	3 x/week 6 weeks (Total: 54 min)	sBP: ↓ 13 dBP: not significant	

Table 2.2 Continued.

Reference	Study Design	Intervention Participants	BP Category	Mode	Training Intensity	Training Time.	Intervention Frequency /Duration	BP change mmHg	Other Measures
McGowan et al, (2006)	Cohort	17	HTN - medicated	Unilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 3 min	3 x/week 8 weeks (Total: 192 min)	sBP: not reported dBP: not reported mBP: not significant	
McGowan et al, (2007a)	Cohort	11	NTN	Unilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 3 min	3 x/week 8 weeks (Total: 288 min)	sBP: ↓ 5 dBP: not significant	
McGowan et al, (2007b)		9	HTN - medicated	Unilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 3 min	3 x/week 8 weeks (Total: 192min)	sBP: ↓ 9 dBP: not significant	
Miller et al, (2008)	RCT	25	NTN	Bilateral IHG	30-40% MVC	Exercise: 4 x 2 min Rest: 1 min Home and Lab based	3 x/week 8 weeks (Total: 192 min)	sBP: ↓ 10 dBP: ↓ 3	
Wiles, Coleman & Swaine, (2010)	RCT	11	NTN	Bilateral leg extension	75% HRpeak (~10% MVC)	Exercise: 4 x 2 min Rest: 2 min	3 x/week 8 weeks (Total: 192 min)	sBP: ↓ 4 dBP: ↓ 3 mBP: ↓3	
		11	NTN	Bilateral leg extension	95% HRpeak (~21% MVC)	Exercise: 4 x 2 min Rest: 2 min	3 x/week 8 weeks (Total: 192 min)	sBP: ↓ 5 dBP: ↓ 3 mBP: ↓3	
Devereux, Wiles & Swaine (2010)	Crossover	13	NTN	Bilateral leg extension	95% HRpeak (~24% MVC)	Exercise: 4 x 2 min Rest: 3 min	3 x/week 4 weeks (Total: 96 min)	sBP: ↓ 5 dBP: ↓ 3 mBP: ↓3	

Table 2.2 Continued.

Reference	Study Design	Intervention Participants	BP Category	Mode	Training Intensity	Training Time.	Intervention Frequency /Duration	BP change mmHg	Other Measures
Baross, Wiles & Swaine (2012)	RCT	10	Pre-HTN	Bilateral leg extension	70% HRpeak (~8% MVC)	Exercise: 4 x 2 min Rest: 2 min	3 x/week 8 weeks (Total: 192 min)	sBP: not significant dBP: not significant mBP: not significant	
		10	Pre-HTN	Bilateral leg extension	85% HRpeak (~14% MVC)	Exercise: 4 x 2 min Rest: 2 min	3 x/week 8 weeks (Total: 192 min)	sBP: ↓ 11 dBP: not significant mBP: ↓5	
Millar et al, (2013)	Cohort Controlled	13	HTN-medicated	Unilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 4 min	3 x /week 8 weeks (Total: 192 min)	sBP: ↓ 5 dBP: not significant mBP: ↓ 3	HRV – linear and non linear
Badrov et al, (2013a)	RCT	12	NTN	Unilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 4 min	3 x /week 8 weeks (Total: 192 min)	sBP: ↓ 6 dBP: not significant mBP: not significant	HRV Resistance Vessel Function
		11	NTN	Unilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 4 min	5 x /week 8 weeks (Total: 320 min)	sBP: ↓ 6 dBP: not significant mBP: not significant	
Badrov et al, (2013b)	RCT	12	HTN - medicated	Unilateral IHG	30% MVC	Exercise: 4 x 2 min Rest: 1 min	3 x /week 10 weeks (Total: 240min)	sBP: ↓ 8 dBP: ↓ 5 mBP: ↓ 6	Serial subtraction Cold pressor CV reactivity
Gill et al, (2014)	Cohort Controlled	8	NTN	Bilateral leg extension	20% MVC	Exercise: 4 x 2 min Rest: 3 min	3 x /week 3 weeks (Total: 72min)	sBP: ↓not significant dBP:↓not significant mBP:↓not significant	
		9	NTN		30% MVC			sBP:↓ 3.6 dBP:↓ 4 mBP:↓ 3.9	

Table 2.2 Continued.

Reference	Study Design	Intervention Participants	BP Category	Mode	Training Intensity	Training Time.	Intervention Frequency /Duration	BP change mmHg	Other Measures
Garg et al, (2014)	Cohort	30	NTN	Bilateral IHG	30% MVC	Exercise: 5 x 3 min Rest: 5 min	3 x /week 10 weeks (Total: 450min)	sBP:↓ 10 dBP:↓ 3	
Hess et al, (2016)	Cohort	10	NTN	Unilateral IHG	5% MVC	Exercise: 4 x 2 min Rest: 1 min	3 x /week 6 weeks (Total: 144min)	sBP: ↓ 4 dBP: ↓ 5	Heart Rate Cont BP
		10	NTN	Unilateral IHG	10% MVC	Exercise: 4 x 2 min Rest: 1 min	3 x /week 6 weeks (Total: 144min)	sBP: ↓ 5 dBP: ↓ 0.9	
Wiles et al, (2017)	RCT	28	NTN	Isometric wall squat	95% HR_{peak}	Exercise: 4 x 2 min Rest: 2 min	3 x /week 4 weeks (Total: 96min)	sBP:↓ 4 dBP:↓ 3 mBP:↓ 3	HR TPR SV
Pagonas et al, (2017)	Cohort Controlled	24	HTN	Bilateral IHG	30% MVC	Exercxise: 4 x 2min Rest: 1 min	5 x /week 12 weeks (Total: 480min)	sBP:↓ not significant dBP:↓ not significant	ABP TPR Peripheral artery elasticity index

Although some acute programme variables have been used repeatedly, the optimum muscle group, training intensity and session protocol have not been definitively established. The most common protocol (4 x 2-min contractions, 3 x per week) has resulted in reductions in sBP and dBP in some studies (Wiley *et al.*, 1992b; Wiles *et al.*, 2008), and no significant reductions in others (Stiller-Moldovan *et al.*, 2012; Baross *et al.*, 2013). Contraction intensity and duration can be manipulated to evoke similar physiological and training responses (Lawrence *et al.*, 2014), but little has been published with regards to manipulating these factors. It is important to develop a variety of effective training protocols that adhere to the training principle of variation, for successful longer term exercise prescription.

A relationship between working muscle mass and physiological response has been proposed (Mitchell *et al.*, 1980), suggesting that using larger muscle mass, such as during isometric leg flexion or extension exercise rather than isometric handgrip (IHG), could elicit greater BP reductions. When compared, sBP and dBP reductions following isometric leg training are similar or greater than those elicited through IHG training, even when performed at lower intensities (Howden *et al.*, 2002). This may be caused by the recruitment of additional motor units in a larger muscle mass, increasing the sympathetic response to the working muscles (Mitchell *et al.*, 1980). In addition, the muscle fibre arrangement within the quadriceps muscle group may result in higher intramuscular pressure than other muscle groups. Adaptations may also be intensity dependant (Wiles *et al.*, 2008).

Despite the proven effectiveness of IET, Millar *et al.* (2014) highlighted concerns that methodologies used in much of the IET research to date is neither cost nor time efficient. Many studies have required lab attendance for each exercise session and have made use of large and impractical equipment such as an isokinetic dynamometer (Wiles *et al.*, 2008; Howden *et al.*, 2002; Baross *et al.*, 2012; Wiles *et al.*, 2010; Devereux *et al.*, 2011) or an expensive programmable digital handgrip dynamometer (Mitchell *et al.*, 1980; Millar *et al.*, 2007). The need for supervision and equipment availability in these protocols has called into question the cost-benefit relationship of this training modality and the external validity of research at a population level. Future research must address these barriers against participation if this exercise intervention is to become widely accepted.

Although reductions in single resting BP measurements following a programme of IET have been well reported (Table 2.2) there is a dearth of research reporting ABP responses to training of this type. Given that ABP monitoring provides a better predictor of cardiovascular outcome (Beevers, 2001; Fagard *et al.*, 2008; Pickering *et al.*, 2006) future IET research must use ABP recordings to assess changes in diurnal BP as a marker of cardiovascular health.

2.5 Physiological stimulus and responses associated with isometric exercise

Much research has focussed on establishing the optimal training conditions for BP reductions while the exact mechanisms responsible for measured reductions remain equivocal. In order for IET to be recommended as a health intervention, the physiological mechanisms for the observed benefits must be better understood.

Isometric exercise acts as a stimulus, which can be manipulated to create a physiological response. Combined, these represent the acute cascade of events that is initiated at the onset of IE. However, repeated stimulation of acute responses through IET may act upon a range of cardiovascular pathways, which may induce the physiological mechanisms responsible for the observed physiological adaptations, as portrayed in Figure 2.2.

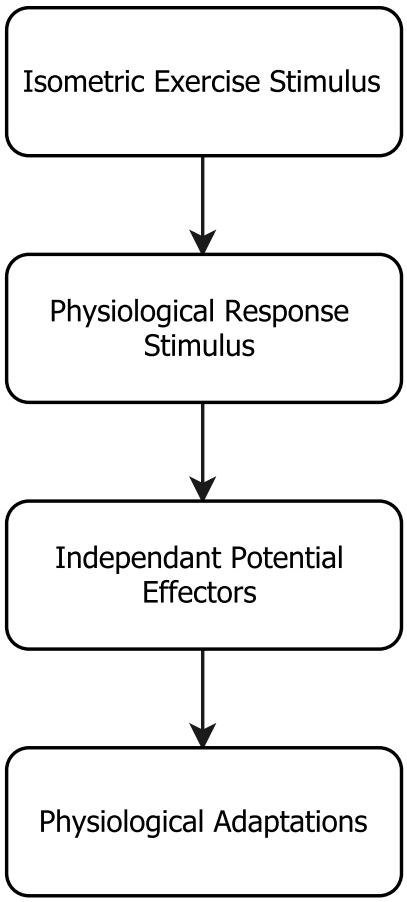

Figure 2.2. The concept of the link between IET as a stimulus that elicits a physiological response. The physiological response may stimulate a number of independent effectors, which when repeated are the mechanisms leading to physiological adaptation.

A number of mechanisms for BP reductions observed through IET have been proposed. Pathways studied to date include endothelial function (McGowan *et al.*, 2007; Badrov *et al.*, 2013a), oxidative stress (Peters *et al.*, 2006), structural vascular adaptations (Baross *et al.*, 2013), autonomic modulation (Taylor *et al.*, 2003), and haemodynamic activity (Wiles *et al.*, 2010). Determinants of arterial BP ($\dot{Q}$ and TPR) and their control mechanisms are undoubtedly involved in the regulation of chronic BP improvements. Attempts have been made to define mechanisms; however, the influence of cardiovascular regulatory pathways remains unclear.

In order to help establish the potential effectors to a programme of IET, it is useful to better understand the physiological response to a single isometric contraction stimulus and a single session of IE as it is possible that physiological responses elicited during a single session of IE may be exaggerated through repeated training, which may lead to chronic adaptations.

Compared with research into IET, research exploring acute responses to IE differs substantially with regards to the isometric stimulus, parameters measured, and the time point at which measures are taken. The acute BP response in a range of relevant studies is presented in Table 2.3. However, it is noteworthy that most research involves the measurement of cardiovascular and haemodynamic responses during or following a single IE contraction, rather than in response to a session of IE.

Table 2.3: Evidence of acute haemodynamic responses measured during and following IE

Reference	N=	BP Category	Exercise mode	Isometric intensity	Training duration	BP method	BP during contraction (mmHg difference from baseline)	BP Post IE (mmHg difference from baseline)	Time taken for BP recovery	Further Variables Reported
Carlson *et al,* (2017)	120	NTN Pre-HTN	IHG	5% MVC 10% MVC 30% MVC	4 x 2 min (3 min rest)	Continuous (beat-to-beat)	sBP:↑ ~19-38 dBP:↑ ~9-21 (↑intensity = ↑BP)	Not Reported	Not Reported	HR ↑ RPP ↑
Goldring *et al,* (2014)	23	NTN	Wall Squat	Various knee joint angles 135-95°	2 min	Continuous (beat-to-beat)	sBP:↑ ~14-76 dBP:↑ ~0-36 (↑intensity = ↑BP)	Not Reported	Not Reported	HR ↑
Olher *et al,* (2013)	12	HTN (medicated)	IHG	30% MVC 50% MVC	4 x 5 x 10 seconds	Single Measure (Pre, During, Post)	sBP: ↑ 5-7 dBP: ↑ 0-2	Not reported	> 45 min < 60 min	HR RPP
Araujo *et al,* (2011)	41	NTN (Medicated cardiac patients)	IHG	30 % MVC	Exercise: 4 x 2 min Rest: 1 min	Single measures Pre/Post	sBP/dBP: ↑ significant increase (mmHg not reported)	Not reported	Baseline restored ~1 min	HR ↑
Millar *et al,* (2010)	12	NTN & Pre-HTN	IHG	30% MVC	Exercise: 4 x 2 min Rest: 1 min Exercise: 8 x 1 min Rest: 30 sec Exercise: 16 x 30 sec Rest: 15 sec	Single measures Pre/Post	Not reported	sBP: ↑ 7 dBP: ↑ 2 (not significant) sBP: no change dBP: ↑ 3 (not significant) sBP: no change dBP: ↓ 2 (not significant)	<30 min	HR ↑ ECG
Millar *et al,* (2009)	18	Pre-HTN	IHG	30% MVC	Exercise: 4 x 2 min Rest: 1 min	Single measures Pre/post	Not reported	sBP: ↓~ 3 dBP: ↓	5 min post = PEH sustained	HR ↑ ECG HR complexity

Table 2.3 continued.

Reference	N=	BP Category	Exercise mode	Isometric intensity	Training duration	BP method	BP during contraction (mmHg difference from baseline)	BP Post IE (mmHg difference from baseline)	Time taken for BP recovery	Further Variables Reported
Wiles *et al*, (2008)	15		Double Leg Knee Extension	10-30% EMG_{peak}	5% EMG_{peak} increase every 2 min until exhaustion/completion	Single measures every 60 seconds	sBP:↑ linear with intensity dBP: Not Reported	Not Reported	Not Reported	HR ↑ EMG
Fisher *et al*, (2007)	8	NTN	IHG	15 % MVC 30 % MVC 45 % MVC 60 % MVC	Exercise: 1 min Rest: 3 min	Continuous (beat-to-beat)	sBP: ↑ 4 dBP: ↑ 3 sBP: ↑ 9 dBP: ↑ 6 sBP: ↑ 24 dBP: ↑ 16 sBP: ↑ 36 dBP: ↑24	Not reported	Not reported	ECG Strain gauge pneumograph Neck suction BRS ↓
Wiles *et al*, (2005)	10	NTN	Single Leg Extension Single Arm Flexion (transducer) Single Arm Flexion (Scales)	30%	Exercise: 2 x 2 min Rest: 2 min	Single measures every 30 seconds	sBP:↑ ~24-33 dBP: ↑ baseline not reported	Not Reported	Not Reported	HR ↑ RPE: Borg CR-10
Fisher & White, (1999)		Pre-HTN	Dominant leg Calf raise (pre-6 week IET) Dominant leg Calf raise (post-IET)	30% MVC	2 min	Continuous (beat-to-beat)	sBP: ↑ 21mmHg dBP: ↑12 mmHg sBP: ↑ 13 mmHg dBP: ↑ 9 mmHg	Not reported	Not reported	ECG Fatigue index

Table 2.3 continued.

Reference	N=	BP Category	Exercise mode	Isometric intensity	Training duration	BP method	BP during contraction (mmHg difference from baseline)	BP Post IE (mmHg difference from baseline)	Time taken for BP recovery	Further Variables Reported
Iellamo *et al,* (1999a)	10	NTN	IHG Leg Extension Leg Extension	30% MVC 30% MVC 15% MVC	Exercise: 3 min Post exercise circulatory occlusion (PECO): 4 min	Continuous (beat-to-beat)	mBP: ↑ 12 mBP: ↑ 23 mBP: ↑ 13	PECO mBP:↑ 8 PECO mBP:↑ 19 PECO mBP:↑ 8	<4 minute	ECG Oxygen uptake ↑ BRS Minute ventilation
Iellamo *et al,* (1999b)	11	NTN	ILE	30% MVC	Exercise: 4 min x 1 PECO: 4 min	Continuous (beat-to-beat)	sBP: ↑26.5 dBP: ↑15.5	PECO sBP: ↑22 PECO dBP: ↑13	<4 minute	HRV LFnu: ↑ HFnu:↓ BRS: ↓ HR: ↑
Iellamo *et al,* (1997)	10	NTN	Knee Extension	40% MVC	1 min	Continuous (beat-to-beat)	sBP: ↑ 38 mmHg dBP:↑ 23 mmHg	mBP: ↓ 8.4 sBP/dBP: not reported	>1 minute	HR ↑ Oxygen uptake↑ Minute ventilation ↑
Eshani *et al,* (1981)	14	NTN	IHG	20% MVC 40% MVC 60% MVC	2-min 2-min 45-seconds	Single measure (Pre, During)	sBP: ↑16-44 dBP: ↑15-39	Not reported	Not reported	ECG Cardiac function (echo)
Crawford *et al,* (1979)	27	NTN	IHG	15% MVC 50% MVC	Until Fatigue (Recorded last 30 seconds)	Not Specified	sBP ↑ 10-26mmHg	Not reported	Not reported	HR ↑ Cardiac function (echo) Atropine

Table 2.3 continued.

Reference	N=	BP Category	Exercise mode	Isometric intensity	Training duration	BP method	BP during contraction (mmHg difference from baseline)	BP Post IE (mmHg difference from baseline)	Time taken for BP recovery	Further Variables Reported
Martin *et al,* (1974)	26	NTN	IHG	30 % MVC Control Propranol olPractolo l, Atropine	3 min	Continuous catheterisation (Central venous pressure)	Control trial: sBP: ↑ 27 dBP: ↑26	Not reported	Not reported	LVET ↓ SV ↓ CI / $\dot{Q}$↑ HR ↑ TPR ↑
Fisher *et al,* (1973)	72	Heart disease (medicated)	IHG	25% MVC + 75% MVC	5-min + 1-min	Continuous Cardiac catheterisation (Pulmonary arterial & systemic systolic pressure)	MPAP: ↑ 5.9-7.3 sBP: ↑24-26 ↑% MVC = ↑mmHg	Not reported	Not reported	HR ↑ CI / $\dot{Q}$ ↑ SV ↑ TPR Cardiac function (echo)
Kiveloff & Huber (1971)	19	NTN & HTN & medicated	Extremities, buttocks, abdomen. Discrete and simultaneous	Not reported	30 sec 10 sec 6 sec	Not specified	sBP:↑ ~16 dBP:↑ 24 sBP:↑2-22 dBP:↑4-28 sBP:↑4-32 dBP:↑8-26	Not reported	<90seconds (contraction duration dependant)	Pulse pressure ↑
Kivowitz *et al,* (1971)	22	Heart Disease	IHG	25% MVC	5-min	Continuous Cardiac Catheterisation	sBP: ↑ 22 dBP: ↑ 12	Not reported	<10 secs	HR ↑ CI ↑ TPR ↑ Cardiac function (echo) A-V_{02} difference

2.5.1 Cardiovascular stimulus induced by acute IE and recovery

An isometric contraction is associated with a sharp increase in BP (Araujo *et al.*, 2011; Iellamo *et al.*, 1997; Iellamo *et al.*, 1999b; Fisher *et al.*, 2007; Fisher and White, 1999; Martin *et al.*, 1974; Eshani *et al.*, 1981; Kivowitz *et al.*, 1971), which may be mediated by increases in $\dot{Q}$, changes in TPR or both. The chronotropic and BP responses to IE are linear, therefore higher intensity IE appears to elicit greater increases in HR and BP (Carlson *et al.,* 2017(Lind and McNicol, 1967; Fisher *et al.*, 2007; Devereux *et al.*, 2011; Goldring *et al.*, 2014; Crawford *et al.*, 1979). Muscle fibre recruitment and BP increases are positively associated with isometric contraction intensity, while maximum contraction duration is negatively associated with contraction intensity (Hietenan, 1984). Heart rate and BP responses during IE are higher than those measured at the same level of oxygen uptake, minute ventilation and arterio-venous oxygen difference measured in dynamic aerobic exercise (Asmussen, 1981).

The acute recovery responses to an isometric stimulus are of relative importance and may also assist in understanding the associated chronic health benefits. Cessation of isometric muscle contraction and release of the tension developed results in sudden reperfusion of blood to previously occluded muscle mass and a period of post exercise hyperaemia (Halliwill *et al.*, 2013). A transient pressure undershoot is observed in arterial pressure as HR and BP begin to fall immediately and rapidly (Millar *et al.*, 2009a; Araujo *et al.*, 2011; Moraes *et al.*, 2012; Hill *et al.*, 1989). This stimulus provides the mechanism for a hypotensive effect (MacDonald, 2002) observed in the recovery period, which can be sustained for 5-60 minutes (Olher *et al.*, 2013; Mediano *et al.*, 2005; Hill *et al.*, 1989). This response is more exaggerated in pre-hypertensive and hypertensive individuals compared with normotensive exercisers, following aerobic, resistance, isometric and combined exercise modalities (MacDonald, 2002; Cardoso *et al.*, 2010).

2.5.2 Acute cardiac functional response to an isometric stimulus

Isometric handgrip testing is commonly used to measure cardiac performance to increased load by replicating the conditions of HTN. The relationship between peak LV afterload and end systolic pressure is determined by myocardial contractility and

individuals with normal cardiac function will display an increase in afterload, which will result in a compensatory increase in contractility during IE (Sagiv, 2012). This intrinsic change in inotropy in response to a sudden increase in aortic pressure is known as the Anrep effect (Opie, 2004). In addition to increases in HR and BP in a healthy heart, an isometric stimulus has been shown to cause an increase in LV end systolic volume and a reduction in LV diameter shortening, factors which were most marked at an IHG intensity of 50% MVC compared with IHG at 15% MVC (Crawford *et al.*, 1979). This response indicates a drop in SV, which was greater with a higher intensity contraction. The sBP and LV end systolic volume ratio increases significantly from resting values during acute IE in healthy elderly (Sagiv, 2012). In contrast, Laird *et al.* (1979) reported no change in LV systolic and diastolic dimensions in normotensive participants performing a 3-minute IHG contraction at 25% MVC, therefore a measured increase in cardiac index was attributed to increased HR. Flessas *et al.* (1976) measured no change in LV end diastolic pressure and a decrease in EDV during IHG exercise in healthy participants, using cardiac catheterisation, suggesting a reduction in SV. It is important to recognise that such research has employed isometric contractions as a method of eliciting an increase in LV afterload. Therefore research investigating the acute cardiac response has mostly consisted of a single IHG contraction, as opposed to a session of multiple IE bouts.

Ludbrook *et al.* (1980) state that the RV contributes to mediating an upward shift in LV pressure-volume during IE. The LV and RV experience a disproportionate pressure change during IE primarily due to the difference in chamber volume, however this is in keeping with the structural and functional interrelationships of the two ventricles. Alterations in RV volume and pressure adjust stress across the interventricular septum, causing marked changes in LV pressure and volume (Ludbrook *et al.*, 1980). During IE, there are elevated systolic and end diastolic pressures in the RV (Ludbrook *et al.*, 1980). The acute cardiac responses following a session of IE are unknown and may provide an important mechanistic link for reduced BP following IET.

2.5.3 Peripheral vascular response to IE

The peripheral vascular response to IE may also contribute to acute cardiovascular outcomes, given that mean BP is the product of TPR x $\dot{Q}$. Restricted blood flow in

relation to the metabolic demands of an isometric task results in the accumulation of metabolites, activating the metaboreflex. This may increase vasoconstrictor nerve activity and increase TPR during the contraction, accompanied by a rapid increase in both sBP and dBP (Hietanen, 1984; Martin *et al.*, 1974). Greater intensity and duration of contraction will result in a wider activation of muscle fibres, causing a greater occlusion of resistance vessel blood flow (MacDougall *et al.*, 1984; Williams *et al.*, 2007), which also accounts for the linear relationship between contraction intensity and duration, and HR and BP.

Increased regional blood flow in the working muscles causes an increase in localised mechanical shear rate, stimulating the release of flow induced vasoactive agents by the vascular endothelium (Tinken *et al.*, 2010). Nitric oxide is released by the vascular endothelium to instigate vasodilation in an attempt to reduce TPR and restore normal blood flow.

A sudden dramatic drop in BP could be attributed to a reduction in venous return and removal of mechanical resistance to blood flow, thus reducing TPR. In addition, parasympathetic activation is associated with the release of acetycholine, which induces endothelial dependant NO synthesis in the vasculature and contributes to vasodilation. Post exercise vasodilation can be sustained for around two hours (Halliwill *et al.*, 2013), a function that may regulate TPR, and sustained post exercise hypotension. A primary mechanism for this response may be histamine H_1 and H_2 activation during recovery (Halliwill *et al.*, 2013).

2.5.4 Acute autonomic response to IE

As explained in section 2.3, autonomic control of cardiovascular responses is mediated by three neurogenic control mechanisms: the arterial baroreflex, central command and the exercise pressor reflex (Smith *et al.*, 2006). Isometric exercise is associated with an inhibited blood flow to working muscles, resulting in an increase in intramuscular pressure (Smith *et al.*, 2006) and a pressor reflex response to local circulatory occlusion (Mitchell and Wildenthal, 1974). Stimulation of mechanoreceptors generates somatosensory signals, which are transmitted to the central nervous system via afferent fibres and efferent responses are delivered via central command, triggering the

heightened cardiovascular responses observed during IE (Seals, 1993; Franke *et al.*, 2000).

During an isometric contraction motor unit recruitment and firing rates increase to maintain tension in the contracting muscle (Vaz *et al.*, 1996). Afferent mechanoreceptor activity causes the cardio-acceleratory centre to increase sympathetic excitation, and the cardio-inhibitory centre to stimulate parasympathetic activity. Stimulation of the sympathetic cardioaccelerator nerves initiates the release of catecholamines by the adrenal medulla and sympathetic nervous system. Increased circulating noradrenaline binds to β1-andrenoceptors on the cardiac cell membrane, triggering an acceleration of spontaneous SA node depolarisation and an increase in HR. Shortening of the myocyte action potential duration increases inotropy, causing an increase in SV, $\dot{Q}$, and an increased rate of relaxation (Levick, 2003).

Autonomic nervous system control has been evidenced during both isometric leg (Iellamo *et al.*, 1999a) and IHG contractions (Stewart *et al.*, 2007a; Millar *et al.*, 2009a). The contribution of the HF component of HRV was markedly reduced during isometric leg contraction, while the LF component increased, indicating an increase in sympathetic activation coupled with vagal withdrawal (Iellamo *et al.*, 1999a). In addition, during IHG, Watson *et al.* (1980) measured a 17% increase in circulating plasma adrenaline, a 27% increase in plasma noradrenaline in the resting arm and localised plasma adrenaline increase of 97%, suggesting both a system wide and localised increase in adrenergic stimulation, and sympathetic control of the ANS during IE.

It has been suggested that acute post exercise reductions in BP and HR are associated with a shift in autonomic regulation. Millar *et al.* (2009b) measured an increase in parasympathetic modulation and a reciprocal withdrawal of sympathetic activity indicating a shift in sympathovagal balance during recovery from IE. This acute improvement in autonomic regulation has also been measured at rest following a period of IET. Taylor *et al.* (2003) noted a downward trend of LF:HF ratio following 10 weeks of IET in concert with reductions in resting BP, while Millar *et al.* (2013) detected adaptations in HR complexity, a measure also associated with increased

cardiac vagal modulation. These findings suggest that in addition to acute improvements in autonomic regulation, repeated stimulation through IET may produce chronic improvements in autonomic modulation, which could be mechanistically linked to reduced BP.

The release of all catecholamines has been shown to increase during resistance training (Kraemer and Ratamess, 2005), with the magnitude of release dependant on the force of contraction, volume of muscle stimulation, exercise dose and rest intervals (Kraemer *et al.*, 1987). When measured in relation to IE, Watson *et al.* (1980) found that a session of IHG training elicited increases in both plasma noradrenaline and plasma adrenaline in both exercising and resting limbs, indicative of a sympathetic response. Measures taken following a programme of dynamic resistance training confirm a reduction in resting plasma catecholamine release (Guezennec *et al.*, 1986) and sympathodarenal activity is reduced in response to the same pre-training absolute workload (McArdle *et al.*, 2010) indicating a withdrawal of sympathetic activation and increase in vagal tone.

2.5.5 Acute adrenergic stimulation

Various hormonal factors act to control BP both at rest and under conditions of physical stress. These hormonal pathways are commonly manipulated in the pharmacological treatment of HTN and CVD. Medications which inhibit or block chemical activity (ACE inhibitors, aldosterone receptor blockers) are used to decrease arterial pressure, LV afterload, blood volume, pre-load and reverse vascular hypertrophy (Del Colle *et al.*, 2007; Law *et al.*, 2009; Leosco *et al.*, 2013). Any changes in adrenergic stimulation could help to elicit the driving factors behind isometric training induced BP reductions.

A reduction in the sympathetic response at rest and to a stressor would reduce noradrenaline production thereby inhibiting the subsequent RAAS cascade (Tsuda, 2012). This pathway could be a contributory factor to the measured chronic reductions in resting BP through IE, in association with improved autonomic modulation. Such hormonal adaptations could result in reduced pathogenesis of pre- HTN. Cornelissen

and Fagard (2005) state that chronic exercise related reductions in BP are a result of reduced vascular resistance, mediated by the sympathetic nervous system and RAAS, favourably affecting cardiovascular risk factors.

2.5.6 Baroreceptor reflex response

Vagal activation during acute recovery from exercise is associated with increased BRS. Iellamo *et al.* (1999b) carried out an isometric leg exercise protocol, and continued to occlude blood flow for 4 minutes upon cessation of the voluntary contraction. They measured reductions in HR while elevated BP was maintained, concluding that BRS was restored during post exercise ischaemia to allow HR to decrease, and suggesting that a vagally mediated baroreflex mechanism overpowering metaboreflex-induced sympathetic activation may be responsible. Isometric exercise causes the baroreflex response to reset at a higher point of activation so that the higher BPs associated with IET can be tolerated (Fisher *et al.*, 2006). Baroreceptor sensitivity has been shown to decrease during an isometric contraction (Iellamo *et al.*, 1999), suggesting that $\dot{Q}$ and BP responses are controlled by sympathetic activation, and the withdrawal of parasympathetic activation is accompanied by the withdrawal of baroreflex control of BP.

2.6 Possible mechanisms underpinning reductions in arterial blood pressure following isometric exercise training

2.6.1 *Cardiac structure and function*

Despite acute HR responses, changes in resting $\dot{Q}$ are not generally reported following a period of exercise training of any modality (Pescatello *et al.*, 2004a). Reductions in resting sBP and dBP following IET have not been associated with changes in SV and $\dot{Q}$ (Wiles *et al.*, 2010; Devereux *et al.*, 2010); however, few studies record these variables. Many studies also report no statistically significant change in HR (Peters *et al.*, 2006; Wiles *et al.*, 2010) and only one study has reported significant reductions of 4.8 ± 5.9 b·min^{-1} ($p<0.05$) following an 8-week isometric leg training (ILT) protocol (Baross *et al.*, 2012). A further study showed that IHG training reduced pulse pressure

(p<0.01 (Badrov *et al.*, 2013a), which is a further predictor of cardiovascular disease and all-cause mortality (Franklin *et al.*, 1999). These findings suggest that mediators of $\dot{Q}$ may not be mechanistically responsible for BP adaptations following IET. However the methods used in previous research to measure $\dot{Q}$ may not have been sensitive enough to demonstrate changes.

The effect of IET on the structure and function of the myocardium has not been widely researched, yet known adaptations following aerobic exercise training may shed light on the potential impact of IET.

Aerobic exercise is associated with increases in LV mass and improvements in contractile function. This response differs to pathological LV concentric remodelling, which is commonly seen in hypertensive hearts (Mihl *et al.*, 2008). Exercise training causes an increase in cardiac myocyte length of ~7% (Moore and Palmer, 1999a) causing enlargement of the ventricular cavity and increased wall thickness. This cardiomyocyte growth is stimulated by an increase in blood plasma and the release of insulin-like growth factors. LV filling time is enhanced and myocardial diastolic function improves with aerobic fitness. Improved LV size, contractile function and subsequent increased SV, lead to lower resting and sub-maximal HR's and associated improvements in parasympathetic activity (Libonati, 1999; Woodiwiss and Norton, 1995). As with the LV, aerobic exercise will lead to eventual augmentation in RV chamber size as both chambers must receive and eject greater blood volumes. Adaptations to the RV will contribute to a lower resting and exercising HR. Similarly to the LV, impaired RV function is a recognised consequence of HTN and CVD (Haddad *et al.*, 2008). Aerobic exercise is advocated to improve cardiovascular health including cardiac function (Libonati, 2013). Furthermore, studies have revealed that by remaining physically active into old age, diastolic function can be maintained and the age related decline in myocardial performance can be diminished (Libonati, 1999).

The use of physical activity to improve cardiac function and structure in an existing state of disease has produced mixed findings. A programme of aerobic exercise training can elicit improvements in cardiovascular fitness, and parasympathetic reactivity in diseased populations (Lucini *et al.*, 2002). In HTN, reductions in resting sBP and dBP are well reported; however, there is disparity in results regarding LV

hypertrophy with studies showing no change, an increase, or a decrease in LV volume following exercise (Libonati, 2011).

Although the effects of long term IET on chronic cardiac structure and function have not been widely explored in either healthy or diseased hearts, research into power trainers, weight lifters and hammer throwers, who incorporate an isometric element into their training may be of slight relevance. Keul *et al.* (1981), found that LV volume does not increase in athletes who train isometrically, as it does through dynamic endurance training. Instead, static exercise has been linked to increases in LV wall thickness, resembling concentric LV hypertrophy, which may or may not be accompanied by changes in LV volume (Spirito *et al.*, 1994). Such adaptations may have negative implications, however comparisons between untrained individuals performing IET and power athletes should be made with caution. The BPs reached during contraction, myocardial afterloads, and volume of training experienced in power athletes far exceed any loads involved in health based IET training protocols studied to date. Martin *et al.* (1974) found that the effects of IE on LV performance were dependant on the intensity of the contraction as well as the muscle mass involved.

Gandhi (2016) assessed cardiovascular and echocardiographic parameters following 5 weeks of IHG training and reported statistically significant increases in inter-ventricular septum thickness, LV posterior wall thickness, and LVEF, indicating a possible increase in LV mass. Furthermore, a reduction was measured in LV end systolic diameter and LV end systolic volumes, which with a significant reduction in SV would indicate a reduction in $\dot{Q}$. The study also reported a significant reduction in RPP, which is likely caused by a reduced afterload and reduced HR, which together improve myocardial efficiency. Hietanen (1984) stated that isometric training does not elicit any myocardial damage to a healthy heart. The myocardial response to IE in higher risk populations is unclear.

The myocardium and arteries adapt their structure in response to an increase in load (Mayet and Hughes, 2003). Therefore it is likely that changes in cardiac structure are an effect of elevated BP, caused by the increase in afterload. The progression of raised BP can occur prior to measurable decline in cardiac structure and function, as cardiac remodelling occurs (Cohn *et al.*, 2000). This supports the concept that cardiac

structural and functional maladaptations are an effect rather than a cause of raised BP. However, it remains of importance to understand whether interventions that reduce BP are also able to induce improvements in cardiac structure and function, as such findings may demonstrate wider CV adaptation and greater risk reduction.

2.6.2 Peripheral vascular adaptations

The anti-hypertensive effects of exercise training, including IET are mostly considered to be due to a reduction in TPR (Carlson *et al.*, 2014) mediated by structural and neurohumoral factors (Pescatello *et al.*, 2004a). Total peripheral resistance can be affected by blood vessel length, lumen diameter and arterial elasticity (Tortora and Derrickson, 2012). Pescatello *et al.* (2004a) suggest that, of these factors it is changes in lumen diameter that will evoke the biggest reduction in TPR.

Improved endothelial function, decreased sympathetic nervous activity and vascular remodelling may all have an influence on TPR (Millar *et al.*, 2014) and should all be considered as potential mechanisms for reduced BP. Reductions in vasoconstrictor hormones, such as endothelin-1 (Maeda *et al.*, 2001) and increases in vasodilator hormones, such as NO (Jungersten *et al.*, 1997) and histamine (Halliwill *et al.*, 2013) are recognised adaptations to aerobic exercise programmes, which have favourable effects on TPR. These pathways may also be implicated in inducing BP reductions through IET.

Following an 8-week bilateral ILT protocol, Baross *et al.* (2012) measured changes in femoral artery diameter alongside reductions in sBP and dBP. Millar *et al.* (2009b) suggested that NO bioavailability is improved and endothelium dependant vasodilation is increased in response to reactive hyperaemia encountered during IET, leading to reduced TPR and therefore decreased BP. Similarly, Badrov *et al.* (2013a) report an increase in resistance vessel endothelial function following IHG training. McGowan *et al.* (2007) measured improvements in NO dependent vasodilation in the trained arm only following unilateral IHG in medicated hypertensives, suggesting a localised response to trained muscles. Peters *et al.* (2006) also reported significant reductions in oxidative stress through 6-weeks of IHG in hypertensive participants. This was evidenced by a reduction (-266%, $p<0.05$) in exercise induced oxygen-centered

radicals and an increase in resting concentrations of antioxidant glutathione (+61%, $p<0.05$), suggesting that IET induced the upregulation of antioxidant activity, mediating BP regulation. Oxidative stress affects the delivery of vasoactive substances to the blood vessel wall therefore improvements may be associated with reduced TPR.

2.6.3 Autonomic regulation

Taylor *et al.* (2003) noted a downward trend of LF:HF ratio following 10 weeks of IET in concert with reductions in resting BP, while Millar *et al.* (2013) detected adaptations in HR complexity, a measure also associated with cardiac vagal modulation. These findings suggest that in addition to acute improvements in autonomic regulation, repeating this stimulus may provide chronic improvements in autonomic modulation, which may be mechanistically linked to reduced BP.

Improvements in cardiovascular and autonomic reactivity to an acute isometric stimulus have also been measured following IET. In addition to reductions in resting BP following IET, Fisher and White (1999) and Gandhi (2016) found that mean rises in sBP, dBP and mBP during an acute isometric training session were lower than pre-training values, which may be an important safety consideration in higher risk populations. Somers *et al.* (1992) reported an attenuation in muscle sympathetic nerve activity (MSNA) responses to a 2-min IHG contraction following a 6-week unilateral handgrip training programme and Sinoway *et al.* (1996) measured a reduction in noradrenaline spill-over response to an acute session of IHG, following a 4-week training programme. A reduction in sympathetic activation and the associated noradrenaline secretion may contribute to attenuated vasoconstriction and improved TPR (Hamer, 2006).

2.6.4 Neurohumoral changes

Elevation in the concentrations of inflammatory cytokines, CRP and IL-6 occurs with ageing (Rohde et al, 1999), however maintaining a good level of physical fitness is strongly associated with lower levels of inflammation (Hjelstuen *et al.*, 2006; Geffken *et al.*, 2001). TNF- α and IL-6 concentrations of physically active individuals have

been shown to be lower than age and gender matched inactive controls (Reuben *et al.*, 2003).

It has been suggested that a reduction in cytokine production may diminish circulating inflammatory cytokine concentrations, which may inhibit the acute phase cascade response leading to reductions in hepatic CRP production (Petersen and Pedersen, 2005). Interleukin-6 has been identified as a myokine, defined as a cytokine that is produced by skeletal muscles (Pedersen *et al.*, 2003). IL-6 is expressed in contracting skeletal muscle fibres during acute exercise (Steensberg *et al.*, 2002; Hiscock *et al.*, 2004). The anti-inflammatory properties of IL-6 are recognised, and the protein exerts an inhibitory effect on the production of inflammatory proteins TNF- α and IL-1, and induces the production of anti-inflammatory proteins IL-1ra and IL-10 (Steensberg *et al.*, 2003). It is possible that the acute anti-inflammatory effects of myokines during exercise will protect against chronic systemic low-grade inflammation, mediating the health benefits of exercise (Petersen and Pedersen, 2005).

A programme of aerobic exercise training can reduce concentrations of inflammatory markers in healthy (Toft *et al.*, 2000) and diseased (Goldhammer *et al.*, 2005) populations. Combined resistance and aerobic training has been shown to reduce CRP in both young and old participants, with the greatest reductions achieved in those who were physically inactive at baseline, and had the highest baseline CRP concentrations (Stewart *et al.*, 2007b). There is a limited body of evidence that demonstrates reductions in markers of inflammation following resistance training in diseased populations (Stewart *et al.*, 2007b), however the efficacy of IET in inducing favourable inflammatory responses is unknown.

2.6.5 Summary

This review highlights the importance of pre-HTN as a precursor to the development of HTN, which is an established risk factor for increased CVD morbidity and mortality. As recognised in hypertensive populations, pre-HTN may be associated with altered haemodynamic and autonomic modulation, as well as markers of impaired cardiac structure and function compared to individuals with optimal BP. As such,

populations with pre-HTN should be targeted with non-pharmacological interventions for the primary prevention of HTN and improved health.

A common barrier for adherence to current policy recommendations for physical activity is lack of time. Isometric exercise training is a potential alternative exercise intervention since it requires little time commitment and has been proven to reduce BP in normotensive, pre-hypertensive and hypertensive populations. In addition, IET requires no specific venue, costly equipment or supervision. However, despite this, many existing trials have involved supervised laboratory based interventions and home based IET requires further research. In addition, existing studies are limited by small sample sizes of normotensive or medicated hypertensive populations, despite pre-HTN being accepted as a moderate risk category.

Despite the widely accepted BP reductions following IET, the mechanisms are not completely understood. Previous research has focused heavily on haemodynamic outcomes following IET, with limited measures of cardiovascular parameters. As such, determining the acute continuous haemodynamic and cardiac autonomic responses to IE and in recovery, may provide potential mechanistic pathways for the known chronic adaptations. In addition, limited research has been performed assessing the acute myocardial response to IE. Any change in cardiovascular regulation will likely affect cardiac performance.

Despite being recognised as a more reliable predictor of cardiovascular outcome; the use of ABP monitoring is typically superseded by single resting BP measurement. Future IET research, that uses ABP monitoring as a measure of BP adaptations, is required. In addition, the wider cardiovascular implications of the BP reductions measured following IET require further research. The effects a programme of IET has on cardiac autonomic, systemic inflammation, vascular bio-markers and myocardial performance is unclear.

2.7 Aims

2.7.1 Thesis aims

The aim of this thesis was to investigate the acute cardiovascular responses and chronic adaptations following IE, performed using an isometric wall squat protocol. Therefore, a number of empirical studies, sectioned into acute and chronic investigations were completed and the primary aims of each study are outlined below.

2.7.2 Acute investigations

Study 1. To assess the cardiac autonomic and haemodynamic responses at rest, continuously during a single IE session and in recovery.
Study 2. To investigate the cardiac structural and functional responses as measured via a spectrum of echocardiographic indices pre and immediately post a single IE session.

2.7.3 Chronic investigations

Study 3. To evaluate changes in resting and ambulatory BP pre and post a home-based IET programme.
Study 4. To investigate potential physiological mechanisms and wider cardiovascular adaptations following a programme of IET, with specific reference to haemodynamic function, cardiac autonomic modulation and bio-chemical markers of inflammation and vascular function.
Study 5. To evaluate the impact of IET on cardiac structure and function. Specifically, left ventricular diastolic and systolic function will be assessed using standard echocardiograhic techniques.

2.8 Hypotheses

2.8.1 *Acute investigations*

Study 1

1: *H1:* There will be a progressive increase in BP during IE followed by immediate post exercise hypotension.

2: *H1:* There will be an increase in sympathetic activation and a reduction in parasympathetic modulation during IE. In recovery, there will be a reduction in sympathetic modulation and an increase in parasympathetic modulation.

3: *H1:* Post IE alterations in haemodynamic and cardiac autonomic modulation are associated with improved baroreceptor reflex control mechanisms.

Study 2

1: *H1:* IE will significantly improve diastolic function, measured by E/A ratio during recovery.

2: *H1:* IE will be associated with a reduced estimated LV filling pressure during recovery.

2.8.2 *Chronic investigations:*

Study 3

1: H1: Four weeks of IET will significantly reduce 24-hour ambulatory BP.

2: H1: Four weeks of IET will elicit a reduction in HR and increase SV, with no change in $\dot{Q}$.

Study 4

1: H1: Four weeks of IET will improve cardiac autonomic modulation with a predominance of parasympathetic over sympathetic activity.

2: H1: Four weeks of IET will significantly increase BRS.

3: H1: Four weeks of IET will significantly reduce plasma concentrations of IL-6, TNF-α and hs-CRP.

4: H1: Four weeks of IET will significantly reduce plasma concentrations of intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) and asymmetric dimethylarginine (ADMA).

Study 5

1: H1: Four weeks of IET will significantly reduce estimated LV filling pressure.

2: H1: Four weeks of IET will significantly alter isovolumetric relaxation time.

3: H1: Four weeks of IET will significantly reduce the myocardial performance index.

CHAPTER 3:

General Methods

3.1 Overview

The aim of this Chapter is to provide an understanding of the research approach, research methodology, participants studied and variables measured and to collate common procedures used throughout the studies described in this thesis. Details of measurement and recording will be provided with regards to the following variables: HR, BP, TPR, $\dot{Q}$, SV, HRV, BRS, cardiac function as well as information regarding the validity and reliability of the variables. The isometric training protocol, and subsequent isometric wall squat training prescription will also be explained.

3.2 The research approach

A quantitative, experimental approach was adopted for the research conducted in this thesis. Study 1 and 2 examined the acute effects of a single session of isometric wall squat training, at a pre-prescribed knee-joint angle. The acute cardiovascular responses of BP, HR, TPR, $\dot{Q}$ and SV were measured on a beat-to-beat basis pre, during, and following a single session of IE. Cardiac autonomic function was also measured continuously to ascertain sympathovagal modulatory responses during IE and in recovery. The acute effects of a single session of IE on haemodynamic and autonomic function are presented in Chapter 4. Cardiac function was measured before and after a single IE session and these findings are presented in Chapter 5.

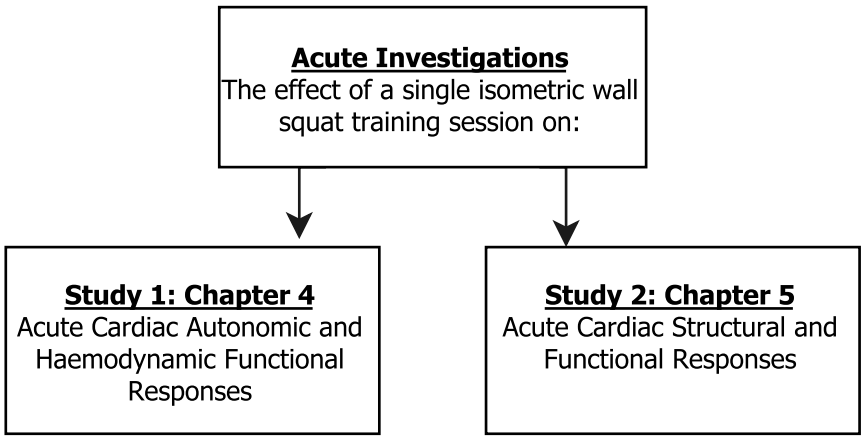

Figure 3.1. Schematic 1 of studies contained within this thesis.

Studies 3, 4 and 5 investigated the effects of a 4-week, home-based isometric wall squat intervention (IET) on haemodynamic, neurohumoral and myocardial variables in order to establish any chronic functional adaptations following a period of training. Chapter 6 explores the effects of the 4-week IET programme on resting haemodynamic function and changes in ABP. Chapter 7 examines adaptations in cardiac autonomic regulation and in bio-markers of inflammation and vascular function pre and post isometric training. Chapter 8 reports the cardiac structural and functional responses to 4-weeks of isometric training.

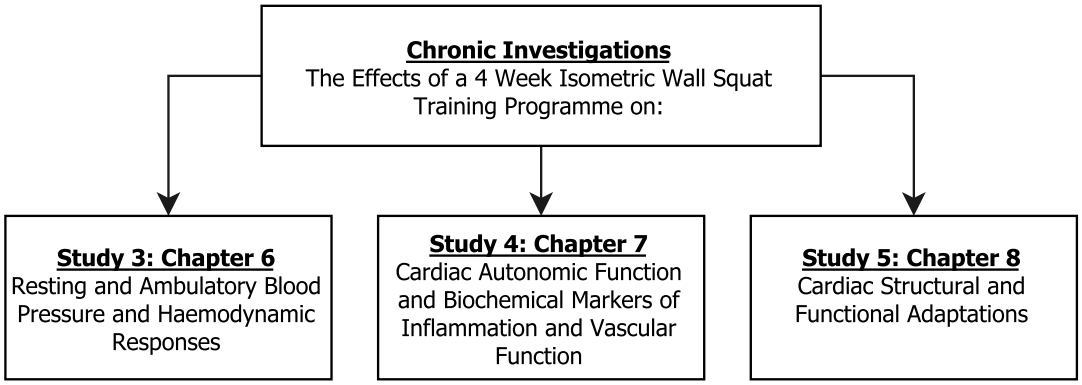

Figure 3.2. Schematic 2 of studies contained within this thesis

With the exception of the ABP monitoring used in Chapter 6, all experimental procedures were conducted in a laboratory environment within the Section of Sport and Exercise Sciences at Canterbury Christ Church University. The home-based isometric training intervention was completed in a location of the participants' choice, with portable equipment provided to them. All studies were approved by the Canterbury Christ Church University Ethics Committee and all procedures were conducted according to the Declaration of Helsinki (2013). A copy of the letter of approval is presented in Appendix 1.

