The Role Of A Haemodynamic Stimulus In Isometric Exercise Training: Implications For Cardiovascular Adaptations.

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Abstract

The purpose of this thesis was to explore the role of exercise induced blood flow haemodynamics in the cardiovascular adaptations associated with isometric exercise training, with focus on resting blood pressure adjustment in normotensive participants. Using a cross-sectional study, it was identified that significant relationships were present between (i) blood flow, (ii) shear stress, and (iii) shear pattern responses (measured in the femoral artery), during and immediately following isometric bilateral leg extension exercise of increasing intensity. Based on these findings, it was feasible to suggest that the haemodynamic response to high intensities of acute isometric exercise might provide a physiological challenge to the cardiovascular system, that upon repeated exposure via isometric exercise training, may induce cardiovascular adaptation and resting blood pressure reductions. Subsequent to this, a randomised controlled trial established that performing isometric exercise training to a 'high haemodynamic stimulus' did not induce significantly greater adaptation in resting blood pressure than when performing isometric exercise training to a 'low haemodynamic stimulus' or control. When the training group (high and low combined) were compared to the control, significant reductions in resting blood pressure were observed. Furthermore, non-invasive cardiovascular variables that were considered as possible physiological mechanisms for resting blood pressure adaptation following isometric exercise training did not correlate with within group resting blood pressure changes. Whilst these findings suggest that a haemodynamic challenge may not be the primary stimulus responsible for inducing resting blood pressure adaptation following isometric exercise training, these results do demonstrate the effectiveness of isometric exercise training for potential health gains via reductions in resting blood pressure in normotensives. Importantly, these findings have progressed the current understanding surrounding isometric exercise training induced resting blood pressure reductions and will allow future research to narrow their focus upon other physiological variables that may be the stimuli for blood pressure adaptation.