3.3 Participant information

3.3.1 Inclusion criteria

Pre-hypertensive males (aged 30-65) were recruited for each of the studies. Pre-HTN

was determined based on three resting sBP measurements of ≥120 mmHg and <140 mmHg or dBP measurements of ≥80 mmHg and <90 mmHg, in accordance with protocols described in section 3.6. Recruitment was also subject to a 12 lead ECG that was checked by a consultant cardiologist. Volunteers with a resting BP outside of these ranges were excluded from participation and were advised to visit their GP if resting BP exceeded these ranges. All participants were non-smokers and free from injury or disease that could conceivably affect their wellbeing or the results of the study during the experimental period. Participants were screened using a self-reported standardised health and medical questionnaire, including details of personal and family heredity health. Participants were free from any clinically diagnosed cardiovascular condition/disorder and were not taking any medication, and had no immediate family history of CVD (See Appendix 2: Physical activity readiness questionnaire). All participants were considered to be physically inactive, meaning that their exercise participation rate was below public health recommendations of 150 minutes of moderate physical activity per week (Britain, 2004). Women were excluded from the research due to known confounding variation in cardiovascular and BP variables during the menstrual cycle (Dunne *et al.*, 1991; Sato *et al.*, 1995).

3.3.2 Recruitment

All participants were either members of staff from Canterbury Christ Church University, or were associated contacts that gained knowledge of the research through word of mouth. All staff members were offered free anonymous basic health screenings, which included BP measurement. Participants who met the inclusion criteria based on the results of their health check, were given a participant information sheet detailing the research and were invited to take part. The participant information sheets can be found in Appendix 3. The participant information sheets for both studies contained a written explanation of the testing protocols, testing requirements, and purpose of investigation. It was explained that some minor discomfort might be experienced during IE but that some health improvements could potentially be made through IET. As the exercise training intervention formed a cross over trial, all participants would receive the intervention either pre or post a control period.

Participation was voluntary and included no monetary or material reward. Feedback

and advice regarding the health and fitness benefits of physical activity would be provided at the end of the study and details of the full cardiovascular assessment performed during the research were provided to participants as an incentive.

Prior to study commencement, all participants signed an informed consent form (Appendix 4) confirming that they understood the information sheet, had been given the opportunity to ask questions, understood that participation was voluntary, and that they agreed to take part in the study.

3.3.3 Sample size

Sample size calculations were used to ensure that the research was adequately powered and was ethical. If a sample size is too small, a clear finding may not be produced, whereas if a sample size is larger than necessary, resources beyond those required may be wasted for no additional gain (Hopkins, 2006). Determination of the sample size necessary for research requires consideration of the smallest worthwhile effect to be detected, Type I and Type II error rates, and the study design (Hopkins, 2001). A good level of reliability of the dependant variable is also important as this will make detection of true changes more evident, therefore requiring a smaller participant sample to be recruited (Hopkins, 2001). In this thesis, sample size (n) was calculated using the following equation where s is within-subject variation and d is the smallest worthwhile change to be detected (Hopkins, 2001), using the equation $n=16\ (s^2/d^2)$.

Sample size calculation was specifically conducted for use in a randomised cross-over control trial and considers within-subject variation. Within-subject variation concerns the reproducibility of values obtained from the same participant by the same experimenter on the same equipment (Hopkins, 2000). Within subject standard deviation can be expressed as a percentage of the mean value, also known as Coefficient of variation (CoV), and change recorded outside of this range is considered as 'real' significant change.

The equation used gives a study power equivalent of 0.80 with a significance level of $p=0.05$. This is equivalent to a Type II error rate of 20% and a Type I error rate of 5%, suggesting an 80% certainty of finding a statistically significant ($p<0.05$) worthwhile

change.

Previously published data from within our laboratory was used to determine the smallest worthwhile change value (d). Wiles, Coleman and Swaine (2010) found significant reductions in resting BP measures following an 8-week isometric double-leg training programme. Table 3.1 shows the change scores expressed as a percentage of the mean baseline value ([change/mean]100), which were then used in the sample size equation. Data from the control group from Wiles, Coleman and Swaine (2010) was also used to calculate the CoV's for each of the variables. Table 3.1 shows the estimated number of participants required for this thesis.

Table 3.1. Previously published data (Wiles *et al.*, 2010) used for sample size calculation and smallest detectable change based upon the methods of Hopkins (2001).

	Change Post isometric training	Baseline Value	*d* (% of mean score)	*s* = resting CoV	*n*= no. of participants =16 (s^2/d^2)
sBP (mmHg)	-5	122	-4.3%	4.6%	18
dBP (mmHg)	-3	69	-3.8%	4.0%	18
mBP (mmHg)	-3	89	-2.8%	3.5%	25

The main primary outcome variable selected in this thesis was the measurement of BP pre and post an isometric wall squat training intervention and control period. Based on the sample size estimation in Table 3.1 a sample size of 25 was selected for this thesis. Additional variables were measured in order to gain an insight into the potential mechanisms that regulate BP changes. Previous research has found that participants with a higher baseline BP experience the greatest reductions (Lawrence *et al.*, 2014), therefore this sample size, based on data from a normotensive population, was expected to be adequate to measure any changes in BP. Furthermore, as demonstrated in Table 2.2, page 28, previous IET studies have used much smaller sample sizes. Previous research has highlighted a drop out of up to 20% of participants in clinical randomised control trials (Wood *et al.*, 2004). In order to account for potential attrition, an over recruitment target of 20% was selected, to account for the frequency

of commitment required by participants. As such a target recruitment of 30 was set and due to circumstances outside the control of the investigator, not all participants completed all studies, as demonstrated in Figure 3.3.

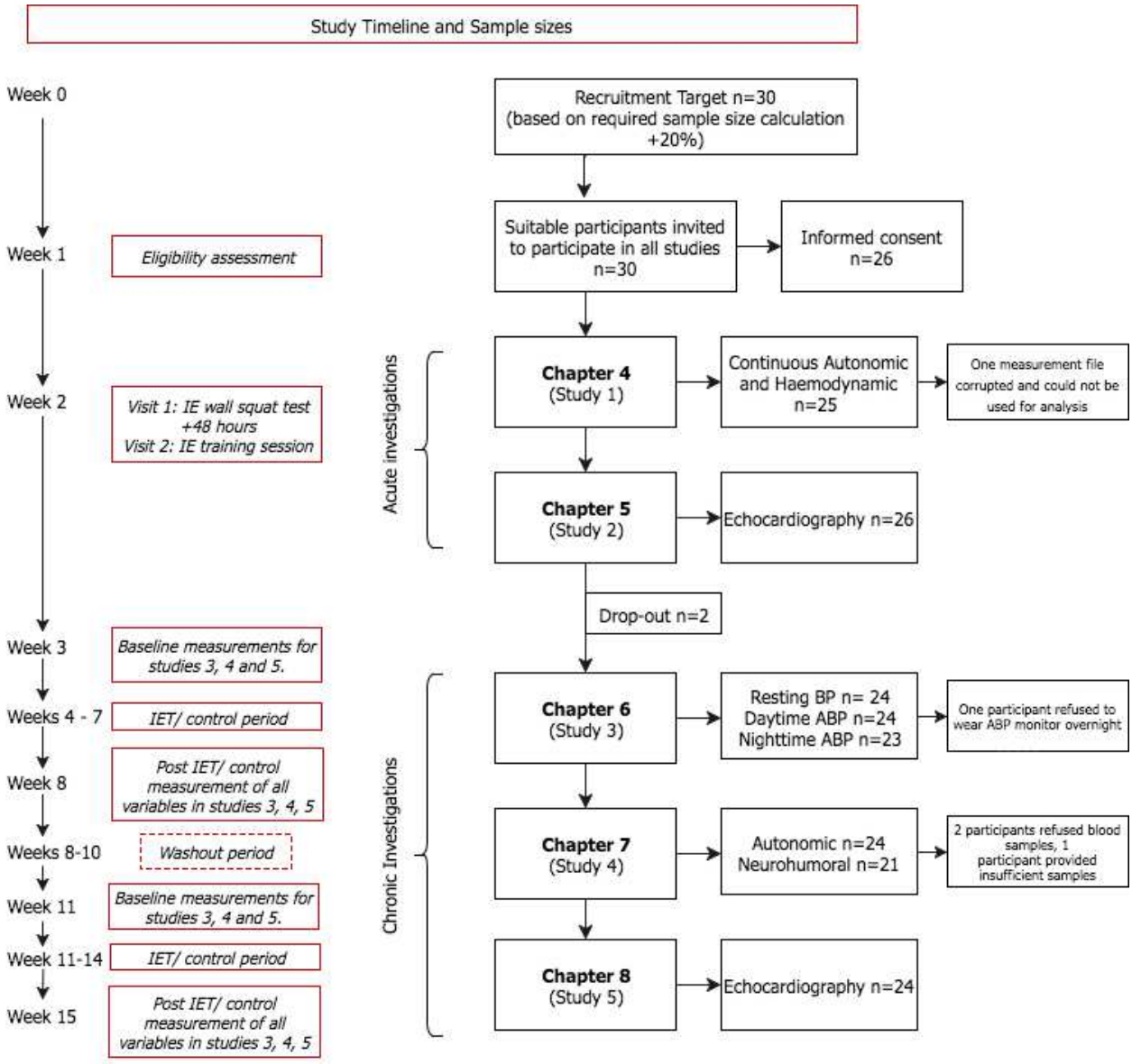

Figure 3.3. Data collection timeline and sample sizes used in all studies of this thesis

3.3.4 Testing requirements

Prior to each visit to the laboratory, participants were requested to maintain an abstinence from alcohol and caffeine for 24 hours, and from food for 4 hours, as these factors have previously been shown to influence HR and/or BP measurements (Potter *et al.*, 1986; Shapiro *et al.*, 1996; James, 2004). Participants were also required to avoid involvement in physical activity, including IET, for 48 hours prior to each visit, due to possible enduring recovery effects of an acute exercise session (Rezk *et al.*, 2006). Throughout the IET intervention period, participants were required to maintain their pre-participation dietary habits and routine level of physical activity (other than the inclusion of the prescribed isometric protocol). Adherence to these requirements was verbally confirmed with each participant prior to the start of each laboratory visit before any measures were taken.

3.3.5 Familiarisation

Following recruitment and consent, all testing protocols and measurement procedures were verbally explained to each participant. Participants were familiarised with isometric wall squat exercise, although a full test was not completed at this stage. Once wall squat training intensity had been prescribed (3.10.3), a familiarisation IET session was completed in the laboratory prior to the acute IE session for study 1, and 2 and prior to home-based training in studies 3, 4 and 5.

3.4 Measurement of the variables studied

This thesis includes the measurement of a number of cardiovascular and haemodynamic variables and this section will outline the techniques used to measure these parameters. The validity and reliability of each measure will be discussed with reference to relevant previous research.

3.4.1 Cardiac autonomic assessment

The Task Force® Monitor (TFM) (CNSystems, Graz, Austria) is a multi-use device employed in this thesis for recording a range of measures both at rest and during IET.

The validated monitoring system provides non-invasive and continuous evaluation of the cardiac ANS. In this thesis, the TFM was used for the continuous non-invasive beat-to-beat monitoring and automatic online calculation of all cardiovascular, haemodynamic and HRV parameters (Fortin *et al.*, 1998; Valipour *et al.*, 2005). The TFM is shown in Figure 3.4.

Figure 3.4. The Task Force® monitor.

The TFM enables the continuous measurement of BP using the vascular unloading technique (Fortin *et al.*, 1998; Gratze *et al.*, 1998), which is automatically corrected to oscillometric BP values obtained at the brachial artery of the contralateral arm. Beat-to-beat stroke volume is recorded with impedance cardiography (ICG) (Fortin *et al.*, 1998; Gratze *et al.*, 1998). A 6-channel ECG is included for R-R interval determination (Valipour *et al.*, 2005; Fortin *et al.*, 2001) and the beat-to-beat values are used for the real-time calculation of HRV by an autoregressive model (Fortin *et al.*, 2001; Bianchi

et al., 1997) and are displayed as 3-dimensional sliding power spectra (Fortin *et al.*, 2001). Baroreceptor sensitivity (BRS) is automatically evaluated via the sequence method and displayed on-line. The TFM meets the requirements of the CE mark (CE 0408, TUeV Austria, Vienna) and the Food and Drug Administration (FDA) clearance 510(k) (n^o:K014063).

A selection of haemodynamic parameters measured are indexed to participant body surface area (BSA). Participant stature in centimetres (cm) using a stadiometer (Seca 213, Seca GmbH & Co. Kg., Hamburg, Germany) and body mass in kilograms (kg) using mechanical column scales (Seca 710, Seca GmbH & Co. Kg, Hamburg, Germany) were measured during the first lab visit, and each subsequent laboratory visit commenced with the measurement of participant body mass.

3.4.2 Continuous blood pressure monitoring

Single BP measurements using a sphygmomanometer are deemed sufficient in clinical practice and can provide adequate assessment of seated resting BP (Williams *et al.*, 2004). However this method does not account for sudden transient changes in the circulation, such as in response to an exercise stimulus. The recording of beat-to-beat fluctuations in arterial pressure enable evaluation of cardiovascular control mechanisms (Benditt *et al.*, 1996; Low, 1996), although intra-arterial measurement is not commonly used due to potential complications and the effect on autonomic tone (Harms *et al.*, 1999; Stevens, 1966), therefore non-invasive methods are preferential.

Compared with the Finapres®, the TFM employs an improved version of the vascular unloading technique to measure continuous BP at the proximal limb of the index or middle finger (Hirschl *et al.*, 1996; Parati *et al.*, 2003; Parati *et al.*, 1989). Within the finger cuff, blood flow is detected by infrared light sensors and pressure is exerted by inflation or deflation of the cuff in order to keep blood flow and pulsation constant. The pressure required to maintain constant blood flow corresponds to real arterial pressure (CNSystems, 2014b). Multiple digital feedback loops and high fidelity signal processing exert system control and ensures artifact and vasomotor activity rejection. An algorithm is used to translate plethysomographic signals into BP information based

on the changes in blood volume (Fortin *et al.*, 2006b).

The arteries in the fingers are responsible for thermoregulation and therefore possess a heightened susceptibility to vasoconstriction and vasodilation in accordance with environmental temperature and blood volume of the participant. Although arterial pressure in the fingers may not correspond with pressure in the larger arteries, continuous BP is automatically corrected to oscillometric BP values obtained at the brachial artery of the contralateral arm, providing true arterial BP values as opposed to finger arterial pressure (Fortin *et al.*, 1998; Fortin *et al.*, 2006b). Oscillometric BP monitoring is explained in 3.6.

The continuous BP device of the TFM has been systematically tested against intra-arterial BP monitoring, and the Finapres. All methods reveal comparable results during both rest and autonomic function testing. The TFM is advantageous as it offers continuous, non-interrupted BP recording while the Finapres requires recalibration during testing. In addition, the oscillometric BP device used on the TFM has been evaluated against the protocol of the American national standard for electronic or automated sphygmomanometers (ANSI AAMI SP10-1992) and has also been validated against other BP measurement devices such as the Dinamap® BP monitor (Fortin *et al.*, 2001), also used in this thesis.

3.4.3 Impedance cardiography

Impedance cardiography (ICG) is a non-invasive technology to measure the total conductivity of the thorax and how it changes over time in relation to the cardiac cycle (Fortin *et al.*, 2001; Fortin *et al.*, 2006a). A number of cardiodynamic parameters can be determined continuously, including SV, $\dot{Q}$ and thoracic fluid content (Ventura *et al.*, 2000; Drazner *et al.*, 2002). Invasive measures of beat-to-beat $\dot{Q}$, such as the thermodilution technique, are still considered the gold standard measure (Harms *et al.*, 1999; Fortin *et al.*, 2006a). However, these methods are expensive and require a high level of expertise, specialist facilities and carry inherent risks including mortality (Harms *et al.*, 1999; Parrot *et al.*, 2004; Guyatt, 1991). For this reason, invasive techniques are unsuitable for research purposes and are highly unnecessary in healthy participants. Therefore non-invasive measures provide a more suitable alternative.

The method of measuring transthoracic impedance cardiography has been advanced since its conception in the 1940s and now incorporates individual differences in participants for more accurate measures. It is accepted that the shape of the thorax changes according to weight, therefore the TFM incorporates the calculation of each individuals BSA to more accurately quantify SV measurements. The TFM estimates changes in blood volume in the aorta and changes in fluid volume in the thorax, thus utilising ICG methodology (Fortin *et al.*, 2006a). Online and continuous quantification of LV SV is calculated by measuring the maximum rate of thoracic electrical impedance during ventricular ejection, which is divided by the base impedance and multiplied by the LV ejection time (ET) and volume constant of the chest. The volume constant of the chest is determined by the individuals' age, height, weight and body surface area (BSA) (Valipour *et al.*, 2005; Fortin *et al.*, 2006a).

The non-invasive and risk free measure of ICG by the TFM has produced recordings of $\dot{Q}$ (Fortin *et al.*, 2006a) and other haemodynamic parameters comparable to those of the thermodilution technique (Fortin *et al.*, 2006a; Drazner *et al.*, 2002; Parrot *et al.*, 2004). The TFM uses a lower measurement current (400uA) than other available non-invasive ICG devices and meets the standard EN 60601-1 for class CF devices (Albert *et al.*, 2004), and is therefore deemed safe for use in all populations by the International Electrotechnical Commission.

The TFM ICG electrodes consist of two electrode bands manufactured at predetermined distances and set onto an adhesive strip. These electrodes allow easy reproducibility of placement for consecutive measures and are therefore advantageous over spot electrodes for research purposes. An electrode is placed on the nape of the neck and two other electrodes are placed on the thorax (left and right side) in line with the xiphoid process (Fortin *et al.*, 2006a), as shown in Figure 3.5. This creates the non-invasive uniform high frequency alternating current field within the thorax that is required for ICG measurement (Fortin *et al.*, 2006a). The electrodes cover a large area and the electrode design allows limited participant disturbance, adding to the high reproducibility reported in validation studies by Fortin et al, (2006) ($r=0.971$, $N=20$, $p< 0.001$) and Fortin et al, (2001) ($r=0.963$, $N=42$, $p<0.001$).

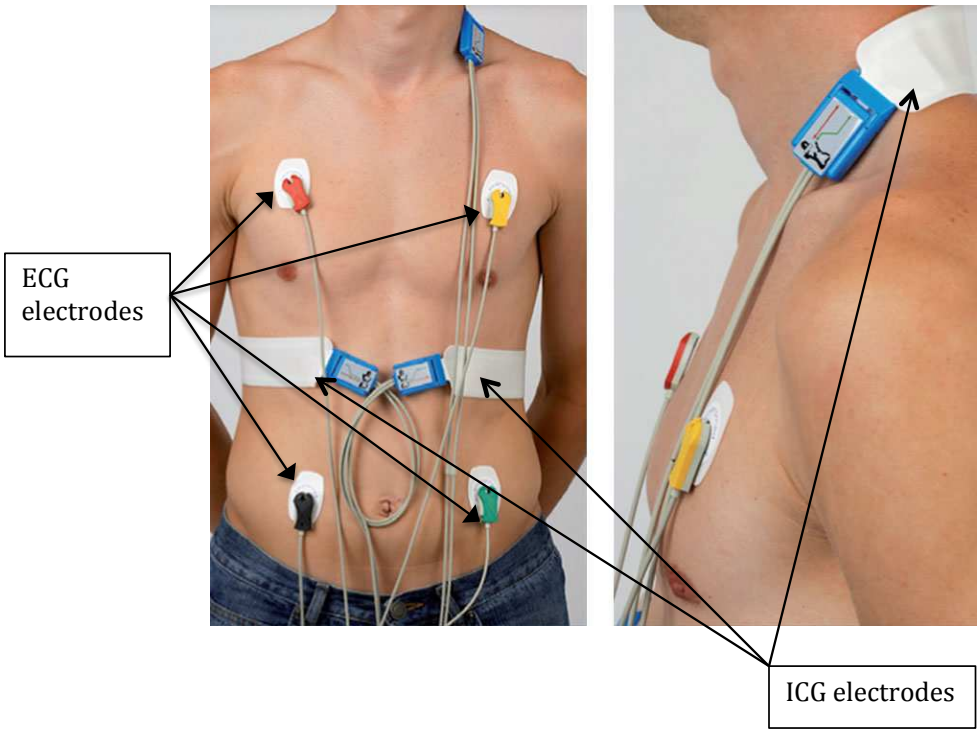

Figure 3.5. The Task Force® Monitor impedance cardiography and electrocardiography electrode placement.

The TFM also calculates total peripheral resistance (TPR) and total peripheral resistance index (TPRI) according to Ohm's law, where TPRI equals mean BP divided by cardiac index (CI) (Fortin *et al.*, 2006a).

3.4.4 Heart rate variability

Heart rate variability is widely accepted as a non-invasive marker of cardiac autonomic activity (Blaber *et al.*, 1995; Malik *et al.*, 1996), reflecting heart-brain interactions and ANS dynamics (Shaffer *et al.*, 2014). Both ECG and power spectral analysis methods provide an index of autonomic modulation (Akselrod *et al.*, 1981). A healthy heart is characterised by significant beat-to-beat variability, symbolising its capacity to adapt to transient changes in autonomic input (Pagani *et al.*, 1997).

Heart rate variability is a reflection of sympathetic and parasympathetic input and demonstrates the end organ response in the SA node, which is determined by nerve firing, cardiac adrenergic receptor sensitivity, and post-synaptic signal transduction

(Sandercock *et al.*, 2005). However, HRV cannot quantify the intensity of a stimulus, as it is a marker of neural efferent activity (Floras, 2009).

To maintain homeostasis, neural hierarchy elicits modifications in myocardial performance, vascular tone and R-R intervals (Lanfranchi and Somers, 2002). The oscillating changes in R-R intervals are the result of continuous changes in sympathetic and vagally mediated impulses. The frequency and amplitude of impulses can be assessed to distinguish sympathetic and parasympathetic nervous activity. It is widely accepted that the LF component represents sympathetic outflow to the heart, while the HF component represents parasympathetic outflow via the vagus nerve. It is proposed that the ratio of LF:HF components provides a measure of cardiac sympathovagal balance (Pomeranz *et al.*, 1985).

The TFM uses power spectral analysis to measure HRV. Each R-R interval is degenerated into a sum of waves (sinusoidal) of different amplitudes and frequencies. The results are displayed with the magnitude of variability as a function of frequency (power spectrum) (Ditor *et al.*, 2005). The power spectrum reflects the amplitude of R-R interval fluctuations at different oscillation frequencies (Akselrod *et al.*, 1981), allowing frequency specific oscillations to be studied (Akselrod *et al.*, 1981). The TFM uses a combination of published detection algorithms (Pumprla *et al.*, 2002), and uses the output data to calculate real-time HRV by an autoregressive model (Li *et al.*, 1995). Data is displayed as three-dimensional sliding power spectra (Gratze *et al.*, 1998).

The total power of the LF, HF and an additional VLF band is expressed in absolute values (ms^2) and the cumulative values are expressed as PSD. Physiological correlates of very low frequency component remains unknown and require further investigation (Malik *et al.*, 1996) therefore to assess the distribution of power spectral variance, the VLF component is discarded, as this is considered unnecessary noise and the remaining LF and HF measures can be expressed in normalised units (nu), by dividing the power of every LF and HF component by the total power and multiplying the ratio by 100 (Sharma *et al.*, 2015).

The QRS-algorithm used by the TFM has been evaluated with the MIT/BIH databases,

containing 24-hours of real world ECG data, including the broadest possible range of waveforms, achieving a detection rate of all included data of 98.87%, demonstrating the reliability of the device (Fortin *et al.*, 2001).

3.4.5 Baroreceptor reflex sensitivity

The baroreflex is responsible for short-term BP regulation and used a feedback loop with cardiac, vascular and cerebral components (Valipour *et al.*, 2005). Transient increases and decreases in BP are mediated by baroreflex adjustment of HR, myocardial contractility and peripheral resistance.

Baroreflex sensitivity (BRS) is a measure of how much control the baroreflex has on HR at a given moment and demonstrates the degree of health or disease (Swenne et al, 2013). It is understood that BRS decreases with age (Pinna *et al.*, 2000), HTN (Gribbin *et al.*, 1971) and CVD, and is also an independent predictor of cardiac mortality (Mortara *et al.*, 1997). Invasive measurement of BRS involves the intravenous administration of an alpha-adrenoreceptor stimulant or vasoactive drug such as phenylephrine (Laitinen *et al.*, 1999; Kardos *et al.*, 2001). The linear regression line between increases in sBP and reflex lengthening of the pulse interval (PI) represents the strength of the BRS (Pinna *et al.*, 2000). This technique involves arterial cannulation in order to record beat-to-beat arterial BP (Schwartz *et al.*, 1992). As with most invasive measures, application is expensive, and can only be carried out in an in-patient setting. Non-invasive alternatives are necessary for research purposes and for use in healthy volunteers.

The TFM uses the sequence technique method to quantify BRS non-invasively, which is a widely used practice (Robbe *et al.*, 1987; Bertinieri *et al.*, 1985). Therefore spontaneous baroreflex control of the heart can be established using sophisticated algorithms and computer technology.

Baroreflex sensitivity measures the coupling between the R-R interval and sBP. This marker demonstrates the ability of the ANS to react to BP changes by altering the R-R interval. When a change in BP is detected, sympathetic or parasympathetic activity triggers the HR to adjust accordingly, allowing the sensitivity of the baroreceptor

reflex to be detected (CNSystems, 2014a).

In association with measures of beat-to-beat PI and BP, the TFM identifies a series of successive increases (hypertension/ bradycardia or +PI /+sBP) or decreases (hypotension/ tachycardia or –PI / -sBP) in sBP and lengthening of the PI (Valipour *et al.*, 2005; Di Rienzo *et al.*, 2001). The slope of the regression line between the sBP and PI values in each sequence is taken as an index of the BRS control of the heart (Di Rienzo *et al.*, 2001). Research has demonstrated that the interactive sequences of sBP and PI are real physiological events as opposed to chance interactions (Valipour *et al.*, 2005).

3.4.6 Task Force® monitor reliability

The most accurate measurements of autonomic function are invasive procedures, however these are impractical in non-clinical and outpatient settings and as such are reliant on small sample sizes. Measuring HRV non-invasively can provide a good index of cardiac autonomic modulation, however some early methods of measuring HRV non-invasively returned poor reproducibility, with co-efficient of variation ranging from 1-235% (Sandercock *et al.*, 2004). Measurement of HRV requires participants to be stationary and for recording periods to be free from ectopic beats and irregularities.

Inter-individual and intra-individual reliability of the TFM has been previously performed over four separate trials at 2 week intervals, demonstrating low intra-individual and moderate intra-individual CoV (Goswami *et al.*, 2009).

3.5 Preliminary Study: Reliability of the Task Force® Monitor

3.5.1 Preliminary Study: Introduction

Many devices are available for recording and measuring health parameters such as heamodynamic and cardiac autonomic function, both of which are subject to transient changes, even at rest, due to the highly reactive nature of these variables (Mancia *et al.*, 1983). Many devices do not allow for continuous measurement, restricting data to

discrete time points within a study period, which does not represent an entire intervention or time interval. In addition, some devices are designed to perform just a small number of measurements, meaning that multiple devices may be needed to gain a comprehensive recording of autonomic and haemodynamic function.

The TFM is able to provide continuous, beat-to-beat monitoring of a range of haemodynamic and cardiac autonomic parameters. The aim of this preliminary study was to assess the reliability of repeated measures of continuous haemodynamic function (HR, beat-to-beat sBP, dBP and mBP, LVET, oscillometric sBP and dBP) and cardiac autonomic function (PSD, VLF, LF, HF, BRS), taken over a number of days using the TFM device.

3.5.2 Preliminary Study: Method

3.5.2.1 Participants

Twenty healthy male participants volunteered to participate in the preliminary study (age 21 ± 2, height 179 ± 6cm, weight 83 ± 11kg). All participants provided written informed consent and the study received ethical approval from the institution.

3.5.2.2 Procedure

Participants attended the laboratory on three days of the week, with each visit separated by 48 hours. All visits commenced at the same time on each day, following a >4 hour fast and a >24 hour abstinence from caffeine or alcohol. The visit protocol was precisely replicated on each occasion.

Participants rested in a seated position for 15 minutes. Height was measured in centimetres (cm) using a stadiometer (Seca 213, Seca GmbH & Co. Kg., Hamburg, Germany) and body mass was measured in kilograms (kg) using mechanical column scales (Seca 710, Seca GmbH & Co. Kg, Hamburg, Germany). Once the TFM was set up, participants were asked to remain as still, quiet and relaxed as possible and rested in a supine position with the lights turned off, a 5-minute recording period was used for analysis following 15 minutes of supine rest.

3.5.2.3 Data analysis

Prior to analysis the data was checked for the assumptions using parametric tests. Mean values were calculated for all test variables for each trial. Within-participant variation, expressed as a CoV, was derived by log-transformed two-way analysis of variance as described by Atkinson and Nevill (2001), together with the confidence intervals for a normal distribution (Tate and Klett, 1959).

3.5.3 Preliminary Study: Results

On each of the three laboratory visits, cardiac autonomic function was recorded for 5-minutes. The reliability as CoV and CI of all measures taken is shown in Table 3.2.

Table 3.2. Reliability of cardiac autonomic recording of the Task Force® Monitor

	CoV %	Confidence Limit (Lower-Upper)
PSD (ms^2)	4.08	3.08-6.03
VLF (ms^2)	12.46	9.48-18.20
LF (ms^2)	10.9	8.24-16.12
HF (ms^2)	9.58	7.24-14.17
HR ($b \cdot min^{-1}$)	5.9	4.49-8.62
sBP beat-to-beat (mmHg)	3.08	2.34-4.50
mBP beat-to-beat (mmHg)	4.29	3.26-6.27
dBP beat-to-beat (mmHg)	5.66	4.30-8.27
BRS ($ms \cdot mmHg^{-1}$)	9.3	6.9-13.5

Note: PSD, power spectral density; VLF, very low frequency; LF, low frequency; HF, high frequency; HR, heart rate; sBP, systolic blood pressure; mBP, mean blood pressure; dBP, diastolic blood pressure; BRS, baroreceptor sensitivity.

3.5.4 Preliminary Study: Discussion

The results of this preliminary study show that the TFM provides a reliable measure of cardiac autonomic and haemodynamic function in a non-clinical population, a finding in line with those previously demonstrated in a clinical population (O'Driscoll, 2009). These reliability findings support the use of the TFM for research purposes.

3.6 Oscillatory blood pressure monitoring

Oscillometric BP monitoring methods are employed during continuous (TFM) resting (see 3.7) and ambulatory (see 3.8) BP measurement within this thesis. An automated oscillometric BP monitor consists of a pneumatic upper arm cuff containing a transducer, an air hose and a monitor. Manual or automatic activation of the BP monitor initiates a sequence of cuff inflation and deflation controlled by a microprocessor, in a cycle which takes ~20-30 seconds. The cuff initially inflates to suprasystolic pressure, high enough to occlude the underlying brachial artery. The transducer in the upper arm cuff detects oscillations of the arterial wall. The amplitude of minute pressure oscillations within the cuff are measured and inflation ceases when oscillations are no longer detected. The cuff then deflates progressively in increments of 5 mmHg, every time two pressure pulsations of equal amplitude are detected, known as stepped-deflation (GE Medical Systems, 2002). Systolic BP is detected where oscillation amplitudes increase most rapidly, at the point where blood begins to pass once again down the previously occluded artery. Diastolic BP is detected where oscillation amplitudes decrease most rapidly. The cuff fully deflates when oscillation amplitudes cease to exist below dBP.

An appropriately sized cuff is important for accuracy in BP measurement, and the margin of error tends to be larger when the cuff used is too small (Bovet *et al.*, 1994). A cuff width of 46% of the participants' upper arm is the ideal size (Marks and Groch, 2000) and the length to width ratio should be 2:1 (Pickering *et al.*, 2005).
In addition, postural factors have also been shown to affect BP readings. Supine dBP tends to be 5 mmHg lower than when a person is seated (Pickering *et al.*, 2005), BP readings may be higher if the back is not supported (Cushman *et al.*, 1990) or if the legs are crossed (Peters *et al.*, 1999), and if the arm is hanging down below the level of the right atrium (Pickering *et al.*, 2005).

3.7 Resting blood pressure measurement

Resting BP measures were performed using an automated BP monitor 'Device for Indirect Non-invasive Automatic Mean Arterial Pressure' (Dinamap® Pro, GEMedical Systems, Slough, Berks, UK). This device operates using the oscillometric technique,

whereby oscillations of the arterial wall are created as pulsatile blood flows through an artery.

Each participant rested in a seated position for 15 minutes with their back and left arm supported by a chair and side table, and their feet placed flat on the floor with legs uncrossed. The participant was asked to remove all clothing from their left arm, to avoid a tourniquet effect. Significant differences have been noted in the BP measurements in each arm (Pickering *et al.*, 2005), therefore the left arm was used at all times for resting and ABP measurement (See 3.8) in this thesis to ensure uniformity of measurement conditions. Once the participants arm circumference had been measured using an ergonomic circumference measuring tape (Seca 201, Seca GmbH & Co. KG., Hamberg, Germany), an appropriately sized pneumatic arm cuff was placed around the participants supported arm, covering the left brachial artery, approximately 1.5 cm above the anticubital fossa and level with the heart.

Blood pressure measurement was activated using the 'manual' mode of the Dinamap and three BP measurements were taken, each separated by 5 minutes of further seated rest. The average of the three readings was calculated and used for analysis (Pickering *et al.*, 2005; Mancia *et al.*, 2013). Participants were recruited to or excluded from the studies using this method of BP measurement.

3.7.1 Reliability of Dinamap® pro

The Dinamap® pro has been tested in accordance with the European Society of Hypertension International Protocol (O'Brien *et al.*, 2002), which was revised from the British Hypertension Society protocol (O'Brien *et al.*, 1993). As such, the Dinamap® (GE Medical) is listed by the British Hypertension Society (2016a) as a validated monitor for clinical use. Requirements of these guidelines stipulate that devices must display a mean difference of ≤5 mmHg to a comparison device and a standard deviation of ≤8 mmHg for both sBP and dBP (O'Brien *et al.*, 1993). In addition, devices must achieve an accuracy grade A or B for both sBP and dBP on the BHS grading system (2016b) in order to be recommended for clinical use.

It is detailed in the operation manual for the Dinamap® Pro Series (100-400) that the

monitor comparisons fall within guideline differences when compared with intra-aortic values from invasive central aorta catheter measurement, therefore meeting the American National Standards Institute/Association for the Advancement of Medical Instrumentation SP10 1992 requirements for accuracy (Systems, 2002; Baker, 1986).

Devices in the Dinamap® series have been previously criticised for underestimating sBP and dBP (O'Brien *et al.*, 1990), as well as overestimating dBP compared with sphygmomanometer readings (Beaubien *et al.*, 2002). However these inaccuracies were found to be in keeping with other semi-automated devices (Lewis *et al.*, 2002), and are still accepted to eliminate investigator bias, by which readings taken using the manual auscultatory method may be rounded up or down based upon the expectations of the measurer (Coe and Houghton, 2002).

The accuracy of the Dinamap® Pro monitor, used within this thesis, is in keeping with international protocol guidelines. A validation study of the device found sBP accuracy to be -2.5 (±5.4) mmHg, and dBP to be 0.5 (±4.5) mmHg when 9 single arm measurements carried out using manual auscultation by two independent observers were compared against the device in 33 patient volunteers (Reinders *et al.*, 2006).

3.8 Resting electrocardiogram

A 12-lead resting ECG was performed prior to participation in the research. This provided basic screening for cardiovascular abnormalities and all ECG recordings were verified as ‘normal’ by a consultant cardiologist prior to participation in the research.

3.9 Transthoracic echocardiography

Clinical assessment of cardiac function and structure can be performed invasively and non-invasively. The most accurate tools involve invasive investigation through cardiac catheterisation under local anaesthesia, allowing measurement of intra-cardiac pressure and flow within the myocardium. However, the use of catheterisation for diagnosis has been reduced in the favour of non-invasive alternatives such as echocardiography, due to cost effectiveness, potential complications, patient comfort and a reduced risk of mortality (Hadian and Pinsky, 2006).

Transthoracic echocardiography (TTE) is the most popular non-invasive tool used to measure cardiac-structure and function. The affordability of equipment and ability to transport a device to be used anywhere has led to TTE being used over other non-invasive alternatives such as MRI. Ultra-high frequency sound waves used in TTE are reflected from the heart walls and valve cusps and collated during millisecond listening intervals between pulses. Echocardiography is used to evaluate cardiovascular anatomy, cardiac function and haemodynamic properties of the heart and major blood vessels as well as blood flow velocity during systole and diastole, providing information on contractility and compliance. However, echocardiography is a skilled technique and highly operator dependant. Image quality can vary between individuals due to differences in anatomical orientation of the myocardium, acoustic windows, body composition, and/or disease. These indiscriminate physical characteristics cause changes in tissue density and the degree of ultrasound penetration, which may introduce error into measurements.

To gain images of cardiac structures, a piezoelectric transducer is placed on the thorax and images are obtained through the chest using sound wave technology. A number of measures of echocardiography are employed in clinical practice to provide an overall evaluation of the heart and great vessels (Ryan *et al.*, 2008). Two-dimensional (2D) and motion-mode (M-mode) measures provide real time visualisation of cardiac structures from multiple tomographic planes (Libby *et al.*, 2008) to provide quantification of cardiac systolic and diastolic function (Ryan *et al.*, 2008). The distance of ultra-sound echoes along a vertical axis represents the depth of echo producing structures and the brightness indicates the intensity of the returning echo (Gottdiener *et al.*, 2004). In addition, both pulsed and continuous wave Doppler colour-flow imaging measures enable the quantification of cardiac haemodynamic variables and flow disturbances such as gradients or pressure (Libby *et al.*, 2008; Ryan *et al.*, 2008). Transthoracic echocardiography was performed in Chapter 5 and Chapter 8 of this thesis, using a commercially available Vivid-q portable ultrasound system (GE Healthcare, Milwaukee, Wisconsin) with a 1.5 – 3.6 MHz phased array transducer. All images were acquired by the same sonographer and participants were examined in the left lateral decubitus position. To assess specific parameters in relation to the cardiac cycle, a three lead ECG was used continuously throughout the examination. Images were stored in digital format and offline measurements were

made using commercial software on a proprietary workstation (EchoPAC; V.113.0.x, GE Healthcare). Measurements were averaged over three cardiac cycles.

Previous researchers have reported small CoV values for intra and inter-observer reliability in echocardiography assessment, which demonstrates high reproducibility in the technique (Ladipo *et al.*, 1980; Otterstad *et al.*, 1997; Pollick *et al.*, 1983; Stefadouros and Canedo, 1977). A single, experienced operator was used for echocardiographic image acquisition primarily due to the logistics (volume and timing) of data collection. The CoV values for cardiac structural and functional measures for the sonographer ranged from 1.9% - 5.9% (O'Driscoll, 2009). In addition, prior research between the research sonographer and another experienced operator has been reported in a clinical population (O'Driscoll *et al.*, 2014). To ensure standardisation of image acquisition between baseline and post intervention measures, the cardiac sonographer was able to review the baseline images whilst simultaneously completing the post intervention examination. All required data was captured successfully.

Cardiac structural and functional measurements were recorded as recommended by current guidelines (Lang *et al.*, 2015). Left ventricular end diastolic diameter (LVIDd) and left ventricular end systolic diameter (LVIDs) were measured from parasternal long axis recordings of the LV with the cursor positioned at the tips of mitral valve (MV) leaflets, as demonstrated in Figure 3.6. Measurements of relative wall thickness and LV mass index (p79) were used to establish LV geometry and participants were classified as having either normal LV geometry, concentric remodelling, eccentric hypertrophy or concentric hypertrophy. In accordance with current guidelines (Lang *et al.,* 2015), normal LV geometry was defined as LV mass index ≤ 115 gm/m^2 and relative wall thickness ≤ 0.42. A relative wall thickness of >0.42 was considered to represent concentric remodelling.

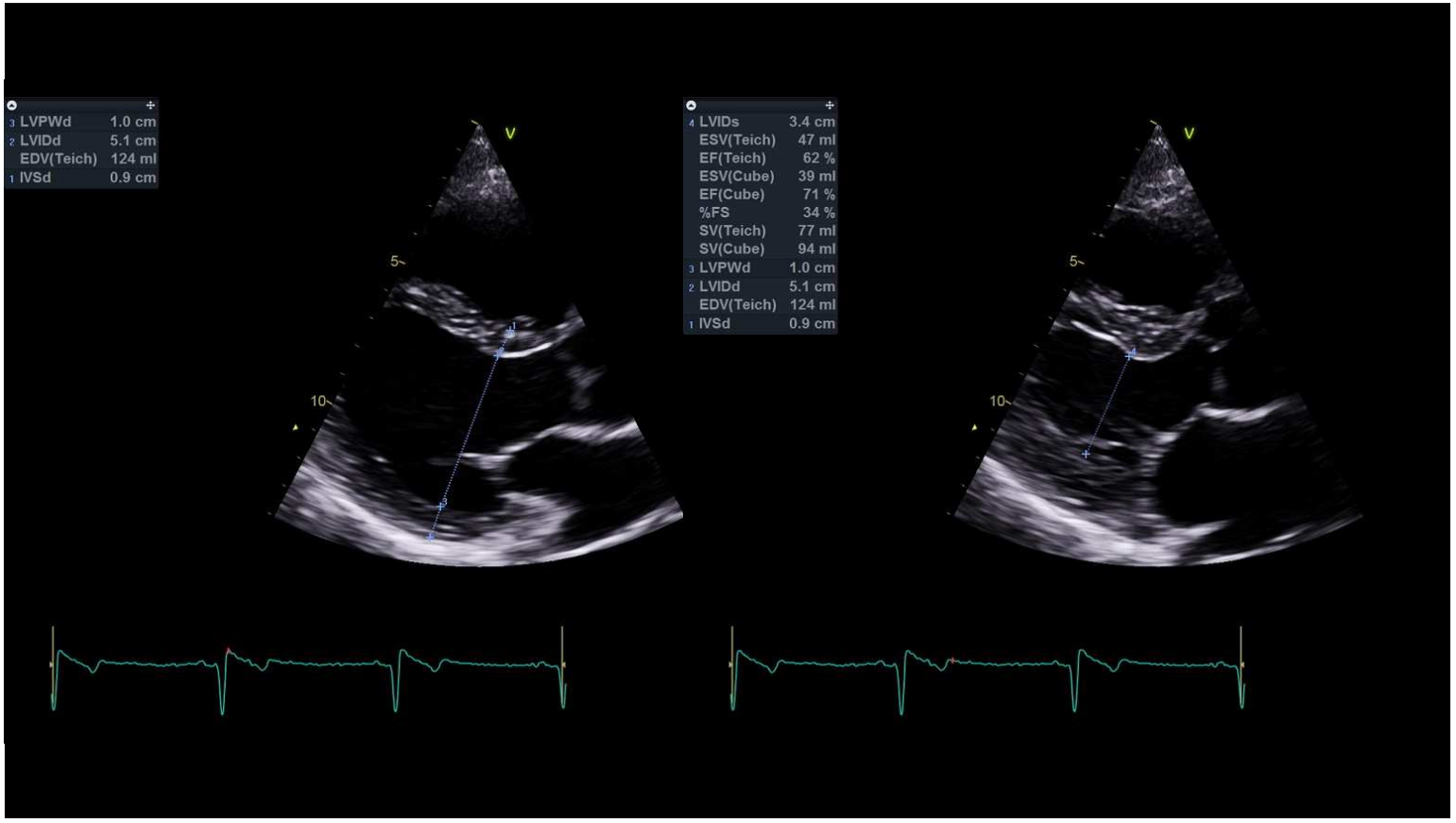

Figure 3.6. Parasternal long axis view of the LV. Note: Left image illustrates cardiac dimensions measured at end diastole and right image illustrates cardiac dimensions measured at end systole.

LV mass was calculated from measurements of septal and posterior wall thickness and LV cavity size at end diastole. LV mass was calculated according to Devereux *et al.* (1986) using the following equation, which was also indexed to BSA to provide LVMI.

$$\text{LV mass} = 0.8\ (1.04\ (\text{LVIDd} + \text{IVSd} + \text{PWd})^{3} - \text{LVIDd}^{3}) + 0.6$$

Relative wall thickness was calculated as

$$\text{RWT} = 2 \times \text{PWd} / \text{LVIDd}$$

LV end diastolic and systolic volumes were determined from the apical 4-chamber and 2-chamber views and averaged. The modified biplane Simpson's technique and standard formula were used to give LV ejection fraction (LVEF) (Schiller *et al.*, 1989) using the equation

$$\text{EF} = 100 \times (\text{LVEDV} - \text{LVESV}) / \text{LVEDV}$$

This method correlates strongly with cineangiography (Folland *et al.*, 1979) and has demonstrated good reliability (Gottdiener *et al.*, 1995; Himelman *et al.*, 1988).

Transmitral inflow was assessed using pulsed wave Doppler recordings with the sample volume placed at the mitral valve leaflet tips in the apical 4-chamber view, parallel to flow. Peak velocity of early (E), peak velocity of late filling (A), the early to late diastolic filling ratio (E/A ratio) and E- deceleration time (ms) were measured. Figure 3.7 illustrates a typical pulsed wave Doppler trace of the LV blood flow through the mitral valve during diastolic filling.

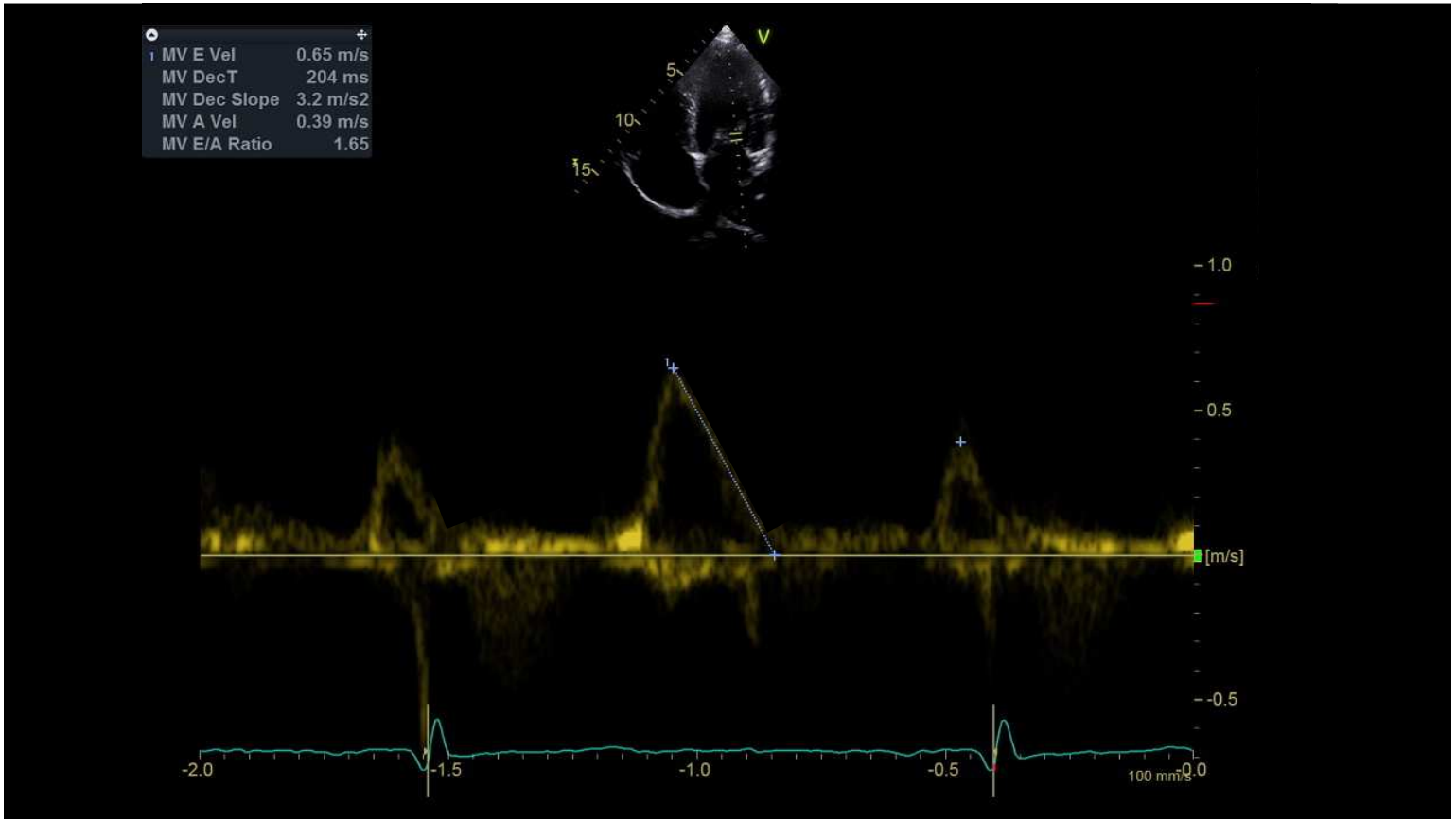

Figure 3.7. Pulsed wave Doppler trace of the LV blood flow through the mitral valve during diastolic filling.