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Commonly Used Abbreviations

ΔASR - delta antegrade shear rate

ΔBF - delta blood flow

ΔOSI - delta oscillatory shear index

ΔRSR - delta retrograde shear rate

ΔSR - delta shear rate

AD – artery diameter

AO – arterial occlusion

ASR – antegrade shear rate

AVI – audio video interleave

BF – blood flow

BP - blood pressure

BV – blood velocity

CI – confidence interval

CL – confidence limits

CON – control group

CV – coefficient of variation

DBP - diastolic blood pressure

DIIET - discontinuous incremental isometric exercise test

ECG – Electrocardiogram

EMG – Electromyography

EMGamp – EMG amplitude

EMGfreq – EMG frequency

eNOS - endothelial nitric oxide synthase

FMD – flow mediated dilatation

HI – high intensity exercise training group

HR - Heart rate

HRV - heart rate variability

IET - isometric exercise training

IHET - isometric handgrip exercise training

ILEET - isometric leg extension exercise training

IVRT – isovolumic relaxation time

LO – low intensity exercise training group

LVET – left ventricular ejection time

LVOT – left ventricular outflow tract

MAP - mean arterial blood pressure

MASR - mean antegrade shear rate

MBF - mean blood flow

MOSI - mean oscillatory shear index

MRSR - mean retrograde shear rate

MSNA – muscle sympathetic nervous activity

MSR - mean shear rate

NO -nitric oxide

O_2 - Oxygen

OSI – oscillatory shear index

PASR - peak antegrade shear rate

PBF - peak blood flow

PE-MASR - post exercise mean antegrade shear rate

PE-MBF - post exercise mean blood flow

PE-MRSR - post exercise mean retrograde shear rate

PE-MSR - post exercise mean shear rate

PE-PASR - post exercise peak antegrade shear rate

PE-PBF - post exercise peak blood flow

PE-PSR - post exercise peak shear rate

PRSR - peak retrograde shear rate

PSR - peak shear rate

PE-PRSR - post exercise peak retrograde shear rate

PE-MOSI - post exercise mean oscillatory shear index

RBP – resting blood pressure

REI – relative exercise intensity

RMSE – root mean square error

ROI – region of interest

ROS – reactive oxygen species

RSR – retrograde shear rate

SBP - systolic blood pressure

SS – shear stress

SR – shear rate

SV – stroke volume

TPR - total peripheral resistance

$\dot{Q}$- cardiac output

Vmax – peak systolic antegrade blood velocity

Vmean – mean blood velocity

Vmin – peak retrograde blood velocity

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Chapter 1

Introduction

Chapter 1: Introduction

1.1 The current position on exercise and hypertension.

Currently, around one in three adults in England and Scotland are classified as having high blood pressure, otherwise known as hypertension. This is defined as a systolic blood pressure of 140 mmHg or over, or a diastolic blood pressure of 90 mmHg or over (Allender et al, British Heart Foundation 2012). The World Health Report (2002) estimates that around 11% of all disease burden in developed countries is caused by raised blood pressure, and that over 50% of ischemic heart disease and almost 75% of stroke in developed countries is due to systolic blood pressure being in excess of 115 mmHg. Furthermore, the National Institute for Health and Care Excellence (NICE) guidelines on hypertension management in adults report that every 2 mmHg rise in systolic blood pressure is associated with a 7% increased risk of mortality from ischemic heart disease and a 10% increased risk of mortality from stroke. Whilst clinical management of hypertension is one of the most common interventions in primary care, accounting for approximately £1 billion in drug costs alone in 2006 (NICE Guidelines), there is a need to find more favorable and cost effective solutions to help reduce the prevalence of a worldwide hypertension epidemic.

Millar et al (2014) stated that the traditional objective of clinical practice has been to achieve a resting blood pressure (RBP) of < 140 / 90 mmHg, as a RBP greater than this is a significant risk factor for cardiovascular diseases such as coronary artery disease, stroke or heart failure. The latest treatment guidelines for prevention of hypertension recommend a number of non-pharmacological lifestyle changes such as smoking cessation, weight loss, exercise training, healthy diet, moderation of alcohol consumption and reduced sodium intake (Calhoun et al, 2008; Chobanian et al, 2003; Go, 2013; NICE Hypertension guidelines, 2011).

Exercise training is a well established physiological stimulus which reduces primary and secondary cardiovascular events (Tinken et al 2010). There are a number of meta-analyses that have demonstrated the benefits of physical activity on reducing RBP (Braith & Stewart, 2006; Cornelissen & Fagard, 2005; Cornelissen et al, 2011; Cornelissen & Smart, 2013; Halbert et al, 1997; Kelley, 1997; Kelley & Kelley, 2000; Kelley et al, 2001).

Current guidelines produced by the American College of Sports Medicine (ACSM) on exercise and hypertension (Franklin & Fagard, 2004) recommends > 30 minutes of at least moderate intensity activity preferably on most days of the week, composed of aerobic exercise supplemented with dynamic resistance exercises. In support of this recommendation, a recent meta-analysis performed by Cornelissen & Smart (2013) using healthy adults demonstrated that aerobic endurance exercise elicited a 3.5 mmHg reduction in resting systolic blood pressure (SBP), with a 2.5 mmHg reduction in diastolic blood pressure (DBP), whilst dynamic resistance exercise training induced 1 mmHg reduction in SBP, and a 3.2 mmHg reduction in DBP.

The use of isometric exercise has provided an alternative method of exercise training, that although less popular than traditional aerobic and dynamic resistance exercise methods, demonstrates greater reductions of 10.9 mmHg in SBP and 6.2 mmHg in DBP (Cornelissen & Smart, 2013). These findings have led Cornelissen & Smart (2013) to conclude that the data from a small number of isometric exercise training (IET) studies suggests that this form of exercise training has the potential for the greatest reductions in RBP. Indeed to contexualise this, evidence suggests that a 5 mmHg reduction in resting SBP is estimated to reduce mortality from coronary heart disease by 9%, stroke by 14% and all causes by 7% (Stamler et al, 1989), and a 5 mmHg in resting DBP in hypertensives is associated with a 34% reduction in stroke risk and a 21% reduction in coronary heart disease risk (MacMahon et al, 1990). It is plausible to suggest that IET may provide a more favorable, cost effective treatment for hypertension than the exercise recommendations traditionally prescribed, and it may ultimately improve long term mortality risk in this specific population.