Tissue Doppler imaging (TDI) was used to record wall motion velocities within the myocardium to assess both systolic and diastolic function. In an apical 4-chamber view, the sample volume was placed at the level of the lateral and septal mitral annulus of the LV and RV free wall. The peak velocity of the systolic myocardial wave (S'), peak early diastolic (E') and late diastolic (A') velocities were recorded, with LV values averaged, as shown in Figure 3.8. The LV mitral E/E' ratio were used to estimate LV filling pressure (Ommen *et al.*, 2000). Validation studies using simultaneous catheterisation and Doppler echocardiography to assess myocardial function, filling pressures and LVEF have reported moderate correlations (R=0.539-0.842, $p<0.001$) (Tei *et al.*, 1997; Homma *et al.*, 2001).

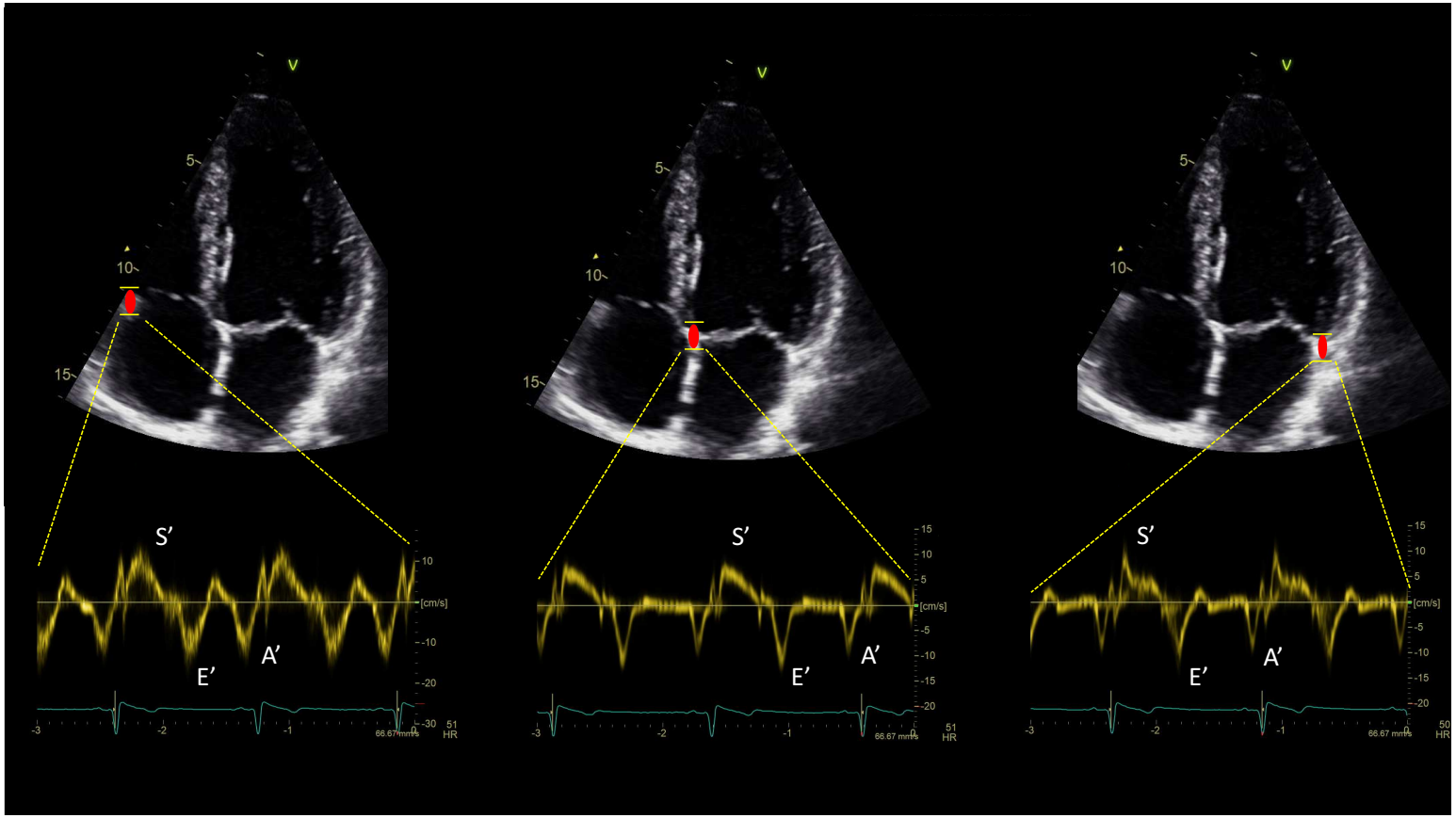

Figure 3.8. Tissue Doppler imaging of the right ventricular free wall, septral mitral annulus and lateral mitral annulus (left to right), for S', E' and A'.

3.10 Isometric exercise and isometric exercise training

The isometric protocol used throughout this thesis was isometric wall squat exercise. A single IE session was examined in Studies 1 and 2 (Chapters 4 & 5) while the effects of 4-weeks of isometric wall squat training, completed three times per week, were examined in Studies 3, 4 and 5 (Chapters 6, 7 & 8). Previous IET interventions have used a fixed percentage of MVC to inform the intensity of IET using hand grip exercise, or leg exercise on an isokinetic dynamometer (Table 2.2, page 28). However, isometric wall squat exercise cannot be performed at a single voluntary maximal intensity to establish a fixed % of MVC, therefore training intensity must be otherwise determined. Electromyographic activity (EMG) is a measure of the level of activation of skeletal muscles. It has been shown that HR and BP responses increase with greater EMG activity (Wiles *et al.*, 2008) and that greater knee flexion increases the EMG activity of the quadriceps (Escamilla, 2001; Kvist and Gillquist, 2001; Bevilaqua-Grossi *et al.*, 2005). Goldring *et al.* (2014) showed that a greater cardiovascular response was produced when wall squats were performed with an increased degree of knee flexion down to 90°, therefore advocating the manipulation of knee joint angle as a means to alter training intensity in isometric wall squat exercise.

3.10.1 Establishing Knee Joint Angle for all studies

Knee joint angle for IE was prescribed individually for all participants. Required knee joint angle was determined as the angle needed to elicit 95% of the maximum HR attained during an incremental isometric wall squat test (see section 3.10.2). The incremental isometric wall squat test protocol and how this is used to establish knee joint angle, is explained below.

A goniometer was used during the incremental isometric test and acute IET session to measure the participants' knee joint angle (MIE Clinical Goniometer, MIE Medical Research Ltd., Leeds, U.K.). Such a device has previously been used to quantify knee joint angle during squatting exercise (Youdas *et al.*, 2007). The device consisted of a 360° gauge from which 2 mounted arms, (one stationary and one moveable) rotate around the gauge, attached by a central rivet. The device used was made from clear

plastic and displayed 1° increments to obtain maximum accuracy in measurement of the knee joint, as shown in Figure 3.9.

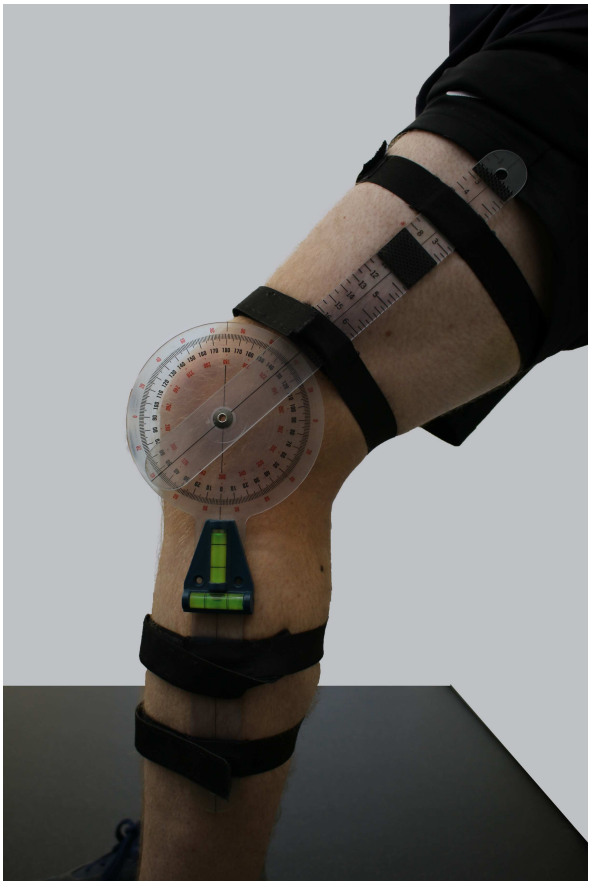

Figure 3.9. Goniometry of the knee joint

To position the goniometer, participants were seated with their left knee at 90°. Loose clothing was adjusted in order to gain an optimal view of the limb and the relevant bony landmarks of the knee, hip and ankle were palpated so that the goniometer could be accurately aligned. The stationary arm of the goniometer was aligned with the middle of the lower leg, which was the stationary segment of the joint, on the lateral midline of the fibula using the malleolus and fibular head for reference. The moving arm was placed on the moving segment of the joint on the midline of the femur with the greater trochanter used for reference. The fulcrum of the goniometer was aligned with the axis of rotation of the joint, which was on the sagittal plane, aligned with the lateral epicondyle of the femur. A spirit level was attached to the device to ensure that the lower leg remained vertical during the exercise, and four 25mm elastic Velcro straps, which passed around the participants' upper and lower leg, were fitted to the

goniometer to ensure that the device stayed in place during the exercise, ensuring that compression of muscle did not occur (See Figure 3.9). The alignment of the device was rechecked during exercise. The knee joint angle measured was the internal angle between the femur and fibula.

3.10.2 The incremental isometric wall squat test protocol

Once the goniometer had been accurately fitted, participants were instructed to position themselves upright with their feet flat on the floor and back resting against a fixed wall. The experimenter instructed subtle movements in raising and lowering the back and shuffling their feet forwards/backwards until the start knee joint angle of 135° was reached. The experimenter ensured that the lower leg remained vertical at all times during the test by observing the spirit level attached to the goniometer. The incremental wall squat test comprised five increments, each of which was held for 2-minutes. At the end of each stage the participant was instructed to lower their back down the wall and move their feet out slightly to decrease the knee joint angle, taking on average approximately 2-5 seconds. Participants moved directly from one stage to the next stage without recovery time or contraction release during the test. The incremental stages involved holding an isometric wall squat at the angles of 135°, 125°, 115°, 105°, 95° (Goldring *et al.*, 2014), as shown in figure 3.10.

Figure 3.10. The five knee joint angles of the incremental test.

During each increment of the test, feet and back positions were measured using a standard metre rule. Feet position was measured as the direct distance from the wall to the back of the left heel and back position was measured as the direct distance from the floor to the lower back, defined as the lowest point of contact that the participants back had with the wall. These measurements were later used to determine isometric exercise training position (See 3.10.3).

Participants continued with the test until competition (10-minutes) or volitional exhaustion. They were informed of elapsed time throughout each stage and received verbal encouragement to complete the test, or to hold a contraction for as long as they felt they could. Participants were reminded to breathe normally to avoid performing a Valsalva manoeuvre. Continuous BP and HR data was recorded throughout the incremental test and rate of perceived exertion was recorded at the end of each increment (See 3.11).

3.10.3 Establishing isometric exercise training intensity

Following the incremental test, data collected from the final completed stage of the test was used to establish a training angle. Based upon previous work demonstrating significant reductions in resting BP (Wiles *et al.*, 2010), 95% of the participants maximum HR during the incremental test was selected as the target training intensity. Thus the knee joint angle required to elicit this response was calculated. Prior research has demonstrated that when incremental isometric exercise test constant electromyography (EMG) was used to determine IE intensity, a 'steady state' HR was achieved in the final 30 seconds of each 2 minute 10, 15, 20, 25, and 30% EMG peak (Wiles *et al.*, 2008). This physiological response established the potential for IE training prescription via a linear relationship between EMG and HR. Subsequent to this, research has demonstrated that knee joint angle during a wall squat produced a reliable inverse curvilinear relationship with HR (Goldring *et al.*, 2014). As such, following the incremental isometric wall squat test, knee joint angle was plotted against mean HR for the last 30-seconds of each stage. The relationship produced was then used to calculate each participants knee joint training angle that would elicit a target of 95% peak heart rate (HR_{peak}, defined as the mean HR of the final 30 seconds achieved during the incremental test) as used in previous research (Devereux *et al.*,

2010; Wiles *et al.*, 2010).

Once the training angle had been calculated, the floor and wall positions measured at each stage angle during the incremental test were used to work out the floor and wall positions needed to elicit the prescribed angle.

3.10.4 Bend and squat device

The clinical goniometer used during the laboratory testing is not suitable for home-based training as it requires accurate fitting and could be easily misaligned by the participant, which would lead to IET being completed at an imprecisely determined angle. It has been previously established that the floor and wall positions of a participants' feet and lower back can be used to recreate a target knee-joint angle (Goldring, 2014). Therefore, a simple bend and squat device (made in house, Section of Sport and Exercise Sciences, Canterbury Christ Church University) was created that would facilitate precise positioning of the feet and back to repeatedly recreate a target knee joint angle to be used in IET.

The bend and squat device consisted of two arms, labelled 'floor' and wall', connected by a hinge. The arms are length-adjustable and display a measurement scale marked in centimetres. The floor segment could be set from 34-54 cm, and the wall arm could be set from 50-83 cm, lengths determined by (Goldring, 2014). The two arms were adjusted to create the desired wall and floor measurements for each participant, which was placed at a right angle on the floor with the appropriately labelled arms against the floor and wall. A removable metal arm could be attached through the floor arm segment, parallel to the wall, which the back of the heels could rest against to maintain the correct floor position. A rounded plastic lip was created at the top of the wall arm segment, marking the correct wall position. This could not support the weight of the participant, but provided a tactile reference point for the correct position, and prevented any further movement down the wall.

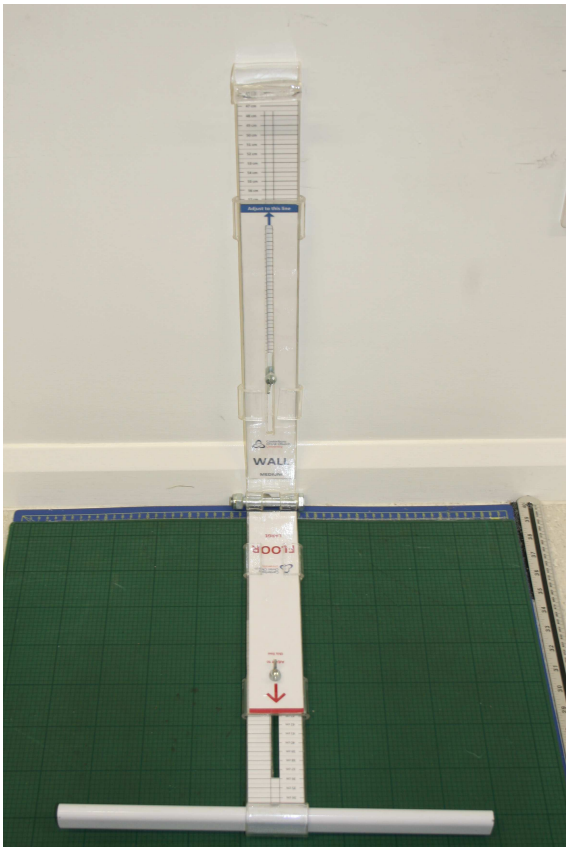

Figure 3.11. Image of the bend and squat device.

3.10.5 Isometric exercise training session

The IET session protocol utilised in this thesis lasted a total of 14-minutes, including recovery periods. Isometric contractions of 2-minutes are widely used in research (Table 2.2, page 28), and have proven effective in reducing resting BP (Wiles *et al.*, 2017; Millar *et al.*, 2008; Wiley *et al.*, 1992a). Rest periods between 2-minute contractions have varied from 1-3 minutes, and rest periods of 2-mintutes were selected for this thesis based upon prior isomeric wall squat research by Goldring (2014). Figure 3.12 shows the training session protocol used once in Study 1 and 2 (Chapters 4 and 5) and three times per week in Study 3, 4 and 5 (Chapters 6, 7, 8).

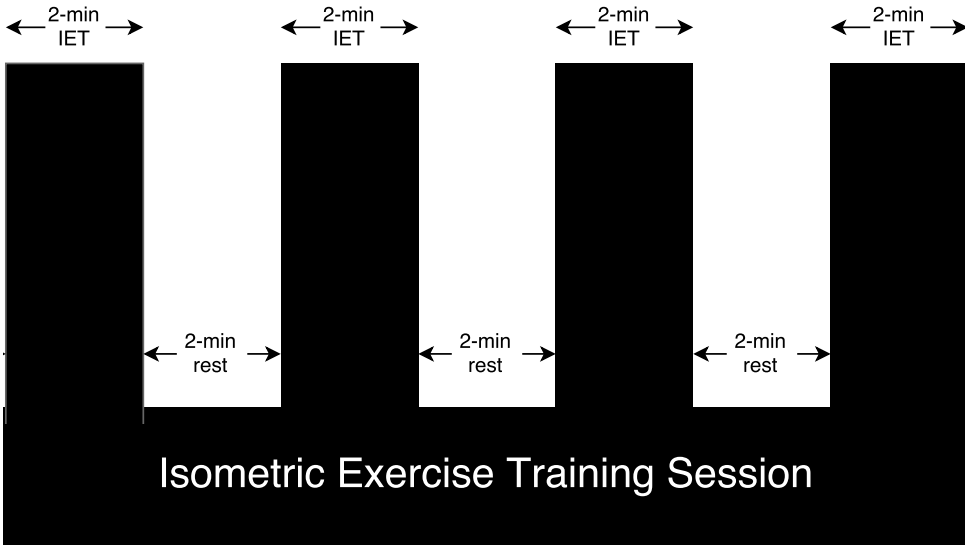

Figure 3.12. The contraction and rest periods of a single session of IET.

3.10.6 Home-based isometric exercise training

Home-based IET followed the session protocol displayed in Figure 3.12 (above). Training was completed 3 times per week for a period of 4-weeks, which is the most commonly used frequency in IET research (see Table 2.2, page 28) and an intervention duration used by (Devereux *et al.*, 2010).

To ensure that the prescribed angle continued to produce the required intensity, all participants were given a HR monitor, which consisted of a watch (Polar RS400, Polar Electro Oy, Kempele, Finland) and matching chest strap (Wearlink V2, Polar Electro Oy, Kempele, Finland) to wear during IET at home. Participants were shown how to use and adjust the bend and squat device and HR monitor and were provided with a training manual, containing training and equipment instructions, safety advice and a diary to record their training HR data from the HR monitor (Appendix 5: Training session manual). A target HR range was calculated for each participant, using a modified limits of agreement equation (Hopkins, 2000; Coleman *et al.*, 2005). Previous research found that normotensive participants spent 67% of the overall 8 minutes of isometric wall squat training (4x2 minutes) within this target-training zone, which included at least the final 30 seconds of each exercise bout (Goldring, 2014). If a participants mean self-reported HR did not fall within the target HR range for 2 consecutive training sessions, they would be requested to re-attend the laboratory to repeat the incremental isometric wall squat test in order to re-establish a training angle.

All participants completed a familiarisation session of IET in the laboratory under the supervision of experimenters, during which time the training angle created by the bend and squat was confirmed as correct using a goniometer. This session also provided confirmation that the angle elicited a HR within the target HR range.

After each training session, participants uploaded their recorded HR data to a personalised online spread sheet, which could be accessed only by themselves and the experimenter. Training data was regularly reviewed by the experimenter to ensure HR remained within the prescribed target zone during every IET session.

3.11 Rate of perceived discomfort (RPD) measurement

In addition to physiological responses, the 'Borg CR10 scale' (1998) scale was employed as a measurement of subjective somatic symptoms, to gauge a participants level of effort, exertion, fatigue and pain. The scale ranges from 0 to 10, and localised discomfort in the quadriceps muscle could be defined using descriptive verbal anchors where 0 is 'nothing at all' and 10 is 'extremely strong' (see Appendix 5). The scale is non-linear therefore the 'moderate' anchor is not in the middle of the scale, but instead at number 3 at the lower end of the scale. The CR10 scale has been previously used during isometric leg exercise to provide an indication of discomfort (Wiles *et al.*, 2005) and perceived exertion (Pincivero *et al.*, 2000). A degree of localised pain or discomfort in the contracting muscle, particularly during fatiguing contractions, is expected during IE (Lind, 2011) due to the occlusion of muscle blood flow and accumulation of metabolic biproducts (Folland *et al.*, 2002). Discomfort during exercise may limit an individuals ability to continue exercising, thus forcing the voluntary termination of exercise. In this thesis, the CR10 scale was used during the incremental isometric wall squat test to gauge each participants' ability to complete the test, or as an indication of when they were nearing volitional exhaustion. During home based training, participants recorded RPD values along with HR, at the end of each exercise. This was to ensure that the perceived training intensity matched the lab-based training intensity and that participants perceived the same relative intensity throughout the IET programme.

CHAPTER 4:

Study 1

Continuous Cardiac Autonomic and Haemodynamic Responses to a Single Isometric Exercise Session in Pre-Hypertensive Males

4.1 Introduction

Systemic arterial HTN remains a significant global public health problem. Traditional approaches to lowering BP have been through the use of pharmacotherapy. More recently, there has been a shift in focus towards lifestyle modification in the first instance, for pre-hypertensive and stage 1 hypertensive populations with no apparent comorbidities (Public health England, 2014). The role of aerobic exercise training as a lifestyle modification for BP reduction is well established, with positive cardiac (Baggish and Wood, 2011b), vascular (Dinenno *et al.*, 2001), and neurohumoral adaptations (Ray and Hume, 1998), all of which are considered as potential mechanisms for improved arterial haemodynamics (Pescatello *et al.*, 2004a). However, a programme of IET has also been shown to reduce resting BP in normotensive (Wiles *et al.*, 2010; Millar *et al.*, 2008), pre-hypertensive (Baross *et al.*, 2012; Wiley *et al.*, 1992a) and hypertensive populations (Peters *et al.*, 2006; Taylor *et al.*, 2003), and reductions observed are greater than traditional aerobic exercise and dynamic resistance training programmes (Cornelissen and Smart, 2013). A meta-analysis of 11 short term IE interventions, including both IHG and ILT protocols by Inder et al, (2016), revealed sBP reductions of 5.20 mmHg (95% CI − 6.08 to − 4.33, P<0.00001), dBP reductions of 3.91 mmHg (95% CI − 5.68 to − 2.14, P<0.0001) and mBP reductions of −3.33mmHg (95% CI −4.01 to − 2.66, P<0.00001). A meta-analysis of the use of BP lowering drugs from 147 studies (Law *et al.*, 2009), estimated that a single treatment induced reductions of 5.9 mmHg (sBP) and 3.1 mmHg (dBP), therefore greater reductions typically require the prescription of 2 or more anti-hypertensive drugs.

In addition to resting BP reductions, IET has been associated with reductions in BP for which a number of potential mechanistic adaptations have been proposed. Central adaptations following IET have involved improvements in cardiac autonomic regulation (Taylor *et al.*, 2003) that may affect $\dot{Q}$, while peripheral changes such as an increase in femoral artery diameter (Baross *et al.*, 2013), increased resting endothelium dependant vasodilation in trained limbs (McGowan *et al.*, 2006b) and improved resistance vessel function (Badrov *et al.*, 2013a), may contribute to reduced TPR. As a consequence, modifications in $\dot{Q}$ and TPR may lower BP, which may in turn have a

positive effect on CVD risk factors (Qureshi *et al.*, 2005a; Julius *et al.*, 1990; Greenlund *et al.*, 2005).

Existing research concerned with the adaptations influencing BP reductions appears to focus on the responses to IET training interventions, shown in Table 2.2, page 28. Previous acute studies have tended to focus on one off bouts of IE, as shown in Table 2.3, page 36. However the acute responses during a conventional 4 x 2 minute session of IE have received little attention. The acute effects of IE based on existing research are outlined in Chapter 2.5.1, page 40. Typically, the onset of IE is associated with a pressor response, which has been suggested to be greater than aerobic exercise (Rowell and O'Leary, 1990). This is determined by peripheral mechanoreceptor activation in the working musculature and is accompanied by increases in central command (Goodwin, 1972; Schibye *et al.*, 1981). A resultant feed-forward mechanism stimulates intensity matched (Mitchell, 2012) increases in autonomic and cardiovascular function (Williamson, 2010). As such, IE is associated with a rapid rise in HR, sBP and dBP (Lind and McNicol, 1967), and a subsequent increase in both cardiac preload and afterload. Gallagher *et al.* (2006) confirmed that BP control during a static contraction as the result of an upward shift in baroreflex BP control, induced by the over activity of central command and enhanced activation of the exercise pressor response. The HR and BP responses during an isometric contraction have been shown to rise with increasing intensity, Rowell (1993) and Mitchell et al (1980) found that IE using a larger muscle mass resulted in faster HR and BP responses than in smaller muscle groups.

Previous research has highlighted a linear relationship between training intensity (by EMG% peak) and HR and sBP responses during a 2-minute double leg extension contraction and also found that a relative steady state was reached by the end of the exercise period during a continuous incremental IE test, allowing IE training prescription based upon % peak HR (Wiles *et al.*, 2008). To overcome the need for use of an isokinetic dynamometer during training an alternative isometric leg training protocol was developed by (Goldring *et al.*, 2014). An inverse linear relationship between knee joint angle and HR and BP responses during an isometric contraction was established, leading to the prescription of training intensity based upon knee joint angle (Goldring *et al.*, 2014; Wiles *et al.*, 2017; Taylor *et al.*, 2017).

In an attempt to identify potential stimuli that might be responsible for causing the reduction in BP following IET, it is important to understand the acute responses to IE. Existing research of this nature has focussed on performing measures during recovery from IE. It has been previously demonstrated that a single session of IHG exercise (4 x 2-min bilateral contractions) elicits acute improvements in cardiac autonomic regulation during recovery, accompanied by post exercise systolic hypotension (Millar *et al.*, 2009a). In addition, McGowan *et al.* (2006b) reported acute reductions in endothelium dependant vasodilation in the brachial artery of the training arm immediately following IHG. It has been suggested that sympathetic stimulation during an isometric contraction is mediated by metaboreflex control of HR, while the arterial baroreflex has been implicated in HR recovery (Iellamo *et al.*, 1999b), under the control of central command (Goodwin, 1972). There is currently a lack of evidence to elucidate the role of central and peripheral responses continuously during an IE session and in recovery, yet the acute central and peripheral haemodynamic and cardiovascular responses to IE may be mechanistically linked to the reductions in resting BP following IET.

As demonstrated in Table 2.2, a programme of IET is associated with reductions in resting BP. It is therefore postulated that repeated stimulation of the acute physiological responses to IE may determine longer term physiological adaptations. To date, IHG training has been the most commonly prescribed IE intervention, primarily due to the ease of prescription to older and physically inactive adults. However, research has suggested that a larger muscle mass may influence the magnitude of BP reductions (Howden *et al.*, 2002; Seals, 1989), which may be due to a greater physiological response stimulus during a single session (Mitchell et al, 1980). As such, an increasing number of research studies have utilised isometric leg training (Wiles *et al.*, 2010; Baross *et al.*, 2013; Devereux *et al.*, 2010), which has produced notable reductions in BP, of a similar level to IHG training, even when performed at a lower relative percentage of maximal voluntary contraction (Millar *et al.*, 2013).

The aim of this study was to investigate the transient cardiac autonomic, central and peripheral haemodynamic responses; measured continuously pre, during and immediately following a single isometric wall squat session in a pre-hypertensive population.

4.2 Methods

4.2.1 Study population

Twenty-five physically inactive pre-hypertensive males (age 44.8 ± 8.4 years; height 178.1 ± 5 cm; body mass 89.9 ± 2.43 kg; BSA 2.1 ± 1 m^2), volunteered to take part in the study. Participants reported no history of CVD, were non-medicated and had a seated resting sBP of ≥120 mmHg and ≤140 mmHg and/or dBP of ≥80mmHg and ≤90mmHg (Dinamap® Pro, GEMedical Systems, Slough, Berks, UK). Inclusion in the study was subject to a normal cardiovascular examination and electrocardiogram, which were checked and confirmed by a consultant cardiologist.

Participants were required to attend the laboratory on 3 occasions. Participants abstained from food for at least 4 hours prior to each laboratory visit, and did not consume caffeine or alcohol for 24 hours before each visit. During the first visit, BP status was confirmed (3.7, page 73) and eligible participants completed an isometric wall squat test to establish an appropriate exercise session intensity (see 3.10.1-3.10.3, pages 83-86). The second visit took place a minimum of 48 hours after the first visit and participants were familiarised with the isometric wall squat training session (see 3.10.5, page 88). Data collection for the present study was conducted during a third laboratory visit. This investigation conformed to the Declaration of Helsinki principles and was approved by the institutional research ethics committee (Ref: 12/SAS/122). All participants provided signed informed consent before testing.

4.2.2 Study protocol

All testing was conducted in a controlled laboratory environment. Autonomic and haemodynamic assessment was performed using the Task Force® Monitor (TFM) using methods outlined in 3.4, page 62. Following 15 minutes of supine rest, baseline autonomic and haemodynamic assessment was performed over a period of 5 minutes. Following this, once a steady state HR had been reached in a standing position, participants completed a single IE session. The session consisted of four, 2-minute wall squats, each interval separated by 2-minutes of rest, as shown in figure 4.1 and outlined in 3.10.5. Intensity was determined by a prescribed isometric wall squat knee

joint angle (mean 106 ± 7 º), which had been calculated based on HR and BP responses to an incremental isometric wall squat test performed during their first laboratory visit (see 3.10.2, page 85). Continuous beat-to-beat monitoring and automatic online calculation of all cardiac autonomic and haemodynamic parameters was performed throughout the exercise session, and during a subsequent 5-minute recovery period. Intervention marks enabled the separation of the cumulative data into independent stages of the IE session outlined in Figure 4.1.

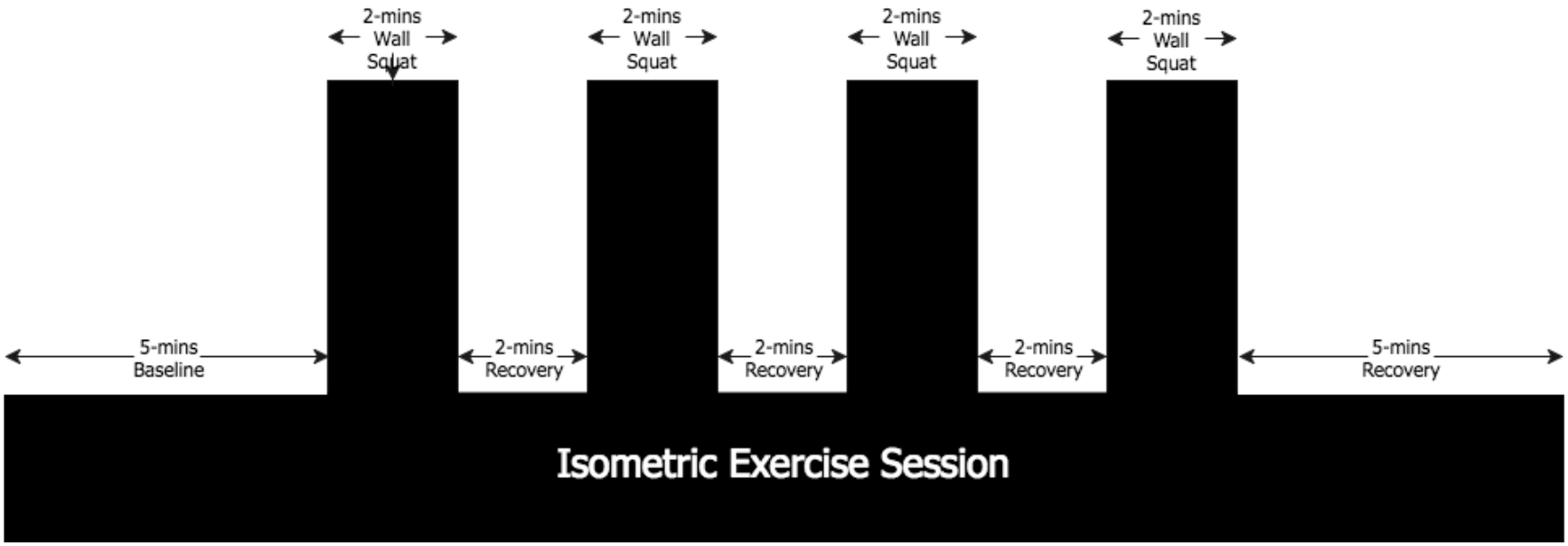

Figure 4.1: Data recording periods at baseline, during isometric wall squat contractions and in recovery of a single session of IE.

4.2.3 Data analysis

Unless otherwise stated, continuous variables are expressed as mean ± standard deviation. All data were analysed using the statistical package for social sciences (SPSS 22 release version for Windows; SPSS Inc., Chicago IL, USA). Data were assessed for conformity with parametric assumptions. A repeated measures analysis of variance (ANOVA) was performed, followed by Bonferroni post hoc tests for multiple comparisons. A p value of <0.05 was regarded as statistically significant.

4.3 Results

Baseline demographic information is shown in Table 4.1.

Table 4.1: Baseline participant demographic characteristics. Mean and SD

Characteristic	Mean ± SD
Age (years)	44.8 ± 8.4
Height (m)	178.1 ± 5
Weight (kg)	89.14 ± 2.43
Systolic blood pressure (mmHg)	132.63 ± 5.61
Diastolic blood pressure (mmHg)	77.61 ± 9.37
Resting heart rate ($b \cdot min^{-1}$)	62.84 ± 12.84

4.3.1 Cardiac autonomic response

All participants completed the entire training session at their prescribed individual specific training angle. Cardiac autonomic function at baseline, during each bout of IE and in recovery is shown in Figure 4.2 and Table 4.2. Isometric exercise training produced a statistically significant change in mean R-R PSD of HRV between baseline and recovery time points (3339 ± 2187 ms^2 to 3634 ± 2221, p<0.001). Figure 4.2A, demonstrates that there was a significant stepwise reduction in R-R PSD from baseline to IE2 (1872 ± 1034 ms^2, p<0.02), IE3 (1094 ± 596 ms^2, p<0.001), and IE4 (586 ± 408 ms^2, p<0.001), followed by a significant increase in R-R PSD above baseline from

IE4 to recovery (p<0.001). Absolute HF (ms^2), LF (ms^2) and very low frequency (VLF ms^2) HRV data is shown in Table 4.2. All frequencies decreased significantly between baseline and IE3 and IE4 (p<0.05), then increased significantly following IE4 into recovery (p<0.001). When analysing HRV in normalised units, LFnu increased during the first bout of IE, and remained above baseline during all 4 training bouts (59.9 ± 16.6 to 70.5 ± 14.7); however, this was not statistically significant. There was a significant decrease in LFnu during the recovery period compared to during IE (70.1 ± 15.9 to 46.3 ± 14.3, p<0.001) and compared to baseline (70.1 ± 15.9 to 59.9 ± 15.6, p<0.05). An inverse response was recorded in HFnu (see Figure 4.2B). The LF/HF ratio increased during the first bout of IE and remained above baseline throughout the training session, followed by a significant reduction from the final IE bout into recovery (4.4 ± 4.1 to 1.1 ± 0.7, p<0.05) (see Figure 4.2C).

Baroreceptor reflex sensitivity (BRS) decreased significantly (p<0.001) between baseline (19.9 ± 10.33 $ms \cdot mmHg^{-1}$) and all four bouts of IE (IE1, 9.6 ± 4.9 $ms \cdot mmHg^{-1}$; IE2, 7.8 ± 3.4 $ms \cdot mmHg^{-1}$; IE3, 6.05 ± 2.2 $ms \cdot mmHg^{-1}$; IE4, 5.2 ± 2.8 $ms \cdot mmHg^{-1}$). During recovery BRS increased significantly above baseline (60 ± 53.08 $ms \cdot mmHg^{-1}$, p<0.001), as shown in Figure 4.2D.

Table 4.2: Haemodynamic and autonomic parameters at baseline, during IE and in recovery.

Parameter	Baseline N=25	IE 1 N=25	IE 2 N=25	IE 3 N=25	IE 4 N=25	Recovery N=25
VLF (ms^2)	853.63 ± 117	665.28 ± 117.1	404.15 ± 62*	217.57 ± 27.71**	151.28 ± 20.03**	898.45 ± 131.31§§
LF (ms^2)	1352.07 ± 116.96	1559.83 ± 117.11	983.9 ± 61.99	624.89 ± 27.71*	304.04 ± 20.03**	1184.61 ± 131.31§§
HF (ms^2)	1133.64 ± 256.87	780.51 ± 160.59	484.12 ± 71.39	251.64 ± 40.69*	131.04 ±25.56*	1553.39 ± 244.28§§
SI ($ml \cdot m^2$)	42.65 ± 2.13	35.94 ± 1.41*	37.88 ± 1.7	37.87 ± 1.69	37.61 ± 1.68	45.63 ± 2§
CI ($L \cdot min \cdot m^2$)	2.6 ± 0.09	2.99 ± 0.1*	3.29 ± 0.13**	3.6 ± 0.13**	3.9 ± 0.13**	3.17 ± 0.17*§§
TPRI ($dyne \cdot s \cdot m^2 \cdot cm^5$)	2983.46 ± 133.66	2306.56 ± 122.79	2985.58 ± 148.66	2802.90 ± 129.88	2749.19 ± 129.83	2170.08 ± 199.39**

Note: Data is mean ± SEM. VLF = very low frequency; LF = low frequency; HF = high frequency; SI = stroke index; CI = cardiac index; TPRI = total peripheral resistance index. $P<0.05$, ** $P<0.001$ between baseline and all stages; § $P<0.05$, §§ $P<0.001$ between IE4 and recovery.

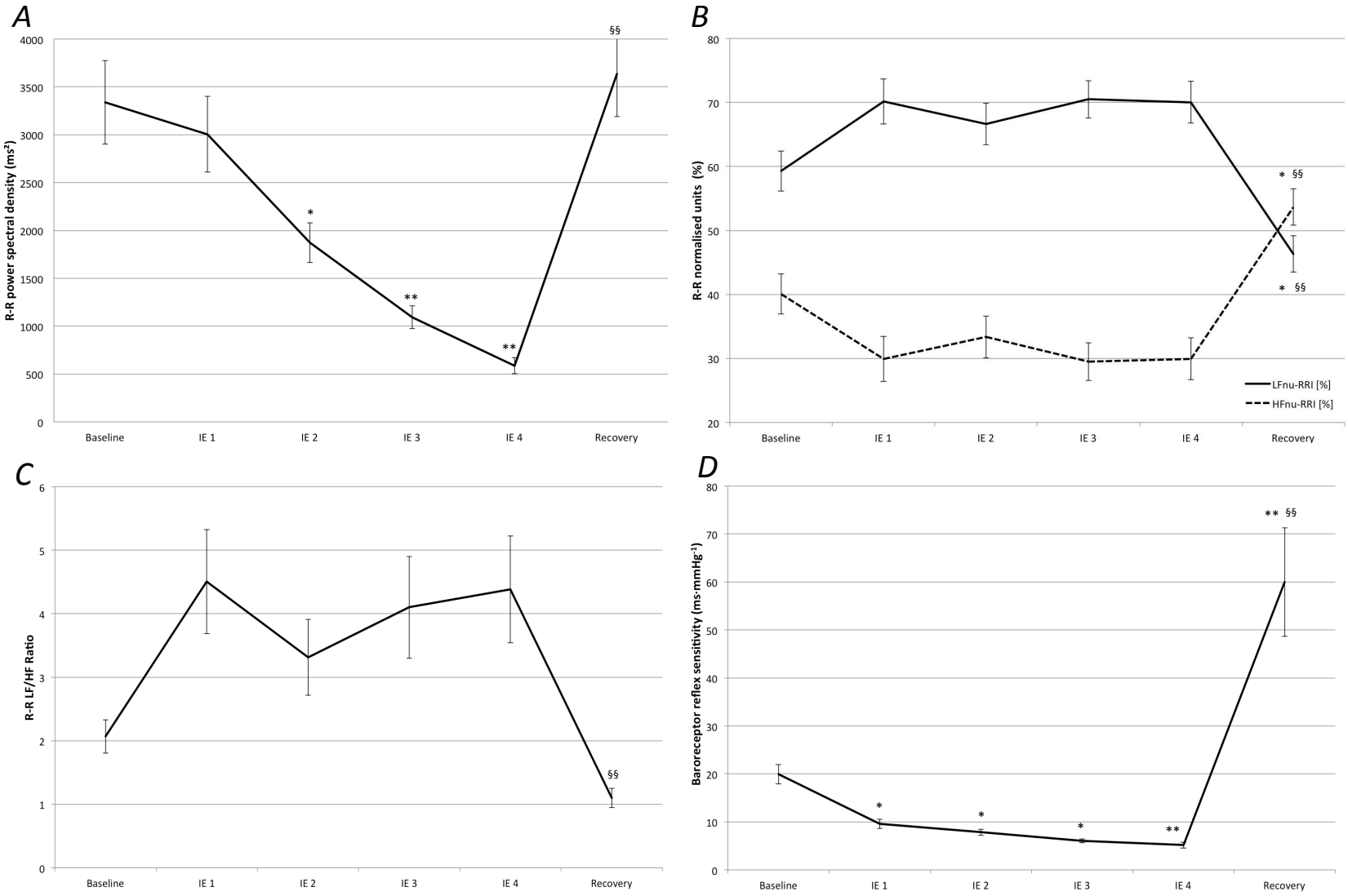

Figure 4.2: Cardiac autonomic responses at baseline, during 4 bouts of IE and in recovery and standard error of mean. Note: A, Power spectral density; B, LFnu and HFnu; C, LF/HF ratio; D, Baroreceptor reflex sensitivity.

4.3.2 Haemodynamic response

Haemodynamic parameters at baseline, during each period of IE and in recovery are shown in Figure 4.3 and Table 4.2. A significant stepwise increase in sBP occurred during the IE session from baseline (132.6 ± 5.6 mmHg) to IE1 (141.5 ± 15.7 mmHg), IE2 (145.9 ± 17.5 mmHg), IE3 (152.4 ± 15.8 mmHg), and IE4 (165.9 ± 21 mmHg) (all $p<0.05$). Following cessation of the IE session, there was a significant reduction ($p<0.001$) in sBP from 165.9 ± 21 mmHg in IE4 to 109.4 ± 19.5 mmHg during recovery, which was also significantly lower than baseline sBP ($p<0.001$). The same trend was observed in dBP, with significant increases from baseline and all periods of the IE session ($p<0.001$) followed by a significant reduction from IE4 into recovery ($p<0.001$), which was also significantly lower than baseline dBP ($p<0.001$). The mBP response during the IE session demonstrated a similar pattern to sBP and dBP with the same differences ($p<0.05$) (see Figure 4.3A).

There was a statistically significant stepwise increase in HR from baseline through each IE interval (all $p<0.001$), followed by a significant reduction in HR from IE4 into recovery from 108.5 ± 17 to 70.3 ± 14.8 $b \cdot min^{-1}$ ($p<0.001$). In the recovery periods between IE bouts, mean HR was 68.3 ± 11.8 $b \cdot min^{-1}$ between bout 1 and 2; 73.4 ± 12 $b \cdot min^{-1}$ between bouts 2 and 3; and 77.9 ± 13.1 $b \cdot min^{-1}$ between bout 3 and 4. As a consequence of the HR and BP responses, there was a significant linear increase in RPP from baseline through all IE bouts, followed by a significant decrease in RPP from IE4 into recovery ($p<0.001$) to below baseline (See Figure 4.3B).

TPR (Figure 4.3C) demonstrated an initial increase during IE1, followed by a stepwise decrease during the remaining IE intervals, and was significantly lower during the recovery period compared with baseline ($p<0.05$). TPR indexed data is presented in Table 4.2.

Stroke Volume (SV) decreased significantly from baseline to IE1 ($p<0.05$) and remained below baseline throughout the IE training session. In recovery, SV significantly increased ($p<0.05$) and was higher than baseline (Figure 4.3D). Stroke index data is presented in Table 4.2. At each IE interval, there was an increase $\dot{Q}$ from baseline ($p<0.05$). During recovery, there was a significant reduction in $\dot{Q}$ and $\dot{Q}I$

compared with IE4 (p<0.05) as well as was a significant difference in $\dot{Q}$ and $\dot{Q}I$ between baseline and recovery (p<0.001) as shown in table Figure 4.3D and Table 4.2.

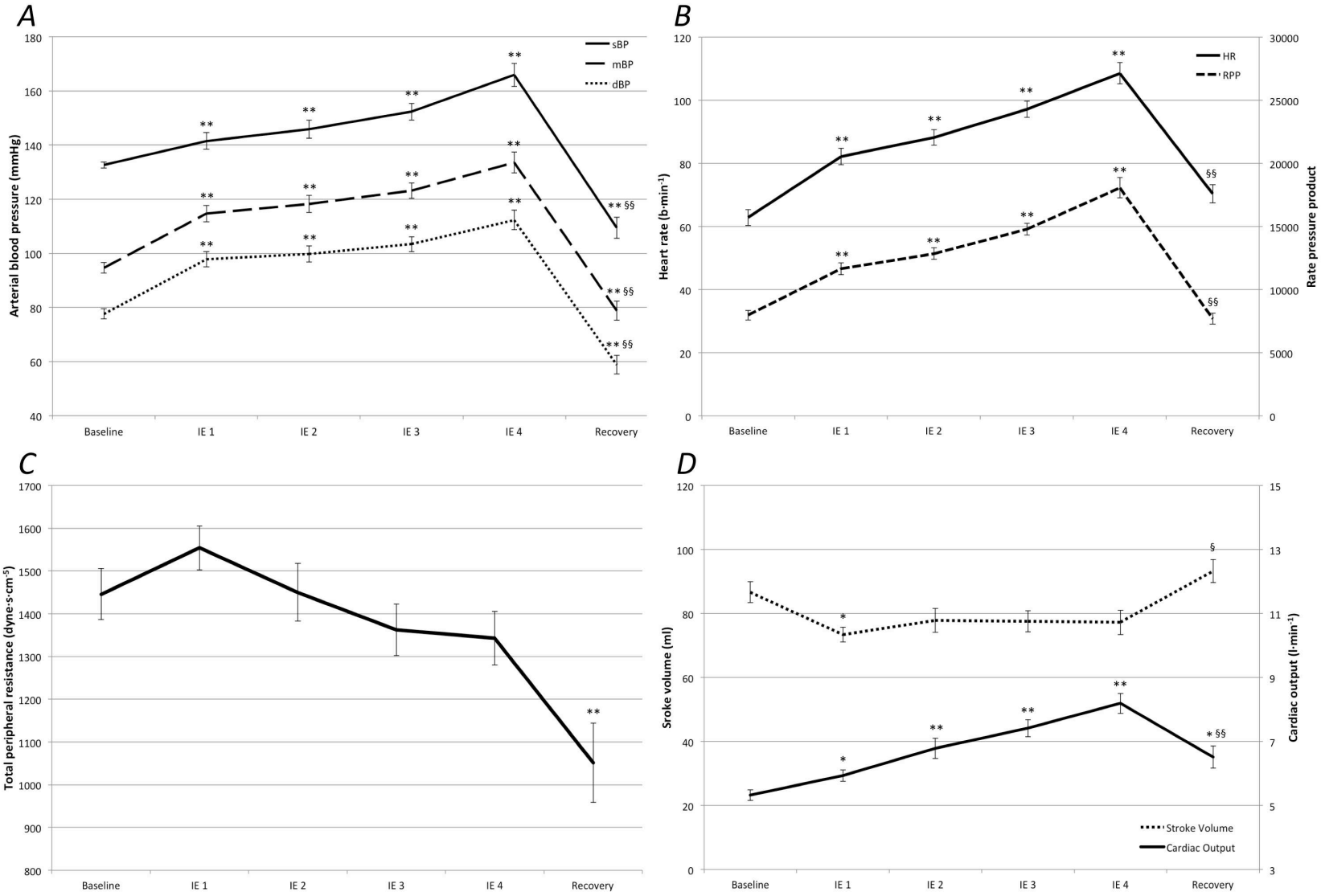

Figure 4.3: Heamodynamic responses at baseline, during 4 bouts of IE and in recovery and standard error of mean. Note: A, arterial systolic, mean and diastolic blood pressure; B, Heart rate and rate pressure product; C, total peripheral resistance; D, Stroke volume and cardiac output. * p<0.05, ** p<0.001 between baseline and all stages. $^{\S}p$<0.05, $^{\S\S}p$<0.001 between IE4 and recovery.

4.4 Discussion

This was the first study to explore the continuous cardiac autonomic and haemodynamic regulatory responses during a single isometric wall squat exercise session and in recovery.

The results demonstrate a stepwise reduction in the total power of HRV during each of the 4 bouts of IE. During the isometric contractions, a greater proportion of the frequency domain parameters remained in the LF (ms^2) band, indicating greater sympathetic activity and parasympathetic withdrawal (Akselrod *et al.*, 1981; Pomeranz *et al.*, 1985). This response is supported by a reciprocal increase and decrease in LFnu and HFnu respectively, and changes in the LF:HF ratio. This response was similar to reductions in HRV observed during aerobic exercise (Casties et al, 2005; Arai et al, 1989; Casadei et al, 1995), the magnitude of which appear to be intensity dependant (Sandercock and Brodie, 2006).

Cessation of IE resulted in an overall increase in HRV above baseline, resulting from increases in the VLF, LF and HF components. Compared with baseline and during IE, recovery was associated with a greater proportion of power spectrum in the HF (ms^2) domain, indicating predominant parasympathetic modulation and sympathetic withdrawal. The results indicate a shift in autonomic regulation between isometric contraction and recovery, which has been previously demonstrated following a number of IE protocols (Iellamo *et al.*, 1999b; Stewart *et al.*, 2007a; Millar *et al.*, 2009a). When compared to aerobic exercise, Martinmaki and Rusko (2008) demonstrated that overall LF (ms^2) and HF (ms^2) increased upon cessation of aerobic exercise; however baseline was still not restored following 10 minutes of recovery. Furthermore, during the first 5 minutes of recovery from aerobic exercise, increases in HRV can be attributed to an increase in the LF component of HRV (Kaikkonen *et al.*, 2008; Casties *et al.*, 2006), suggesting sustained sympathetic activity in the recovery period following aerobic exercise, which may be related to residual circulating catecholamines (Paulev *et al.*, 1984). The parasympathetic response following IE may be associated with upregulation of the NO pathway, a response that would facilitate vagal cholinergic activity and heightened antagonism of cardiac sympathetic activity (Paterson, 2001). Baroreceptor synapses in the cardiac vagal neurone pathway in the

medulla are positively regulated by an intrinsic NO mechanism. The three-fold increase in BRS (19.9 ± 10.3 ms·mmHg^{-1} to 60.04 ± 53.1 ms·mmHg^{-1}) measured during recovery from IE supports this concept.

Alongside a reduction in parasympathetic activation during IE, there was also a step-wise decrease in vagally controlled BRS, which marks the active resetting of baroreceptors and accounts for the directionally opposite, sympathetically controlled increases in HR and BP (Iellamo, 2001) in an attempt to maintain adequate muscle perfusion. Iellamo *et al.* (1999a) suggest that a reduction in BRS during an isometric contraction may be dependent on muscle mass and intensity, therefore a greater muscle mass activation, such as the large muscle group and relatively high contraction intensity used in this study, may have enabled a greater engagement of the muscle metaboreflex, eliciting a reflex inhibition of cardiac vagal tone and increase in sympathetic nerve activity (Iellamo, 2001; Hietanen, 1984; Asmussen, 1981; Iellamo *et al.*, 1999a).

The three-fold increase in BRS during the recovery period contrasts findings from dynamic resistance and aerobic training (Niemela *et al.*, 2008; Heffernan *et al.*, 2008; Somers *et al.*, 1985), which have reported a reduction in BRS, sustained for 20-60 minutes following acute exercise.

The differences in the acute cardiac autonomic response between exercise modes, may in part, explain the greater exercise induced BP reductions following IE compared to aerobic exercise. Furthermore, these acute responses may also be important mechanisms producing greater BP reductions following a programme of IE training compared to traditional aerobic exercise.

Sympathetic activation by central command and metaboreceptors during IE, followed by excitation of the cardiovascular centres, induced linear increases in HR, sBP and $\dot{Q}$, a response previously reported by Stewart *et al.* (2007a) during a single 2-minute isometric contraction.

Aerobic exercise is associated with an increase in sBP and a plateau or small decrease in dBP, as the exercise stimulus imposes a volume load (preload) on the heart (Pollock

et al., 2000). In contrast, IE induced an initial significant rise in both sBP and dBP in the first bout, followed by a continued increase during the remaining bouts that was significantly greater compared to baseline. There was also a significant rise in TPR in the first IE bout compared to baseline, followed by a gradual non-significant decrease in the remaining IE bouts. The rise in dBP in the first IE bout is likely due to the increase in $\dot{Q}$ and TPR. However, in the remaining IE bouts, the small continued rise in dBP despite small progressive reductions in TPR may be explained by the continued rise in $\dot{Q}$ in association with impaired left ventricular diastolic function (Weiner *et al.*, 2012) and/or increased end-diastolic pressure, which is supported by the reduced SV seen during IE. The disproportionate rise in sBP and dBP indicates an increased pressure load (afterload) on the heart (Pollock *et al.*, 2000; Lind and McNicol, 1967).

A step-wise increase in $\dot{Q}$ was primarily mediated by a linear increase in HR, since SV significantly decreased at the onset of IE and remained plateaued until recovery. This is in contrast to aerobic exercise, which demonstrates an increase in SV due to increased preload (Rodeheffer *et al.*, 1984). A reduced SV has been noted during the Valsalva manoeuvre and isometric handgrip testing when there is an increase in intrathoracic pressure, cardiac afterload and LV end-systolic volume (Weiner *et al.*, 2012; Pollock *et al.*, 2000; Stefadouros *et al.*, 1974; Flessas *et al.*, 1976).

The recorded increase in LFnu suggests that during IE, the sympathetic activation is responsible for both HR and BP, while parasympathetic withdrawal (reduced HFnu) inhibits a compensatory increase in SV that would reduce HR while maintaining $\dot{Q}$. Administration of atropine has been shown to block this response, supporting the notion that vagal withdrawal is responsible for increases in HR and BP (Martin *et al.*, 1974). Parasympathetic inhibition during IE results in a reduced capacity to buffer against high BPs, evidenced by a reduction in BRS.

The recovery period was associated with a significant decrease in arterial BP compared with baseline. Post IE arterial BP reductions of 17.4% (23.2 ± 18.1 mmHg), 23.7% (18.7 ± 16.9 mmHg) and 16.5% (15.8 ± 15.5 mmHg) below baseline were demonstrated for sBP, dBP, and mBP, respectively. This represents a greater degree of post exercise hypotension compared to unilateral IHG exercise, which has revealed reductions of 3 mmHg sBP (Millar *et al.*, 2009a), and following acute aerobic exercise

which has elicited reductions of ≈14 mmHg sBP and ≈9 mmHg dBP (MacDonald, 2002). The recovery BP response to isometric wall squat training could be mediated by the significant post exercise changes in TPR and autonomic regulatory responses (HRV and BRS) as these parameters have not previously been reported following an acute bout of IE. The magnitude of BRS gain and BP reduction in recovery demonstrates parasympathetic reactivation, and the extent of response observed in this research could be explained by the type of isometric contraction employed during the training. It is well established within other exercise modalities (Pescatello *et al.*, 2004b; MacDonald *et al.*, 2000), and other isometric protocols (Iellamo, 2001), that the muscle mass and exercise intensity (Hietanen, 1984) have a profound effect on the BP response in recovery.

Modulation of TPR is implicated in the early haemodynamic response to an IE contraction. The reduction in TPR during successive intervals of IE suggests that arterial dilatation occurs, and that the release of sympathetic neurotransmitters may be superseded by a vascular reaction. An isolated muscle contraction during IE results in an increase in sympathetic activation, increasing venous compliance and blood flow to the working muscle, and as such a number of peripheral and vascular mechanisms may offer explanation to the subsequent effect on TPR; increased concentrations of NO and adenosine triphosphate (ATP) and other potential vasodilators may act to down regulate the release of noradrenaline produced by sympathetic activation.

It has been previously suggested that accumulation of exercise-mediated vasodilator NO within the static leg musculature, through increased cell metabolism may cause an attenuated vascular response to vasoconstriction during IE (Lawrence *et al.*, 2014), thus causing a reduction in TPR. During IE, O_2 uptake increases to meet added demands of cell metabolism. A drop in PO_2 in the capillaries and arterioles during an isometric contraction triggers the detection of hypoxic conditions and induces the release of ATP from red blood cells into the lumen via purinergenic signalling, which may indirectly assist with relaxation of smooth muscle (Burnstock, 2009; Mortensen *et al.*, 2009; Ellsworth, 2004).

In addition to the recognised function of NO, it has been suggested that other endothelial cells may be able to induce the hyperpolarisation of vascular smooth

muscle (Cohen and Vanhouette, 1995). An endothelium-derived hyperpolarising factor (EDHF) has been implicated in vasodilation (Sandow, 2004), and acts independently of NO (Luksha *et al.*, 2009). Transmitted via electrical coupling through myoendothelial gap junctions between endothelial and vascular smooth muscle to contractile cells in the vascular wall, EDHF causes cells to relax, allowing the blood vessels to expand in diameter.

When the IE contraction is released, there is sudden perfusion of previously occluded muscle mass and a transient pressure undershoot (MacDougall *et al.*, 1984). A short period of reactive hyperaemia, following ischaemic conditions in the contracted muscle, has been shown to cause acute increases in blood flow and shear rate and a drop in resistance in recovery from an IHG session (McGowan *et al.*, 2006a). An increase in NO synthesis, in response to the shear stress induced by hyperaemic blood flow, triggering vasodilation (Tinken *et al.*, 2010), is a potential mechanism for reduced TPR. However, it has been suggested (Halliwill *et al.*, 2013) that a primary mechanism for sustained post-exercise vasodilation may be histamine H_1 and H_2 receptor activation. A reduction in TPR, via vasodilation demonstrates sympathetic inhibition, while a reduction in HR demonstrates parasympathetic reactivation during recovery, a finding supported by the measured changes in HRV observed in the present study. Redistributed blood flow accounts for restored SV in recovery through increased venous return, and a reduced $\dot{Q}$ is a consequence of restored parasympathetic HR control. These combined responses result in a reduction in arterial BP, and have been a suggested mechanism for post exercise hypotension during recovery from exercise (Pescatello *et al.*, 2004b).

4.4.1 Clinical implications

The acute cardiac autonomic and haemodynamic responses to IE may be important in determining the chronic BP reductions measured through a period of ≥4 weeks of IET (Wiley *et al.*, 1992a; Taylor *et al.*, 2003; Devereux *et al.*, 2010), and may also serve to predict the extent of such responses (Liu *et al.*, 2012; Badrov *et al.*, 2013b).

Impaired autonomic function is an independent predictor of all-cause mortality (Gerritsen *et al.*, 2001) and is implicated in the development of HTN (Schroeder *et al.*,

2003). In addition BRS is considered to have strong prognostic value for cardioprotection (La Rovere *et al.*, 2008b). Isometric exercise is associated with a reduced HRV and residual predominance of sympathetic over parasympathetic activity with an attenuated BRS. In recovery there is a directionally opposite autonomic response with a residual increase in parasympathetic over sympathetic activity and increased HRV and BRS. These transient autonomic responses indicate an improvement in cardiac autonomic modulation, which differ from aerobic exercise and may be important mechanisms producing greater reductions in BP following IE training programmes. Prior research has demonstrated that an >8-week period of IHG training can elicit improvements in cardiac vagal activity (Millar *et al.*, 2013; Taylor *et al.*, 2003), however, few studies have reported the transient BRS response. Isometric exercise training and regular exercise induced hypotension may stimulate the baroreceptors to reset to a lower operating range, which may be an important mechanistic pathway in reducing BP.

Endothelial dysfunction is implicated in a range of cardiovascular diseases (Panza *et al.*, 1990) and may precede their development (Deanfield *et al.*, 2007). Pre-hypertension is associated with impaired vascular reactivity (Giannotti *et al.*, 2010). This study shows a reduction in TPR during IE training and in recovery indicating a transient improvement in endothelial function.

4.5 Conclusion

This study demonstrates an increase in sympathetic activation during an isometric muscle contraction, followed by a shift in sympathovagal balance, with a residual predominance of parasympathetic modulation and improved haemodynamic cardiovascular control during recovery. The acute improvements seen may be mechanistically linked to the IE induced reductions in arterial BP, and may aid to increase understanding of the mechanisms behind chronic BP reductions following a period of IET. Future research is needed in order to ascertain the importance of these acute responses in long-term BP control, as well as any implications these may have on cardiovascular health.

CHAPTER 5

Study 2:

Acute Cardiac Structural and Functional Responses to a Single Isometric Exercise Session in Pre-Hypertensive Males

5.1 Introduction

There is a large body of evidence showing that pre-HTN augments the risk of CAD and CVA, independently of other cardiovascular risk factors (Lewington *et al.*, 2002; Qureshi *et al.*, 2005a). Furthermore, the risk of developing HTN is markedly higher in pre-hypertensive than normotensive populations (Leitschuh *et al.*, 1991; Vasan *et al.*, 2001b).

Evaluation of cardiac structure and function across large populations of normotensive, pre-hypertensive and hypertensive patients, free from heart disease and heart failure, reveals a progressive decline in structural and functional parameters as BP increases (Santos *et al.*, 2016). Impaired cardiac structure is a further predictor of cardiovascular morbidity and mortality.

In pre-hypertensive patients, deterioration in cardiac structure is displayed as LV hypertrophy, as a result of greater LV wall thickness (Santos *et al.*, 2016; Manios *et al.*, 2009; Drukteinis *et al.*, 2007), which demonstrates an association between pre-HTN and target end organ damage (Santos *et al.*, 2016). In addition, measurements of diastolic function, E/A ratio, E' and E/E', considered an early measure of LV dysfunction, are significantly lower in pre-HTN than in NTN (Santos *et al.*, 2016). Diastolic dysfunction has been previously considered a pathophysiological mid point between HTN and heart failure (Di Bello *et al.*, 2010) and as such it is important to recognise this prior to irreversible damage. However, despite evidence of impaired diastolic function, systolic function is usually maintained in pre- HTN (Drukteinis *et al.*, 2007).

Behavioural risk factors, such as smoking, poor dietary habits and physical inactivity, account for 80% of CAD and CVA deaths (WHO, 2010c). Physical inactivity is directly associated with raised BP (WHO, 2010b). Inversely, participation in regular physical activity and maintenance of physical fitness are associated with a decreased likelihood of developing HTN (Chase *et al.*, 2009; Carnethon *et al.*, 2010; Diaz and Shimbo, 2013) and a decreased risk of cardiovascular disease (Eckel *et al.*, 2013). In addition, an inadequate myocardial load as a result of physical inactivity, is known to cause atrophy of the myocardium, which leads to reduced LV mass, impaired cardiac

compliance and reduced SV (Perhonen *et al.*, 2001), resulting in impaired overall cardiac structure and function. However, physical activity interventions in hypertensive participants, as recommended by international guidelines (Chobanian *et al.*, 2003; Pescatello *et al.*, 2004a) have been shown to result in considerable improvement in cardiac structure and function (Libonati, 2013).

The cardiovascular benefits and adaptations induced by physical activity are well documented. With regards to cardiac health, lifelong participation in physical activity is associated with reduced age related cardiac structural and functional degeneration, such as development of diastolic dysfunction (Arbab-Zadeh *et al.*, 2004) and impaired myocardial contractility (Gielen *et al.*, 2010). Uptake of physical activity can induce a number of cardiovascular benefits. With regards to cardiac structure and function, physical activity can induce physiological cardiac remodelling, reverse cardiac deterioration and induce coexistent improvements in systolic and diastolic function (Baggish and Wood, 2011a).

Existing physical activity guidelines recommend participation in aerobic activities for 150 minutes per week (Pescatello *et al.*, 2004a); however, IET has been recognised for its cardiovascular benefits, particularly in reducing BP (Chrysant, 2010; Carlson *et al.*, 2014; Inder *et al.*, 2016; Lawrence *et al.*, 2014). Isometric exercise training has been shown to elicit greater reductions in resting BP than traditional aerobic training interventions (Cornellissen and Smart, 2013) and is effective even following short-term training interventions (Wiley *et al.*, 1992a; Millar *et al.*, 2013; Wiles *et al.*, 2010)(see Table 2.2).

Observed BP reductions following IET are associated with a favourable decrease in the risk of cardiovascular events and all-cause mortality in pre-hypertensive and hypertensive groups (Lewington *et al.*, 2002). However the mechanisms responsible for both acute and chronic BP reductions are unclear. Vascular (McGowan *et al.*, 2006a) and autonomic (Iellamo *et al.*, 1999a; Millar *et al.*, 2014) mechanisms are considered to be important pathways, indeed Chapter 4 demonstrated that a single session of IE induces a step-wise increase in BP and $\dot{Q}$ and reduced and plateaued SV. The increase in $\dot{Q}$ appears to be due to a sympathetically mediated chronotropic and inotropic response and plateaued SV due to an increase in afterload (Pollock *et al.*,

2000). The cardiac autonomic responses during IE demonstrating predominant sympathetic activity support this concept. Of interest, in the recovery period there is evidence of exercise induced hypotension supported by a significant reduction in TPR and normalisation of $\dot{Q}$ and SV. These recovery cardiovascular responses are associated with a directionally opposite cardiac autonomic response with a residual predominance of parasympathetic over sympathetic activity and a significant increase in BRS. Prior research also supports these findings and has identified these acute responses as important mechanisms for the BP reductions following a programme of IET. However, the effects of an acute IE session that uses typical acute programme variables employed in many IET prescriptions on cardiac structure and function are unknown.

Early research used single isometric handgrip contractions during cardiac catheterisation to assess cardiac function (Flessas *et al.*, 1976; Kivowitz *et al.*, 1971; Fisher *et al.*, 1973). Kivowitz *et al.* (1971) recognised a relationship between SV and end diastolic pressure, confirming that a reduction in SV was associated with an increase in end diastolic pressure while increased SV did not change end diastolic pressure. Flessas *et al.* (1976) reported decreased end diastolic pressure, EDV and end systolic volume (ESV) while left ventricular ejection fraction (LVEF) remained constant. Due to the invasive nature of these procedures, research is largely limited to patients with existing cardiovascular disease making it difficult to infer the same conclusions in a healthy population.

Previous research investigating cardiac responses to IE has been applied only during IHG exercise, possibly due to ease of measure, primarily as a method to produce a clinically relevant increase in LV afterload, and as such has been limited to a single IHG contraction. Therefore, the cardiac responses following multiple IE bouts remain un-researched. The cardiac structural and functional responses to a single IE session may, in part, provide a further mechanistic link to the observed reductions in arterial BP seen following IE training.

Therefore, the aims of this study were to establish the effects of an acute bout of isometric wall squat exercise on cardiac structure and function in a pre-hypertensive population.

5.2 Methods

Participants for this study were made up of the same cohort used in Chapter 4 following the inclusion criteria outlined in 3.3.1 page 57.

5.2.1 Study protocol

A single isometric wall squat exercise session at a prescribed training angle (3.10.1-3.10.3, pages 83-86) was completed, as outlined in 3.10.5, page 88, and as completed in Chapter 4.

Transthoracic echocardiography was performed before and immediately following IE, with participants lying in the lateral decubitus position, as per the protocol described in 3.9, page 75.

5.2.2 Data analysis

Data was analysed using the statistical package for social sciences (SPSS 22 release version for Windows; SPSS Inc., Chicago IL, USA). Following tests for normality, a paired samples T-test was used to compare baseline and post IE measurements. A chi-squared test was used to compare categorical data. A p value <0.05 was regarded as statistically significant.

5.3 Results

All of the 26 participants completed the training session at their prescribed training angle. Echocardiographic images suitable for analysis were obtained in all participants at baseline and immediately following acute IE session. All measures are presented as mean ± SD.

5.3.1 Cardiac structure

Cardiac structural changes are shown in Table 5.1. There were significant decreases in LV posterior wall thickness (0.99 ± 0.1 vs. 0.9 ± 0.1 cm, p=0.013), relative wall thickness (0.4 ± 0.06 vs. 0.36 ± 0.05, p=0.027) and LVIDs (3.4 ± 0.2 vs. 3.09 ± 0.3 cm, p=0.002), following IE. Interventricular septal thickness, LVIDd and LV length did not change from baseline to post IE. The proportion of participants with normal LV geometry increased significantly following IE (69.2% vs. 92.2%, p=0.035).

5.3.2 Cardiac function

Cardiac functional responses are shown in Table 5.1. Following IE there was a significant increase in LVEF (60.8 ± 3 vs. 68.3 ± 4 %, p<0.001), in addition to a significant decrease in ESV (52.4 ± 8.5 vs. 46.7 ± 9.7, p<0.001) and increase in EDV (122.24 ± 24.1 vs. 128.68 ± 24.6, p<0.05). Changes in systolic function were measured as an increase in LV tissue Doppler S' (0.09 ± 0.01 vs. 0.19 ± 0.04, p<0.001) and an increase in E' (0.12 ± 0.02 vs. 0.15 ± 0.03, p<0.001), which resulted in a significant decrease in estimated filling pressure following IE (E/E' ratio 6.08 ± 1.87 vs. 5.01 ± 0.82, p=0.006). There were no significant changes in variables of global diastolic function.

Table 5.1: Left and right ventricular function from standard and tissue Doppler echocardiography

Structural Parameters	Pre-IE	Post-IE	P Value
LV internal diameter diastole (cm)	4.98 ± 0.4	5.09 ± 0.47	0.42
LV internal diameter systole (cm)	3.4 ± 0.2	3.09 ± 0.3	0.002
IVSd (cm)	0.98 ± 0.1	0.93 ± 0.1	0.16
LVPWd (cm)	0.99 ± 0.1	0.9 ± 0.1	0.013
Relative wall thickness	0.4 ± 0.06	0.36 ± 0.05	0.018
LV mass (g)	177.8 ± 31.7	164.6 ± 26.8	0.16
LV mass index ($g \cdot m^2$)	86.3 ± 15	80 ± 13.8	0.18
LV geometry			
Normal (n=)	18	24	0.035
Concentric remodelling (n=)	8	2	
LV length (cm)	8.9 ± 0.6	8.8 ± 0.7	0.7
Global LV diastolic function			
Peak E velocity ($cm \cdot s^{-1}$)	0.7 ± 0.1	0.74 ± 0.2	0.32
Peak A velocity ($cm \cdot s^{-1}$)	0.5 ± 0.2	0.51 ± 0.2	0.82
Peak E/A ratio	1.48 ± 0.3	1.53 ± 0.4	0.69
Isovolumic relaxation time (ms)	77.2 ± 15	82.1 ± 23	0.67
Global LV systolic function			
Left ventricular ejection fraction (%)	60.8 ± 3	68.3 ± 4	<0.001
ESV (ml)	52.4 ± 8.5	46.7 ± 9.7	<0.001
EDV (ml)	122.24 ± 24.1	128.68 ± 24.6	0.036
LV tissue Doppler			
Peak E' ($m \cdot s^{-1}$)	0.12 ± 0.02	0.15 ± 0.03	<0.001
Peak A' ($m \cdot s^{-1}$)	0.1 ± 0.02	0.11 ± 0.02	0.05
Peak S' ($m \cdot s^{-1}$)	0.09 ± 0.01	0.19 ± 0.04	<0.001
LV filling pressures			
E/E' ratio	6.08 ± 1.87	5.01 ± 0.82	0.006

5.4 Discussion

This is the first study to investigate the acute effects of isometric wall squat training on cardiac structural and functional variables in a pre-hypertensive population.

A single session of IE resulted in acute improvements in cardiac structure, including significantly reduced LV end systolic internal diameter, LV posterior wall thickness and relative wall thickness (RWT). At baseline, 8 participants displayed signs of concentric LV remodelling, in comparison to normative values (Lang *et al.*, 2015); however, during recovery, this was reduced to just 2. The baseline LV wall thicknesses measured in this study are in line with dimensions reported in a large pre-hypertensive sample (IVSd, 0.99 ± 0.13cm; LVPWd 0.89 ± 0.11cm) (Santos *et al.*, 2016). An increase in SV was reported in Chapter 4, and a non-significant increase in LVIDd, may demonstrate a greater stretch of the ventricle, in accordance with Starlings Law, which may explain the measured reduction in LVPWd and RWT following IET.

Into recovery, preload appears to remain elevated; as SV becomes elevated, and $\dot{Q}$ remains higher than baseline (Chapter 4, page 104). A reduced TPR was reported during recovery (Chapter 4, page 104), which may be responsible for the exercise induced hypotension, as longitudinal and radial systolic function improved following acute IE. Left ventricular end diastolic dimensions did not change, yet there was a significant reduction in LVIDs demonstrating increased contractility, mediating an increase in LVEF and SV.

A chronic increase in afterload induced by raised BP can cause pathological remodelling and increased LV wall thickness as a result of fibrosis (Kahan and Bergfeldt, 2005) yet aerobic exercise training in hypertensive patients is has been demonstrated to reduce RWT and BP (Hinderliter *et al.*, 2002; Kokkinos *et al.*, 2005; Turner *et al.*, 2000), in addition to increases in SV, $\dot{Q}$ and LVEF (Rinder *et al.*, 2004). For these reasons, aerobic exercise training is widely recommended as a lifestyle modification and treatment option to improve cardiovascular health. The cardiac structural and functional responses to a single session of IE are noteworthy, as repetition of this training may lead to a similar chronic response. Isometric exercise training is known to reduce resting BP, and requires a relatively small time commitment compared with aerobic exercise alternatives, therefore additional benefits and cardiovascular adaptations that occur following IET may be of interest.

Weiner *et al.* (2012) measured the cardiac structural and functional responses during a 3-minute IHG contraction in healthy participants. The isometric stimulus produced an increase in afterload, evidenced by increases in BP and LV ESV. During IE Weiner *et al.* (2012) also reported a reduction in SV and an increase in HR and contractility as a result of increased sympathetic tone, which is supported by the results presented in Chapter 4. Sympathetic activation may result in beta-adrenergic stimulation, which increases cardiac calcium (Ca^{2+}) signalling by cardiomyocytes, leading to a potential increase in excitation coupling, assisting in the release of NO, to promote cardiac contractility. Importantly, although this is a positive acute response, chronic sympathetic over activation, associated with HTN, leads to excessive concentrations of Ca^{2+} and insufficient bioavailability of NO (Ziolo et al, 2001), which is required for Ca^{2+} handling to prevent pathological remodelling of the myocardium (Opie, 2004).

Therefore reduced sympathetic activation and increase parasympathetic modulation measured in recovery from IE (Chapter 4) (Taylor *et al.*, 2017) may therefore be an important mechanism of an IE stimulus.

Weiner *et al.* (2012) demonstrated a decline in diastolic function during IHG exercise, evidenced by a reduction in early diastolic filling accompanied by an augmentation in late diastolic filling during contraction. However, these findings were measured during IHG whereas measurements in the present study were taken during recovery from multiple bouts of IE. Given the similarities in haemodynamic responses during IE, it is possible that isometric wall squat training induced a similar cardiac response to Weiner *et al.* (2012) during contraction. However, recovery from isometric wall squat training resulted in an increase in SV and LVEF, demonstrating improved contractility, as well as significant improvements in estimated filling pressure, which is a strong predictor of premature mortality in patients with HTN (Sharp *et al.*, 2010).

Recovery from acute IE (handgrip and wall squat exercise) is associated with improved cardiac autonomic regulation, demonstrated as increased parasympathetic activation, and post exercise hypotension (Millar *et al.*, 2009a; Taylor *et al.*, 2017) as well as post exercise hyperaemia and associated sheer stress, mediating increased NO bioavailability (Tinken *et al.*, 2010), all of which may be responsible for post exercise hypotension. Vagal stimulation is associated with the release of acetylcholine which stimulates endothelial cells to release endothelial nitric oxide synathase (eNOS), which leads to the synthesis of NO and vasodilation. This occurs in the vascular endothelium and may cause a reduction in TPR, which may reduce cardiac afterload. Results from Chapter 4 support this concept, which may lead to the improvements in LV haemodynamics. Nitric oxide in cardiac myocytes has been shown to improve cardiomyocyte Ca^{2+} signalling and LV contractile function (Pironti *et al.*, 2016). Bioavailability of NO has been reported to effect LV relaxation as well as modulating fundamental events of myocardial excitation-contraction coupling (Paulus and Shah, 1999).

The role of NO, an important regulator of vascular function, has been proposed as a potential mechanism for BP reductions following IET (Lawrence *et al.*, 2014; Millar *et al.*, 2014). Indeed, greater physical fitness is positively associated with cardio myocyte

contractile capacity and endothelial function (Kemi *et al.*, 2004). Therefore, repeated activation of this acute response may lead to the up-regulation of NO, as significant improvements in NO-dependant vasodilation have been shown following programmes of IET (McGowan *et al*, 2007).

5.4.1 Clinical implications

Hypertension is associated with a progressive decline in cardiac performance, which is the leading modifiable risk factor for mortality (WHO, 2012) and pre-HTN is associated with an increased risk of HTN and CVD (Vasan *et al.*, 2001b; Chobanian *et al.*, 2003). Isometric exercise training has been shown to elicit greater reductions in resting BP compared with other non-pharmacological lifestyle changes (Millar *et al.*, 2014). The acute favourable improvements in LV remodelling and LV function following IE advocate the need to assess if similar responses endure alongside BP reductions following a period of IE training of this type. It is possible that acute changes in cardiac structure and function may be mechanistically linked to longer term BP reductions, however further research is needed to test this hypothesis.

5.5 Conclusion

This study suggests that IE acutely improves LV remodelling, LV systolic function and estimated LV filling pressure. Regularly inducing these adaptive changes, through adherence to regular IET may result in chronic improvements in cardiac structure and function. This could also provide a mechanistic explanation for the chronic reductions in resting arterial BP frequently measured following a period of IET (Millar *et al.*, 2014; Carlson *et al.*, 2014; Inder *et al.*, 2016).

CHAPTER 6:

Study 3

Resting and Ambulatory Blood Pressure Responses to Short-term, Home-based Isometric Exercise Training in Pre-Hypertensive Males. A Randomized Cross-Over Controlled Trial.

6.1 Introduction

The global prevalence of high BP and its associated risk of accelerated CVD (Mancia *et al.*, 2013), is accompanied by a continuous need to develop effective treatment therapies that can maintain BP to within optimal levels. Pre-hypertension precedes the development of HTN and is associated with an increased risk of future HTN, as well as an increased risk of CVD (Vasan *et al.*, 2001a). There is growing evidence that this population could benefit dramatically from reduced BP, as such current guidelines recommend the use of non-pharmacological interventions in the early treatment of elevated BP (Chobanian *et al.*, 2003). Physical activity (PA) is a current recommendation for BP maintenance and an active lifestyle is associated with the maintenance of optimal BP compared to those who are physically inactive (Cornelissen and Smart, 2013). Aerobic exercise is currently recommended as a primary treatment option to maintain and reduce resting BP (Pescatello *et al.*, 2004a). However, in spite of known health benefits, 33% of adult males in England are physically inactive and participation rates in physical activity decline with age (Townsend *et al.*, 2015). Moreover, physical inactivity is considered the 4th biggest risk factor for global mortality (WHO, 2010a). It is therefore important that future interventions seek to minimise barriers that currently impede engagement and adherence to exercise training.

Isometric exercise training has been proposed as a time efficient method of producing clinically relevant reductions in resting BP (Wiley *et al.*, 1992a; Wiles *et al.*, 2008; Taylor *et al.*, 2003; McGowan *et al.*, 2006b) (see Table 2.2, page 28). A meta-analysis of IET interventions lasting 3-10 weeks (Inder *et al.*, 2016) reported mean resting sBP reductions of 5.2 mmHg (95% CI -6.08 to -4.33, $p<0.001$) and mean resting dBP reductions of 3.91 mmHg (95% CI – 5.68 to -2.14, $p<0.001$). These findings can be considered clinically significant, as a reduction of 5 mmHg (sBP or dBP) is considered the minimal important difference for appreciable benefits by the World Health Organisation Guideline Development Group (NICE, 2011), and is associated with a 10% reduction in the risk of mortality, CVA and MI. However, the risk of cardiovascular events doubles for every 20 mmHg (sBP) and 10 mmHg (dBP) increase in BP >115 mmHg and >70 mmHg in sBP and dBP respectively (Lewington *et al.*, 2002). Therefore greater reductions in BP equate to greater risk reduction (Whelton *et*

al., 2002).

Although reductions in resting BP have been widely reported, there have been few IET intervention studies that have used ABP monitoring (Inder *et al.*, 2016; Millar *et al.*, 2014). In addition, existing research is focussed on medicated hypertensive, and healthy normotensive participants respectively (Stiller-Moldovan *et al.*, 2012; Somani *et al.*, 2017). Ambulatory blood pressure monitoring involves measurement of BP at randomised intervals over a 24-hour period and this method is proven more effective in the prediction of BP related cardiac damage (Protogerou *et al.*, 2014) and is also a stronger predictor of cardiovascular events than single measurement procedures (Ohkubo *et al.*, 2005; Verdecchia *et al.*, 1998; O'Brien, 2011; Verdecchia, 2000; Verdecchia *et al.*, 1994; Staessen *et al.*, 1999).

Circadian variation and factors such as respiration, emotion, exercise, temperature and meals can affect sBP by up to 20 mmHg (Beevers, 2001). During sleeping hours, particularly when individuals are supine, there is an increase in vagal activity, which reduces HR, $\dot{Q}$, respiratory rate and BP. Upon wakening, a withdrawal of vagal tone and a surge of adrenergic activity causes a rapid increase in BP and HR, marking the highest point in diurnal internally regulated BP (Opie, 2004). There is typically a gradual decrease in BP throughout the day; however, BP will adapt based on environmental stimulation, such as mental stressors or physical demands. Overall, acute variations in BP are smaller than fluctuations in HR due to baroreflex compensatory mechanisms (see 2.3.5, page 19). Compared with resting BP measurement, ABP monitoring provides a more accurate indication of true BP, as it accounts for diurnal variation in BP in addition to the presentation of white coat or masked HTN (Verdecchia, 2000). Blood pressure variability (Hansen *et al.*, 2010; Pickering *et al.*, 2006) and the extent of BP dipping during nocturnal hours (Fagard, 2009) can also be measured through ABP monitoring and as such ABP monitoring is recognised as more accurate in predicting CVD risk and mortality than resting BP measurement (Pickering *et al.*, 2006).

A longitudinal study by Licitra *et al.* (2012) performed ABP measurements on 107 pre-hypertensive patients and assessed progression to HTN over a 6-year period. Follow up data at the end of the study revealed that almost half (n=43) of those studied went on to

develop drug treated HTN. Despite this, ABP monitoring is reportedly under-used in intervention and outcome studies (O'Brien, 2011) namely due to the higher cost and time commitments associated with this measure. As such, future research is required to further explore the effects of IET on ABP.

Previous IET interventions have utilised IHG and ILT protocols, 3 times per week for 4-12 weeks, with each training session most commonly consisting of 4 x 2 minute contractions (Inder *et al.*, 2016; Lawrence *et al.*, 2014). Laboratory attendance has been essential during ILT as many leg training methodologies have made use of expensive and inaccessible isokinetic dynamometers (Baross *et al.*, 2012b; Devereux *et al.*, 2011; Wiles *et al.*, 2010), while handgrip programmes have made use of programmable handgrip dynamometers (Ray and Carrasco, 2000; Peters *et al.*, 2006; Millar *et al.*, 2007; McGowan *et al.*, 2006b), which although comparatively much less expensive, remain relatively unaffordable to the general public. The onset of IE is accompanied by a rapid increase in sBP, dBP and HR, as outlined in Chapter 4, and as such IE has been prescribed cautiously, particularly in populations with increased CVD risk, due to perceived dangers of this type of exercise (Carlson *et al.*, 2014).

However, this type of training has the potential to overcome time barriers and certain cost barriers related to exercise participation. Some studies have effectively used a combination of laboratory and home-based IET (Millar *et al.*, 2009a; Millar *et al.*, 2009b; Stiller-Moldovan *et al.*, 2012; McGowan *et al.*, 2006b; Millar *et al.*, 2007). Recent research has successfully employed a purely home-based training approach (Wiles *et al.*, 2017) using a very inexpensive device in a healthy population and demonstrated BP reductions of 4 mmHg and 3 mmHg in sBP and dBP respectively. Such an intervention may successfully reduce exercise barriers, such as time, cost and wanting to avoid the judgement of others, which are currently responsible for limited participation in PA (Salmon, 2001), in addition to eliciting measurable BP reductions.

As detailed in Chapter 4, acute IE was associated with significant increases in BP, with a significant hypotensive response in the 5-minute recorded recovery period. The recovery response to acute IE may have been mediated by post exercise changes in autonomic modulation, such as BRS and increased vagal activation. It has been suggested that repeated stimulation of the acute haemodynamic pathways may

contribute to the widely reported changes in resting BP following IET and help to explain the mechanistic changes, which result in chronic resting BP reductions.

Therefore, the primary aim of this study was to investigate the effects of a short-term programme of isometric wall squat training on resting and ambulatory BP. The secondary aim was to evaluate any changes in resting haemodynamic variables including TPR, SV and $\dot{Q}$ in order to ascertain any physiological adaptations, which may underpin reductions in BP in response to IET. A pre-hypertensive population was selected for experimentation and all IET took place in an unsupervised home-based setting.

6.2 Methods

6.3.1 Participants

Twenty-four pre-hypertensive males (age 43.75 ± 7.3 years; height, 178.1 ± 7 cm; weight, 89.7 ± 12.8 kg) were recruited to participate in this randomised cross-over controlled trial, as per the recruitment information and eligibility criteria described in 3.3.1, page 58. Ethical approval was granted by the ethics committee at Canterbury Christ Church University and all participants provided written informed consent before participation. The study adhered to the principles of the Declaration of Helsinki (2013).

6.2.2 Overall study design

Sample size calculations and participant numbers are described in 3.3.3, page 59. All participants (n=24) completed a 4-week IET intervention, either preceded (n=12) or followed by (n=12) a 4-week control period. The two conditions were separated by a 3-week washout period outlined in Figure 6.1. Participants were required not to alter daily dietary and physical activity routines and to inform the researchers of any changes to their health during the 12-week overall study period. These details were verbally confirmed with each participant upon each laboratory visit.

The purpose of randomisation is to avoid selection bias whilst ensuring a balanced allocation of participants to concurrent groups of a study. Participants were randomised

by the experimenter to perform either IET intervention, or control period first, using a simple randomisation approach based on order of entry into the study. It is acknowledged that this approach is limited, as it does not account for experimenter bias, and does not blind the experimenter to the research condition. However, given that the eligibility criteria during recruitment was reasonably narrow, there was very little risk of encountering differences in group demographic or baseline characteristics and as such balance between groups was achieved.

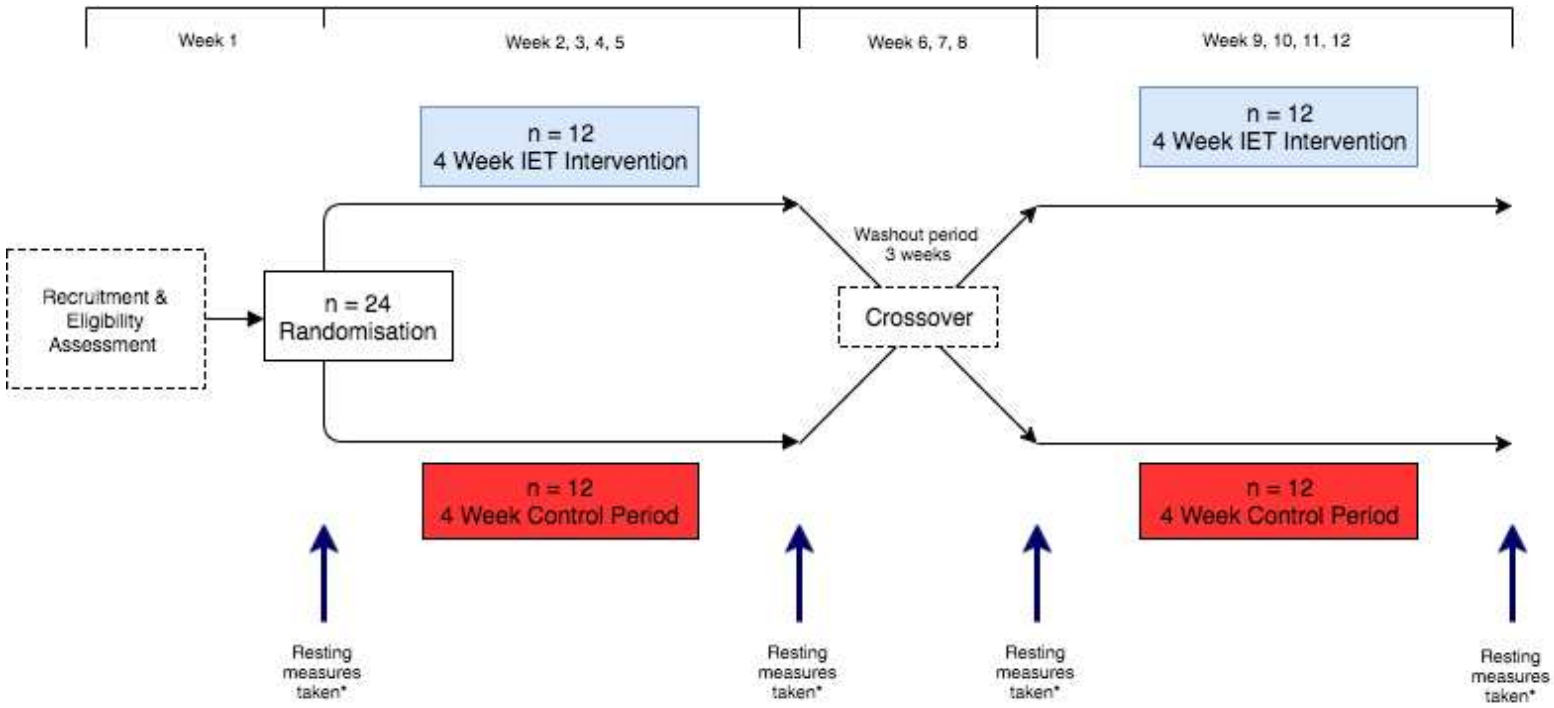

Figure 6.1. Visual representation of the randomised crossover study design.

6.2.3 Laboratory procedures

Participants observed a 4-hour fast and abstained from caffeine and alcohol for 24-hours prior to each visit to the laboratory. Time of day remained constant for each laboratory visit. Participants visited the laboratory on 6 separate occasions. During the first visit, resting BP status was confirmed (3.7, page 73) and participants completed an isometric wall squat test to establish an appropriate IE session intensity (see 3.10.1-3.10.3, pages 83-86). During the second visit, participants performed a single IE session in the laboratory (see 3.10.5, page 88) using a bend and squat device (see 3.10.4, page 86) at their prescribed training intensity. A goniometer (Figure 3.9, page 84) was used during this session to confirm that the appropriate training angle was elicited by the bend and squat device. The remaining 4 laboratory visits occurred pre and post IET and control periods, during which resting measures were taken, as indicated in Figure 6.1.

Each of these 4 visits followed the same procedures. Height (m) and body mass (kg) were also recorded at each visits as some variables are indexed to BSA. Resting BP measurement was performed as per the methods outlined in 3.7 (page 73) following 15-minutes of seated rest. Resting haemodynamic function was recorded in a supine position over a continuous 5-minute measurement period using the TFM, following 15-minutes of supine rest.

6.2.4 Ambulatory blood pressure monitoring

Ambulatory BP recordings were performed using a Welch Allyn 6100 ambulatory BP monitor (Welch Allyn Inc. Skaneateles Falls, NY, USA). The monitor employs the oscillometric method (see 3.6, page 73) and determines pressure by sensing the cessation of pressure waves in the artery when occluded by pressure in the cuff (Welch Allyn, Ambulatory Blood Pressure Monitoring Hardware Manual). The cuff inflates at pre-programmed intervals throughout the day and night to capture diurnal variation in participant BP. All BP readings are stored on the device, and uploaded to a computer using Welch Allyn CardioPerfect^{TM} Workstation software for Windows$^{®}$ (Welch Allyn Inc. Skaneateles Falls, NY, USA) for evaluation.

6.2.5 Ambulatory blood pressure monitoring protocol

Upper arm circumference was measured and an appropriately sized pneumatic cuff was wrapped around the participants left arm, underneath loose fitting clothing. The cuff was connected by a plastic tube to the monitor unit, which was worn on a belt around the participant's waist. Participants were advised to avoid tight fitting clothing, heavy lifting, strenuous exercise and eating or drinking differently to their normal lifestyle and were asked not to remove the cuff during the 24-hour period of wear. For their own comfort, participants were advised to avoid excessive hand movement, flexing muscles and whole body movement where possible during BP readings. A manual reading was taken in the laboratory to ensure participant comfort and device function, which also activated the device to commence the 24-hour monitoring cycle. The monitor was programmed to measure BP at intervals of 20/30 minutes during the daytime and 30/60 minutes overnight. After 24-hours, the participant returned the monitor to the laboratory, and the data was downloaded using the relevant software.

Measurements taken between 08.00 and 22.00 were regarded as daytime measurements, and measurements taken between 24.00 and 06.00 were night-time measurements (Fagard *et al.*, 2009). Participants wore the same monitor for repeated measures. A reading was considered acceptable if 14 measurements were successfully obtained during day-time hours and 6 measurements were recorded during night-time. The ABP measurement period was repeated if the number of measurements obtained was inadequate. One participant elected not to wear the monitor during night-time hours, however full recordings were obtained for waking hours in this case.

6.2.6 Reliability of Welch Allyn 6100

The Welch Allyn 6100 has been tested in accordance with international protocol from the European Society of Hypertension (O'Brien *et al.*, 2002). This was the first ambulatory device to receive a double A grade as well as passing international protocol validation (Jones *et al.*, 2004). Accuracy of the device was measured within 0 (± 7) mmHg for sBP and -1 (± 6) mmHg for dBP of the device used for comparison (Goodwin *et al.*, 2007) advocating it as a validated tool for clinical use.

6.2.7 Isometric exercise training

Participants completed 4-weeks of home-based exercise training (see 3.10.6, page 89). A single training session consisted of 4 x 2-minute isometric wall squat contractions, separated by 2-minutes of rest (see 3.10.5, page 88) and training sessions were completed 3 times per week over a period of 4-weeks.

In order to monitor adherence and haemodynamic responses, participants were provided with a Polar HR monitor and a Borg RPD scale (Borg, 1998), along with a training manual, which included detailed instructions on how to use the equipment and training programme (see Appendix 5: Training Session Manual). During each session, participants were asked to record RPD and HR at rest and at the end of each exercise bout. Participants input this data into an online database, which could be accessed only by the participant and experimenter. Training data was monitored daily by the experimenter to confirm that HR responses fell within the target zone throughout the intervention period. All participants remained within their target training zone throughout the intervention, therefore no adjustments in intensity were required.

6.2.8 Control condition

During the control condition, participants were requested to maintain their habitual dietary and physical activity daily routines. During this period participants did not visit the laboratory and no measures were taken. Adherence to these conditions was verbally confirmed during the subsequent laboratory session.

6.2.9 Data analysis

Following a Shapiro-Wilk test for normality, a two-way repeated measure ANOVA was performed with a Bonferroni post-hoc test, for the comparison of outcome measures between and within groups. A correlation coefficient for repeated observation was calculated between body mass data and variables indexed to BSA, as described by Bland and Altman (1995). Data is presented as mean ± SD unless otherwise stated. Bean plots were constructed to ascertain the distribution of ABP data across the sample. All statistical analyses were performed using IBM SPSS 22

software (SPSS Inc., Chicago, Illinois, USA). An alpha level of <0.05 was set as the threshold for statistical significance.

6.3 Results

All 24 participants completed 12 training sessions during the 4-week IET intervention. Resting BP and daytime ABP was obtained from all participants however one participant elected not to wear the ABP monitor during night-time.

Resting BP and haemodynamic function data is presented in Table 6.1 and ABP is presented in Table 6.2. There was no significant change in height or weight in either the control (p=0.12) or IET condition (p=0.653). In addition, a correlation of repeated observations revealed that a change in body mass did not account for any variable indexed to BSA (SI, r=0.09; $\dot{Q}$I, r=0.167; TPRI, r=0.193). Resting and ABP were not significantly different at baseline between conditions.