Therefore, this research thesis aims to contribute to the existing literature surrounding the use of IET to reduce RBP, so that this form of exercise training might be better understood in an attempt to promote the greatest exercise derived cardiovascular benefits to health. The remainder of this review will critically examine the previous literature to date, with specific reference to the physiological training stimulus for RBP reductions after IET.

1.2 The potential for isometric exercise training to reduce resting blood pressure.

From the 1970's to the present day, isometric exercise has been established as a successful training method for RBP adaptation. One of the first initial studies was

performed by Kiveloff & Huber (1971), who reported that whole body isometric efforts lowered resting SBP and DBP in a group of hypertensives after 5 - 8 weeks of training. Further to this, Buck & Donner (1985) found that in a population sample of > 4000 men, those who performed regular isometric exercise in their occupation had a lower incidence of hypertension. Taken together, the potential implications of these two studies warranted further investigation into the cardiovascular health benefits isometric exercise may provide.

More recently, Wiley et al (1992) explored the effects of IET on RBP with an experimental study design. Participants with either a high normal RBP or hypertension performed isometric handgrip exercise training (IHET) at 30% maximum voluntary contraction (MVC) 3 times a week for 8 weeks, or at 50% MVC 5 times a week for 5 weeks. Training sessions for the group performing IHET at 30% MVC were composed of 4 x 2 min isometric handgrip contractions, whilst the group performing IHET at 50% MVC completed 4 X 45 sec isometric handgrip contraction per training session. Results demonstrated significant reductions in both resting SBP and DBP in both exercise training groups (30% MVC: SBP 12.5 mmHg; DBP 14.9 mmHg, 50% MVC: SBP 9.5 mmHg; DBP 8.9 mmHg). Importantly, these results also demonstrated that whilst performing IHET to a greater exercise intensity (50% MVC) may induce quicker reductions in RBP within a shorter time frame (5 weeks versus 8 weeks at 30% MVC), performing IHET at 50% MVC induced fatigue at a quicker rate than when IHET is performed at 30% MVC. This suggests that isometric contractions performed at higher exercise intensities may be difficult for most individuals to maintain. This is reflected in the training prescription of Wiley et al (1992) as IHET contractions at 30% MVC were maintained for 2 minutes, as opposed to only 45 seconds when 50% MVC contractions were performed. Consequently, as a result of these factors, the 4 x 2 min IHET at 30% MVC became accepted as the standard exercise prescription protocol in later studies to induce a significant reduction in RBP. These studies can be viewed in Table 1 and Table 2 (pages 5-8), which document the methods of isometric exercise prescription and outcomes for every IHET study that has been performed to date that has explored RBP adaptation

Table 1. *Studies examining the effects of isometric handgrip exercise training and isometric arm flexion on resting blood pressure in normotensive participants.*

Authors & Year	**Study Design**	**Participants (n)**	**Age (years ± SD)**	**Initial RBP status**	**Exercise mode & Exercise intensity**	**Training Intervention (Frequency; duration)**	**RBP results.**
Wiley et al (1992)	Cohort	10	29-52	Normotensive	Alternating unilateral IHG 4 x 45 sec, 1 min rest, 50% MVC	5 x/week; 5 weeks	SBP -10 mmHg DBP -9 mmHg
Ray & Carrasco (2000)	Cohort	Ex: 9 Sham: 7 Con: 8	19-35	Normotensive	Unilateral IHG 4 x 3 min, 5 min rest, 30% MVC	4 x/week; 5 weeks	DBP -5 mmHg MAP -4 mmHg
Howden et al (2002)	Cohort	Ex: 8 Con: 8	21 ± 1	Normotensive	Bilateral arm flexion 4 x 2 min, 3 min rest, 30% MVC	3 x/week; 5 weeks	SBP -12 mmHg
McGowan et al (2007a)	Cohort	Ex: 11	28 ± 14	Normotensive	Unilateral IHG 4 x 2 min, 4 min rest, 30% MVC	3 x/week; 8 weeks	SBP -5 mmHg
Millar et al (2008)	RCT	Ex: 25 Con: 24	66 ± 6	Normotensive	Alternating unilateral IHG 4 x 2 min, 1 min rest, 30-40% MVC	3 x /week; 8 weeks	SBP -10 mmHg DBP -3 mmHg