Table 6.1. Resting BP and heamodynamic data, pre and post control and IET period.

	Control (n=24)		Isometric Exercise Training (n=24)	
	Pre	Post	Pre	Post
Resting BP				
sBP (mmHg)	132.2 ± 5.4	132.5 ± 4.9	132.4 ± 5.6	120.1 ± 5.7**
dBP (mmHg)	81.9 ± 6.25	81.7 ± 6.5	81.4 ± 6.9	75.2 ± 6.2**
mBP (mmHg)	101.2 ± 6.3	101.1 ± 6.5	101.1 ± 6.6	93.1 ± 5.8**
Resting Haemodynamic Variables				
Heart Rate ($b \cdot min^{-1}$)	61.06 ± 7.52	60.07 ± 7.12	60.07 ± 7.39	59.56 ± 8.18
Stoke Volume (ml)	84.04 ± 14.2	84.46 ± 13.98	84.11 ± 13.9	90.57 ± 15.24**
Stroke Index ($ml \cdot m^2$)	41.45 ± 7.72	41.57 ± 7.52	41.43 ± 7.66	44.45 ± 7.88**
Cardiac Output ($l \cdot min^{-1}$)	5.09 ± 0.87	5.05 ± 0.98	5.01 ± 0.82	5.38 ± 1.19*
Cardiac Index ($l \cdot min^{-1} \cdot m^2$)	2.51 ± 0.44	2.48 ± 0.52	2.46 ± 0.43	2.63 ± 0.55*
TPR ($dyne \cdot s \cdot cm^{-5}$)	1508 ± 358	1516 ± 357	1524 ± 308	1348 ± 311**
TPRI ($dyne \cdot s \cdot m^2 \cdot cm^5$)	747 ± 199	748 ± 196	751 ± 164	664 ± 165**

Note: Values are mean ± SD. * p<0.05, ** p<0.001 values between pre and post IET condition. There were no significant differences within control group. TPR, total peripheral resistance; TPRI, total peripheral resistance index.

As shown in Table 6.1, significant differences in all measures of resting BP were observed between the pre and post conditions of the intervention. Following 4-weeks of IET there were significant reductions (mean and 95% CI) in resting sBP (12.35 mmHg; 95% CI, 10.94-14.23, $F(1, 23)=226.13$, $p<0.001$), dBP (6.24 mmHg; 95% CI, 4.01-8.12, $F(1, 23)=37.22$, $p<0.001$), and mBP (8.01 mmHg; 95% CI, 6.04-9.64, $F(1, 23)=81.04$, $p<0.001$). In the control condition there were no changes in resting BP (sBP, $p= 0.252$; dBP, $p=0.671$; mBP $p=0.653$).

Resting SV (+6.45 ± 4 ml, $p<0.001$) and $\dot{Q}$ significantly increased (+0.36 ± 0.67 $l \cdot min^{-1}$, $p=0.014$), while TPR was significantly reduced (-175.85 ± 184.68 $dyne \cdot s \cdot cm^{-5}$, $p<0.001$) following IET. When indexed to BSA, changes in SI ($p<0.001$), $\dot{Q}I$ ($p=0.005$) and TPRI ($p<0.001$) were also significant. There were no changes in the control condition in SV ($p=0.357$), SI ($p=0.616$), $\dot{Q}$ ($p=0.684$), $\dot{Q}I$ ($p=0.367$), TPR ($p=0.837$) and TPRI ($p=0.941$). There were no changes in resting HR in the control or IET condition.

As shown in Table 6.2, following IET 24-hour mean ambulatory sBP, dBP and mBP reduced by 11.83 mmHg (95% CI, 10.26 - 13.52, $F(1, 22)=121.09$, $p<0.001$), 5.9 mmHg (95% CI, 3.05 – 6.29, $F(1, 22)=65.98$, $p<0.001$) and 5.9 mmHg (95% CI, 4.13

– 7.82, $F(1, 22)=36.38$, $p<0.001$) respectively. There were significant changes in night-time and day-time sBP, mBP and dBP (all $p<0.001$) (night sBP, $F(1, 22)=52.85$; night dBP, $F(1, 22)=12.63$; night mBP, $F(1, 22)=7.35$; day sBP, $F(1, 23)=71.66$; day dBP $F(1, 23)=36.79$; day mBP, $F(1, 23)=59.73$. There were no changes in the control condition for 24-hour ABP (sBP, $p=0.864$; dBP, $p=0.899$; mBP $p=0.817$), daytime ABP (sBP, $p=0.635$; dBP, $p=0.132$; mBP, $p=0.253$), or night-time ABP (sBP, $p=0.449$; dBP, $p=0.564$, mBP, $p=0.994$).

In addition, there were significant reductions in PP and RPP, in overall 24-hour ambulatory measures (PP, $p<0.001$; RPP, $p<0.001$), daytime (PP, $p=0.002$; RPP, $p<0.001$) and night time (PP, $p=0.007$; RPP, $p<0.001$) measures following IET. Night-time HR was significantly reduced following IET ($p=0.014$), however there were no changes in overall 24-hour HR ($p=0.62$), or daytime HR ($p=0.274$). There were no changes in the control condition for PP (24 hour, $p=0.427$; day, $p=0.886$; night, $p=0.184$) or RPP (24 hour, $p=0.262$; day, $p=0.127$; night, $p=0.993$).

There was a significant reduction in 24-hour systolic BPV ($p<0.001$) and 24-hour mean BPV ($p<0.001$), night-time mean BPV *(*$p=0.005$) and night-time diastolic BPV ($p=0.009$) following IET. There were no changes in day-time BPV following IET and no changes in BPV in the control period.

Table 6.2. Twenty-four hour ambulatory, daytime and night-time BP, HR, PP and RPP data and 24-hour, daytime and night-time BPV data.

	Control (n=24)		Isometric Exercise Training (n=24)	
	Pre	Post	Pre	Post
24 hour ambulatory BP$^{\otimes}$				
sBP (mmHg)	130.6 ± 5.9	130.7 ± 6.7	131.4 ± 5.6	119.6 ± 6.3**
dBP (mmHg)	73.4 ± 8.1	73.3 ± 7.8	74.3 ± 7.9	68.3 ± 6.7 **
mBP (mmHg)	91.7 ± 8.8	91.6 ± 7.9	91.3 ± 8.4	85.4 ± 6.24**
HR ($b \cdot min^{-1}$)	72.9 ± 6.1	70.3 ± 7.3*	71.1 ± 7.7	69 ± 7.3
PP (mmHg)	55.8 ± 9.1	55.3 ± 9.9	55.9 ± 12.1	50.1 ± 6.8**
RPP	9212 ± 952	9115 ± 885	9249 ± 1101	8130 ± 953**
Day Ambulatory BP 08.00-22.00				
sBP (mmHg)	133.9 ± 9.7	134.2 ± 10.4	136.8 ± 11.1	122.9 ± 6.7**
dBP (mmHg)	77.4 ± 7.9	76.2 ± 6.6	78.5 ± 7.1	72.9 ± 5.5**
mBP (mmHg)	96.1 ± 9	95.3 ± 7.2	97.3 ± 7.6	89.9 ± 5.4**
HR ($b \cdot min^{-1}$)	74.5 ± 3.9	72.9 ± 7.9	75.3 ± 7.2	74 ± 7.6
PP (mmHg)	55.6 ± 9	55.6 ± 9.9	56.4 ± 13	49.8 ± 6.2*
RPP	10016 ± 983	9773 ± 1021	10245 ± 1155	9047 ± 879**
Night Ambulatory BP 00.00-06.00$^{\otimes}$				
sBP (mmHg)	112.4 ± 9.5	112.8 ± 8.8	113.6 ± 9.8	104.2 ± 6.9**
dBP (mmHg)	59.9 ± 6.8	59.2 ± 8.1	61.7 ± 3.9	56.8 ± 5.6**
mBP (mmHg)	76.4 ± 6.9	76.3 ± 7.2	76.7 ± 6.5	72.8 ± 5.9**
HR ($b \cdot min^{-1}$)	63.9 ± 5.5	63.3 ± 5.8	65.4 ± 6.4	62.1 ± 7.2*
PP (mmHg)	52.7 ± 8.7	53.9 ± 10	51.4 ± 9.2	47.6 ± 5.5*
RPP	7189 ± 932	7188 ± 788	7461 ± 976§	6466 ± 840**
24 Hour Ambulatory BPV$^{\otimes}$				
sBP (mmHg)	14.4 ± 2.1	14.1 ± 3.6	15.1 ± 3.2	13.2 ± 2.6**
dBP (mmHg)	11.3 ± 1.9	11.7 ± 2.6	11.8 ± 3.1	11.3 ± 2.2
mBP (mmHg)	12.5 ± 2	12.3 ± 2.5	13.5 ± 2.8	11.9 ± 2.3**
Day Ambulatory BPV				
sBP (mmHg)	9.9 ± 1.2	16.2 ± 24.2	10.1 ± 2.8	9.3 ± 2.7
dBP (mmHg)	7.8 ± 1.6	11.6 ± 11.2	8.4 ± 2.4	8.4 ± 2.4
mBP (mmHg)	8.1 ± 1.7	12.2 ± 15.1	9.2 ± 2.1	8.6 ± 2.2
Night Ambulatory BPV$^{\otimes}$				
sBP (mmHg)	10.6 ± 2.6	9.8 ± 9.3	10.7 ± 4.1	9.5 ± 3.9
dBP (mmHg)	8.5 ± 3.6	9.3 ± 2.9	9.5 ± 3.5	7.1 ± 2.8*
mBP (mmHg)	9.3 ± 3.4	9.4 ± 3.1	9.7 ± 3.7	7.2 ± 3.3*

Note: $^{\otimes}$ n=23 participants; * $p<0.05$, ** $p<0.001$ within groups; § $p<0.05$ between groups. HR, heart rate; PP, pulse pressure; RPP, rate pressure product; BPV, blood pressure variability.

Following the control period, there were small significant reductions in 24-hour HR (p=0.031), but no other significant changes were measured following the control condition. However, night-time RPP (p=0.003) was significantly different between the pre control and pre IET conditions.

Bean plots (Figure 6.2) provide a visual comparison of univariate data between conditions for 24-hour BP. Each small line represents one observation of that batch and a per batch mean is provided to allow easy comparison, while density traces summarise the distribution of the batches, giving an overall representation of responders to the intervention.

Figure 6.3 shows the group mean sBP, mBP and dBP of the pre and post IET ABP at each hour of the ambulatory measurement periods. Mean sBP, mBP and dBP are lower at each time point post IET. This depicts diurnal variation and visually demonstrates the mean dipping pattern in BP.

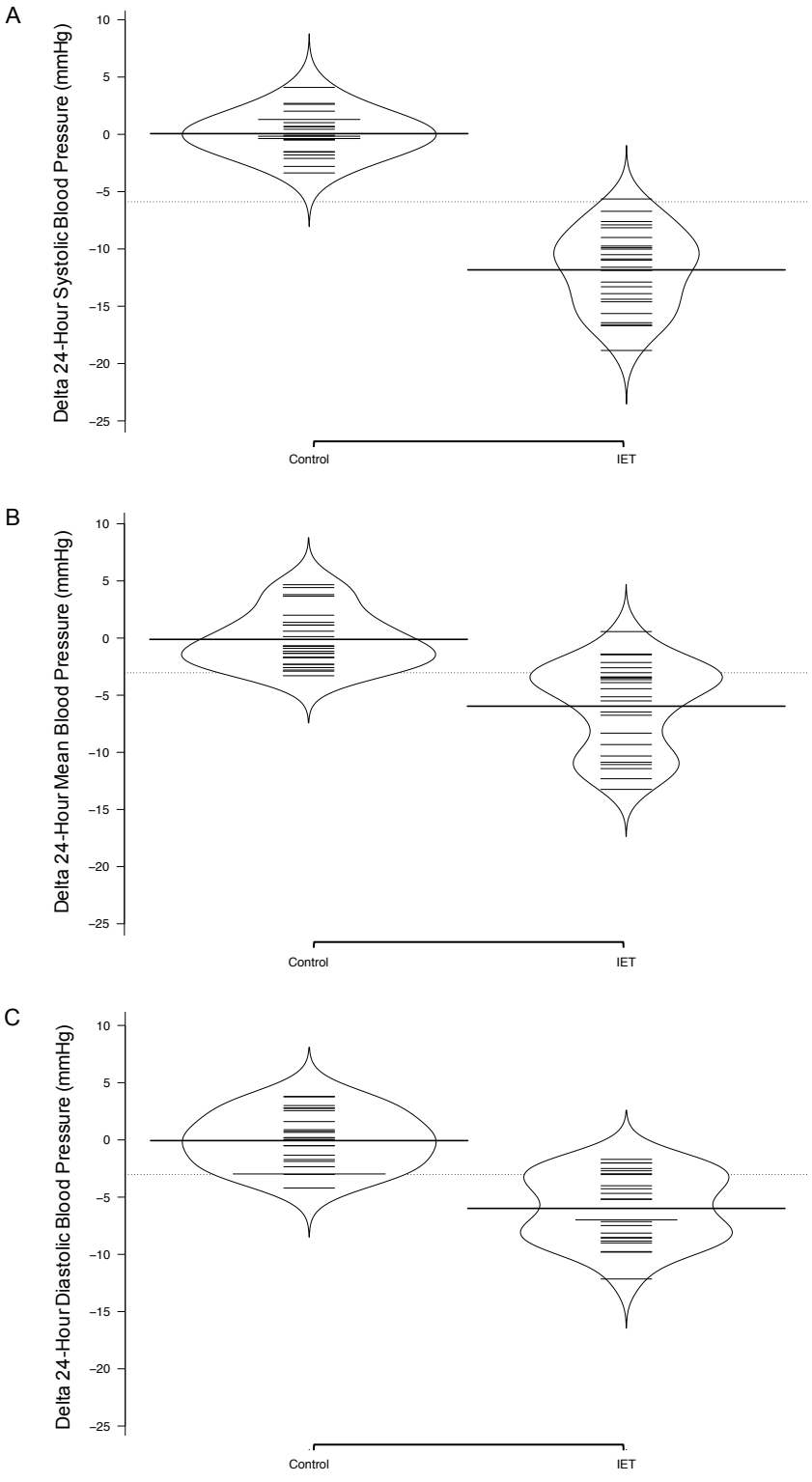

Figure 6.2. Bean plots of ambulatory BP change following control and IET. Note: A, delta of systolic BP; B, delta of diastolic BP; C, delta of mean BP.

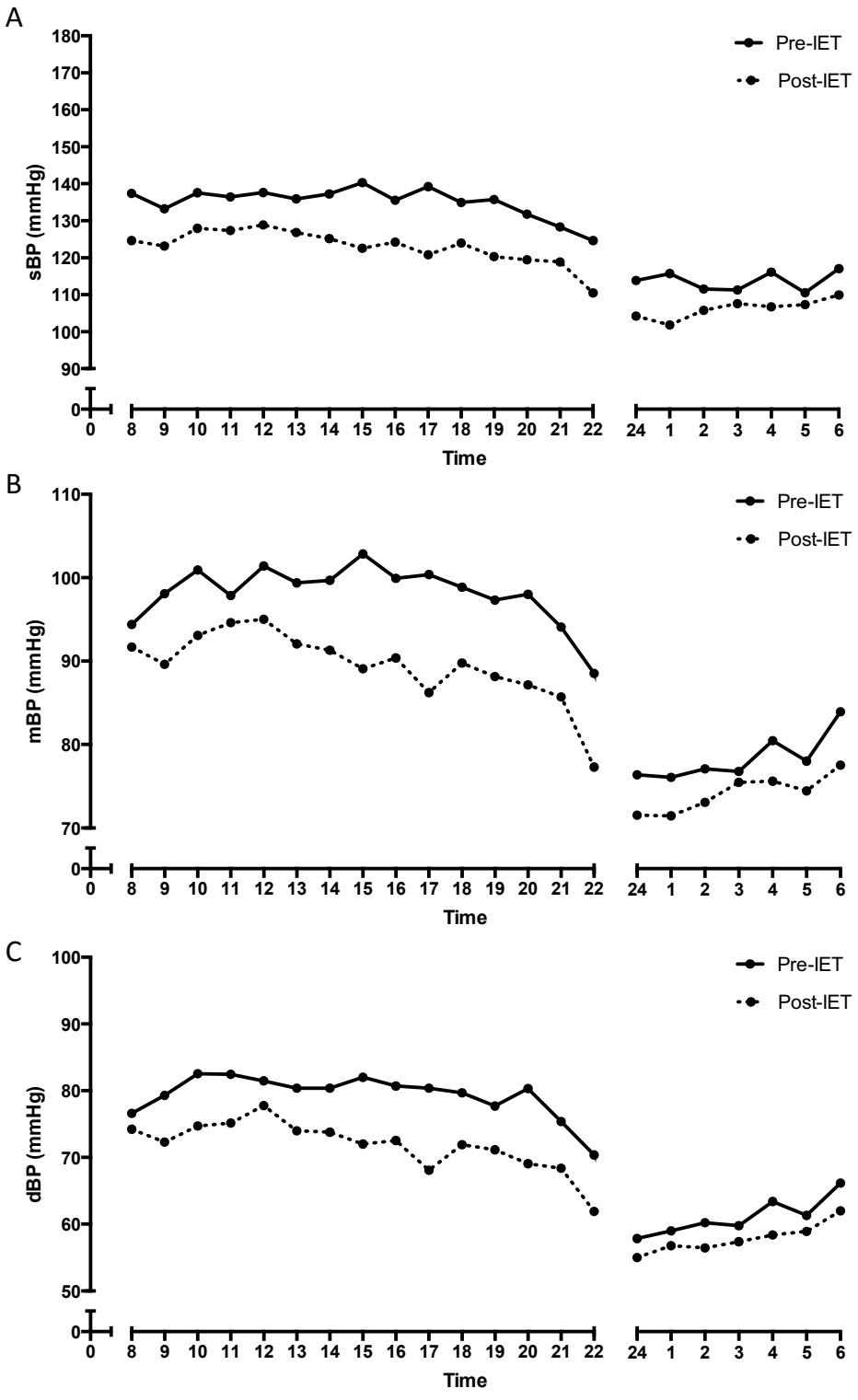

Figure 6.3. Mean BP at each hour throughout the ambulatory period of measurement pre and post IET. Note: A, systolic BP; B, mean BP; C, diastolic BP

6.4 Discussion

This study demonstrates that a 4-week home-based isometric wall squat training intervention significantly reduced resting and ambulatory BP. The results confirmed that following IET there were significant reductions in ambulatory sBP (-11.83 mmHg) dBP (-5.57 mmHg) and mBP (-5.67 mmHg). Given that BP reductions of 5 mmHg are considered the minimal clinically important difference for therapeutic interventions (NICE, 2011), the reductions measured in this study may be associated with reduced risk of future HTN, stroke and CVD (Lewington *et al.*, 2002; Whelton *et al.*, 2002; Cook *et al.*, 1995). Furthermore, 24-hour ambulatory sBP at baseline was 131 ± 5 mmHg and this was reduced to 119 ± 6 mmHg. When each case was individually considered, half of the participants (n=12) could be reclassified as having optimal BP (sBP >120 mmHg, dBP >80 mmHg) following IET, a change associated with additional significant risk reduction (Vasan *et al.*, 2001b). There were no significant changes in BP during the control period. Furthermore, the BP reductions from this study are of a similar magnitude to BP reductions reported following aerobic training interventions (Borjesson *et al.*, 2016), a training type which is already recommended to reduce and maintain BP (Pescatello *et al.*, 2004a).

There were also significant reductions in day-time ABP (sBP, dBP and mBP) and night-time ABP (sBP, dBP and mBP), both of which carry prognostic information for CVD, independently from resting BP (Fagard *et al.*, 2008). Night-time ABP is considered a superior predictor of outcome for all cause (Dolan *et al.*, 2005) and CVD mortality (Fagard *et al.*, 2008) and a greater BP decline overnight, known as dipping, is thought to result in improved prognosis (Verdecchia *et al.*, 1994). Ambulatory measurement of nocturnal BP that is 10-20% lower than day-time BP represents a normal dipping pattern (O'Brien *et al.*, 1988). Individuals who experience a smaller dip in BP overnight have an increased risk of all-cause mortality and CVD events (Fagard *et al.*, 2009). Indeed, it has been shown that a 5% reduction in dipping of nocturnal BP is associated with a ~20% increased risk of CV mortality, even in normotensive individuals (Ohkubo *et al.*, 2002). At baseline in this study, 22 participants were classified as dippers, and just two were non-dippers (4% nocturnal BP decrease). Following IET, overall mean dipping percentage did not significantly change, however when cases were examined individually, both non-dipping participants at baseline had

a mean night time BP dip of 16% following IET, which may be associated with reduced risk of adverse cardiovascular outcome (Verdecchia *et al.*, 1994).

Variability of BP (BPV) also has important prognostic value (Dolan and O'Brien, 2010) and lower BPV is associated with a reduced risk of cardiovascular events, particularly stoke risk (Rothwell, 2010). Short term fluctuations in BP may have prognostic relevance in predicting organ damage (Parati *et al.*, 1987) and cardiovascular events (Mancia *et al.*, 2001) to a greater extent than resting or ambulatory BP. Baroreflex control is responsible for minimising acute variations in BP, therefore large BPV may be indicative of poor BRS and impaired short term BP regulation in response to diurnal variations in activity (Conway *et al.*, 1984; Mancia *et al.*, 1986). A reduction in average BP is the target outcome of most anti-hypertensive interventions for preventing CV morbidity and mortality (Schillaci *et al.*, 2011); however it has been suggested that the most effective interventions are those that lower BPV in addition to BP (Dolan and O'Brien, 2010). Variability of BP has been shown to increase with age and BP levels (Mancia *et al.*, 1983), suggesting that the pre-hypertensive cohort in this study may display a greater BPV compared to normotensive participants. Reductions in 24-hour BPV following 4-weeks of IET may therefore be linked to improved short-term BP regulation and BRS.

Pulse pressure (PP) represents the force that the heart generates as it contracts and is calculated as the difference between sBP and dBP. High PP is a marker of increased arterial stiffness (Franklin *et al.*, 1997b), and is associated with carotid atherosclerosis (Franklin *et al.*, 1997a), increased LV mass (Pannier *et al.*, 1989) and is an additional predictor of future cardiac events (Millar *et al.*, 1999; Verdecchia *et al.*, 1998) independently of other traditional risk factors (Madhaven *et al.*, 1994; Verdecchia *et al.*, 1998). Therefore the significant reductions in PP reported in this study are of clinical importance and are strongly related to the significant changes in sBP and dBP. Lowered PP demonstrates an improvement in the capacity of large conduit arteries to minimise pulsatility, which may indicate an improved cushioning capacity of the arteries, a reduction in arterial stiffness and/or improved arterial compliance and distensibility (Safar *et al.*, 2003).

Resting BP reductions have been repeatedly demonstrated following programmes of IHG (Millar *et al.*, 2013; McGowan *et al.*, 2007), and ILT (Baross *et al.*, 2013; Devereux *et al.*, 2010), as displayed in Table 2.2 (page 28) and meta-analytical research has revealed mean resting sBP reductions of 5.2 mmHg and dBP reductions of 3.91 mmHg (Inder *et al.*, 2016). Limited research exists into the effects of IET on ABP; Stiller-Moldovan *et al.* (2012) reported non-significant reductions of ~2 mmHg in ambulatory sBP and dBP in medicated hypertensive participants after an 8-week IHG programme. However, the mean BP of the intervention participants (n=11) was well controlled to 113.9 ± 12.7 mmHg (sBP) and 60.7 ± 11.6 mmHg (dBP) prior to the intervention, values considered optimal (Mancia *et al.*, 2013). It is conceivable that BP had plateaued at this level and patients were resistant to experiencing any further BP lowering. By the same measure, Somani *et al.* (2017) measured an ambulatory sBP reduction of 4 mmHg and no change in ambulatory dBP in young normotensive participants. Although BP was lowered, the low baseline BPs may have limited further BP lowering during 8-weeks of IHG training. The study can also be criticised for the lack of a control condition, which limits the ability to attribute BP reductions to the training stimulus directly.

The reductions in both resting and ambulatory BP measured in the current study are greater than those previously reported. Fundamental methodological differences in IET protocols, such as muscle group used in training and participant baseline BP may account for this. It is likely that the physically inactive, pre-hypertensive cohort included in this study were more responsive to the physiological stimulus of IET, resulting in greater BP reductions. Indeed, a meta-analysis by Millar *et al.* (2007) demonstrated that more pronounced BP reductions occurred from IET in participants with higher baseline BP. Further to this, MacDonald (2002) report that the greatest acute hypotensive response, following varying exercise types, occurs in pre-hypertensive participants, which may be predictive of a chronic adaptations. This is noteworthy, as the acute response to exercise may be predictive of chronic response; Liu *et al.* (2012) observed a relationship between the magnitude of acute and chronic haemodynamic responses to aerobic exercise in pre-HTN. The acute hypotensive response and adjustment in autonomic regulation in recovery from IE reported in Chapter 4 may therefore also be indicative of the chronic responses measured in this

Chapter. Similarly, in relation to IE, Badrov *et al.* (2013b) found that reactivity to a cardiovascular stress task was predictive of chronic BP responses to a 10-week IHG intervention. The ability to improve short term BP reactivity may be associated with reductions in ABP.

The most commonly used IET interventions have involved use of a handgrip protocol (Millar *et al.*, 2008) and previous leg training interventions have used leg extension exercise on an isokinetic dynamometer (Wiles *et al.*, 2010). Activation of a larger muscle mass is accompanied by greater central and peripheral drive in order to induce a greater cardiovascular response (Galvez *et al.*, 2000; Mitchell *et al.*, 1980), and it has been suggested that a greater muscle mass may induce a greater (pressor) response during an acute isometric contraction of a fixed 2-minute duration (Hietanen, 1984; Seals, 1989). Compared to other IET protocols, this may induce greater production and accumulation of metabolites, which has been implicated in the reduction of TPR, which is suggested as a possible mechanism of the anti-hypertensive effects of IET (Millar *et al.*, 2013; Carlson *et al.*, 2014). The reduction in global TPR following IET demonstrates a vascular response to training. Previous research has found improvements in vascular structure and function following IET (Baross *et al.*, 2012; McGowan *et al.*, 2006b; Badrov *et al.*, 2016); however, improvements have been limited to the training limb only. When isometric arm flexion and isometric leg extension interventions were compared, they appeared to elicit comparable results (Howden *et al.*, 2002). However, leg extension exercise was performed at an intensity of 20% MVC while arm flexion was performed at 30% MVC, supporting the concept that engagement of larger muscle masses may have induced greater cardiovascular and haemodynamic responses.

Wiles *et al.* (2017) elicited BP reductions (sBP -4 ± 5 mmHg, dBP -3 ± 3 mmHg, mBP – 3 ± 3 mmHg) in normotensive participants, following 8-weeks of isometric wall squat training. Once again the extent to which BP could be lowered in a normotensive population was likely limited, but the authors suggested that the wall squat contraction style may induce a different response to other contraction styles in the same muscle group. It was proposed that leg extensions isolate the quadriceps (Delavier, 2010) and result in a relatively small lower limb mass engagement during contraction. In

comparison, the constant position maintained during performance of a wall squat, requires the isometric engagement of additional muscle groups (Contreras, 2013).

The mechanisms that regulate reductions in resting BP are not well understood (Millar *et al.*, 2013). However, given that mBP is a product of TPR and $\dot{Q}$ (which is determined by HR and SV), any reductions in BP are likely to be mediated by one, or a combination of these haemodynamic variables (Pescatello *et al.*, 2004a). Changes in TPR following IET are thought to be the result of improved vascular function (Millar *et al.*, 2014), yet it is unclear if this is due to structural vessel remodelling or functional changes in vasodilatory capacity. Following IET, increases in NO-dependant vasodilation have been indirectly measured in the training limb (McGowan *et al.*, 2006b; McGowan *et al.*, 2007) and improvements in peak reactive hyperaemic blood flow have also been measured in the resistance vessel vasculature (McGowan *et al.*, 2007; Badrov *et al.*, 2013a) in hypertensive participants. During acute IE, release of a contraction is associated with a period of reactive hyperaemia and increased blood flow, which stimulates a reduction in TPR (Chapter 4) via vasodilatory pathways. It is therefore possible that repeated stimulation of this acute physiological reaction is responsible for chronic improvements in the vasculature. Indeed, the reduction in TPR measured in this Chapter supports previous findings of improved vascular function following IET (McGowan *et al.*, 2007; Badrov *et al.*, 2013a). The respective roles of improved oxidative stress (Peters *et al.*, 2006) and histamine H_1 and H_2 receptor activation have also been suggested to alter vascular function which may contribute to BP lowering (Luttrell and Halliwill, 2017; Romero *et al.*, 2017).

Impaired vascular function and chronic oxidative stress can lead to increases in inflammation and neurohumoral activation, affecting short and long term BP control mechanisms such as autonomic activation and the RAAS. Cardiac autonomic function, representing the beat-to-beat variations in parasympathetic and sympathetic nervous system control of HR, has been shown to improve following IET (Millar *et al.*, 2013; Taylor *et al.*, 2003). Aerobic exercise training is associated with increased vagal modulation (Cornelissen and Fagard, 2005) and recovery from IE is also associated with increased vagal modulation, as well as increased BRS, as demonstrated in Chapter 4. Cardiac autonomic regulation and neurohumoral activation, must also be explored

following isometric wall squat training, to understand the chronic effect of autonomic, peripheral, and central BP control mechanisms.

There is a dearth of research that has assessed both TPR and Q̇ simultaneously and it has been suggested that changes in BP are more likely to be caused by TPR (Millar *et al.*, 2013). However, reductions in HR have also been reported (Wiles *et al.*, 2017; Baross *et al.*, 2012) meaning that changes in Q̇ may also act as a mediating variable. This study reported a significant reduction in TPR, in addition to significant increases in SV and Q̇, with no significant change in HR. This response may have been caused by cardiac functional or structural adaptation, and it is noteworthy that RPP, an indirect measure of myocardial workload, was significantly reduced following IET, which suggests there may be an improvement in cardiac performance. Chapter 5 demonstrated acute improvements in LV remodelling and estimated LV filling pressure following a single session of IE; however, no prior research has studied the myocardial response to IET, therefore cardiac structural and functional variables must be studied in more detail in order to ascertain the potential role of cardiac performance as a mechanism for BP reductions.

6.4.1 Clinical implications

Although resting BP reductions of 2 mmHg have been deemed to be clinically relevant (Chobanian *et al.*, 2003; Pescatello *et al.*, 2004a), reductions of 5 mmHg are considered the minimal important difference (NICE, 2011). A sBP reduction of 5 mmHg equates to a 9% reduced risk of CAD mortality (Stamler *et al.*, 1989), while a 5 mmHg reduction in dBP could reduce the risk of CAD by 29% and CVA by 46% (Macmahon *et al.*, 1990). In addition, the use of ABP monitoring is considered superior to resting BP measurement (Verdecchia, 2000). The magnitude of BP reductions in this study, combined with the validity of experimental procedures used, may be associated with important clinical implications. This advocates the need for further research of this method of IET for lowering BP in wider populations beyond the present study.

Current guidelines recommend non-pharmacological lifestyle changes to reduce above optimal BP (WHO, 2010b). Of those recommendations, the reductions measured

following IET in this study are comparable to aerobic exercise training (Borjesson *et al.*, 2016) and greater than salt reduction (NICE, 2011) and weight reduction (Neter *et al.*, 2003). Although pharmacological treatments are not currently recommended to pre-hypertensive patients for BP control, average BP reductions from one BP lowering drug in stage 1 HTN are 9.1 mmHg (sBP) and 5.5mmHg (dBP) (Law *et al.*, 2009) and most patients are required to take a combination of 2 or more drugs in order to attain optimal BP (NICE, 2011). Although focussed on a different population, findings presented compare favourably to those produced through pharmacology, therefore the effect of IET in a stage 1 hypertensive population is of interest for future research.

6.5 Conclusion

This home-based intervention demonstrates the effectiveness of IET as a tool to reduce resting and ambulatory BP in pre-hypertensive individuals. This study provides justification for future research to consider isometric wall squat training as a potential alternative and/or adjunctive intervention to pharmacological therapies in stage 1 HTN. In order to investigate the mechanisms of BP reduction, future research must establish the effects of IET on cardiac autonomic modulation, neurohumoral activity, and cardiac structure and function, to assess the wider health implications of IET. The longer-term responses to such a training programme also remain un-researched.

CHAPTER 7:

Study 4

Neurohumoral Responses to a 4-week Home-Based IET Intervention in Pre-Hypertensive Males. A Randomised Cross-Over Controlled Trial

7.1 Introduction

Reductions in resting BP following a programme of IET are well established (Millar *et al.*, 2008; Wiley *et al.*, 1992a; Wiles *et al.*, 2017). Recent research has demonstrated that an isometric wall squat training programme significantly reduced resting BP in a normotensive population (Wiles *et al.*, 2017). Results presented in Chapter 6 of this thesis extended this work and confirmed the ability of the isometric wall squat protocol to reduce resting BP in a pre-hypertensive population; more importantly the IET intervention produced clinically significant reductions in ABP, which may have prognostic implications for long term BP control and improved outcome prognosis.

Research has linked reductions in BP to improved cardiovascular health (Stevens *et al.*, 2016), reduced risk of CVD morbidity and mortality and other adverse comorbidities (Lewington *et al.*, 2002). Pre-hypertension is associated with wider health implications (Qureshi *et al.*, 2005a), such as autonomic dysfunction (Carthy, 2014; Duprez, 2008) and increased inflammation (Chae *et al.*, 2001; Chrysohoou *et al.*, 2004). In addition to widely recognised reductions in BP (Pescatello *et al.*, 2004a), aerobic exercise training has been shown to improve ANS function (Pagani *et al.*, 1988) and reduce inflammation. Furthermore, lifelong adherence to aerobic exercise training has been shown to attenuate sympathetic activity and inhibit progressive and age related increases in resting BP (Stewart *et al.*, 2007b). Similarly, aerobic exercise training can reduce concentrations of inflammatory markers (Goldhammer *et al.*, 2005; Toft *et al.*, 2000; Kasapis and Thompson, 2005) and individuals who remain physically active demonstrate a lower age matched expression of such markers (Reuben *et al.*, 2003). However, the effects of IET on neurohumoral factors have not been investigated. While the mechanisms for BP reductions are not fully understood, changes in short and long term BP control mechanisms such as the ANS and cytokine responses, may be of significance.

The autonomic nervous system has been implicated in the development of HTN and progression of CVD (Julius *et al.*, 1991). Sympathetic nervous activity is responsible for stimulating heart function and constricting blood vessels, thus leading to a rise in BP. In addition excessive sympathetic activation has been demonstrated prior to a diagnosis of HTN, through measures of MSNA (Grassi and Esler, 1999) and HRV

(Singh *et al.*, 1998). Hypertension (Millar *et al.*, 2013) and pre-HTN (Carthy, 2014) are associated with reduced cardiac autonomic modulation, demonstrated through HRV, by proportionately elevated LF (a marker of sympathetic activation) and blunted HF (a marker of parasympathetic activation). Similarly, raised BP is related to elevated levels of circulating inflammatory markers (Schillaci *et al.*, 2003) and although lower than in HTN, concentrations of inflammatory markers in pre-HTN have been demonstrated to be greater than in a normotensive populations (Kim *et al.*, 2008; Kasapis and Thompson, 2005; Petersen and Pedersen, 2005).

Raised BP increases sheer stress on endothelial cells (Chappell *et al.*, 1998), which causes an increase in neurohumoral activation and the endothelial expression of inflammatory markers known as cytokines (Chae *et al.*, 2001; Kim *et al.*, 2008). This causes vessels to become inflamed and increases the vessels susceptibility to plaques, platelet activation and hypercoagulability (Cook-Mills and Deem, 2005), through an increase in adhesion molecules. All of these factors contribute to accelerating the atherosclerotic process, augmenting the risk of CV events (Ross, 1999). Autonomic dysfunction has been directly related to an imbalance in bioactivity of endothelial factors (Amiya *et al.*, 2014) and catecholamine circulation is associated with increases in cytokine expression (Kan *et al.*, 1999). This highlights the relationship between autonomic and neurohumoral factors, suggesting that improvements in either factor could be reciprocal. A large-scale study by Chrysohoou *et al.* (2004) suggested that pre-HTN may be a pro-inflammatory condition, as there was an association between pre-HTN and inflammatory markers linked to the atherosclerotic process, independently of additional risk factors. Indeed, pre-HTN is associated with subclinical atherosclerosis (Washio *et al.*, 2004) and increased intima-media thickness of the carotid and brachial arteries (Toikka *et al.*, 2000). Therefore the inflammatory response may be associated with the cascade from optimal BP to clinically elevated BP and wider CVD implications.

The baroreflex is responsible for short-term BP control. Acute increases or decreases in BP detected by baroreceptors trigger a response to attenuate BP change. Baroreceptor sensitivity quantifies the efficiency of this mechanism, which is inversely related to BP, and positively related to HRV (Hesse et al, 2007). The baroreflex is vagally mediated therefore increases in parasympathetic modulation may lead to

improvements in BRS and assist in longer term BP control, yet the effects of IET on this pathway are currently unknown.

The acute parasympathetic and BRS response in recovery following IE reported in Chapter 4, support the idea that adaptations in cardiac autonomic modulation and BRS may play an important mechanistic role affecting the reductions in BP commonly observed following IET. Prior research also supports this concept (Millar *et al.*, 2009a; Iellamo *et al.*, 1999b) and repeated stimulation of this response may be linked to chronic adaptations. Previous IET studies using IHG training have demonstrated improvements in cardiac autonomic function and increased vagal stimulation, alongside clinically important reductions in BP (Millar *et al.*, 2013; Taylor *et al.*, 2003). However, the effects of an isometric wall squat training programme, using a larger muscle mass, on cardiac autonomic regulation have not been studied. In addition, no prior research has assessed the effects of an IET intervention on biomarkers of inflammation and vascular function.

The aims of this study were to establish the effects of a 4-week isometric wall squat training programme on resting cardiac autonomic modulation, and biomarkers of inflammation and vascular function in pre-hypertensive males. Improvements in cardiac autonomic function and inflammation may also be indicative of wider cardiovascular health benefits of IET.

7.2 Methods

This randomised cross-over trial included 24 males, aged 30-65. Recruitment, eligibility and participant demographic is provided in Chapter 6 (6.2.1, page 125). All participants randomly completed both a 4-week isometric wall squat training programme and a 4-week non-exercise control period, also described in Chapter 6 (6.2.7-6.2.8, page 129).

7.2.1 Procedures

Resting autonomic function was measured as per the methods described in Chapter 3 (3.4, page 62) and blood samples were taken as per the protocols outlined below, pre and post IET control conditions.

All laboratory visits occurred at the same time of day and occurred following at least 4-hours of fasting and abstinence from caffeine and alcohol for at least 24-hours. Post IET measures were taken 48-hours following the final training session to account for any residual acute physiological responses to training.

7.2.2 Blood Sampling

Venous cannulation was performed by a trained experimenter in adherence with World Health Organisation Guidelines (2010d). Cannulation was performed 30 minutes before recording of cardiac autonomic data to reduce any confounding influence on autonomic function and resting BP. A 20 ml sample of venous blood was obtained before and after the isometric training intervention and control periods. Samples were transferred from the syringe into test tubes centrifuged at 15000 rpm for 15-minutes to separate the blood plasma. Plasma was carefully extracted via pipet into 2 ml storage vials, which were clearly labelled and placed in purpose designed plastic storage containers within a securely padlocked freezer at -80°C at Canterbury Christ Church University. Once all samples had been collected, plasma vials were transported via specialist courier to St George's Healthcare NHS Trust, London, where they were subsequently analysed.

7.2.3. Cardiac autonomic assessment

Resting cardiac autonomic function was recorded in a supine position for a continuous 5-minute measurement period, following 15-minutes of supine rest using the TFM, as outlined in Chapter 3 (3.4, page 62). Spurious readings were screened for and manually removed. Reported data represents the average reading during the 5-minute measurement periods.

7.2.4 Data analysis

Following a Shapiro-Wilk test for normality, a two-way repeated measured ANOVA was performed comparing the control and intervention conditions for all variables. Bonferroni post-hoc tests were conducted to compare the outcome measures between and within groups.

Blood biomarkers were positively skewed and therefore log transformed (ln) prior to analysis. The threshold for statistical significance was set at <0.05 and all data is presented as mean ± SD unless otherwise stated. All data analysis was conducted using IBM SPSS 22 software (SPSS 22 release version for Windows; SPSS Inc., Chicago IL, USA).

7.3 Results

All participants completed the isometric wall squat training programme, totalling 96-minutes of isometric wall squat contraction time, made up of 12 sessions over a 4-week period. Cardiac autonomic function data was collected from all participants, pre and post intervention and control periods as shown in Figure 7.1.

In the IET condition, there were significant increases in overall PSD (3371 ± 2001 ms^2 to 4402 ± 2216 ms^2, $p<0.001$) and HF power spectral density (873 ± 921 ms^2 to 1413 ± 1037 ms^2, $p<0.001$) and a significant reduction in LF/HF ratio (3.23 ± 3.08 to 1.36 ± 0.86, $p<0.001$), but there was no change in LF power spectral density ($p=0.185$). There was a shift in sympathovagal modulation with a significant increase in HFnu (35.56 ± 22.45% to 47.41 ± 16.15%, $p=0.003$) and parallel reduction in LFnu. There was also a significant increase in BRS (17.47 ± 7.13 $ms \cdot mmHg^{-1}$ to 25.76 ± 9.68 $ms \cdot mmHg^{-1}$, $p<0.001$).

There were no differences in the pre-control or pre-IET values for any autonomic variables. There was no change in VLF following the intervention (1194.47 ± 739 ms^2 to 1360.22 ± 778 ms^2, $p=0.138$) or control (1143.34 ± 546 ms^2 to 1175.03 ± 509 ms^2, $p=0.307$) periods. No significant changes were measured in any variable following the

control period (LF, p=0.715; HF, p=0.506; VLF, p=0.307; LFnu and HFnu, p=0.802; LF:HF ratio, p=0.785; PSD, p=0.142; BRS, p=0.938).

Blood samples for all 4 time-points were collected in 21 of the 24 participants; 2 participants requested not to take part in this element of the study, while a sample could not be obtained from one participant at one time-point, thus excluding the comparative data for this participant. Inflammatory and vascular biomarker responses are shown in Figure 7.2.

Following IET there was a significant reduction in IL-6 (p=0.025) and ADMA (p=0.048), yet there were no changes in hs-CRP (p=0.749) tumor necrosis factor alpha (TNF-α) (p=0.417), ICAM (p=0.899) and VCAM (p=0.129). There was no significant difference in the control condition in any of the inflammatory markers (IL-6, p=0.328; TNFa, p=0.2; hs-CRP, p=0.138; ICAM, p=0.386; VCAM, p=0.978; ADMA, p=0.271) and no differences in pre-control and pre-IET values.

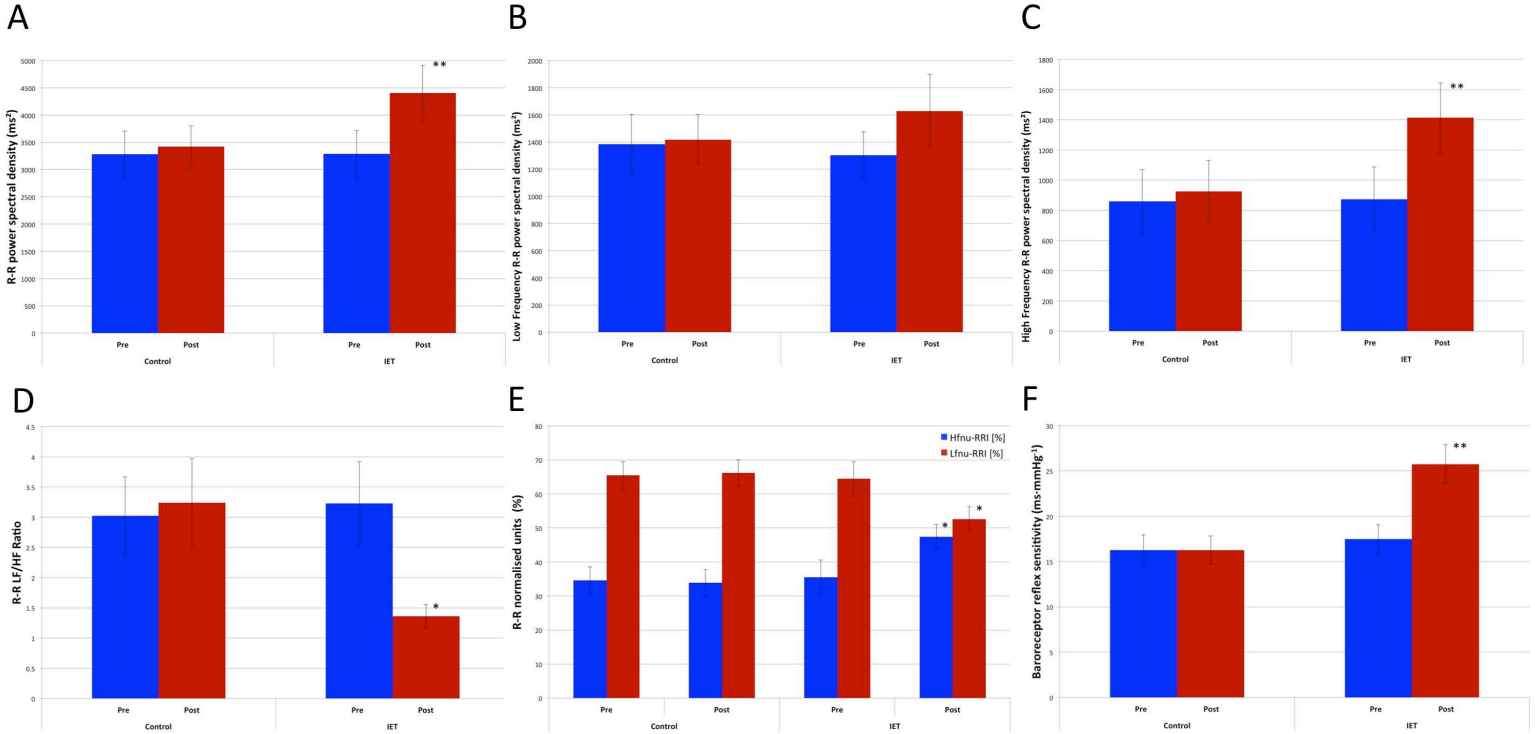

Figure 7.1: Cardiac autonomic responses to a 4-week IET intervention. A, power spectral density; B, low frequency power spectral density; C, high frequency power spectral density; D, LF/HF ratio; E, LFnu and HFnu; F, baroreceptor reflex sensitivity. $p<0.05$ *, $p<0.001$ **.

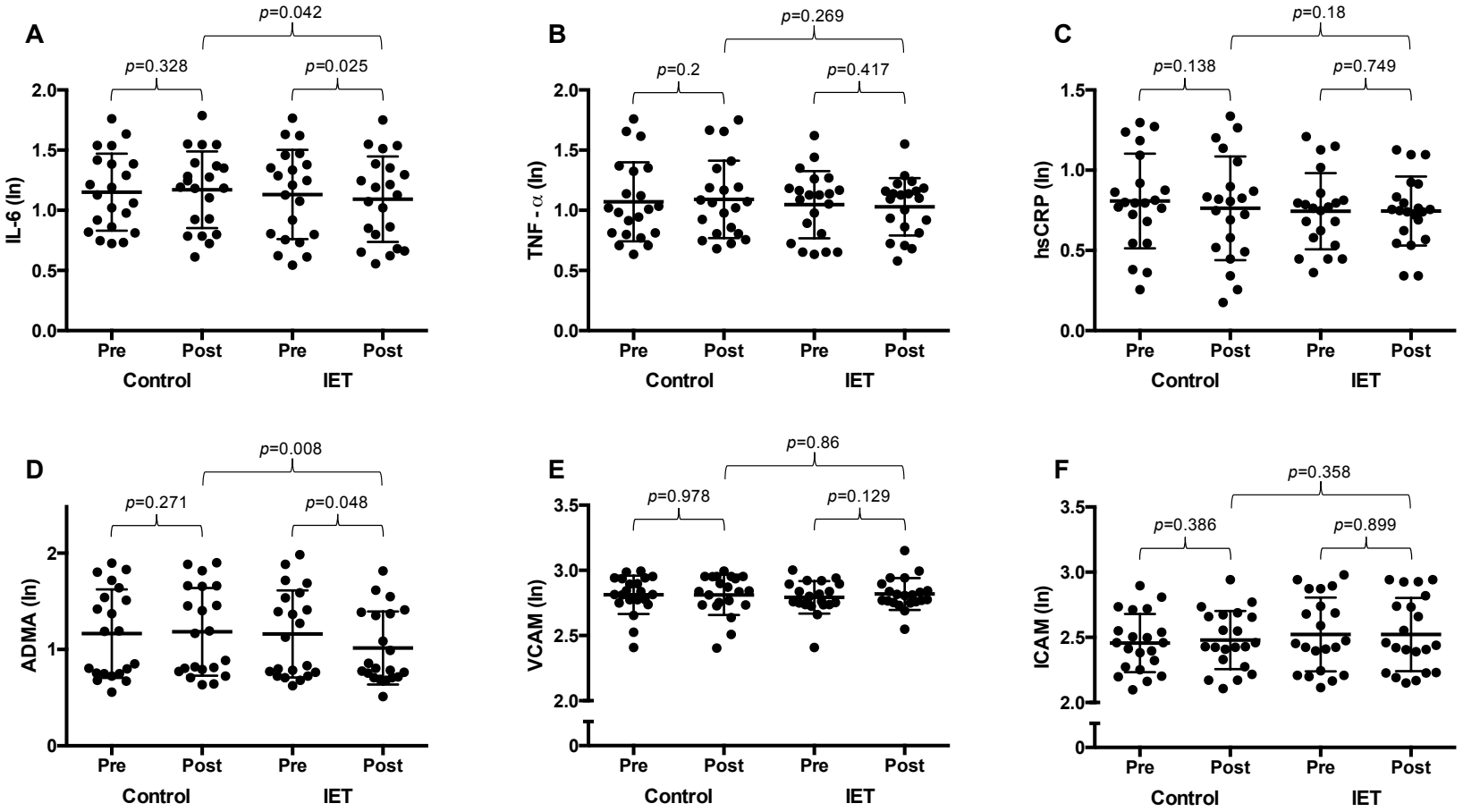

Figure 7.2. Inflammatory and vascular biomarkers pre and post control and IET period. P values within and between conditions.