Badrov et al (2013)	RCT	Ex: 12	19-45	Normotensive	Alternating unilateral IHG 4 x 2 min, 1 min rest; 30% MVC	3 x/week; 8 weeks	SBP -6 mmHg
		Ex: 11 Con: 9			Alternating unilateral IHG 4 x 2 min, 1 min rest; 30% MVC	5 x /week; 5 weeks	SBP -6 mmHg

RBP resting blood pressure, *Con* control, *DBP* diastolic blood pressure, *Ex* exercise, HR_{peak} peak heart rate, *IHG* isometric handgrip exercise training, *MAP* mean arterial blood pressure, *MVC* maximal voluntary contraction, *n* number of participants, *RCT* random controlled trial, *SBP* systolic blood pressure, *SD* standard deviation, *Sham* sham group. Blood pressure values are reported as means.

Table 2. *Studies examining the effect of isometric handgrip exercise training in pre hypertensive and hypertensive participants*.

Authors & Year	**Study Design**	**Participants (n)**	**Age (years ± SD)**	**Initial RBP status**	**Exercise mode & Exercise intensity**	**Training Intervention (Frequency; duration)**	**RBP results.**
Wiley et al (1992)	RCT	Ex: 8 Con: 10	20-35	Pre-hypertensive	Unilateral IHG 4 x 2 min, 3 min rest periods, 30% MVC	3 x/week; 8 weeks	SBP -13 mmHg DBP -15 mmHg
Taylor et al (2003)	RCT	Ex: 9 Con: 8	69 ± 6	Medicated hypertensives	Alternating IHG 4 x 2 min, 1 min rest, 30% MVC	3 x /week; 10 weeks	SBP -19 mmHg MAP -11 mmHg
McGowan et al (2006)	Cohort	Ex: 17	67 ± 6	Medicated hypertensives	Unilateral IHG 4 x 2 min, 4 min rest, 30% MVC	3 x /week; 8 weeks	MAP 0 mmHg
Peters et al (2006)	Cohort	Ex: 10	52 ± 5	Hypertensive	Alternating unilateral IHG	3 x /week; 6 weeks	SBP -13 mmHg DBP -2 mmHg
McGowan et al (2007b)	Cohort	Ex: 7	62 ± 11	Medicated hypertensives	Alternating unilateral IHG 4 x 2 min, 1 min rest, 30% MVC	3 x /week; 8 weeks	SBP –15 mmHg
		Ex: 9	66 ± 19		Unilateral IHG 4 x 2 min, 4 min rest, 30% MVC		SBP -9 mmHg

Stiller – Moldovan et al (2012)	RCT	Ex: 11 Con: 9	60 ± 9	Medicated hypertensives	Alternating unilateral IHG 4 x 2 min, 1 min rest, 30% MVC	3 x /week; 8 weeks	0 mmHg
Millar et al (2013)	Cohort	Ex: 13 Con: 10	66 ± 6	Medicated Hypertensives	Unilateral IHG 4 x 2 min, 4 min rest, 30% MVC	3 x /week; 8 weeks	SBP -5 mmHg MAP -3 mmHg
Badrov et al (2013)	RCT	Ex: 12: 12 Con: 12	51-74	Medicated hypertensives	Alternating unilateral IHG 4 x 2 min, 1 min rest, 30% MVC	3 x /week; 10 weeks	SBP -8 mmHg DBP -5 mmHg MAP -6 mmHg

RBP resting blood pressure, *Con* control, *DBP* diastolic blood pressure, *Ex* exercise, HR_{peak} peak, *IHG* isometric handgrip exercise training, *MAP* mean arterial blood pressure, *MVC* maximal voluntary contraction, *n* number of participants, *RCT* random controlled trial, *SBP* systolic blood pressure, *SD* standard deviation. Blood pressure values are reported as means.

Table 3. *Studies examining the effect of isometric leg extension exercise training on normotensive and pre hypertensive participants*.