7.4 Discussion

This is the first study to measure the cardiac autonomic responses to a programme of isometric wall squat training and the first study to measure biomarkers of inflammation and vascular function following any IET intervention. The results of this study demonstrate an increase in parasympathetic control of the ANS following IET. In addition, significant reductions were measured in IL-6 and ADMA, which are important markers of inflammation and vascular function, respectively. These findings may indicate wider cardiovascular health benefits associated with IET, in addition to significant BP reductions reported in Chapter 6.

7.4.1 Effects of IET on autonomic function

This study demonstrated that a 4-week isometric wall squat training programme significantly altered cardiac autonomic modulation, evidenced by an increase in HRV. There was a significant increase in the HF power component of HRV which resulted in a residual increase in HFnu and a reduction in the LF:HF ratio. This demonstrates an increase in parasympathetic modulation and proportional withdrawal in sympathetic activity, ultimately changing the sympathovagal balance (Malik *et al.*, 1996). This finding is supported by Taylor *et al.* (2003); who, following a 10-week IHG training protocol, reported a significant increase in the HF component of HRV. Differences in the group studied, which included elderly medicated hypertensive patients (n=13), and an IHG training programme, may account for the greater magnitude of findings in the present study.

Improvements in autonomic function have also been noted by other means of assessment. Following a 4-week rhythmic handgrip training intervention, Sinoway *et al.* (1996) measured an increase in MSNA and a reduction in arterial noradrenaline at rest in healthy young males. This may have been due to a reduction in the sympatho-excitatory effects of central command and may suggest lowered sympathetic activity at rest. When the acute response to the rhythmic isometric handgrip stimulus was measured post-training, adrenaline and noradrenaline spill-over was lower than pre-training values. In addition, a steady state was reached earlier in the session and MSNA response during training was attenuated, which was suggested to reflect an

overall reduction in sympathetic outflow. Following training, the production of metabolic bi-products (lactate) was reduced, as was mechanoreceptor activation and sympathetic nervous system activation. However, given that the tension generated during training was unchanged, it was suggested that the recurrent bouts of exercise during the 4-week period led to mechanoreceptor desensitisation (Sinoway *et al.*, 1996).

Millar *et al.* (2013) reported increases in non-linear measures of HRV, yet there was no significant changes in time domain or spectral analysis of HRV parameters following 8-weeks of IHG training in 9 elderly, medicated hypertensive patients. Despite a lack of change in spectral HRV, it was suggested that the findings represent an increase in the complexity of HR, which may represent sympathetic withdrawal or improved cardiac vagal modulation. Methodological differences in IET protocols may account for the lack of change in HRV as Millar *et al.* (2009a) permitted a greater recovery period (4-minutes) between IE bouts, which may have limited the accumulated exercise response in the training limb. It was established in Chapter 4 that the 2-minute recovery period was inadequate to restore resting haemodynamic or autonomic state between sequential bouts. Therefore, the IE stimulus used by (Millar *et al.*, 2009a) may have resulted in a lower absolute workload and reduced physiological response with each bout, compared with the present study. Furthermore, given that sympathetic activation and BP tend to demonstrate a gradual increase with age (Ng *et al.*, 1993), it is possible that the ability to reduce these in an elderly population is limited. In addition, the cohort studied by Millar et al, (2013) were medicated for hypertension. Many anti-hypertensive medications are aimed at reducing sympathetic activity (Del Colle *et al.*, 2007). Therefore, it is possible that an IET programme in a medicated population may not produce the pronounced alterations in autonomic modulation seen in non-medicated populations, due to the pharmacology. Anti-hypertensive medication may make the ANS less receptive to further improvement.'

A number of previous studies have reported no changes in autonomic function following IET through measures of HRV (Wiles *et al.*, 2010; Badrov *et al.*, 2013b) and MSNA (Ray and Carrasco, 2000). This could be explained by the participant demographic, as all studies included young, physically active and normotensive participants who are unlikely to have displayed any signs of autonomic dysfunction,

potentially limiting the extent to which improvement was possible. However, although no changes in autonomic function were reported, all three studies reported significant reductions in resting BP, suggesting that an alternative mechanistic pathway may be responsible for attenuated BP in such populations.

Pre-hypertension is associated with impaired cardiac autonomic function (Duprez, 2008), which is displayed as a greater LF/HF ratio compared to normotensive populations, and greater LF power than in both NTN and HTN (Wu *et al.*, 2008). Autonomic dysregulation is associated with an overall increased risk of mortality (Dekker *et al.*, 1997; Tsuji *et al.*, 1994), which is exacerbated when coupled with raised BP and existing CVD (Gerritsen *et al.*, 2001). Low HRV is associated with an increased risk of mortality and a 1SD decrement in LF power has been associated with a 1.7 times greater risk of all-cause mortality (hazard ratio 1.7; 95% CI 1.37-2.09) (Tsuji *et al.*, 1994). The increase in HRV, HF and shift in sympathovagal balance measured in this study indicates an improvement in overall autonomic regulation, which may demonstrate significant reduction in CVD risk.

The ANS has an important impact on vascular function as the parasympathetic and sympathetic systems innervate vascular walls, regulating contraction and wall tension in response to efferent reflexes and central command (Toda and Okamura, 2003). As such, an association between impaired endothelial function and autonomic dysregulation measured via HRV has been suggested (Amiya *et al.*, 2014). Sympathetic stimulation impairs flow-mediated dilatation of the vascular endothelium (Hijmering *et al.*, 2002), subsequently stimulating the expression of endothelial cytokines, which contribute to the atherosclerotic process (Steffens and Mach, 2004). Furthermore, it has been demonstrated that high concentrations of plasma noradrenaline were associated with impaired endothelial function (Kaplon *et al.*, 2011). An increase in HF power, indicting an increase in parasympathetic stimulation, as measured in the current study, may be associated with an improved vasodilatory capacity and improved endothelial function (Borovikova *et al.*, 2000). This is supported by the reduction in TPR reported in Chapter 6, which may indicate improvements in vascular tone. This may have been caused by, or resulted in changes in biochemical activity, as concurrent reductions in concentrations of the inflammatory marker IL-6 and marker of vascular function ADMA were also reported.

7.4.2. Effect of IET on inflammatory and vascular biomarkers

This is the first study to assess the effect of IET on biomarkers of inflammation and vascular function, despite a number of studies reporting positive changes in autonomic function and BP. The 4-week home based IET programme, resulted in significant reductions in IL-6 and ADMA, however there were no significant changes in TNF-α, hs-CRP, ICAM or VCAM. Inflammation is a causal factor in the development of endothelial dysfunction (Yudkin *et al.*, 1999) and pre-HTN has been directly associated with elevated levels of CRP (King *et al.*, 2004; Chrysohoou *et al.*, 2004; Santos *et al.*, 2016), TNF-α (Chrysohoou *et al.*, 2004), ICAM and IL-6 (Chae *et al.*, 2001) compared with NTN. Although changes were not observed in all markers, it is possible that any reduction in inflammation has a positive role in reducing CVD risk, as inflammation plays a central role in all phases of the atherosclerotic process (Libby, 2002; Chrysohoou *et al.*, 2004; Petersen and Pedersen, 2005).

Acute exercise is associated with an increase in the production of IL-6 (Petersen and Pedersen, 2005). As a myokine, it is produced by contracting muscle fibres, which also stimulates the production of additional anti-inflammatory cytokines IL-10 and IL-a (Pedersen *et al.*, 2003). During exercise, IL-6 is thought to inhibit the production of other pro-inflammatory cytokines such as TNF-α (Petersen and Pedersen, 2005). It has been suggested that repeated stimulation of transient changes in IL-6 during acute exercise bouts may act to down regulate the production of CRP protein in the liver (Stewart *et al.*, 2007b). However, given that no changes in hs-CRP or TNF-α were observed, the reduction in IL-6 may have been the result of a training effect of IET over the 4-week period.

As a pro-inflammatory cytokine, IL-6 has been shown to stimulate CRP synthesis (Bautistaa *et al.*, 2001; Goldhammer *et al.*, 2005; Yu and Rifai, 2000), therefore it is conceivable to expect that changes in the markers would occur simultaneously. However, it is possible that the reductions in IL-6 reported in this study were inadequate to induce a change in CRP, or that the study duration was too short for this pathway to be affected by any change in IL-6. Previous research has reported reductions in CRP without concurrent reductions in IL-6 or TNF-α following both aerobic and resistance training protocols (Stewart *et al.*, 2007b). Other research (Kohut

et al., 2006) reported reductions in both CRP and IL-6 following an aerobic training programme over 10 months and no change in inflammation following a time-matched comparative resistance and flexibility training condition. However, the intensity in the resistance training protocol was not matched to the aerobic intensity that was clearly defined by the authors. Pedersen *et al.* (2003) express the importance of exercise intensity, duration and recruited muscle mass in relation to exercise induced IL-6 production, which may support an advantageous role of isometric leg training as a large muscle mass is involved. Goldhammer *et al.* (2005) suggest that further research is required to ascertain whether exercise may stimulate the synthesis of further, unidentified factors that act to attenuate IL-6 or CRP synthesis independently of one another. This may explain the lack of concurrent reductions in these markers following IET.

C-Reactive protein, is considered one of the most important markers of inflammation in predicting CVD and outcome risk (Ridker, 2003; Ridker *et al.*, 2000), and is an independent risk factor for HTN (Bautistaa *et al.*, 2001). Although studies have shown that resistance training (Castaneda *et al.*, 2004; White *et al.*, 2006) and aerobic training (Goldhammer *et al.*, 2005; Gielen *et al.*, 2003) are effective interventions for reducing CRP in diseased populations, other findings have suggested that CRP is not affected by exercise training (Kelley and Kelley, 2006), supporting the lack of change in this pre-hypertensive population. In addition, it must be noted that the pre-hypertensive population studied was otherwise healthy and CRP levels were normal at baseline and may therefore be resistant to change. It has also been demonstrated that both young and old participants who are physically active have lower concentrations of CRP, and that CRP is reduced following a 12-week combined aerobic and resistance exercise training intervention in previously physically inactive young and old participants (Stewart *et al,* 2007b). Given these measured changes, it is possible that the current 4-week IET intervention was of inadequate duration to induce changes in CRP concentrations.

Interleukin-6 and CRP result in the down regulation of NO production by inhibiting eNOS (Libby, 2002). This is detrimental to vasodilation, and chronic over expression of IL-6 and CRP causes endothelial dysfunction (Fichtlscherer *et al.*, 2000). Furthermore, ADMA also acts as a NO synthase inhibitor. Asymmetric

dymethlyarginine interferes with L-arginine in the production of NO, thus inhibiting normal endothelial function. The resultant impeded vasodilation is positively associated with raised BP (Riccioni *et al.*, 2015). Reductions in biomarkers IL-6 and ADMA through IE may have increased NO bioavailability and therefore vasodilatory capacity.

This concept is supported by the measured improvement in TPR in Chapter 6, as well as an increase in parasympathetic activation, which is also associated with vasodilatory capacity (Amiya *et al.*, 2014). This mechanistic pathway, which is associated with an increase in the bioavailability of NO, has been proposed as a possible mediator of the IET effect on BP (Lawrence *et al.*, 2014). Prior research supports the influence of improved endothelial function following IET, measured indirectly via flow-mediated dilatation (McGowan *et al.*, 2007; Badrov *et al.*, 2013b), however this is the first study to provide biochemical evidence to suggest such a pathway. The reductions in ADMA in the current study are supported by similar findings following aerobic exercise training interventions (Riccioni *et al.*, 2015; Mittermayer *et al.*, 2005).

The well-defined acute phase response of cytokine activity suggests an agonistic relationship between many markers of inflammation. During exercise, there is a dramatic increases in the release of IL-6 from an exercising limb (Pedersen and Hoffman-Goetz, 2000) inhibiting the production of pro-inflammatory cytokines, such as TNF-α, which may impede atherosclerosis development (Pedersen *et al.*, 2003) and stimulating the appearance of anti-inflammatory cytokines such as IL-10 and IL- α. However, IL-6 remains a marker of inflammation, and elevated concentrations in a state of rest are associated with a pro-inflammatory response and the development of CVD (Petersen and Pedersen, 2005). Repeated stimulation of the anti-inflammatory environment induced by IL-6 during acute exercise may be responsible for a chronic reduction in inflammation (IL-6) following IET.

Cytokines IL-6 and TNF-α, released by the endothelium, stimulate the release of adhesion molecules ICAM and VCAM. A graded relationship between BP and the inflammatory markers ICAM and IL-6 has been described, in healthy males (Chae *et al.*, 2001), as well as a relationship between the expression of vascular adhesion molecules, other CVD risk factors (Demerath *et al.*, 2001) and raised sympathetic

activation (Mousa *et al.*, 2010). The presence of these adhesion molecules contributes to subendothelial inflammation, adherence of mononuclear leukocytes to the vascular surface and subsequent atherosclerosis (Ross, 1999). Despite a reduction in sympathetic activation, there was no significant change in ICAM or VCAM following IET, however the lack of change in TNF-α may provide an explanation for this. Hjelstuen *et al.* (2006) measured an inverse association between the volume of participation in physical activity and TNF-α concentrations in medicated hypertensive males, however these findings account mainly for aerobic and lifestyle physical activities which are not specifically defined or controlled, and apply to a period of life beyond the 4-weeks duration of the current intervention. A longer IET intervention period may reveal a positive change in ICAM and/or VCAM. A previous intervention in patients with chronic heart failure resulted in reductions in both ICAM and VCAM following a 12-week cycle ergometer aerobic exercise training intervention, however it must be noted that the duration of this programme exceeds the current 4-week IET intervention, and the participants displayed elevated markers of endothelial dysfunction at baseline (Adamopoulos *et al.*, 2001).

Release of IL-6 is commonly associated with a similar TNF- α response (Petersen et al 2005), however following 4-weeks of IET there was no change in TNF- α. Tumor necrosis factor alpha amplifies and prolongs an inflammatory response by activating the release of cytokines from other cells and is an important biomarker in the pathogenesis of inflammatory diseases (Tracey, 2002). Elevated TNF-α can depress $\dot{Q}$ (Tracey, 2002), which may contribute to a rise in BP as the body tries to maintain homeostatic blood flow. Previous research has demonstrated a reduction in the localised expression of biomarkers TNF-α and IL-6 in patients with chronic heart failure following a 6-month aerobic exercise training intervention (Gielen *et al,* 2003). This finding has also been noted following resistance exercise, which resulted in reductions in skeletal muscle concentrations of TNF-α (Griewe *et al.*, 2001), yet this was not replicated through measurement of plasma TNF-α (Bruunsgaard *et al.*, 2001). These studies were both carried out on elderly participants, who may have had higher baseline TNF-α concentrations, given that inflammation increases with age (Bruunsgaard *et al.*, 2001). However, it is possible that despite no change in plasma TNF-α, changes in muscle concentrations of TNF-α (not measured in this study), may have occurred, in unison with the increase in $\dot{Q}$ measured in Chapter 6.

Inflammation and autonomic function are closely related. The central nervous system is able to activate anti-inflammatory pathways, and neurons in the CNS are capable of synthesising TNF-α, which may in turn participate in neural communication (Tracey, 2002). Experimentally induced parasympathetic stimulation has been shown to inhibit synthesis and reduce concentrations of plasma TNF-α (Bernik *et al.*, 2002; Borovikova *et al.*, 2000). In response to increased parasympathetic activation, the cholinergic anti-inflammatory pathway supresses cytokine release by macrophage nicotinic receptors (Tracey, 2002). The increase in parasympathetic activation, and shift in the sympathovagal balance measured following IET may therefore be a mediator of the anti-inflammatory response (reduced IL-6) and improved vascular function (reduced ADMA), may in turn be linked to the regulation of chronically lowered BP.

7.4.3. Long and short term blood pressure control

The RAAS is responsible for longer term BP control and is thought to play a part in promoting inflammation and progression of atherosclerosis (Montecucco *et al.*, 2009). Activation of the RAAS is heightened in parallel with sympathetic over-activity (Tsuda, 2012). As such, reductions in sympathetic activation following exercise training and associated reductions in the release of catecholamines and inflammatory markers, may cause a reduction in the release of angiotensin and aldosterone (Gielen *et al.*, 2010). Isometric exercise training induced a significant shift in the sympathovagal balance, with an increase in parasympathetic modulation, which may have also contributed to the reported reductions in IL-6 and ADMA. A significant increase in parasympathetic activation, alongside reduced TPR and reductions in IL-6 and ADMA, may all contribute towards improved vasodilation and enhanced venous capacitance, reducing the release of renin and subsequently reducing activation of the RAAS (Bruno *et al.*, 2012). In addition, angiotensin II stimulates the production of IL-6 (Papademetriou, 2002), therefore a reduction in IL-6 may correspond with a reduction in RAAS activity. While no changes were measured in ICAM or VCAM in this study, it is noteworthy that pharmacological treatment that target the RAAS system (ACE-inhibitors) have been associated with the down-regulation of ICAM and VCAM levels

(Pastore *et al.*, 1999), highlighting the complex relationship between bio-markers and BP control mechanisms.

The reductions in systolic, diastolic and mean ABP, and 24-hour BPV in Chapter 6 may be linked to the inflammatory responses. High BP and a wide BPV may produce a greater haemodynamic stress on the vessel wall, inducing an augmented inflammatory response. Raised BPV is associated with increased neurohumoral activation and expression of inflammatory cytokines (Chae *et al.*, 2001), which is related to the progression of end organ damage (Mancia and Parati, 2003). Indeed Kim *et al.* (2008) established a significant association between wide 24-hour ambulatory BPV and raised concentrations of IL-6, which may be associated with the formation of early atherosclerosis, and impaired long term BP regulation.

Improvements in BPV may be due to improved short term BP regulation. Following the current IET intervention, participants displayed a significant improvement in BRS, which may be related to the significant improvements in BP, discussed in Chapter 6. Previous research has reported improvements in BRS following programmes of aerobic exercise training in hypertensive participants (Pagani *et al.*, 1988; Somers *et al.*, 1991) yet this is the first study to examine the effects of IET on BRS. Impaired BRS is associated with higher BP and greater BPV (Smit, 2002) and an elevated risk of CVD, which increases as BRS decreases (Lantelme *et al.*, 2002; Anand *et al.*, 2003). The increase in BRS, along with the reductions in BP and BPV reported in Chapter 6, supports this relationship (Hesse *et al.*, 2007). Improvements in BRS may represent an additional independent risk reduction in the pre-hypertensive sample studied (Anand *et al.*, 2003) as Ducher *et al.* (2006) established that lower BRS was a consistent predictor of BP increase over 5-years, which demonstrates the potential importance of BRS in the pathogenesis of HTN.

Altered BRS function with raised BP may be caused by changes in central autonomic neural pathways (Hunt *et al.*, 2001). A chronic rise in the LF component of HRV has been demonstrated to increase sympathetic outflow and decrease BRS, causing an increase in BP (Lanfranchi and Somers, 2002; Izzo, 2007; Kingwell *et al.*, 1992). The increase in HF following 4-weeks of IET may be responsible for vagally mediated improvement in BRS, improving the acute regulation of BP. Chapter 4 revealed that

following an acute session of IE, there was sympathetic withdrawal, parasympathetic activation, hypotension and a significant increase in BRS, supporting the notion that repetition of the IE stimulus may induce this chronic response.

Changes in vessel structure, altering the transduction of blood flow into vessel as well as stretch and afferent neural information, may also affect BRS (Hunt *et al.*, 2001). Vagal tone induces vasodilation, while sympathetic withdrawal inhibits vasoconstriction, therefore an increase in parasympathetic control may be associated with an improvement in arterial compliance (Lanfranchi and Somers, 2002). Arterial compliance may also be improved by changes in endothelial function and aerobic (Cameron and Dart, 1994) and isometric (McGowan *et al.*, 2006b) exercise training are associated with improved endothelial function. Improved arterial distensibility and mechanoelastic properties of vessels means that baroreceptors in the carotid sinus and aortic arch are more receptive to acute changes in BP. The improved capacity to respond to acute haemodynamic variations by baroreceptors may be a mechanism for maintaining lowered BP following a period of IET. The neurohumoral changes in the current Chapter and TPR response reported in Chapter 6, further support a possible improvement in endothelial function; an improvement that may be directly associated with reduced risk of atherosclerosis, progression of HTN and CVD, however endothelial function and blood flow were not measured in this study.

7.4.5. Clinical implications

Pre-hypertension is associated with impaired autonomic function (Carthy, 2014) increased inflammation (Chae *et al.*, 2001) and reduced vascular function, compared with NTN, all of which contribute to accelerating the atherosclerotic process and increasing the risk of future HTN and CVD (Chrysohoou *et al.*, 2004). The IET induced improvements in autonomic regulation, and reductions in inflammation and vascular biomarkers may therefore represent considerable risk reduction. Furthermore, taken alongside BP reductions reported in Chapter 6, IET as a non-pharmacological anti-hypertensive therapy may induce wider benefits to the cardiovascular system, which may improve outcome prognosis.

Currently prescribed anti-hypertensive medications act to block the activation of the RAAS, reduce inflammation and promote vasodilation to improve TPR. Therefore the ability of IET to induce improvements in these pathways may have important implications in the prescription of non-pharmacological treatments for raised BP. Furthermore, it is possible that these inflammatory and autonomic control pathways are mechanistically linked to the primary outcome variable of reduced BP.

7.5 Conclusion

A 4-week programme of isometric wall squat training in physically inactive pre-hypertensive males resulted in improved cardiac autonomic function demonstrated by increased parasympathetic activation and a shift in sypathovagal balance. Additional improvements in BRS were also measured, alongside previously reported BP reductions (Chapter 6). Changes in inflammation and vascular bio-markers were also demonstrated through reductions in plasma concentrations of IL-6 and ADMA respectively, which may also improve health outcomes, since these factors are independently associated with reduced CVD risk.

Changes in autonomic regulation, inflammation and vascular biomarkers, may be implicated in mediating improvements in BP through IET, however further research is required to ascertain the independent causal pathways for IET induced BP reductions.

CHAPTER 8:

Study 5

Cardiac Structural and Functional Responses to a 4-Week Home-Based IET Intervention in Pre-Hypertensive Males. A Randomised Cross-over Controlled Trial

8.1 Introduction

Optimal BP is associated with a reduced risk of developing CVD and all-cause mortality (Chobanian *et al.*, 2003). Each 20 mmHg increase in sBP over 115 mmHg sBP and 10 mmHg increase in dBP over 75 mmHg doubles the risk of CVD (Lewington *et al.*, 2002). Hypertension is the most commonly diagnosed condition in primary care (James *et al.*, 2014) and as such there is increased focus to reduce BP in pre-hypertensive individuals to reduce the global burden of HTN (Forouzanfar *et al.*, 2017). In pre-clinical populations, lifestyle interventions are recommended to attenuate a rise in BP, including weight management, dietary interventions and physical activity (Mancia *et al.*, 2013; Eckel *et al.*, 2013; Chobanian *et al.*, 2003). Current physical activity guidelines recommend aerobic and dynamic resistance exercise training for 150 minutes per week for all adults (Pescatello *et al.*, 2004a). These guidelines have demonstrated reductions in BP in addition to other cardiovascular risk factors including weight reduction and lowered cholesterol. IET is not currently included in these guidelines. However, at present IET is a Class IIB, level of evidence C exercise based regimen (Brook *et al.*, 2013) with a restricted recommendation, primarily due to few studies investigating the transient BP surge during an acute isometric muscle contraction. Nonetheless, the previous Chapters of this thesis (Chapters 4 and 5) demonstrate that an acute rise in BP during an IE contraction is followed by hypotension in recovery and improvements in cardiac function.

In addition, a 4-week home based programme of IET induced significant reductions in resting and ambulatory BP (Chapter 6), significantly improved cardiac autonomic modulation, and significantly reduced selected inflammatory markers and biomarkers of vascular function (Chapter 7), which is further supported by a reduction in TPR (Chapter 6). These findings support previous research which has shown that IET can result in significant reductions in resting BP (Millar *et al.*, 2013; Inder *et al.*, 2016), produce peripheral vascular adaptations (McGowan *et al.*, 2006b) and induce ANS modulation (Taylor *et al.*, 2003; Millar *et al.*, 2009a).

The acute effects of a single IE session on cardiac structure and function were investigated in Chapter 5. And the results demonstrated that a single session of IE induced acute improvements in cardiac structure and function. Prior research has

shown that acute responses to exercise were indicative of training responses to a specific exercise stimulus (Liu *et al.*, 2012). Therefore, it is possible that repetition of IE in the form of IET may induce chronic myocardial adaptations. However, to date, no study has performed a comprehensive assessment of adaptations in cardiac structure and function following a programme of IET.

A large body of research exists advocating other types of exercise training to improve cardiovascular health and reduce CVD risk in normotensive, pre-hypertensive and hypertensive populations (Whelton *et al.*, 2002). The positive effects of exercise training on BP are well documented and concurrent beneficial changes in cardiac structure and function have been reported including, enhanced diastolic function, reverse pathological hypertrophy and improved calcium uptake in the sarcoplasmic reticulum (Libonati, 2013). Furthermore, a meta-analysis of exercise training programmes in patients with heart failure reported reductions in neurohumoral and sympathetic activation with concomitant reverse LV remodelling (Chen *et al.*, 2012). These results are of particular importance, when considering the results presented in Chapter 6 and Chapter 7 and support the need to investigate any myocardial adaptations evident following IET.

Evidence suggests that a dose dependant relationship exists between the volume of habitual physical activity and the prevalence of CVD (Eckel *et al.*, 2013). Furthermore, individuals who maintain regular participation in physical activity throughout their life have been shown to experience fewer symptoms of age related cardiac structural and functional decline (Libonati, 1999). Animal and human studies have shown that short term programmes of aerobic and interval exercise training can provide protection against cardiomyocyte attrition and induce LV remodelling though increased connective tissue and lengthening of cardiomyocytes (Kemi *et al.*, 2004; Kwak *et al.*, 2006; Moore and Palmer, 1999b; Wisløff *et al.*, 2009). These adaptations improve the contractile capacity of the cardiomyocyte by improving the rate and extent of shortening in systole and relaxation in diastole, therefore improving myocardial performance.

In pre-HTN there are signs of myocardial deterioration, including increased LV remodelling and impaired diastolic function compared with optimal BP (Santos *et al.*,

2016; Drukteinis *et al.*, 2007), as well as evidence of accelerated end organ damage (Mancia *et al.*, 2009). The development of HTN is associated with adverse remodelling, including left ventricular hypertrophy, left atrial enlargement, and left ventricular systolic and diastolic dysfunction (Georgiopoulou *et al.*, 2010; Santos and Shah, 2014), factors which are independently associated with an increased risk of CVD (Levy *et al.*, 1990a).

Despite the well-known improvements in BP following IET, no previous research has assessed the effects of a programme of IET on cardiac structure and function in a pre-hypertensive population. Studies employing strength or resistance training in patients with heart failure and CAD have demonstrated no adverse effects of resistance training (Pu *et al.*, 2001; Haykowsky *et al.*, 2000). In addition, evidence suggests that resistance training may have attenuated the deterioration in LV contractile function as measured by LVEF in chronic heart failure patients (Levinger *et al.*, 2005). Delagardelle *et al.* (2002) measured improvements in LVIDd and LVEF that exceed those measured through endurance training, further advocating the role of resistance exercise training. However, research investigating the chronic cardiac adaptations in resistance-trained athletes has suggested that resistance training may induce concentric remodelling due to an increased pressure load and resultant increased LV wall thickness (Maron, 1986). Although it appears that resistance training would therefore accelerate the process of adverse LV remodelling trained athletes are likely to be regularly exposed to much greater afterloads than could be induced using IE wall squat training. Isometric exercise is known to induce an acute rise in BP during a contraction, thus increasing afterload (Mitchell and Wildenthal, 1974), and as such has not been widely employed in high cardiovascular risk populations.

Recent research using IET in a normotensive population reported no change in TPR or SV, however there were significant reductions in $\dot{Q}$ and HR, suggesting a change in cardiac function (Wiles *et al.*, 2017), while the results of Chapter 6 revealed a reduction in TPR and increases in SV and $\dot{Q}$ in a pre-hypertensive population. Therefore, the aim of the current study was to evaluate the effects of the same 4-week, home-based, isometric wall squat training intervention on cardiac structure and function in a population of pre-hypertensive males.

8.2 Methods

Twenty-four pre-hypertensive male volunteers were recruited for the study as per the procedures outlined in Chapter 3 (3.3.2, page 58), which was a randomised crossover-trial as shown in Figure 6.1 (page 126).

8.2.1 Echocardiographic assessment

Transthoracic echocardiography was performed pre and post intervention and control periods using methods outlined in 3.1.

Cardiac time intervals were measured using tissue Doppler imaging (TDI). Images were obtained in the apical four-chamber view, placing the sample volume at the lateral mitral annulus and septal mitral annulus. Data presented are the mean values obtained from lateral and septal sample volumes. Offline measurements of cardiac time intervals were performed using EchoPAC software (V.113.0.x, GE Healthcare). As demonstrated in Figure 8.1, measurements of IVCT were obtained by measuring from the end of A' wave to the onset of the S' wave. Measurement of isovolumetric relaxation time (IVRT) was obtained by measuring between the end of the S' wave and the onset of the E' wave. Ejection time (ET) was measured from onset to the end of the S' wave. The same approach was used to measure the lateral mitral annulus. Myocardial performance index (MPI) was calculated as (IVCT + IVRT) /ET.

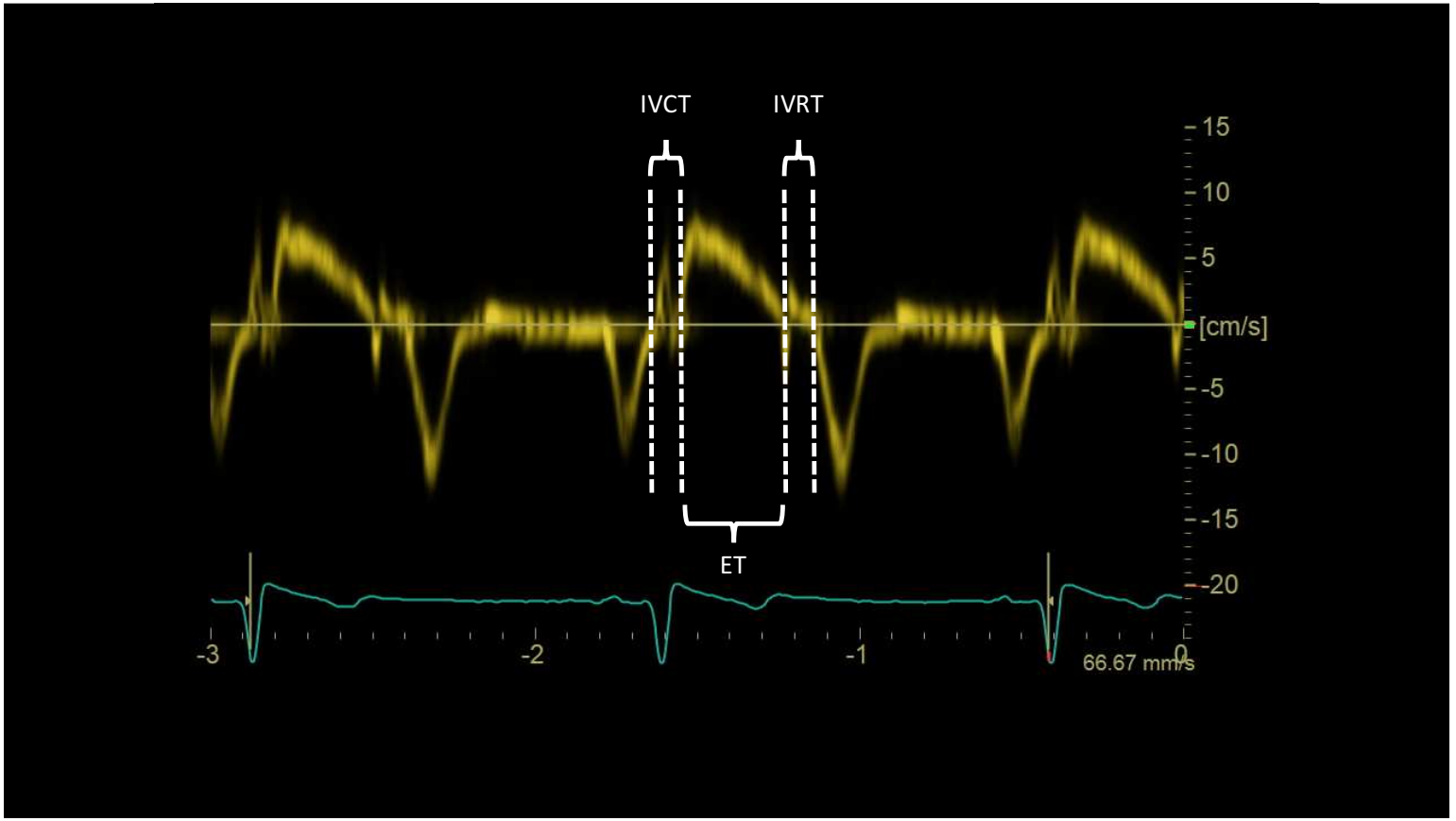

Figure 8.1. Illustration of cardiac time interval measurements from the septal mitral annulus.
Note: IVCT, isovolumetric contraction time; ET; ejection time; IVRT, isovolumetric relaxation time.

8.2.2 Isometric exercise training and control period

The home-based IET intervention and control periods are described in Chapter 6 (6.2.7-6.2.8, page 129).

8.2.3 Data analysis

Following Shapiro-Wilks tests for normality, a two-way repeated measured ANOVA was performed with a Bonferroni post-hoc test for the comparisons of outcome measures between and within groups. The threshold for statistical significance was set at <0.05 and all data is presented as mean ± SD unless otherwise stated. All data was analysed using the statistical package for social sciences (SPSS 22 release version for Windows; SPSS Inc., Chicago IL, USA).

8.4 Results

All 24 participants completed the training intervention and control condition and echocardiographic images suitable for analysis were obtained at all time points.

Control and IET echocardiographic structural, functional and tissue Doppler parameters are detailed in Table 8.1. Following IET there was a significant decrease in LV internal diameter systole (3.28 ± 0.14 cm vs 3.16 ± 0.13 cm, p=0.001), IVSd (0.917 ± 0.088 cm vs 0.875 ± 0.094 cm, p=0.005) LVPWd (0.942 ± 0.088 cm vs 0.908 ± 0.083 cm, p=0.029), RWT (0.384 ± 0.045 vs 0.368 ± 0.037, p=0.036) and LV mass (162.72 ± 28.9 g vs 154.94 ± 30.68 g, p=0.01). This resulted in a significant reduction in the number of participant displaying concentric LV remodelling (16.6% vs 8.3%). There was no change in LVIDd or LA diameter.

Diastolic functional changes included a significant reduction in MV E deceleration time (235.63 ± 52.49 ms vs 204.79 ± 59.62 ms, p=0.027), E/A ratio (1.649 ± 0.418 vs 1.514 ± 0.338, p=0.02) and a significant increase in MV A velocity (0.445 ± 0.133 ms vs 0.513 ± 0.129 ms, p=0.008), however there was no significant change in MV E velocity. Changes in systolic function are indicated by an increase in LVEF (57.32 ±

1.67% vs 58.89 ± 0.053%, p=0.044), as well as an increase EDV (122.21 ± 16.42 mL vs 128.68 ± 16.89 mL, p=0.001) and no change in ESV (p=0.786).

Tissue Doppler parameters revealed significant increases in E' Lateral, E' Septal, and average E' (all p<0.05) and significant reductions in A' Lateral and Average A' (p = 0.043 and p=0.007 respectively), and no significant change in A' Septal (p=0.074). There were no significant changes in S' Lateral (p=0.101), S' Septal (p=0.127) or Average S' (p=0.092).

There were significant reductions in estimated LV filling pressures; E/E' lateral reduced from 5.74 ± 1.16 to 4.52 ± 1.07, (p<0.001), E/E' Septal reduced from 7.42 ± 1.69 to 6.54 ± 2.21 (p=0.026) and Average E/E' reduced from 6.58 ± 1.33 to 5.53 ± 1.55 (p=0.001).

Significant changes were observed in cardiac time intervals; there were significant reductions in IVRT (83.13 ± 10.32 ms vs 76.12 ± 11.15 ms, p=0.006) and IVCT (84.75 ± 10.29 ms vs 72.83 ± 6.41 ms, p<0.001), resulting in an increase in ET (304.63 ± 30.15 ms vs 321.38 ± 20.82 ms, p=0.015). There were consequently reductions in IVCT/ET (p<0.001), IVRT/ET (p=0.005) and MPI (p<0.001).

There were no significant changes in RV functional parameters. There were no significant differences in any variables in the control period, however there was a significant difference between pre IET and pre control values for Average S' (p=0.028).

Table 8.1. Cardiac structure and function: conventional and tissue Doppler parameters

	Control		IET		
	Pre	Post	Pre	Post	*p* value
Structural Parameters					
LV internal diameter diastole (cm)	4.92 ± 0.37	4.9 ± 0.37	4.93 ± 0.37	4.94 ± 0.38	0.378
LV internal diameter systole (cm)	3.28 ± 0.12	3.25 ± 0.13	3.28 ± 0.14	3.16 ± 0.13	0.001
IVSd (cm)	0.925 ± 0.094	0.92 ± 0.083	0.917 ± 0.088	0.875 ± 0.094	0.005
LVPWd (cm)	0.933 ± 0.076	0.945 ± 0.073	0.942 ± 0.088	0.908 ± 0.083	0.029
RWT	0.381 ± 0.041	0.387 ± 0.034	0.384 ± 0.045	0.368 ± 0.037	0.036
LV mass (g)	161.56 ± 27.79	162.08 ± 31	162.72 ± 28.9	154.94 ± 30.68	0.01
LV mass index ($g \cdot m^2$)	77.31 ± 14.96	77.56 ± 16.38	77.87 ± 14.69	74.14 ± 16.17	0.012
LA (cm)	3.67 ± 0.34	3.66 ± 0.34	3.71 ± 0.33	3.68 ± 0.38	0.401
LV Geometry					
Normal	20	20	20	22	0.376
Concentric Remodelling	4	4	4	2	
Global LV diastolic function					
MV Dec T (ms)	217.63 ± 55.21	216.63 ± 58.75	235.63 ± 52.49	204.79 ± 59.62	0.027
E/A ratio	1.645 ± 0.483	1.65 ± 0.497	1.649 ± 0.418	1.514 ± 0.338	0.02
MV E vel (ms)	0.677 ± 0.127	0.712 ± 0.124	0.698 ± 0.172	0.736 ± 0.118	0.192
MV A vel (ms)	0.439 ± 0.123	0.46 ± 0.122	0.445 ± 0.133	0.513 ± 0.129	0.008

Note: LV, left ventricular; IVSd, interventricular septal thickness diastole; LVPWd, left ventricular posterior wall thickness diastole; RWT, relative wall thickness; LA, left atrium; MV dec T, mitral valve deceleration time; E/A , early/late filling; MV E vel, mitral valve early filling velocity; MV A vel, mitral valve late filling velocity. *P* values represent change over time from pre IET to post IET. * $p<0.05$ between pre control and pre IET.

Table 8.1 Continued.

	Control		IET		
	Pre	Post	Pre	Post	*p* value
Global LV systolic function					
LV ejection fraction (%)	57.58 ± 2.39	56.95 ± 2.32	57.32 ± 1.67	58.89 ± 0.053	0.044
ESV (mL)	53.74 ± 10.88	51.89 ± 7.15	52.79 ± 7.65	53.16 ± 8.62	0.786
EDV (mL)	123.58 ± 18.34	120.89 ± 15.18	122.21 ± 16.41	128.68 ± 16.89	0.001
LV Tissue Doppler					
S' Lateral ($m \cdot s^{-1}$)	0.098 ± 0.028	0.096 ± 0.023	0.106 ± 0.014	0.114 ± 0.015	0.101
S' Septal ($m \cdot s^{-1}$)	0.078 ± 0.022	0.088 ± 0.01	0.0860 ± 0.011	0.088 ± 0.007	0.127
Ave S' ($m \cdot s^{-1}$)	0.088 ± 0.019*	0.092 ± 0.014	0.096 ± 0.011	0.101 ± 0.009	0.092
E' Lateral ($m \cdot s^{-1}$)	0.3 ± 0.027	0.132 ± 0.031	0.132 ± 0.026	0.157 ± 0.037	0.003
E' Septal ($m \cdot s^{-1}$)	0.101 ± 0.028	0.1 ± 0.024	0.103 ± 0.024	0.113 ± 0.028	0.003
Ave E' ($m \cdot s^{-1}$)	0.115 ± 0.025	0.115 ± 0.024	0.119 ± 0.023	0.135 ± 0.031	0.001
A' Lateral ($m \cdot s^{-1}$)	0.094 ± 0.026	0.124 ± 0.117	0.096 ± 0.023	0.087 ± 0.018	0.043
A' Septal ($m \cdot s^{-1}$)	0.104 ± 0.015	0.104 ± 0.017	0.106 ± 0.013	0.09 ± 0.02	0.074
Ave A' ($m \cdot s^{-1}$)	0.099 ± 0.019	0.114 ± 0.059	0.101 ± 0.014	0.092 ± 0.016	0.007

Note: LV, left ventricle; ESV, end systolic volume; EDV, end diastolic volume. *P* values represent change over time from pre IET to post IET. * $p<0.05$ between pre control and pre IET.

Table 8.1 Continued.

	Control		IET		
	Pre	Post	Pre	Post	*p* value
Estimated LV filling Pressures					
E/E' Lateral	5.48 ± 1.44	5.67 ± 1.78	5.74 ± 1.16	4.52 ± 1.07	<0.001
E/E' Septal	7.17 ± 2.15	7.63 ± 2.05	7.42 ± 1.69	6.54 ± 2.21	0.026
Ave E/E'	6.28 ± 1.62	6.66 ± 1.78	6.58 ± 1.33	5.53 ± 1.55	0.001
Right Ventricular Function					
TAPSE (cm)	2.2 ± 0.23	2.19 ± 0.25	2.2 ± 0.28	2.278 ± 0.289	0.376
RV S' ($m \cdot s^{-1}$)	0.133 ± 0.019	0.129 ± 0.017	0.113 ± 0.018	0.138 ± 0.02	0.249
RV E' ($m \cdot s^{-1}$)	0.118 ± 0.026	0.12 ± 0.026	0.124 ± 0.027	0.123 ± 0.031	0.793
RV A' ($m \cdot s^{-1}$)	0.115 ± 0.015	0.114 ± 0.016	0.124 ± 0.021	0.123± 0.033	0.867
Cardiac Time Intervals					
IVRT (ms)	82.91 ± 9.34	82.71 ± 8.79	83.13 ± 10.32	76.12 ± 11.15	0.006
IVCT (ms)	82.08 ± 5.11	81.66 ± 6.79	84.75 ± 10.29	72.83 ± 6.41	<0.001
ET (ms)	303.29 ± 26.69	305.38 ± 30.64	304.63 ± 30.15	321.38 ± 20.82	0.015
IVCT/ET	0.272 ± 0.031	0.27 ± 0.04	0.281 ± 0.048	0.227 ± 0.018	<0.001
IVRT/ET	0.276 ± 0.042	0.274 ± 0.044	0.277 ± 0.052	0.238 ± 0.039	0.005
MPI	0.549 ± 0.066	0.544 ± 0.077	0.558 ± 0.089	0.465 ± 0.053	<0.001

Note: TAPSE, tricuspid annular plane systolic excursion; RV, right ventricle; IVRT; isovolumetric relaxation time; IVCT, isovolumetric contraction time; ET, ejection time; MPI, myocardial performance index. *P* values represent change over time from pre IET to post IET. * $p<0.05$ between pre control and pre IET.

8.4 Discussion

This is the first study to examine the effects of an IET programme on cardiac structure and function. A four-week home-based isometric wall squat training programme in pre-hypertensive males resulted in favourable improvements in cardiac structural and functional parameters.

Improvements in cardiac structure are indicated by significant reductions in LVIDs, IVSd, LVPWd and an overall reduction in LV mass and LVMI. Acute IE resulted in reductions in LVIDs, LVPWd and RWT (Chapter 5, page 115), suggesting that repeated stimulation of the acute response may have resulted in chronic structural adaptation to these variables, and that chronic structural adaptations were of a greater magnitude to elicit reductions in LV mass and LVMI.

Established LVH increases the risk of cardiovascular events 6-8 fold (Messerli and Ketelhut, 1991). Greater IVSd and LVPWd are associated with higher sBP (Eliakim-Raz *et al.*, 2016) and IVSd has been shown to predict future systolic HTN in young and healthy normotensives (Grossman *et al.*, 2008). Pre-hypertensives have been shown to exhibit a greater LV mass (Drukteinis *et al.*, 2007), higher incidences of wall motion abnormalities (Di Bello *et al.*, 2010; Santos *et al.*, 2016), and greater age related increase in wall thickness and LV remodelling compared with normotensives (Markus *et al.*, 2008). Thus the measured reductions in LV mass, IVSd and LVPWd may reduce the risk of cardiovascular events. Significant reductions in sBP were measured (Chapter 6) alongside changes in LV geometry, which supports this concept.

Following IET, LVMI and RWT were significantly reduced. Both LV mass and RWT are strong predictors of LV dysfunction, cardiac risk (Li *et al.*, 2001) and mortality (Koren *et al.*, 1991; Levy *et al.*, 1990a), therefore any intervention that is able to slow down or reverse pathological increases in LV mass is desirable (Kokkinos *et al.*, 2007). All participants displayed normal LVMI at baseline (Lang *et al.*, 2015), however of the 24 participants, 4 participants had a RWT measurement >0.42, demonstrating concentric remodelling (Lang *et al.*, 2015). Four-year progression to HTN from pre-HTN can be independently predicted through greater baseline LVMI, RWT for age, and SV (De Marco *et al.*, 2009) suggesting that the reduction in all of these factors

following IET may have a positive impact on attenuating the development of HTN from pre-HTN. Following IET, there was a significant decrease in RWT, and all except two participant displayed normal left ventricular geometry.

Most previous studies reporting improvements in LV remodelling have involved aerobic exercise training programmes of >12 weeks, and have targeted patients with HTN or existing heart failure (Kokkinos *et al.*, 2005; Hinderliter *et al.*, 2002; Rinder *et al.*, 2004; Wisløff *et al.*, 2007; Zheng *et al.*, 2011). These populations may be more receptive to changes in cardiac structure and function, as changes of a greater magnitude are required to revert to optimal conditions. Kokkinos *et al.* (2005) reported a mean reduction of 0.9 cm in IVS thickness following 16-weeks of aerobic exercise training in hypertensive males, which is greater than the 0.3 cm reduction measured following IET. They also measured a reduction of 40g in LV mass, compared with the significant reduction of just 7g following IET; however mean baseline LV mass in the hypertensive male cohort was >300g, compared to the mean baseline of 162g in this study, meaning that their participants may have been more responsive to the exercise training. However, BP reductions of -7 mmHg sBP and -5 mmHg dBP were also reported, which are smaller than those reported following the current IET intervention (Chapter 6).