Authors & Year	**Study Design**	**Participants (n)**	**Age (years ± SD)**	**Initial RBP status**	**Exercise mode & Exercise intensity**	**Training Intervention (Frequency; duration)**	**RBP results.**
Howden et al (2002)	Cohort	Ex: 10 Con: 8	21 ± 1	Normotensive	Bilateral ILEET 4 x 2 min, 3 min rest, 20% MVC	3 x/week; 5 weeks	SBP -10 mmHg
Wiles et al (2010)	RCT	Ex: 11	18-24	Normotensive	Bilateral ILEET 4 x 2 min, 2 min rest, 75% HR_{peak} (~10% MVC)	3 x /week; 8 weeks	SBP -4 mmHg DBP -3 mmHg MAP 3 mmHg SBP -5 mmHg
		Ex: 11 Con: 11			Bilateral ILEET 4 x 2 min, 2 min rest, 95% HR_{peak} (~20% MVC)		DBP -3 mmHg MAP -3 mmHg
Devereux et al (2010b)	Crossover	13	21 ± 2	Normotensive	Bilateral ILEET 4 x 2 min, 2 min rest, 95% HR_{peak} (~20% MVC)	3 x/week; 4 weeks	SBP -5 mmHg DBP -3 mmHg MAP -3 mmHg

Baross et al (2012)	RCT	Ex: 10	55 ± 5	Pre-hypertensive	Bilateral ILEET 4 x 2 min, 2 min rest, 85% HR_{peak} (~14% MVC)	3 x /week; 8 weeks	SBP -11 mmHg MAP -5 mmHg
		Ex: 10 Con: 10			Bilateral ILEET 4 x 2 min, 2 min rest, 70% HR_{peak} (~8 %MVC)		0 mmHg

RBP resting blood pressure, *Con* control, *DBP* diastolic blood pressure, *Ex* exercise, HR_{peak} peak heart rate, *ILEET* isometric leg extension exercise training, *MAP* mean arterial blood pressure, *MVC* maximal voluntary contraction, *n* number of participants, *RCT* random controlled trial, *SBP* systolic blood pressure, *SD* standard deviation. Blood pressure values are reported as means.

As evident in Table 1 and 2 (pages 5-8), the majority of IET research until the new millennium were performed utilising hypertensive participants, with the exception of Wiley et al (1992) who included a normotensive participant group in their work. Given that hypertensive participants are more likely to respond to an exercise training stimulus as they have more "physiological room" for RBP improvement towards normal blood pressure (BP) values, it was a significant finding when in the year 2000, Ray & Carrasco (2000) discovered that IET could also induce RBP reductions (although noticeably smaller) in normotensive participants. Participants performed 4 repetitions of 3 minute isometric handgrip contractions at 30% MVC, 4 times a week for 5 weeks and elicited significant reductions in resting DBP and mean arterial blood pressure (MAP) (-5 mmHg, -4 mmHg respectively). No significant reductions in resting SBP were observed. Millar et al (2009) commented that these findings were likely due to the fact that participants were normotensive, unlike previous IHET studies where the participants had elevated RBP and therefore had, as suggested previously, greater physiological room for improvement. In addition, a study completed by Millar et al (2007) that examined RBP reductions in 43 medicated hypertensive participants after IHET at 30% MVC found a significant correlation between baseline resting SBP and the magnitude of reduction in resting SBP post-training. This suggests that participants who have a higher resting SBP before exercise intervention will experience a greater drop in resting SBP when they undergo a IHET protocol. This may help to explain why Ray & Carrasco (2000) did not observe any reduction in resting SBP after IHET intervention, as participants were normotensive and therefore would have had a lower SBP at baseline. This observed relationship between baseline SBP and the magnitude of SBP reduction post-isometric handgrip exercise intervention should be interpreted with caution, as it is limited to the protocol and participants that Millar et al (2007) utilised in their study. Therefore it is not necessarily representative of other studies that have used different IHET protocols and participants.