A 6-month aerobic exercise training programme in overweight and obese pre-hypertensive and stage 1 hypertensive participants induced reductions in IVSd (5%), LVPWd (3%) and an increase in LVMI (2%), alongside BP reductions of 3.2 mmHg (sBP) and 4.4 mmHg (dBP) (Hinderliter *et al.*, 2002). Although mean baseline values for all cardiac structural dimensions were higher than the cohort of this IET study, relative structural adaptations following 4-weeks of IET are comparable to 6-months of aerobic training (IVSd, -3.3%; LVPWd, -3.9%; LVMI, -3.9%) (Hinderliter *et al.*, 2002). Although IET resulted in smaller reductions in IVSd, similar reductions in LVPWd and greater reductions in LVMI, BP reductions following a comparatively short programme of IET greatly exceed those observed following a long-term aerobic training programme. Differences in findings reported, may be attributed to differences in the participant cohorts, as Hinderliter *et al.* (2002) included stage 1 hypertensive and pre-hypertensive participants, which may have resulted in a different myocardial response to training. However previous research has suggested that larger BP

reductions occur in those with higher baseline BP (Pescatello *et al.*, 2004a). It could therefore be suggested that changes in cardiac structure following IET may not have been the primary mediator of BP reductions reported in Chapter 6, and that differences in the training stimulus were responsible for inducing a much greater magnitude of BP change over a shorter time period via alternate regulatory pathways.

However, in a trial by Rinder *et al.* (2004), un-medicated stage 1 and 2 hypertensive participants undertook a 6-month exercise training programme (comprising 1-month flexibility and 5-month endurance training) or were prescribed daily thiazide diuretic medication. Improvements in BP were reported in both groups; however, it was demonstrated that the exercise group was associated with greater cardiac remodelling (RWT and LVPWd) and greater sBP reductions compared with the pharmacotherapy group. The 6-month exercise programme resulted in BP reductions of 13 mmHg (sBP) and 9 mmHg (dBP), as well as reductions in RWT (0.4 cm) and LVPWd (1.4 cm). Isometric exercise training also resulted in significant reductions in resting BP (see Chapter 6), as well as significant reductions in both RWT and LVPWd. However, all changes were of a smaller magnitude than in the Rinder *et al.* (2004) study, which can be explained by the higher baseline BP of the participant cohort (Pescatello *et al.*, 2004a; Cornelissen and Smart, 2013) and much longer study duration. Furthermore, Rinder *et al.* (2004) reported that only one participant had normal LV geometry, 2 had concentric remodelling and the other 11 had concentric or eccentric LVH. In contrast, 20 of the 24 participants in this study had normal LV geometry, suggesting that the healthy hearts did not need to change to the same extent. It is also important to note that despite reverse LV remodelling and reductions in BP, Rinder *et al.* (2004) measured no cardiac functional changes in either the aerobic or pharmacotherapy group, suggesting that the concurrent cardiac structural, functional and BP changes following IET may be due to a difference in the exercise stimulus.

Wisløff *et al.* (2007) suggested that interval based training was superior to continuous training as rest periods increased the capacity to complete higher intensity bouts. Although their research was carried out on an elderly population with chronic heart failure, the interval structure of the session (4 x 4 min at 90-95% peak HR) and frequency (3 x per week) is comparable to the IET intervention employed in this study. The 12-week aerobic interval training programme elicited 0.7 cm reductions in both

end systolic and end diastolic diameter, which demonstrates considerable reverse remodelling. Comparatively, IET resulted in no change in LVIDd, and a 0.12 cm significant reduction in LVIDs. However, it must be noted that the mean cardiac dimensions in Wisløff *et al.* (2007) study are consistent with moderate LV dilatation compared to normal cardiac dimensions in the present study.

Pathological remodelling through HTN and CVD is associated with the loss and fibrotic replacement of cardiomyoctyes (Weeks and McMullen, 2011), and thickening of the chamber walls, known as concentric hypertrophy. Inversely, in response to a repeated exercise training, such as in elite athletes, a healthy heart increases in cardiac mass, function and contractility, known as physiological remodelling (Ellison *et al.*, 2012). Aerobic exercise training has been shown to induce physiological remodelling both though hypertrophy of individual myocytes (Kemi *et al.*, 2004) as well as the formation of new myocytes (Waring *et al.*, 2014). Animal studies have demonstrated that exercise training can protect against cardiomyocyte attrition and reverse or attenuate myocyte hypertrophy (Kwak *et al.*, 2006). These examples refer to aerobic exercise adaptations in trained hearts, however they may provide an explanation for the cardiac structural changes measured following IET.

A potential mechanism for the remodelling of cardiomyocytes following IET, is the stimulated release of NO during IE. The role of NO has previously been considered important in relation to IE (Millar *et al.*, 2014), due to its role in vascular homeostasis and endothelial function, as a potential mediator of reduced TPR and associated reduction in BP (Peters *et al.*, 2006). However, it has also been suggested that increased NO production within the heart may be responsible for improved myocardial function (Ellison *et al.*, 2012), which may in turn result in lowered cardiovascular risk and contribute to improved BP control.

Upon receipt of an action potential, sarcolemma depolarization triggers Ca^{2+} release from the sarcoplasmic reticulum causing excitation-contraction coupling to occur in the myocardium. While excitation-contraction coupling dictates the rate of inotropy, the reuptake of calcium to the SR following excitation-contraction coupling represents lusitropy. Catecholamine induced Ca^{2+} release promotes positive inotropy and lusitropy, representing improved contractility, however excessive cytosolic calcium of

cardiomyocytes causes an increase in inotropy which can also be associated with LV hypertrophy, and causes lusitropy to decrease (Opie, 2004).

The concentrations of Ca^{2+} are controlled by eNOS, found in the endothelial cells and cardiomyocytes (Feron *et al.*, 1996) and neuronal NO synthase (nNOS), which is localised to cardiac autonomic nerve ganglia and cardiomyocytes (Danson *et al.*, 2005). Both of these enzymes cause NO synthesis, causing vasodilation and relaxation of endocardial cells and their release is triggered via ventricular stretch of endocardial cells and vagal simulation (Balligand *et al.*, 2009). As NO is responsible for Ca^{2+} handling and sensitivity, insufficient bioavailability of NO leads to the release of excessive concentrations of Ca^{2+} (Ziolo *et al.*, 2001) through calcium induced calcium release (Hinch *et al.*, 2004). Reduced availability of NOS in a state of disease or impaired CV health means that the protective function of NO is diminished (Hermann *et al.*, 2006). It has been suggested that a feedback loop exists, whereby increased cytosolic Ca^{2+} (calcium overload) stimulates synthesis of nNOS in the sarcoplasmic reticulum, which in turn inhibits cytosolic calcium levels, promoting vagal bradycardia and reducing contractility (Opie, 2004). Chronic Ca^{2+} elevation, caused by ischaemia, reperfusion or excessive catecholamine stimulation can lead to myocardial cell damage, impaired contractility resulting in the calcification of cardiomyocytes and pathological remodelling (Opie 2004).

The presence of nNOS inhibits the influx of Ca^{2+}, and NO acts to reduce the negative effects of Ca^{2+} overload through a complex cascade of autocrine and paracrine regulatory processes (Massion *et al.*, 2003). Nitric oxide induces positive inotropic responses that depress the production of Ca^{2+} by the sarcoplasmic reticulum resulting in the down regulation of Ca^{2+}, the desensitisation of cardiac myofilaments to Ca^{2+} and attenuation of β-adrenergic effects (Massion *et al.*, 2003). Wisløff *et al.* (2002) found that 6-weeks of aerobic interval training restored contractile function, improved intracellular Ca^{2+} handling and Ca^{2+} sensitivity in the cardiomyocytes of rats.

An exercise training stimulus has been shown to result in an increase in NOS, through the repeated contraction and relaxation of cardiomyocytes (Opie, 2004). The stretch and sheer stress induced by the pressure load applied by the IE stimulus, may result in an increase in the production of eNOS (Balligand *et al.*, 2009), in addition causing the

stretch of ventricles that resulted in the reduction of IVSd and LVPWd. Ingestion of dietary nitrate supplementation, is associated with reduced BP, improved cardiomyocyte calcium signalling and LV contractile function (Pironti *et al.*, 2016). It is therefore possible that IET improves cardiomyocyte calcium signalling, however this requires further research.

Parasympathetic activation is associated with HR regulation and reduced excitation of the SA node, while sympathetically induced catecholamine release leads to increased excitation coupling and the subsequent release of Ca^{2+} (Gordan *et al.*, 2015). A shift in sympathovagal balance and increase in vagal activation, as demonstrated in Chapter 7, as well as by Taylor *et al.* (2003) could therefore provide an explanation for measured cardiac structural changes following IET. Increased vagal excitation and the release of acetylcholine is also associated with nNOS production (Opie, 2004), leading to a subsequent increase in the production of NO (Seddon *et al.*, 2007), which may result in improved intracellular handling of Ca^{2+} and cellular remodelling (Kemi and Wisloff, 2010).

Cardiac remodelling may be responsible for observed functional changes. In the current study, 4-weeks of IET was associated with improvements in contractility, demonstrated by significant increases in LVEF and EDV. In addition, improvements in diastolic and systolic function were also observed, which are associated with improved prognosis.

Left ventricular ejection fraction provides a simple indication of contractile state, relating SV to EDV to demonstrate the percentage of blood leaving the heart with each contraction, thus providing an index of LV fibre shortening and LV systolic function (Opie, 2004). The 1.6% increase in mean LVEF following IET, as a result of significant increases in SV and EDV, shows an improvement in systolic function and improved myocardial contractility. Rinder *et al.* (2004) reported a 2% increase in mean LVEF following aerobic exercise training, although this was not statistically significant. In contrast, following aerobic interval training for 12-weeks in patients with un-medicated HTN, Molmen-Hansen *et al.* (2012) measured a 6% increase in LVEF, and significant reductions in ABP. This increase was also associated with significant increases in both EDV and SV. However, Zheng *et al.* (2011) reported no

change in LVEF following a 6-month moderate intensity aerobic exercise training intervention in medicated hypertensive patients, which could be explained by differences in exercise training stimulus or medication status of participants. This suggests that type and intensity of exercise may be important mechanisms for cardiac functional adaptations.

Markers of diastolic function include E/A ratio, MV E deceleration time and cardiac time intervals (IVRT). E/A ratio demonstrates the ratio of peak blood flow velocity in early and late diastole, demonstrating the capacity of the LV to effectively fill with blood between contractions, thus representing diastole. An E/A ratio between 1-2, with normal LA size and estimated LV filling pressure is indicative of normal diastolic function. Early filling rate (E) is influenced by ventricular compliance and a reduced rate of relaxation and abnormalities in filling patterns represent progressively worse diastolic function (Mottram and Marwick, 2005).

Prolonged MV E deceleration time is an indicator of diastolic dysfunction (Grant *et al.*, 2015); therefore a reduction in deceleration time indicates an improvement in diastolic function (Mottram and Marwick, 2005). Following IET there was a significant reduction in E/A ratio although as with LVEF, this was of a lesser magnitude than those measured by Wisløff *et al.* (2007) following aerobic interval training. In contrast, Zheng *et al.* (2011) reported an increase in E deceleration time and E/A ratio in hypertensive males following an aerobic exercise training programme, however they also reported a reduction in HR, which may account for prolonged MV E deceleration time.

Changes in tissue Doppler imaging provide further indication of cardiac functional improvement following IET. E/E', a measure of LV filling pressure (Ommen *et al.*, 2000) reduced significantly following IET. This variable has been shown to be a strong predictor of cardiovascular events (Sharp *et al.*, 2010), therefore its improvement may indicate improved cardiovascular outcome.

Cardiac time intervals, measuring precise stages of the cardiac cycle, have been shown to change with disease progression. Isovolumetric contraction time represents systolic function, increasing when myoctyes take longer to achieve an equal pressure to that of

the aorta (Biering-Sorensen *et al.*, 2015). Ejection time (ET) is the time taken for blood to be ejected from the ventricle and can be reduced when the LV myocytes are less able to maintain adequate pressure. Isovolumetric relaxation time reflects the time taken for early diastolic filling to occur and is therefore considered an indicator of diastolic function.

Prolonged IVRT is indicative of diastolic dysfunction (Grant *et al.*, 2015), therefore a reduction in IVRT, as recorded following IET (87.32 + 10.19 ms to 73 +6.5 ms), indicates improved diastolic function. Reductions in IVRT have been previously reported following aerobic exercise training interventions in patients with HTN (Zheng *et al.*, 2011; Molmen-Hansen *et al.*, 2012). However, Biering-Sorensen *et al.* (2015) demonstrated that the most accurate prognostic markers relating to cardiac time intervals were IVRT/ET and MPI, both of which account for systolic and diastolic function together within one measure (Masugata *et al.*, 2009). It was suggested that these measures may be of particular use in healthy patients who may display no echocardiographic abnormalities. Over a study duration of 10 years, IVRT/ET and MPI independently predicted adverse cardiovascular outcome (IHD, heart failure or cardiac death) (Biering-Sorensen *et al.*, 2015). Both IVRT/ET and MPI were significantly reduced following IET, which may be associated with reduced risk of adverse outcome, as risk has been demonstrated to increase positively and continuously alongside these variables.

Mechanistically, a reduction in IVCT may be linked to the lower BP recorded in Chapter 6. If aortic pressure is lower, less time is required for LV pressure to overcome the afterload and eject blood, thus shortening IVCT. There were no changes in HR following IET, therefore reductions in both IVRT and IVCT were accompanied by an increase in ET, and as such the overall time of the cardiac cycle remained constant. Hypertension induces a greater afterload at rest when compared to NTN and is therefore associated with prolonged IVRT and IVCT, a shorter ET and greater LV mass (Biering-Sorensen *et al.*, 2016). Therefore, a reduction in resting BP will result in reduced afterload at rest and improved cardiac performance. Indeed, the reduced IVRT and subsequent increase in ET may explain the measured increase in SV as a reduction in preload and longer ET will result in a greater ejection of blood from the heart.

Increases in SV and $\dot{Q}$, measured via echocardiography have previously been reported following aerobic exercise training (Rinder *et al.*, 2004). Following IET there was a significant increase in SV and significant increase in $\dot{Q}$ in Chapter 6. Despite these changes there was no significant change in HR. In contrast, previous research utilising the same isometric wall squat intervention has reported a reduction in resting HR following IE, accompanied by a decrease in $\dot{Q}$ and no change in SV, in a group of young normotensive males (Wiles *et al.*, 2017). Echocardiographic measures were not taken by Wiles *et al.* (2017), but as there was no change in SV, it is likely that the reported reduction in $\dot{Q}$ was caused by reduced HR, suggesting that the reason for this may have been a change in autonomic function and increase in parasympathetic activity in these normotensive participants, rather than cardiac structure or function. However methodological differences in assessment may also account for differences in these findings.

Much of the existing research into the effects of exercise training on cardiac structure and function is limited to long-term studies of aerobic exercise training in patients with existing disease. Given the clear differences between aerobic and IE stimuli and subsequent physiological response, it is likely that the physiological adaptations and their respective mechanisms may differ.

Although both aerobic exercise and IE induce acute increases in $\dot{Q}$, HR, and BP, the RPP response, representing myocardial oxygen consumption (Gobel *et al.*, 1978), during IE is significantly lower than during aerobic exercise at the same relative intensity (Carlson *et al.*, 2017). The recovery response from acute IE (Chapter 4) may have induced a lasting response following training. Repeated sympathetic withdrawal, parasympathetic activation and reduced BP, as well as potential post exercise hyperaemia and associated sheer stress (Tinken *et al.*, 2010) following each bout of IE, may have a more enduring effect on NO synthesis causing a reduction in TPR and a reduction in afterload, thus improving LV performance. Levinger *et al.* (2005) suggested that a reduction in TPR following resistance training results in reduced afterload, causing improvements in LV function and contractile efficiency even without changes in LV geometry.

According to Starlings Law of the heart, an increase in preload, as evidenced by an increase in EDV following IET, causes greater stretch of the ventricle thus enabling a greater ejection. This is supported by the significant increase in SV. Furthermore, an increase in LV stretch will result in the greater expression of eNOS from cardiac myocytes (Balligand *et al.*, 2009). Thus, repeatedly inducing an increase in preload through IET will result in a greater release of eNOS, stimulating an increased bioavailability of NO and associated benefits. Within the myocardium, NO has been shown to modulate fundamental events of cardiac excitation-contraction coupling, resulting in significant effects on cardiac function, including LV relaxation (Paulus and Shah, 1999).

Beckers *et al.* (2008) assigned aerobic and aerobic-resistance combined training programmes to patients with heart failure. It was concluded that cardiac structural and functional improvements were the same for both training types, with reductions in LVIDd and end systolic diameter and increases in EF, confirming that resistance training does not result in any adverse effects on cardiac structure and function. To date, there has been limited research on alternative resistance exercise training types and limited application in populations at risk. However, in a population of athletes, those involved in aerobic activities display chronic LV eccentric enlargement, while those who perform isometric strength training display (to a lesser magnitude) chronic concentric LV hypertrophy (Baggish and Wood, 2011a). It is noteworthy that this applies to elite athletes with extreme training loads and it is unlikely that short term training interventions in previously inactive participants will induce structural remodelling in the same way. Indeed, changes in LV mass suggest that concentric hypertrophy was reduced in some participants following IET, and improved functional parameters including LVEF, cardiac time intervals and MPI, suggest that cardiac contractility and relaxation following IET is improved. A reduction in afterload and increase in preload in a resting state, will ultimately reduce myocardial workload as the heart is working against a smaller resistance to eject blood.

Changes in cardiac structure and function are closely linked to other areas of cardiovascular health. A well-reported association exists between cardiac structure and function, and cardiac autonomic function (Bristow, 1984). Chronic sympathetic over activity and overexposure to catecholamines is associated with pathological cardiac

remodelling (Brum *et al.*, 2002), as previously explained with regards to the role of Ca^{2+}. Sympathetic overactivation and increased vasoconstriction result in an increase in BP by the RAAS causing an increase in afterload. In addition to causing BP increases, this can lead to eccentric LV hypertrophy (Odedra and Ferro, 2006), as the added demand increases excitation contraction coupling, adding to the release of Ca^{2+}, and down regulation of NOS and NO production. In contrast, improvements in any of these cardiovascular variables will cause a cascade response, which may reverse the outlined pathways. A shift in symapthovagal balance and reduced concentrations of IL-6 and ADMA as measured in Chapter 7, may contribute to improved NO bioavailability thus improving vasodilation and reducing BP. Findings from Woodiwiss and Norton (1995) showed that exercise induced improvements in LV function and size were associated with an increase in parasympathetic activation, supporting the combined findings from Chapter 7 and the current Chapter. Neurohumoral activation is related to the severity of LV functional impairment (Torre-Amione *et al.*, 1996), therefore a reduction in makers of inflammation may be linked to improvements in LV function. Overall changes in BP (Chapter 6) may be responsible for improvements in cardiac systolic and diastolic function.

8.4.1 Clinical implications

Hypertension can produce a progressive deterioration in cardiac performance and is the leading risk factor for premature mortality (WHO, 2013). Pre-HTN is associated with a greater risk than optimal BP and accelerated progression to HTN (Vasan *et al.*, 2001a). Hypertension causes adverse cardiac remodelling and accelerates the progression to heart failure, therefore favourable improvements in cardiac structure and function may be mechanistically linked to the clinically important reductions in BP that were measured following IET in Chapter 6.

It is noteworthy that many of the reported changes can be compared to the effects of anti-hypertensive medications that are currently prescribed, that act to increase the bioavailability of NO (Hermann *et al.*, 2006). In addition, favourable LV remodelling responses are similar to those reported previously in patients with hypertension, following a programme of aerobic training or prescribed diuretics (Rinder *et al.*, 2004). Therefore, a non-pharmacological pathway that can induce increases in NO and induce

changes in LV remodelling may be desirable as an anti-hypertensive treatment option. However, further research into the longer-term effects of IET in clinical populations is required to elucidate if this form of exercise training could be used as a substitute for existing pharmacological treatments. In addition, it has been shown that upon cessation of physical activity following an intervention, changes in cardiac structure regressed back to pre-training values after 2-4 weeks (Kemi *et al.*, 2004) therefore the feasibility and extended benefits of performing IET over a longer term remain to be established.

8.5 Conclusion

A 4-week programme of IET was associated with significant improvements in cardiac structure and performance. These responses may be associated with improvements in BP, autonomic function and neurohumoral factors observed following the same training intervention in Chapters 6 and 7. No adverse effects on cardiac structure and function were apparent following this training period and improvements are comparable to those induced by pharmacological interventions in hypertensive populations. These findings further advocate the need for larger scale studies using IET in clinical populations, in addition to longer term assessment of the effects of this training on cardiac structure and function, leading to the possible future prescription of IET as an anti-hypertensive therapy.

CHAPTER 9:

General Discussion

9.1 Overview

In this thesis, two main studies assessed the acute and chronic physiological effects of IE and IET in a population of pre-hypertensive males. Chapter 4 reported the haemodynamic and cardiac autonomic responses before, during and after a single isometric wall squat training session in the laboratory and Chapter 5 assessed echocardiographic measures of cardiac structure and function before and after the same isometric protocol. Following this, Chapter 6 attempted to measure any changes in resting and ambulatory BP and other haemodynamic variables following a 4-week home-based isometric wall squat training programme and a 4-week control period. Chapter 7 assessed any adaptations in cardiac autonomic modulation, inflammatory markers and vascular biomarkers, and Chapter 8 assessed changes in cardiac structural and functional variables in response to the 4-week IET intervention.

9.2 Summary of findings during IE

The results presented in Chapter 4 (pages 101-104), show that during IE there were significant reductions in HRV and significant increase in HR, sBP, mBP, dBP and $\dot{Q}$. The first IE bout resulted in a reduction in SV, which then plateaued throughout the remaining bouts of IE. There was an initial rise in TPR during the first bout, which was followed by a decrease through the remaining bouts of IE, although these changes were not statistically significant.

9.3 Acute physiological responses during an isometric wall squat session

Haemodynamic and autonomic responses to IE are demonstrated in Figure 9.1. At the onset of exercise, an increase in central command (Goodwin, 1972), induced by the exercise pressor response through stimulation of mechanoreceptors (Mitchell, 2012) stimulates a cascade of haemodynamic responses. Isometric exercise results in an increase in sympathetic activity and subsequent parasympathetic withdrawal. Sympathetic activation in IE has been previously demonstrated via an increase in MSNA (Seals and Reiling, 1991) and findings from Iellamo *et al.* (1999b) demonstrated an increase in LF, and a shift in LFnu and HFnu during an isometric contraction. However, despite a reduction in overall PSD, changes in LFnu, HFnu and LF/HF during IE were not significant. Nevertheless, an increase in sympathetic

activation is required to increase HR during IE (Iellamo, 2001), which was responsible for an increase in $\dot{Q}$, as SV was reduced. Increased $\dot{Q}$, TPR, and associated afterload have been reported during an IHG contraction (Stewart *et al.*, 2007a), which may be caused by sympathetically mediated vasoconstriction.

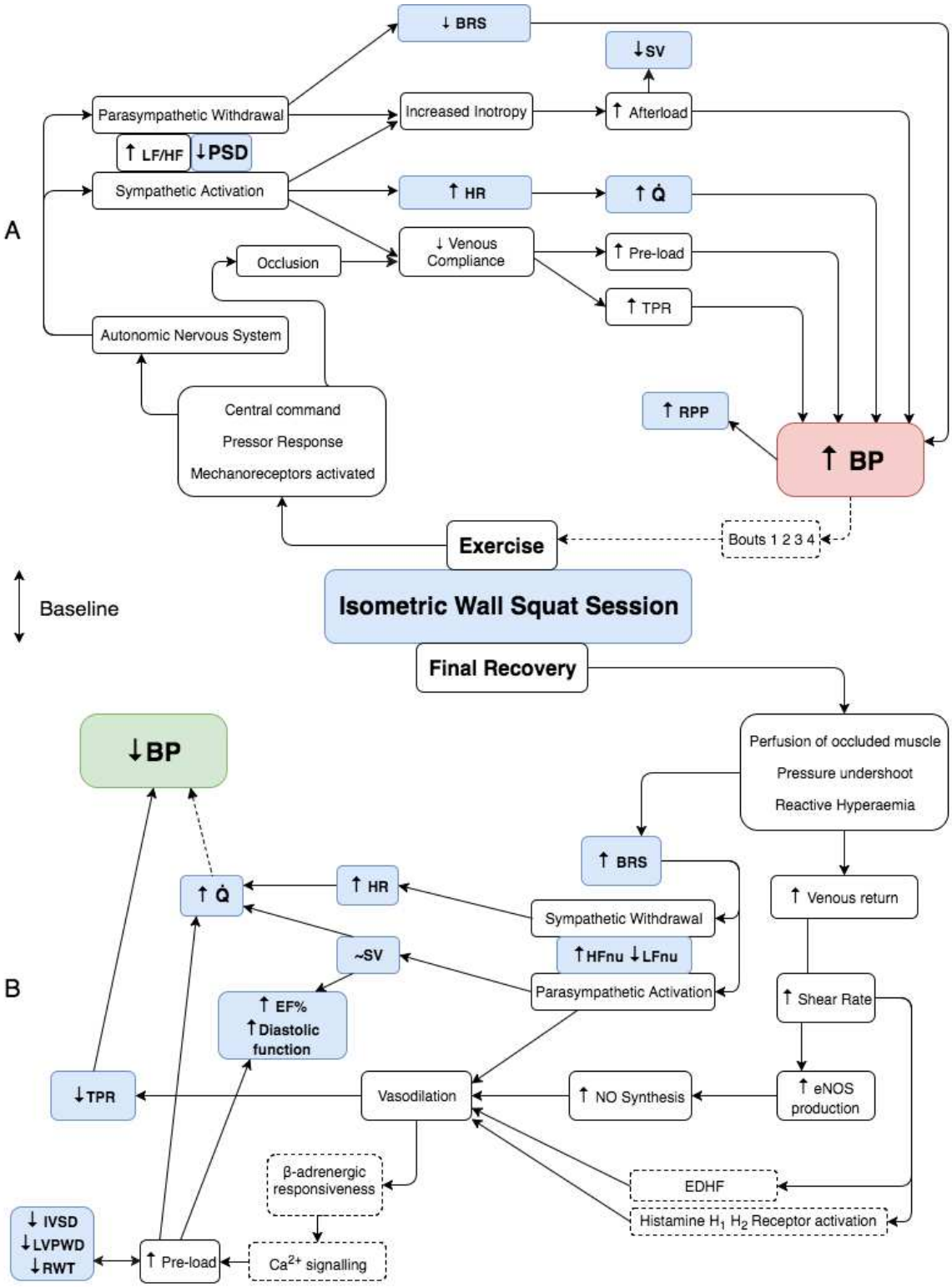

Figure 9.1. Diagram of the acute responses to IE. Note: Section A, the responses during an IE contraction; Section B, the recovery responses to a session of IE. Arrows represent increases and decreases compared with baseline. Variables presented in blue represent statistically significant changes in this thesis. The central hypothesis, BP, is presented in green and red and also represents statistically significant changes.

Parasympathetic activation acts to lower HR, therefore the increase in inotropy and HR could be explained by parasympathetic withdrawal during IE (Iellamo *et al.*, 1999b). Parasympathetic activation stimulates a bradycardic response and aims to maintain $\dot{Q}$ by increasing SV in order to reduce HR (Martin *et al.*, 1974). Therefore, withdrawal of parasympathetic tone may be responsible for the opposite effect (tachycardic response) with an increase in HR and a reduction in SV. Isometric handgrip exercise induces an acute increase in LV afterload (Weiner *et al.*, 2012), this may explain the measured reduction in SV during this type of training (Chapter 4), compared to aerobic exercise which is associated with an increase in SV (Rodeheffer *et al.*, 1984).

Baroreceptor reflex sensitivity is understood to be associated with parasympathetic activation (Hunt *et al.*, 2001). Therefore, withdrawal of parasympathetic tone may explain the resetting of the baroreflex to operate at higher BP and HR. Ichinose *et al.* (2006) demonstrated a time dependant increase in BP modulated by arterial baroreflex function during an isometric contraction, offering explanation for the progressive rise in HR and BP throughout 4 bouts of IE in Chapter 4, as full recovery did not occur during the rest periods between IE bouts. In addition, the overall reduction in BRS documented in Chapter 4 supports pervious findings by Iellamo *et al.* (1999b), who measured a reduction in BRS during a static leg contraction; however, the same study revealed that BRS was not affected during an IHG contraction, suggesting that IE in a larger muscle group elicits an augmented pressor response. This difference in acute IE stimulus and associated physiological responses, could account for overall differences in BP responses to IE training of different muscle groups which will be discussed in 9.7.

9.4 Summary of findings during recovery from IE

Chapter 4 (page 104) details that during recovery from isometric wall squat exercise, HR, RPP, TPR and $\dot{Q}$ were all significantly reduced compared with during IE and sBP, dBP and mBP, were reduced to below baseline values, demonstrating post exercise hypotension.

Autonomic adaptations in recovery included a significant increase in PSD and decrease in LF/HF ratio compared with during IE. There was a shift in sympathovagal balance that was significantly different to baseline and during IE, demonstrating an increase in parasympathetic activation (HFnu) and sympathetic withdrawal (LFnu). In addition, there was a threefold increase in BRS during recovery, which was also significantly different to baseline.

Finally, echocardiographic measures taken immediately following IE, presented in Chapter 5 (page 115), revealed reductions in LVIDs, LVPWD, RWT, and fewer overall cases of concentric remodelling. Functional responses following IE included increases in LVEF, peak E', A' and S', as well as a reduction in E/E', demonstrating a reduced estimated filling pressure.

9.5 Acute physiological responses in recovery from an isometric wall squat exercise session

Cessation of IE is associated with perfusion of previously occluded muscle mass, a transient pressure undershoot (MacDougall *et al.*, 1984) and a period of reactive hyperaemia (Lawrence *et al.*, 2014). This results in an upsurge in blood flow and venous return (Hurley and Gillin, 2015), thus increasing vascular sheer rate and stimulating the release of eNOS, production of NO and subsequent vasodilation (Balligand *et al.*, 2009). Improved endothelial-dependant vasodilation has been previously demonstrated during acute recovery from IHG training, although this was limited to the training limb (McGowan *et al.*, 2006b). Whilst vascular responses were not measured directly in this thesis, a similar response to IHG following isometric wall squat exercise may provide explanation for the reduction in TPR that was recorded in Chapter 4, which is likely to have contributed to the post exercise hypotensive response. Total peripheral resistance was calculated from $\dot{Q}$ measured via impedance cardiography and mean arterial pressure, suggesting that, in contrast to McGowan *et al.* (2006b), the effect of IE may have extended beyond the training limb. If this was the case then additional vasodilatory pathways may also contribute to the observed reduction in TPR during recovery. Cardiac autonomic factors are also likely to influence cardiac function and structure during recovery from IE.

A shift in autonomic regulation was reported in Chapter 4, a response that has been previously measured following acute sessions of IE (Iellamo *et al.*, 1999b; Stewart *et al.*, 2007a; Millar *et al.*, 2009a). The increase in HFnu and reduction in LFnu above baseline, and reduction in LF/HF compared with during IE, mark an improvement in cardiac autonomic regulation via residual predominance of parasympathetic over sympathetic activity. Sympathetic withdrawal results in the down regulation of catecholamine release, attenuating sympathetically mediated HR, BP and vasoconstrictor responses, increasing β-adrenergic responsiveness to enable vasodilation and reduce TPR. Simultaneously, the sudden increase in parasympathetic activation due to the loss of central command and withdrawal of muscle metaboreflex activation, acts upon pathways that work to reduce BP (Pescatello *et al.*, 2004a). Cardiac autonomic factors positively affect vasodilation of cardiac and vascular smooth muscle during recovery. Parasympathetic activation stimulates the release of acetylcholine, which stimulates cholinergic endothelial cells to release eNOS, thus contributing to improved vasodilation, relaxation of smooth muscle, and reduced TPR. In addition, in the myocardium, the parasympathetic release of eNOS promotes a reaction by which adrenergic activation, resulting in the release of enzyme Kinase G, inhibits calcium channels. It is therefore possible that the parasympathetic recovery response to IE, aids in blocking excessive calcium uptake that would eventually lead to adverse remodelling (Opie, 2004).

During IE, SV remained below baseline, however in recovery SV returned above baseline. Echocardiographic images taken during recovery suggest there is an increase in preload; possibly caused by an increase in venous return induced by reactive hyperaemia upon release of the isometric contraction. An increase in LVIDd supports this concept and the increase in LVEF and SV above baseline may be due to a reduced afterload and the Frank-Starling Law. As described by Starlings law of the heart, a greater volume load will cause greater stretch of cardiac myocytes increasing EDV. This stretch can cause the release of myocardial eNOS and nNOS, thus increasing NO production and causing relaxation of the myocardial smooth muscle, which may account for preload induced stretch of cardiomyocytes and subsequent reductions in LVPWd and RWT.

The role of histamine has been implicated in recovery from aerobic exercise and the hypotensive response to acute exercise. Endothelial H_1 and H_2 receptors are activated by an immediate hyperaemic response following exercise, triggering autocrine and paracrine reactions that lead to improved vascular conductance and post exercise hypotension in recovery (Halliwill *et al.*, 2013). Although prior findings refer to acute effects of aerobic exercise, the hyperaemic response following IE is enhanced, and the hypotensive response is evident (Chapter 4), suggesting that this pathway may also be associated with IE.

Following IE, BRS increased threefold compared to baseline. When an IE contraction is released, high BP causes activation of baroreceptors, resulting in increased afferent neuronal firing, a reflex increase in parasympathetic tone and associated decrease in HR and $\dot{Q}$ (Fadel and Raven, 2012). This compensatory increase in BRS, following a reduction in BRS during IE, may therefore be responsible for post exercise hypotension during recovery. A drop in BP during recovery from IE has previously been reported by Millar *et al.* (2009a) and although BRS was not measured, an improvement in cardiac autonomic regulation supports the concept of improved baroreflex mediated vagal outflow (Hunt *et al.*, 2001).

The magnitude of BRS gain during recovery, may be the result of the significant reduction in BRS measured during the isometric wall squat session and the exercise stimulus. Previous research has shown that an IHG contraction does not induce arterial baroreflex resetting, compared with an ILT contraction (Iellamo *et al.*, 1999a). Furthermore Halliwill *et al.* (2013) suggested that during post exercise BRS resetting, muscle afferents play a part in inducing post exercise vasodilation via histamine receptor (H_1 and H_2) activation in the working muscle, therefore the greater muscle mass involved in isometric leg training compared to handgrip training may mediate BRS responses reported in Chapter 4.

The purpose of the acute studies in Chapters 4 and 5 was to ascertain the physiological responses to an acute isometric stimulus as these may, in part, explain the mechanisms that lead to any chronic changes in cardiovascular regulation following a period of IET using the same session protocol.

Indeed prior research has shown that acute responses to an exercise training programme is predictive of a chronic adaptive outcome. The relationship between acute and chronic responses to a training regimen in individuals may also be of value in determining responders vs non-responders to a given exercise intervention. Furthermore the ability to predict the magnitude of a response may be clinically relevant.

However, just as variation in the specific trigger for raised BP exists between individuals (Carretero and Oparil 2000), individual differences in both acute and chronic BP responses may relate to variations in any number of physiological adaptive processes (neural, hormonal, and local vasodilator substance). The current research may allow for prediction of BP outcome responses, however the acute investigations in this thesis (Chapters 4 and 5) did not report additional biochemical variables, therefore limited associations can be made between acute and chronic responses for these markers.

Liu *et al* (2012) measured PEH following a single 20 minute aerobic treadmill exercise session (65% V02max) in a pre-hypertensive population, as well as BP reductions following a 5 week training intervention employing the same training stimulus (4 x session/week). Magnitude of sBP and dBP reductions following the acute session were shown to be predictive of BP reductions following the training intervention, however BP reductions did not correlate with other measured haemodyamic variables, HRV or BRS. In addition, magnitude of sBP reduction was found to be correlated with baseline BMI, which may have been caused by the weight loss induced by aerobic exercise. In contrast, isometric exercise training was not associated with weight reduction. Acute aerobic exercise training responses were also shown to be predictive of chronic responses in patients with CAD (Kiviniemi *et al.*, 2014).

In addition to aerobic exercise research, the presence of PEH following an acute resistance exercise session is also widely accepted (Macdonald *et al*, 1999; 2002), and chapter 4 of this thesis concludes that isometric exercise also induces this hypotensive response. However cardiovascular reactivity measured during a single IHG contraction did not predict BP reductions following a period of IET Badrov *et al*, (2013). Somani *et al* (2017) also noted no correlation between cardiovascular reactivity and the magnitude of IET induced BP reduction in a young normotensive population. It is

noted that these isometric studies observed cardiovascular reactivity to a single isometric stimulus and did not investigate the recovery response to an acute IE training session (4x2 minute contractions).

In spite of multiple reports concluding positive associations between acute and chronic BP responses to exercise, (Taylor *et al.*, 2010) highlight the need to remain cautious in using acute responses to predict chronic outcomes, suggesting that the effect of baseline BP status on the magnitude of PEH can be spuriously exaggerated by data analysis, and may even mask other moderators of BP change which may be of greater importance.

It may therefore follow that the acute stimuli induced during a single isometric session, as demonstrated in figure 9.1, results in a cumulative effect through repetition. These responses are discussed below in 9.6 and 9.7, and portrayed in figure 9.2.

9.6 Summary of responses to a 4-week programme of IET

A 4-week home-based isometric wall squat training programme in pre-hypertensive males resulted in significant reductions in resting and ambulatory BP. Results presented in Chapter 6 show that ambulatory sBP was reduced by 11.83 mmHg, dBP was reduced by 5.57 mmHg and mBP was reduced by 5.67 mmHg. This is one of only a few studies to use ABP monitoring following IET (Stiller-Moldovan *et al.*, 2012; Somani *et al.*, 2017) and the first to apply this methodology to an isometric leg training protocol. Ambulatory readings revealed reductions in PP and BPV following IE, but there were no significant changes in HR at any time point. Haemodynamic assessment at rest revealed significant increases in SV and $\dot{Q}$ following IET.

Findings from Chapter 7 demonstrated an overall increase in HRV (PSD) and a significant increase in parasympathetic regulation. A reduction in LF/HF ratio, and a significant increase in HFnu and decrease in LFnu, demonstrate improved sympathovagal control. Following IET there was a significant increase in BRS which represents an improvement in short-term control of BP. Blood plasma samples revealed reductions in the cytokine IL-6 and the vascular biomarker ADMA following

IET; however, there were no significant changes in other measured factors including TNF-α, hs-CRP, ICAM or VCAM following either exercise or control periods.

Cardiac structural and functional responses to IET were measured in Chapter 8. Following IET, improvements in LV geometry were measured, including reduced LV mass, RWT, LVIDs, IVSd and LVPWd. This led to a reduction in the number of participants displaying concentric remodelling. Furthermore, echocardiographic measures also revealed improvements in myocardial performance, with improved systolic and diastolic function.

9.7 Potential central and peripheral mechanisms for reductions in BP following IET

Many previous studies have reported reductions in resting BP following a programme of IET in normotensive, pre-hypertensive and medicated and un-medicated hypertensive populations, results of which, and details of IET stimuli are outlined in Table 2.2 (page 28). The need to fully comprehend the mechanisms for BP reductions has been expressed in a number of review papers (Lawrence *et al.*, 2014; Millar *et al.*, 2014; Carlson *et al.*, 2014), therefore the intention of this work was to explore cardiac autonomic regulatory pathways and central and peripheral responses following a programme of IET to ascertain potential mechanisms for BP reductions in a pre-hypertensive population. It is recognised that BP is modulated by $\dot{Q}$ and TPR, therefore changes in these must occur in order for BP to be reduced. Figure 9.2 demonstrates the physiological adaptations measured following IET, which may lead to reduced BP via $\dot{Q}$ and TPR mediated by other variables studied.

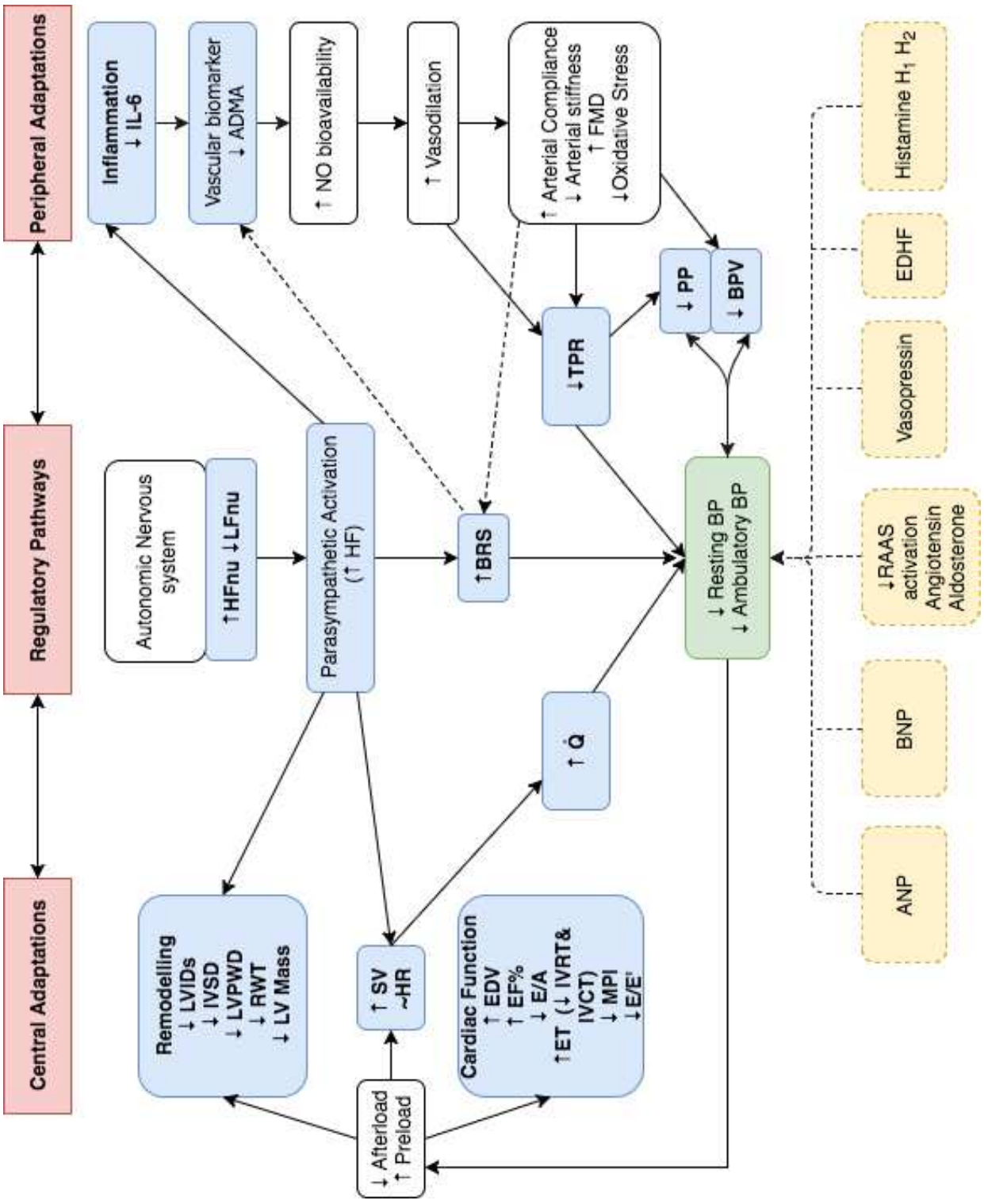

Figure 9.2. Physiological adaptations following IET and potential mechanistic pathways associated with observed changes. Arrows represent a measured increase or decrease in values. Variables presented in blue demonstrated a significant change following IET in this thesis. The variables in green demonstrate significant changes in the central hypothesis. Variables in yellow are additional factors which remain univestigated.

The acute physiological responses to a single session of IE are discussed above (9.2-9.5) and repeated stimulation of these pathways may shed light on the mechanisms responsible for chronic changes measured after 4-weeks of IET in a pre-hypertensive population. Reduced TPR, as measured in Chapter 6, facilitated via neurohumoral and vascular adaptations may explain the antihypertensive effects of exercise (Edwards *et al.*, 2007). The acute ischaemic conditions induced during IE, followed by reactive hyperaemia when a contraction is released, are thought to be the mediating factors which result in adaptations following IET (Hess and Smart, 2017). Metabolites resulting from ischaemia have been associated with managing inflammation (Pedersen and Hoffman-Goetz, 2000), vasodilation and vasoconstriction (Davidge, 2001), and tissue repair (Wang *et al.*, 2014), while the shear stress induced during hyperaemia is implicated in the NO pathway (Balligand *et al.*, 2009). A combination of these mechanisms may be responsible for central and peripheral adaptations, as measured in this thesis.

Consistent with improvements in lumen diameter and endothelium dependent vasodilation following dynamic exercise (Clarkson *et al.*, 1999), improvements in vascular function have previously been reported following IET alongside reductions in resting BP. Changes in vascular structure and function directly impact upon vasodilatory capacity of vessels, thus contributing to lowered TPR. Baross *et al.* (2012) reported increases in femoral artery diameter, femoral mean blood flow and femoral vascular conductance, following isometric leg training. Similarly, McGowan *et al.* (2006b) measured improved flow mediated dilatation (FMD) in hypertensive participants, but not normotensive participants (McGowan *et al.*, 2007), yet Badrov *et al.* (2016) reported improved FMD in young normotensive participants following the same IHG protocol. All of these findings represent improved vascular function and are likely to contribute to improved vasodilation, via the NO pathway, resulting in lowered BP via reduced TPR. However, all previous findings have been localised to the training limb, suggesting that the interplay of additional mechanisms may modulate a global response.

Regulatory cardiovascular control pathways may also directly or indirectly affect TPR, as demonstrated in Figure 9.2. Based on the acute recovery response to IE on

autonomic regulation (Chapter 4) and the cascade of events contributing to post exercise hypotension, it is thought that repeated stimulation of the acute recovery response to IE, results in a chronic shift in sympathovagal balance and an increased predominance of parasympathetic autonomic control (Chapter 7) (Akselrod *et al.*, 1981). This finding has been previously observed following IET by Taylor *et al.* (2003), who observed a trend for a decrease in LF/HF ratio following IET, alongside BP reductions in 9 medicated hypertensive participants. Wiles *et al.* (2010) and Millar *et al.* (2013) measured no change in HRV in young normotensive and medicated hypertensive participants respectively following IET; however, Millar *et al.* (2013) reported an increase in HR complexity, which may also be reflective of cardiac vagal modulation (Millar *et al.*, 2010). Increased parasympathetic control may be responsible for reduced TPR and reduced BP via the pathways outlined in Figure 9.2.

The baroreflex is a vagally mediated BP control mechanism and an increase in BRS at rest was measured after 4-weeks of IET alongside an increase in parasympathetic activity. Aerobic exercise training has been shown to increase BRS (Grassi *et al.*, 1994) in addition to reducing BP and MSNA in un-medicated stage 1 hypertensive participants (Laterza *et al.*, 2007), yet this this is the first study to assess the BRS response following IET. In patients with resistant HTN, long term lowering of BP has been achieved through electrically induced baroreflex activation and sympathetic suppression (Lohmeier and Iliescu, 2015). It is therefore possible that repeated stimulation of the baroreflex with IET forms a mechanism that resulted in chronic resetting of the baroreflex to a lower operating point to assist in maintaining lower resting and ambulatory BP, a concept supported by recent suggestions that the baroreflex may be involved in long term BP regulation and renal function (Lohmeier and Iliescu, 2015).

The arterial baroreflex is responsible for acute BP regulation and therefore may play a part in maintaining a lowered BP. Indeed ABP was significantly reduced (Chapter 6) confirming that reductions in BP were sustained across a 24-hour period in addition to resting measures. Although the BRS response to IET has not been previously investigated, BP reactivity may indirectly indicate BRS by demonstrating capacity to acutely diminish a hypotensive or hypertensive response (Swenne, 2013). Badrov *et al.* (2013b) reported an attenuated sBP response to a single IHG session following 10-

weeks of IHG training which may reflect the improved capacity of the baroreflex to buffer against acute BP rise. Howden *et al.* (2002) measured improvements in resting BP following IHG and ILT interventions, as well as improved orthostatic tolerance following ILT, which is also associated with BRS. Those with lower BRS are likely to experience greater hypotension in a tilt test condition, demonstrating impaired orthostatic tolerance (James and Potter, 1999), and suggesting an inability of the baroreflex to buffer against BP change. This supports the idea that improved BRS following IET may be responsible for attenuated BP reactivity following IET, and also supports reductions in ABP as the acute baroreflex mechanism is able to attenuate BP rise in an ambulatory state.

As such, although the baroreflex is considered a short-term BP control mechanism, vascular and neurohumoral responses following IET may also be linked to improved baroreceptor function. Arterial compliance is positively associated with BRS (Monahan *et al.*, 2001a), therefore previously reported IET induced improvements in arterial structure (Baross *et al.*, 2012) may improve the mechanoelastic properties of arteries, modulating the activation of mechanoreceptors, thus improving baroreflex activation. High PP is associated with arterial stiffness (Franklin *et al.*, 1999) therefore reduced PP, as measured in Chapter 6, may represent reduced arterial stiffness, which in turn may improve arterial compliance. In addition, steeper haemodynamic stress on the arterial wall, caused by persistent alteration in vessel wall tension, may produce increased endothelial stress and contribute to inflammation (Kim *et al.*, 2008), therefore a lowered PP and BPV may be implicated in reduced inflammation. Acute increases in BP induce an inflammatory cytokine response (Pedersen *et al.*, 2003), therefore improved ability to buffer against acute BP rises may assist in attenuating the inflammatory response to stressors, potentially contributing to lower circulating inflammatory markers, as reported in Chapter 7. These factors suggest a positive feedback loop between acute BP regulation via improved BRS, and a reduced inflammatory response, causing improved endothelial function and reduced TPR.

The autonomic nervous system is responsible for the release of neurotransmitters; where sympathetic activation is associated with the release of catecholamines (Esler *et al.*, 1990), while parasympathetic activity is associated with the release of the neurotransmitter acetylcholine (Gordan *et al.*, 2015). A reduction in catecholamines

and increase in acetylcholine is associated with the suppression of inflammation (Tracey, 2002), therefore changes in autonomic regulation are likely to be associated with changes in the neurohumoral response. Chapter 7 reported reductions in the inflammatory cytokine IL-6 and improved vascular biomarker ADMA. Overexpression of both of these factors results in the down regulation of eNOS and nNOS, and reduced NO bioavailability (Achan *et al.*, 2003; Libby, 2002), therefore a reduction in IL-6 and ADMA following IET may be linked to upregulation of NO and associated with improvements in vasodilatory capacity. The release of nNOS has been directly implicated in the central regulation of BP (Togashi *et al.*, 1992), while blockade of nNOS activity has been shown to cause HTN (Toda *et al.*, 2009). Furthermore, an increase in nNOS has been previously demonstrated with exercise training (Song *et al.*, 2009). As a neuronal signalling enzyme, nNOS release via parasympathetic neurons decreases catecholamine release and potentiates acetylcholine (Paton *et al.*, 2002), which may be responsible for reducing adrenergic stimulation at a pre-synaptic level (Massion *et al.*, 2003). Although limited to animal models, inhibition of nNOS has been shown to result in reduced vagal HR control, reduced HRV and reduced baroreflex bradycardia (Choate *et al.*, 2001; Jumrussirikul *et al.*, 1998). This suggests the increase in parasympathetic activation in Chapter 7, may trigger a chain of events leading to improved neuohumoral regulation, improving vasodilatory capacity and reducing TPR.

Reduced ADMA results in an increase in L-arginine production, which is implicated in BP regulation (Gokce, 2004) via nNOS. Although there is a dearth of human studies that have directly assessed NO responses in humans, Sessa *et al.* (1994) demonstrated that aerobic exercise training directly increases L-Arginine derived NO production in dogs, and that acetylcholine administration resulted in greater NO synthesis in trained vs control animals. Furthermore, a causal relationship between ADMA and LV dysfunction has been suggested (Achan *et al.*, 2003) as high concentrations of ADMA (Zocalli *et al.*, 2002) and cytokines (Torre-Amione *et al.*, 1996) have been shown to be associated with depressed LVEF. Improvements in LV function, including LVEF, were measured in Chapter 8, in addition to a reduction in plasma concentrations of ADMA and cytokine IL-6 in Chapter 7. In response to acute exercise, administration of ADMA was shown to increase TPR and BP, and reduce HR and $\dot{Q}$ (Achan *et al.*, 2003). This response reflects the inverse of the current findings, suggesting that a

reduction in ADMA may be associated with a reduction in TPR and BP, an increase in $\dot{Q}$, and associated cardiac functional responses.

A reduction in IL-6 may result in reduced oxidative stress (Wassmann *et al.*, 2004). In addition, IL-6 production is associated with increased angiotensin II production, which causes vasoconstriction and impaired endothelium dependant vasodilation (Wassmann *et al.*, 2004). This suggests that changes in IL-6 production may be associated with reduced RAAS activity, and by extension improved long term BP control, however this requires further investigation.

Markers of inflammation have not been previously measured following a programme of IET; however Peters *et al.* (2006) reported a reduction in oxidative stress alongside sBP reductions of 13 mmHg and dBP reductions of 2 mmHg in hypertensive patients following a 6-week IHG intervention. Oxidative stress causes the oxidation of low-density lipoproteins in the endothelium, causing plaque formation and contributing to atherosclerosis. In addition, oxidative stress down regulates the production of eNOS, therefore affecting the vasodilatory capacity of vessels via NO (Laufs *et al.*, 2005). Repeated stimulation of ischaemic reperfusion that occurs during an acute IE session may mediate reductions in oxidative stress as ischaemia has been shown to cause elevated antioxidant activity following acute aerobic exercise, which may play a role in preventing vessel damage (Santangelo *et al.*, 2003).

Additional factors have been suggested to play a role in the acute regulation of vascular tone, thus potentially mediating BP reductions through reduced TPR. The role of an EDHF, which acts independently of NO to stimulate relaxation of vascular smooth muscle via electrical coupling of endothelial cells through myoendothelial gap junctions (Korthius, 2011), remains a possible pathway. Although its identity remains unknown, when NO synthesis is blocked some hyperpolarisation mediated relaxation of vascular smooth muscle remains (Sandow, 2004), however these pathways require further investigation.

Furthermore, the role of histamine H_1 and H_2 receptors are implicated in recovery from acute IE (Halliwill *et al.*, 2013). Upregulation of acute inflammatory responses, and increased vascular conductance during recovery (Luttrell and Halliwill, 2017), may

have a training effect modulating the endothelial response following repeated stimulation. Recent research has also suggested much wider roles of histamine, which may include metabolism and cellular maintenance (Romero *et al.*, 2016). Research following IET has typically studied the major conduit vessels (Badrov *et al.*, 2016; Millar *et al.*, 2007; Baross *et al.*, 2012). However, the H_1 and H_2 receptor response may be a regulator of microvascular endothelium as the exercise induced capillary permeability may facilitate microcirculation to the skeletal muscle extravascular space (Luttrell and Halliwill, 2017), contributing to global reductions in TPR.

Studies observing IE and cardiac function have been limited to single acute isometric contractions, tested to observe responses to an increase in afterload. It is likely that repeated application of this increase in afterload, is responsible for mediating central physiological adaptations. Combined sympathetic and parasympathetic activation controls the electrophysiological properties of the myocytes, conduction system and SA node mediated by the baroreflex (Swenne, 2013). An increase in myocardial stretch, and neurohumoral changes mediated by regulatory pathways, are therefore likely to be the cause of structural and functional changes.

A parasympathetically mediated increase in acetylcholine release will result in the upregulation of eNOS from endocardial endothelial cells and nNOS from the cardiac autonomic nerve ganglia, resulting in improved cell communication and vasodilation of cardiac muscle. Improved vagally mediated relaxation allows greater stretch capacity in response to increased preload resulting in better dilation and filling of the ventricle, which was evidenced through improvements in diastolic function reported in Chapter 8. Greater ventricular stretch will stimulate shear-induced release of eNOS (Tinken *et al.*, 2010). Upon repeated stimulation, these pathways may also lead to a residual increase in NO bioavailability and contribute to improved vasodilation and relaxation in a resting state.

Aerobic exercise induces improvements in myocardial β-adrenergic responsiveness and improved Ca^{2+} handling (MacDonnell *et al.*, 2005), mechanisms which may prevent calcification of cardiomyocytes, improve LV remodelling and improve cardiac contractility (Pironti *et al.*, 2016). Improvements in β-adrenergic responsiveness are associated with improved regulation of inotropy, lucitropy and chronotropy (Libonati,

2013). These mechanisms offer explanation for significant improvements in cardiac performance measured in Chapter 8. A reduction in calcification and stiffness via improved Ca^{2+} handling, may have caused improved preload induced stretch of the ventricular walls through repeated IE, thus resulting in lengthening of cardiomyocytes, and reductions in LVPWd, IVSd, RWT.

The remodelling effects on the ventricle and lowered BP, resulted in reduced ventricular afterload at rest. This caused an increase in SV demonstrating a greater LV stretch capacity, as shown by an increase in EDV with no change in ESV. This adaptation may be responsible for the increase in $\dot{Q}$, as HR did not change following IE, as reported in Chapter 6.

Improved cardiac time intervals (increased ET and reduced IVRT and IVCT) may also reflect pressure changes in the arteries, as these reflect the time taken for ventricular pressure to match or exceed arterial pressure (Levick, 2003). A reduced IVCT shows that a shorter time was required for pressure within the ventricle to match aortic pressure, and is therefore directly moderated by change in arterial pressure. This suggests that changes in cardiac performance were the result of reduced BP, rather than a mechanism for this.

Blood pressure is mediated by $\dot{Q}$ and TPR ($mBP = TPR \times \dot{Q}$). Following 4-weeks of IET, there was a significant reduction in BP, as well as a significant reduction in TPR, however $\dot{Q}$ was increased. It is therefore logical to assume that the relative reduction in TPR was greater than the relative increase in $\dot{Q}$ making TPR the key factor mediating BP reductions. Improvements in cardiac structure were reported, which may have been mediated by improved cardiovascular control of BP. It is not clear at what stage of the 4-week intervention changes in BP occurred, as early reductions may have played a role in cardiac remodelling through chronically reduced afterload. Daytime ABP is directly associated with LV mass (Polonia *et al.*, 1992); higher IVSd and LVPWd are directly associated with higher sBP (Eliakim-Raz *et al.*, 2016); and IVSd is considered a strong predictor of future systolic HTN (Grossman *et al.*, 2008), suggesting that reductions in BP and LV remodelling may work on a reciprocal basis.

As outlined in Table 2.2, page 28 much of the prior research into IET has focussed on either normotensive or hypertensive participants, therefore differences in intervention outcomes could be attributed to differences in baseline participant characteristics in many cases. Figure 2.1 demonstrated the pathogenesis of above optimal BP from NTN to pre-HTN and HTN, and associated dysregulation of the cardiovascular system that has been previously associated with raised BP. Different causal factors are responsible for increases in BP in different patients (Carretero and Oparil, 2000). Therefore, it is likely that mechanistic pathways responsible for BP reductions are not the same in all populations. Following IET, greater BP reductions occur in pre-HTN than in NTN (Millar *et al.* 2007). This may be because the cardiovascular system is displaying optimal function and is therefore resistant to change. In contrast, established HTN is associated with a number of cardiovascular impairments and structural changes to the cardiovascular system. Such pathways may be in a state of irreversible damage, and may therefore be limited in their capacity to improve. Isometric exercise training in this thesis resulted in reductions in resting and ambulatory BP, in addition to physiological improvement of a number of other cardiovascular variables. Figure 9.3 demonstrates the effect of IET on BP and cardiovascular variables in a pre-hypertensive population. These physiological improvements may demonstrate a reverse in BP increases towards HTN, which may be associated with a reduced risk of CVD. This highlights the potential impact of IET as an intervention to reduce BP and improve wider cardiovascular function in pre-HTN.

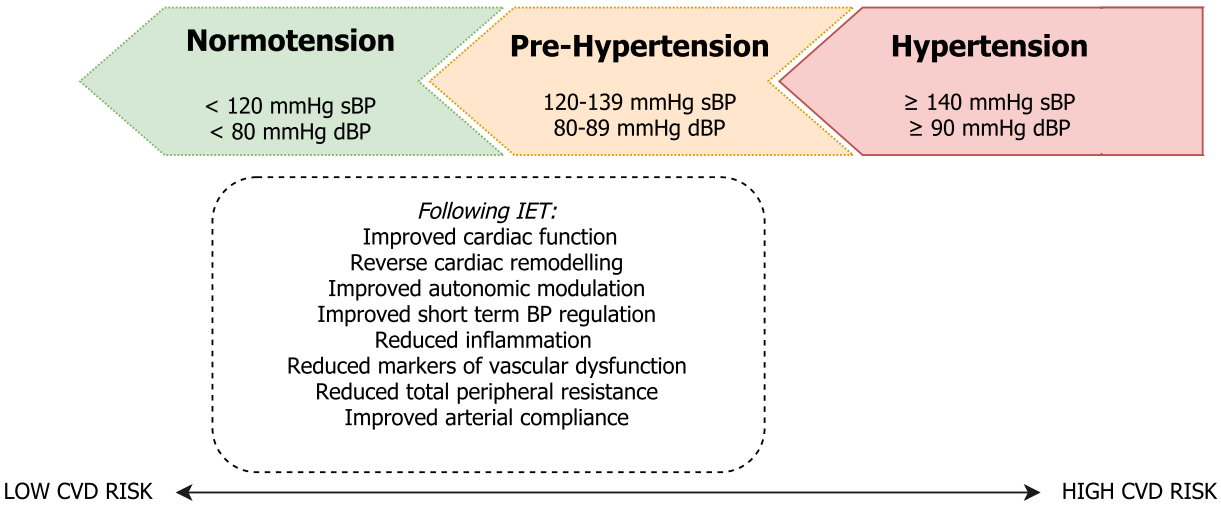

Figure 9.3. Improvements in cardiovascular physiology following IET in pre-HTN.

9.8 Clinical impactions of findings

The primary outcome variable for this thesis was the detection of resting BP reductions. As hypothesised, based on previous findings in this field, sBP, dBP and mBP were significantly reduced following a 4-week IET intervention. Furthermore, these reductions exceed the minimal clinically important difference of 5 mmHg defined by the National Clinical Guideline Centre (2011). A 10 mmHg reduction in sBP and a 5 mmHg reduction in dBP is associated with a 40% lower risk of CVD mortality and a 30% lower risk of IHD and other vascular causes (Lewington *et al.*, 2002). As such, findings from this thesis demonstrate a potential measurable reduction in the risk of future HTN (Vasan *et al.*, 2001a) and CVD.

Additional variables add further support to this risk reduction. Ambulatory BP monitoring is considered superior to resting office measurements (Pickering *et al.*, 2006) and ambulatory reductions may also be associated with reduced future risk (Verdecchia, 2000). Reductions in PP, BPV (Mancia *et al.*, 2001), IL-6 (Lee *et al.*, 2012), ADMA (Miyazaki *et al.*, 1999) and LV Mass (Levy *et al.*, 1990a; Koren *et al.*, 1991) and increases in BRS (Lanfranchi and Somers, 2002) and parasympathetic activation (Gerritsen *et al.*, 2001), may also be associated with improved prognosis following IET, as well as an attenuated risk of progression to HTN.

Aerobic physical activity is currently recommended as a non-pharmacological treatment in pre-HTN, and alongside pharmacotherapies in stage 1 HTN (Pescatello *et al.*, 2015; Mancia *et al.*, 2013; Brook *et al.*, 2013). Dynamic resistance exercise is now included within exercise recommendations to supplement resistance programmes; however no guidelines currently exist for the use of IET to lower BP despite support for the use of IHG training by the American Heart Association (Brook *et al.*, 2013). Findings of this thesis, in addition to prior evidence (Inder *et al.*, 2016; Carlson *et al.*, 2014; Millar *et al.*, 2014; Cornelissen *et al.*, 2011), suggest that BP reductions achieved following IET interventions, are equal to or exceed BP reductions observed following recommended exercise types (Borjesson *et al.*, 2016), yet it is evident that isometric leg training remains under researched in this field.

When compared with other intervention types, the findings of this thesis demonstrate greater reductions in BP than other lifestyle modifications that are currently recommended in the same population. A reduction in salt intake resulted in a sBP reduction of 7.1 mmHg (Sacks and Campos, 2010). An exercise and/or diet induced weight reduction of 5.1kg is associated with reductions of 4.44 mmHg (sBP) and 3.57 mmHg (dBP) (Neter *et al.*, 2003). A reduction in alcohol consumption is associated with BP reductions of 3.31 mmHg (sBP) and 2.04 mmHg (dBP) (Xin *et al.*, 2001). When compared with pharmacological therapies, a meta-analysis by Law *et al.* (2009) demonstrated that multiple drug treatments may be needed to control BP to within optimal ranges. Indeed, it is estimated that daily consumption of two drugs would be required to gain a reduction of 10.2 mmHg (sBP) in an individual with a baseline BP of 130 mmHg (Law *et al.*, 2009). In more advanced HTN, most patients require a minimum of 2 concurrent therapies in order to achieve adequate BP reductions (Law *et al.*, 2009), while some people remain resistant to BP reductions even with daily ingestion of 3-5 adjunct anti-hypertensive medications (Ghofrani *et al.*, 2015). Many anti-hypertensive medications currently prescribed act to increase the bioavailability of NO (Hermann *et al.*, 2006) or block over activity of autocrine and paracrine regulatory pathways; pathways which appear to be mechanistically affected by the IET intervention employed within this thesis. In a pre-hypertensive population, there is scope to employ IET to prevent the progression to HTN and avoid future need for pharmacotherapy. The use of IET in established HTN to compliment other treatments requires further research.

9.9 Limitations

The studies in this thesis were carried out in a physically inactive population of pre-hypertensive males, within the ages of 30-65, therefore the external validity of the findings may be somewhat limited. The inclusion of female participants in future research is essential in order to develop IET as an intervention accessible to a wider population. In addition, both acute and training studies were powered to detect changes in the primary outcome variable of resting BP. Although significant results in some ABP, neurohumoral, cardiac structural and functional variables were detected,

additional variables may also reveal significant findings with the appropriate sample size.

With regards to the acute studies, Chapter 4 recorded the autonomic and haemodynamic recovery responses in the 5-minutes immediately following IE only; therefore the responses beyond this period remain unknown with regards to this isometric wall squat training protocol and longer recovery responses may be of additional interest.

Inherent methodological limitations apply to non-invasive measures of cardiac autonomic modulation, which are acknowledged. However, prior research supports HRV as a valuable tool for measuring cardiac autonomic control in healthy and clinical populations (Akselrod *et al*. 1985; Taskforce of the European society of Cardiology 1996). Guidelines recommend HRV measurements are taken over a minimum duration of 5-minutes. However, IE training methodology dictates that contractions should be just 2-minutes in duration. As such, all IE parameters are reported as mean responses from a 2-minute period while baseline and recovery are taken from a 5-minute recording. Other IE research has recorded HRV over the same truncated period (Millar *et al.*, 2009a), as has research in clinical populations (Sharma *et al.*, 2015).

Although neurohumoral responses to IET were measured in Chapter 7, the acute inflammatory responses to a single session of IE were not measured in this research. Prolonged strenuous exercise has been shown to elicit a pro-inflammatory response (Ostrowski *et al.*, 1999), yet other forms of activity may have an acute anti-inflammatory response. Kaspar *et al.* (2016) reported no change in CRP, IL-6 or IL-10 within 30 minutes of exercise training; however a significant change in IL6/IL10 ratio was reported, suggesting an acute anti-inflammatory response. This acute finding may contribute to chronic improvements following a period of exercise training.

In addition, Chapter 5 measured echocardiographic data during recovery from IET, as the aim of this research was to ascertain the effects of a complete session of IE. However, the progressive cardiac structural and functional responses during the four bouts of the IE session may be of further interest.

Chapters 6, 7 and 8 investigated the effects of a 4-week isometric wall squat training programme. The exercise training was home-based, with the aim to minimise barriers that commonly inhibit participation and adherence to PA interventions. This meant that training was unsupervised; however, regular communications between experimenter and participants, and feedback of training data ensured that participants adhered to the training intervention, and that training was completed at an appropriate intensity. Despite the low equipment costs, the feasibility of this intervention with regards to training prescription and adherence requires further investigation. The current training prescription and reporting method, although effective, is labour intensive and currently requires extensive and time consuming initial assessment within a laboratory, prior to home-based training prescription. This study also offered remote support, and digital training reminders to ensure adherence, which further added to experimenter time commitment.

The training study of this thesis resulted in significant reductions in resting and ambulatory BP. Previous research has demonstrated BP reductions following the same intervention in normotensive participants (Wiles *et al.*, 2017) and IHG interventions have demonstrated BP reductions in hypertensive populations (Millar *et al.*, 2009a), with the greatest reductions observed in those with the highest baseline BP (Millar *et al.*, 2008; Millar *et al.*, 2007). It is therefore likely that this intervention would also elicit BP reductions in a hypertensive population. In addition, research has reported no differences in BP reduction between genders following IET (Somani *et al.*, 2017; Hanik *et al.*, 2012), suggesting that this intervention may also elicit BP reductions of the same magnitude in female participants. The extension of this work into different races is also necessary, as differences in BP responses to anti-hypertensive treatments are well reported, suggesting potential differences in BP reduction mechanisms.

Of the pre-hypertensive cohort in this study, all of the 24 participants were responsive to BP change. However it is recognised that anti-hypertensive interventions do not always elicit BP reductions and that some patients have resistant HTN, or are responsive to some treatments and not to others (Johnson, 2008). Although race has been shown to predict susceptibility to treatments to a certain extent, it does not sufficiently separate between those who will and will not respond well to a given treatment. It has therefore been suggested that the role of a ‘responsive’ genotype,

alongside demographic and lifestyle information will be most effective in predicting the response to pharmacotherapy (Johnson, 2008), which may also be true of predicting future responsiveness to IET in wider populations.

Chapter 7 measured a limited number of neurohumoral markers. The inclusion of IL-10, an anti-inflammatory cytokine that may inhibit the release of TNFa and other pro-inflammatory mediators (van der Poll *et al.*, 1997), may have provided additional information about neurohumoral control through IET. In addition, the roles of atrial ANP and BNP may be of great importance in establishing a causal link between peripheral vascular, autonomic and cardiac responses to IET in the long-term control of BP.

Markers of vascular function were measured in this thesis through plasma blood samples and indirect measurement of TPR. Measurement of flow mediated dilatation and lumen diameter in the femoral artery may have provided additional information regarding the localised vascular response and endothelial function.

9.10 Future directions

This thesis has established the effectiveness of IET on a population of pre-hypertensive males. This is a significant finding, as this form of training may serve as a means of attenuating progression of BP to HTN. Future research must seek to assess the benefits of isometric wall squat training in Stage 1 HTN. Based on previous research using IHG training (Millar *et al.*, 2009b; Taylor *et al.*, 2003), it is likely that the same hypotensive benefits will extend to those with HTN.

This thesis demonstrated reductions in BP after 4-weeks of IET. Previous research has demonstrated comparable findings following interventions of up to 10-weeks (Badrov *et al.*, 2013b; Taylor *et al.*, 2003), however no IET interventions have exceeded this duration. Given that anti-hypertensive intervention strategies, both pharmacological and non-pharmacological, typically require life-long adherence, it is necessary that IET interventions with long term durations (6 or 12 months) are studied to establish whether the benefits of IET progress further, are maintained or cease. In such a case, it is likely that progression or overload may need to be applied in order to continue to

illicit the acute physiological responses measured in Chapters 4 and 5 and maintain the adaptations demonstrated in Chapters 6, 7 and 8.

In line with previous suggestions, it is also important to understand the effects of cessation of IET. Study 2 of this thesis employed a randomised cross-over design and in the group who completed IET in the first stage, BP had returned to baseline during the 3-week control period meaning that any reductions in BP were lost in the washout period. In addition, no other raw variables were significantly different between pre-IET and pre-control conditions, suggesting that no change in any variable endured throughout the 3-week control period. Wiley *et al.* (1992a) also found that BP reductions were lost after a period of 10 days. This further supports the need for a longer-term trial in order to establish any lasting benefits from this training type.

This is the first study to assess neurohumoral adaptations in response to IET. The reductions in inflammatory markers are noteworthy, however as this study was powered to detect changes in resting BP, it is possible that it was not adequately powered to detect changes in all of the selected markers of inflammation and vascular biomarkers. Inflammatory responses may play a part in improved vasodilatory capacity, as previously discussed, and improvements in the NO pathway are likely to be associated with improvements in TPR; however further vasodilatory pathways require exploration. Endothelin, prostaglandin, vasopressin, ANP and BNP are all mediators of vasodilation, which may also contribute to reductions in BP therefore their potential role in IET induced BP reductions could be studied.

9.11 Hypotheses

9.11.1 Acute investigations

Study 1

1:	*H1:*	There will be a progressive increase in BP during IE followed by immediate post exercise hypotension.	ACCEPT
2:	*H1:*	There will be an increase in sympathetic activation and a reduction in parasympathetic modulation during IE. In recovery, there will be a reduction in sympathetic modulation and an increase in parasympathetic modulation.	ACCEPT
3:	*H1:*	Post IE alterations in haemodynamic and cardiac autonomic modulation are associated with improved baroreceptor reflex control mechanisms.	ACCEPT

Study 2

1:	*H1:*	IE will significantly improve diastolic function, measured by E/A ratio during recovery.	REJECT
2:	*H1:*	Post IE induced hypotension is associated with a reduced estimated LV filling pressure during recovery.	ACCEPT

9.11.2 Chronic Investigations

Study 3

1:	*H1:*	Four weeks of IET will significantly reduce 24-hour ambulatory BP	ACCEPT
2:	*H1:*	Four weeks of IET will elicit a reduction in HR and increase SV, with no change in $\dot{Q}$.	REJECT

Study 4

1:	*H1:*	Four weeks of IET will improve cardiac autonomic modulation with a predominance of parasympathetic over sympathetic activity.	ACCEPT
2:	*H1:*	Four weeks of IET will significantly increase BRS.	ACCEPT
3:	*H1:*	Four weeks of IET will significantly reduce plasma concentrations of IL-6, TNF-α and hs-CRP	REJECT
4:	*H1:*	Four weeks of IET will significantly reduce plasma concentrations of ICAM, VCAM and ADMA	REJECT

Study 5

1:	*H1:*	Four weeks of IET will significantly reduce estimated LV filling pressure.	ACCEPT
2:	*H1:*	Four weeks of IET will significantly alter isovolumetric relaxation time.	ACCEPT
3:	*H1:*	Four weeks of IET will significantly reduce the myocardial performance index.	ACCEPT

9.12 Conclusion

Overall, the work presented in this thesis has elicited reductions in resting and ambulatory BP, in a population of pre-hypertensive males, following a 4-week programme of home-based isometric leg training. This finding adds to a body of research supporting the role of IET to reduce BP.

In addition, the acute and chronic effects of IE on cardiac autonomic regulatory pathways and central and peripheral responses were explored, helping to provide mechanistic explanation for BP reductions following this type of exercise training, which have not been previously investigated.

In conclusion, this work advocates the use of isometric wall squat training as a lifestyle modification for the prevention of HTN in a pre-hypertensive population, which may help to reduce future risk of CVD and associated economic burden. Findings also suggest that IET elicits a series of cardiovascular improvements, which may have wider health implications and contribute to improved BP regulation.

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Appendix 1

11 August 2014 Ref: 12/SAS/122

Dr Jamie O'Driscoll
School of Human and Life Sciences
Faculty of Social and Applied Sciences

Dear Jamie

Project Title: ***An investigation into the effects of home based isometric exercise training on arterial blood pressure in a sedentary population with pre-hypertension.***

The Faculty of Social and Applied Sciences Research Ethics Committee reviewed your application during November 2012, and corresponded on the issues raised at various times thereafter.

The Chair of the Committee is content that the amendments and clarifications subsequently submitted now meet the Committee's requirements in full. I am therefore writing to confirm formally that you can commence your research. Any significant change in the question, design or conduct of the study over its course should be notified to the **Research Office**, and may require a new application for ethics approval. You are also required to inform me once your research has been completed.

With best wishes for a successful project.

Yours sincerely

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Canterbury Christ Church University

Section of Sport and Exercise Sciences

Informed Consent & Health and Fitness Questionnaire

Name: ……. Postcode: ……..

Date of Birth: …… Age: …… Sex: ……

Please answer the following questions by *circling* the appropriate response and if necessary providing extra information in the spaces provided.

ANY INFORMATION CONTAINED HEREIN WILL BE TREATED AS CONFIDENTIAL

1. How would you describe your present level of fitness?

Untrained / Moderately trained / Trained / Highly trained

2. Average number of hours spent exercising **…….……per week**

3. How would you describe your present bodyweight?

Underweight / Ideal / Slightly overweight / Very overweight

4. How would you describe your smoking habits?

Non smoker / Previous smoker / Currently smoking

5. How would you describe your alcohol intake?

Never Drink / An occasional drink / A drink every day / More than one drink a day

(Note 1 drink = 1 unit)

6. Have you had to consult your doctor within the last six months? **Yes / No**

If you have answered **yes**, please give details: **…….**

7. Are you presently taking any form of medication? **Yes / No**

If you have answered **yes**, please give details: **……**

8. Do you suffer or have you ever suffered from any of the following?

a. Diabetes	**Yes / No**	b. Asthma	**Yes / No**
c. Epilepsy	**Yes / No**	d. Bronchitis	**Yes / No**
e. Any form of heart complaint	**Yes / No**	f. Serious Back or Neck Injury	**Yes / No**
g. High blood pressure	**Yes / No**	h. Aneurysm [1] or Embolism[2]	**Yes / No**

1: Arterial wall weakness causing dilation. 2: Obstruction in the Artery.

9. Is there a history of heart complaint in your family? **Yes / No**

If you have answered **yes**, please give details: **……**

10. Do you have any allergies? **Yes / No**

If you have answered **yes**, please give details: **……**

11. Do you currently have any form of muscle or joint injury? **Yes / No**

If you have answered **yes**, please give details: **……**

12. Have you had to suspend your normal training/physical activity in the last two weeks? **Yes / No**

If you have answered **yes**, please give details: **……**

An investigation into the effects of home based isometric exercise training on arterial blood pressure in a physically inactive population with pre-hypertension.

INFORMED CONSENT

The full details of the tests have been explained to me. I am clear about what will be involved and I am aware of the purpose of the tests.

I know that I am not obliged to complete the tests. I am free to stop the test at any point and for any reason.

The test results are confidential and will only be communicated to others such as my coach if agreed in advance.

As far as I am aware, there is nothing that might prevent me from successfully completing the tests that have been outlined to me.

Signature of Participant: ……..

Signature of Sport Scientist: ……

Date: ……

PARTICIPANT INFORMATION

A research study is being conducted at Canterbury Christ Church University (CCCU) to ascertain the benefits of a home-based exercise training protocol on reducing arterial blood pressure.

Background

The beneficial effects of continuous aerobic exercise such as walking, jogging or cycling upon cardiovascular health are well documented. However, the effect of regular isometric exercise (the production of force without movement, i.e. a static muscle contraction) on cardiovascular health is less well understood. Isometric exercise has been shown to cause a reduction in resting blood pressure; however this research has been laboratory based and the benefits of this type of exercise training in the privacy and comfort of someone's home has not been established. Furthermore there has been no previous work using the simple isometric wall squat (see figure 1) as an exercise for isometric training. As a result, this study aims to determine whether the simple wall squat exercise can be successfully used to reduce resting blood pressure after 4 weeks of home-based isometric training.

Figure 1. Isometric wall squat exercise

Distinction between Pre-Hypertension and Hypertension

Pre-hypertension was formerly known as high-normal and above-optimal blood pressure. The reason for this change was to highlight the increased risk of cardiovascular disease compared to individuals with normal blood pressure and increase clinical and public health attention on prevention. Current guidelines suggest lifestyle modification for individuals with pre-hypertension, such as exercise, healthy eating, weight loss, and reduced alcohol and salt consumption to help lower resting blood pressure. Individuals classed as hypertensive require medication (in addition to lifestyle changes) to help lower resting blood pressure and are at higher risk of cardiovascular events compared to pre-hypertensive individuals.

What will you be required to do?

If you decide to participate in this study, you will be asked to visit the Sport Science laboratory at Canterbury Christ Church University for 30 minutes on 7 separate occasions and complete a 4-week home-based isometric training programme.

To help us obtain the necessary data for analysis in this study, participants need to be willing to perform:

1. An initial familiarisation visit (30 minutes).
2. A continuous incremental (gradually increasing effort) exercise test (approximately 10 minutes of exercise).
3. On a separate day, a supervised session of isometric exercise in the laboratory, as per the protocol that will be repeated in training.
4. An individual home-based isometric training programme, (8 minutes of exercise, 3 times per week for 4 consecutive weeks) with cardiovascular variables measured before and after the 4 week training period.
5. Provide blood samples at baseline and post-exercise. Participants will need to be fasted for at least 4 hours before testing and can only consume water within this time.
6. Wear a 24-hour ambulatory blood pressure montitor at baseline and post-exercise

To participate in this research you must:

- Aged 30 to 65.
- Be a physically in active male, non-smoker.
- Not have a musculoskeletal injury.
- Have no known medical problems that may impair your ability to participate in the study in any way.
- Have been free from illness/infection for the preceding 2 weeks to testing.
- Not be receiving any treatment for any medical conditions.
- Not be on any drugs or medication that might interfere with the physiological measures of the study and/or not to have been on medication for the preceding 4 weeks prior to testing.
- Have blood pressure >120 and <140 mmHg systolic and >80 and <90 mmHg diastolic.

Familiarisation Visit

Upon arrival to the Sport Science laboratory you will be asked to complete a standard health and fitness questionnaire and will then have an electrocardiogram (ECG) (electrical tracing of your heart muscle) and resting blood pressure measured. Before starting any exercise testing, you will be informed of your resting blood pressure in relation to current UK guidelines (British Hypertension Society 2004). If, for any reason, your blood pressure level is high it will be recommended that you have your blood pressure status confirmed by a qualified medical practitioner. In order to facilitate this process you will be issued with a standard feedback letter detailing your blood pressure results. Should a qualified medical practitioner diagnose high blood pressure then you will be unable to participate in this particular research study.

Once the preliminary health screening has been successfully completed you will be given the opportunity to raise any questions you may have regarding the nature of the study before being asked to sign an informed consent form. Following this you will be introduced to the isometric wall squat exercise, as well as being familiarised with the protocol, equipment and testing procedures to be used in the study.

You will be asked to follow some pre-assessment requirements before each visit to the Sport Science laboratory:

- No caffeine (tea, coffee, fizzy drinks, chocolate) within 4 hours of a visit
- No alcohol within 12 hours of a visit

- No strenuous physical exercise within 24 hours of a visit. If you feel fatigued prior to testing please do not hesitate to inform us straight away and we will rearrange your visit.
- No food within 4 hours of a visit

It is also important that participants are free from any medical condition that could conceivably affect their performance. As such if you fall ill or get injured during the study, please do not hesitate to inform us straight away and we will rearrange your visits accordingly.

Due to the fitting of monitoring equipment it will be necessary to wear shorts and a t-shirt for all tests. Trainers must also be worn for all exercise sessions.

Continuous Incremental Isometric Test

You will be required to visit the Sports Science laboratory to complete an initial continuous incremental isomeric test, which will last approximately 10 minutes. This test will be used to determine how hard you have to work during the 4-week home-based isometric training programme (your individualised training intensity).

The incremental test is split into 2-minute stages and over time the exercise will get harder. You will begin the incremental test at an easy exercise position of 135˚ knee joint angle, see figure 2, A. This position will be held for 2 minutes. After this 2 minute stage the exercise will gradually get harder; the knee joint angle will be decreased by 10˚. The exercise will get harder every 2 minutes until you reach the last stage (see figure 2, E) or until you feel you cannot exercise any longer. It is a continuous test so there will be no rest between stages. Heart rate and blood pressure will be recorded continuously throughout the exercise.

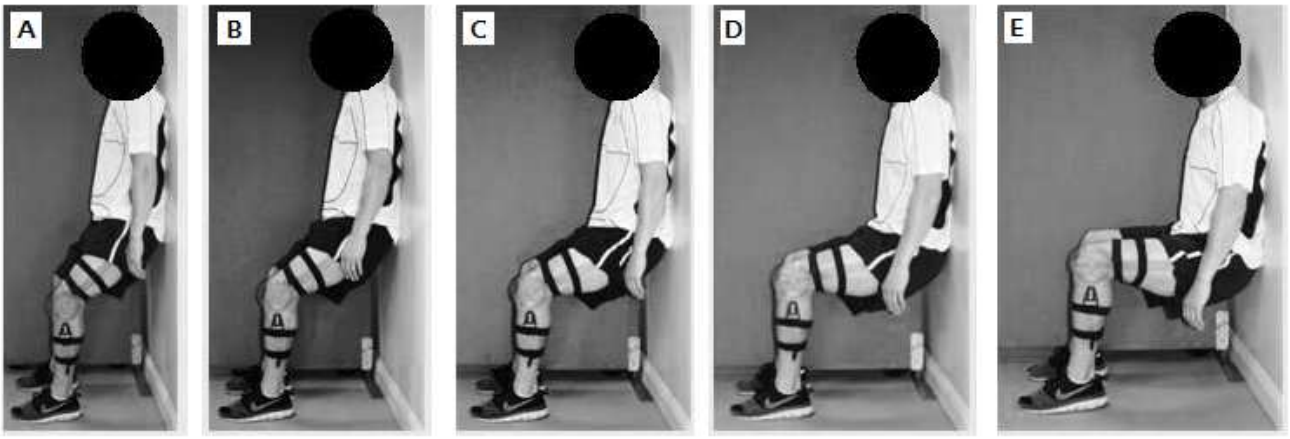

Figure 2. Different isometric wall squat exercise intensities:
A - 135˚, B - 125˚, C - 115˚, D - 105˚, E - 95˚

Laboratory based Isometric exercise

Prior to home-based training, you will complete one session if isometric exercise, as per the procedures of the home based isometric exercise, in the laboratory. This will consist of 4 isometric wall squats in a fixed position each lasting 2-minutes, with a 2-minute rest between squats. This will assess the intensity that is prescribed to you from the incremental isometric test. Heart rate and blood pressure will be recorded continuously throughout the exercise session.

4 Week Home-Based Isometric Training Sessions

You will be required to complete a 4-week home-based isometric exercise training programme consisting of 12 sessions in total. You will need to train 3 days a week and all training sessions will be completed in a location of your choice. Each training session will consist of 4 isometric wall squats at a fixed exercise position. Each isometric wall squat will last 2-minutes and there will be 2-minutes of seated rest between each exercise.

You will be required to come to the Sport Science laboratory before and after the 4-week training period to have resting measures of several cardiovascular variables, such as blood pressure and heart rate.

Potential Risks: Isometric exercise is known to be associated with a rise in blood pressure and heart rate during exercise, but there is currently no evidence to suggest that this presents any risk to pre-hypertensive individuals. The risks of sudden cardiac death or acute myocardial infarction are higher in adults than in younger individuals, which is due to higher prevalence of cardiovascular disease in the older population. In addition, the rates of sudden cardiac death and acute myocardial infarction are higher in sedentary individuals. People with pre-hypertension are at greater risk of cardiac events, stokes, and renal disease compared to individuals with normal blood pressure. Furthermore for those unaccustomed to isometric exercise training, this type of activity may be perceived as being uncomfortable and may also lead to some muscle soreness in the following 48-hour period. This is perfectly normal and will have no long lasting effects. This study has been approved within the University by the faculty of Social and Applied Sciences research ethics committee and complied with the British Association of Sports and Exercise Science (BASES) testing guidelines.

Benefits

The completion of a standardized health screen, which includes the measurement of blood pressure and ECG, provides you with an ideal opportunity to discuss the health and fitness benefits of exercise with a qualified Exercise Scientist. Over the 4 week training period you may well experience improvements in isometric leg strength and cardiovascular variables associated with long term health.

Since this is a training study you will receive personal training using a structured isometric exercise program along with regular feedback on your progress. You will also experience firsthand how to use scientific principles to inform your own training and learn how to exercise at appropriate levels of intensity.

Additionally there will be further opportunities (if you so wish) to become involved in future research investigations with similar benefits.

Feedback

After your involvement in the study is complete, you will receive feedback on your assessment results.

Confidentiality

All data and personal information will be stored securely within CCCU at the Section of Sport and Exercise Science in accordance with the Data Protection Act 1998 and the University's own data protection requirements. Data can only be accessed by Katrina Taylor, and supervisors Dr Jamie O'Driscoll and Dr Jonathan Wiles. After completion of the study, all data will be made anonymous (i.e. all personal information associated with the data will be removed). In line with University insurance policies, your name will be recorded with CCCU's insurance officer Mr Tim Hawkins as being involved in this study.

Dissemination of results

The results of the study may be published, however all participants will be made anonymous.

Deciding Whether to Participate

If you have any questions or concerns about the nature, procedures or requirements for participation do not hesitate to contact me. Should you decide to participate, you will be free to withdraw at any time without having to give a reason.

Any Questions?

Please contact Katrina Taylor

Address: Af50, Section of Sport and Exercise Science,
Canterbury Christ Church University,
North Holmes Road,
Canterbury,
Kent,
CT1 1QU
Tel: 01227 767700 ext. 3145 or 07982715173
Email: kt142@canterbury.ac.uk

Appendix 4
CONSENT FORM

Title of Project: An investigation into the effects of home based isometric exercise training on arterial blood pressure in a physically inactive population with pre-hypertension.

Name of Researcher: Katrina Taylor

Contact details:
Address: Af50, Section of Sport and Exercise Science
Canterbury Christ Church University
North Holmes Road
Canterbury
Kent
CT1 1QU

Tel: 01227 767700 ext. 3145 or 07982715173
Email: kt142@canterbury.ac.uk

	Please initial box
1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.	☐
3. I understand that any personal information that I provide to the researchers will be kept strictly confidential	☐
4. I agree to take part in the above study.	☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Copies: 1 for participant
1 for researcher

Appendix 5
Training Session Manual

Name: …… Participant I.D: ……

Training Session Information

- You will complete 12 training sessions in total over a 4 week period (3 sessions per week).
- Each training session requires you to perform a total of 4 wall squat exercises each lasting 2 minutes.
- Each wall squat will be performed at a specific angle (calculated from your incremental test) so that you reach your target heart rate.
- There will be 2 minutes seated rest between each wall squat.
- Each training session will last 14 minutes in total (see Exercise Protocol).

Your wall squat training angle is: ……

Your target heart rate is: …….....

- You must leave 24 hours between each training session to ensure adequate recovery.
- You should try to ensure that all training sessions are at the same time of the day
-

Equipment
You will be given the following equipment to use whilst exercising at home:

1. Bend and Squat

- The Bend & Squat is a piece of exercise equipment designed to ensure that you are squatting at the correct angle.
- You will need to adjust the Bend & Squat for your specific training angle (*see Bend & Squat Instructions*).
- Your Bend & Squat measurements are:

WALL: …… **FLOOR**: ……..

- You must ensure that the Bend & Squat is always set to these measurements before use.
- For instructions on how to use the Bend & Squat to perform the Isometric Wall Squat Exercise see *page 6*.

2. Heart Rate Monitor

- You will record heart rate throughout the wall squat using the heart rate monitor provided
- At the end of every 2 minute wall squat you need to write down your heart rate on the Data Sheet provided
- At the end of every training session you will need to text or email Katrina Taylor your heart rate data for the 4 exercises.
- Your heart rate monitor will be replaced at the end of each week so that your data can be downloaded.

3. Rate of Perceived Exertion (RPD) Scale

- You will be provided with an RPD scale. This scale is used to measure how much discomfort you feel there is in your legs.
    - At the end of every 2 minute wall squat you need to write down your RPD on the Data Sheet provided

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Data Sheet

Please record your heart rate and RPD at the end of EVERY 2-minute wall squat exercise. Please send me your training data after each session via text message, e-mail or Google sheet.

	Training Session	Resting	Exercise 1		Exercise 2		Exercise 3		Exercise 4	
		Heart Rate	Heart Rate	RPE	Heart Rate	RPE	Heart Rate	RPE	Heart Rate	RPE
WEEK 1	1									
	2									
	3									
WEEK 2	4									
	5									
	6									
WEEK 3	7									
	8									
	9									
WEEK 4	10									
	11									
	12									

RPD Scale

This scale is used to measure how much discomfort you feel there is in your legs.

0	Nothing at all	"No P"
0.3		
0.5	Extremely weak	Just noticeable
1	Very weak	
1.5		
2	Weak	Light
2.5		
3	Moderate	
4		
5	Strong	Heavy
6		
7	Very Strong	
8		
9		
10	**Extremely strong**	**"Max P"**
11		
ǂ		
●	Absolute maximum	Highest possible

Borg CR10 scale

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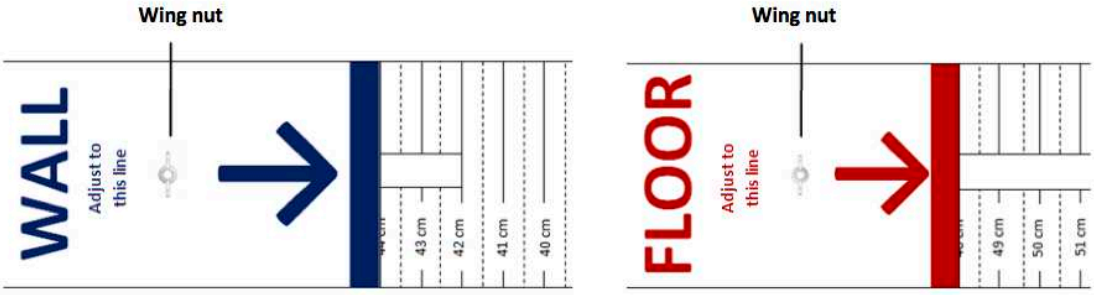
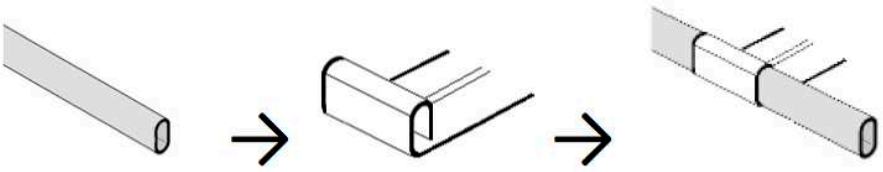
Bend and Squat Instructions

1. Adjust the **WALL** section to the required length by loosening wing nut (*turn anticlockwise*). Then slide the **blue** line to the required measurement and tighten the wing nut (*turn clockwise*). Make sure that the **WALL** section is secure and cannot move.

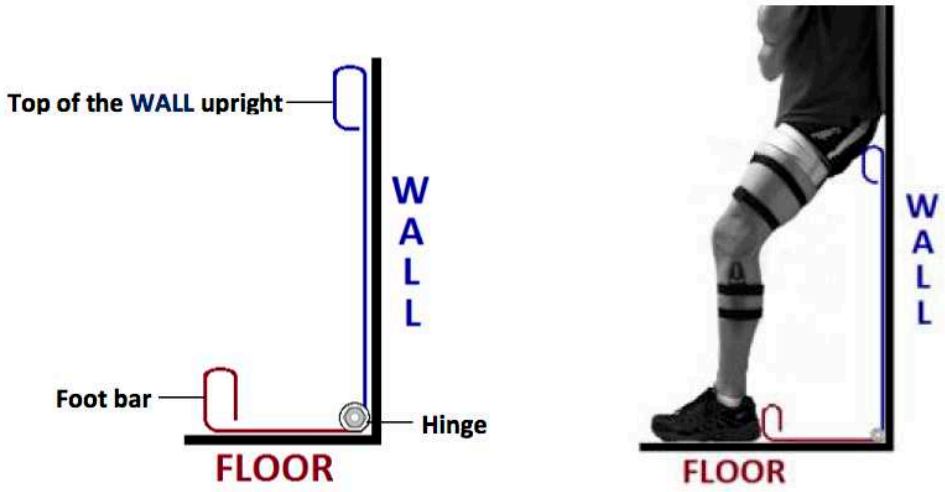


2. Adjust the **FLOOR** section to the required length by loosening wing nut (*turn anticlockwise*). Then slide the **red** line to the required measurement and tighten the wing nut (*turn clockwise*). Make sure that the **FLOOR** section is secure and cannot move.

3. Insert the bar into the slot at the end of the **FLOOR** section.



4. Put the bend and squat at a 90 degree angle against a flat wall, making sure that the hinge is in the corner between the wall and the floor.



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Isometric Wall Squat Exercise

- Stand with your head and back firmly against a flat, sturdy wall that supports the full weight of your body.
- Position your feet shoulder-width apart against the **Bend & Squat** bar with your toes facing forward. Make sure your feet are firmly on the floor, as you may find that they slide forward.
- To perform a wall squat, slowly bend your knees and allow your back to slide down the wall until your bottom is touching the upright of the **Bend & Squat**. ***DO NOT** use the **Bend & Squat** as a seat. It should **NOT** support your body weight*
- Look straight forward and hold this position for 2 minutes.
- Keep your arms crossed throughout the exercise and breathe steadily. ***DO NOT** hold your breath.* Put the laminated **BREATHE** sheet in front of you whilst squatting as a reminder.
- When you have completed the 2 minute wall squat, use your hands to push yourself away from the wall.

Do:

- ✓ Make sure the **Bend & Squat** is set up correctly.
- ✓ Keep your feet shoulder width apart.
- ✓ Keep your arms crossed.
- ✓ Hold the exercise position for 2 minutes.
- ✓ Breathe steadily throughout the exercise.

Do NOT:

- X Sit on the **Bend & Squat**.
- X Slide down/up the wall.
- X Move your feet.
- X Hold your breath.