To explore the effects of IET protocols further in normotensive participants, Badrov et al (2013) specifically investigated variations in training frequency on RBP outcomes. Badrov et al (2013) established that SBP was reduced by the same magnitude in the group that trained 3 times a week for 8 weeks, as the group that trained for 5 times a week for 8 weeks. It was apparent that these observed resting SBP reductions occurred more rapidly (after 4 weeks of exercise training) in the group that trained 5 times a week. This suggested that whilst training frequency may not affect the final magnitude of RBP reduction, these BP reductions may occur at a quicker rate when training frequency is increased. Again these conclusions are

limited to the study of Badrov et al (2013) and need to be explored using a wider range of IET protocols before they can be considered applicable to all forms of IET protocols.

Whilst a large number of the studies exploring the role of IET in RBP reductions have tended to focus upon IHET protocols, work from the research group at Canterbury Christ Church University has explored the use of isometric leg extension exercise training (ILEET) to establish the influence isometric contraction of a larger muscle mass may have on RBP reductions. Furthermore, this work has focused upon performing ILEET in normotensive participants. A summary of these studies can be viewed in table 3 on page 9. Wiles et al (2010) were the first to examine RBP reductions after 8 weeks of performing bilateral ILEET 3 days/week, with each session comprising of 4 contractions held for 2 minutes at a time. This study was unique in that it was the first study to prescribe isometric training intensity using a constant percentage of electromyography peak (%EMG_{peak}) rather than %MVC as seen in previous IET studies. Electromyography is the method used to record and analyse the electrical activity from skeletal muscle during a muscular contraction (Cifrek et al, 2009; Meijers et al, 1976; Merletti & Lo Conte, 1997; Rainoldi et al, 2001). Wiles et al (2010) proposed that when working to a constant %EMG, a more stable cardiovascular response is produced that plateaus within 2 minutes, compared to the likelihood of a continued rise when using constant force (%MVC). Wiles et al (2010) suggested that this makes it possible to determine more precisely the level of the cardiovascular response, which may help to accurately determine the training stimulus. Results showed that in the exercise group that performed high intensity bilateral ILEET (to a constant %EMG that induced a consistent cardiovascular response of 95% of the maximum heart rate [95% HR_{peak}]), resting SBP significantly decreased by 5.2 mmHg. Resting DBP significantly decreased by 2.6 mmHg, whilst MAP decreased by 2.5 mmHg. In the low intensity group that performed ILEET to 75% HR_{peak}, significant reductions in resting SBP, DBP and MAP were observed (-3.7 mmHg, -2.5 mmHg, and -2.6 mmHg respectively). These results established that performing bilateral ILEET to a constant %EMG is an effective exercise training method to induce significant reductions in RBP in normotensive participants.

Based upon the trends indicated in the work of Wiles et al (2010), a subsequent study by Devereux et al (2010b) furthered the understanding of the role of bilateral ILEET in inducing RBP reductions in normotensive participants by establishing that significant RBP reductions can occur after just 4 weeks of IET. Participants performed an identical protocol to that of Wiles et al (2010), but training intervention was performed for 4 weeks as opposed to 8

weeks. Results demonstrated significant reductions in resting SBP, DBP and MAP (-4.9 mmHg, -2.8 mmHg, and -2.7 mmHg respectively). Further analysis of this training intervention revealed significant relationships between the change in resting SBP and training intensity when expressed relative to $\%EMG_{peak}$. In addition, analysis of the EMG signal identified that significant correlations were also evident between changes in resting SBP and MAP variables and the amplitude of the EMG signal measured, and the frequency of the EMG signal measured. Changes in the amplitude and frequency of the EMG signal are thought to reflect the level of fatigue experienced in the working muscle (Devereux, 2010a). Specifically increases in signal amplitude are suggested to represent increased motor unit activity, whilst a decrease in signal frequency represents a decrease in membrane conduction velocity as a result of metabolic changes (in static contractions > 45% MVC), or neural changes (in static contractions <30% MVC) within the muscle (Crenshaw et al, 1997; Gerdle et al, 1997; Korhonen et al, 2005). Thus, participants who trained to a higher exercise intensity (when expressed relative to $\%EMG_{peak}$) and experienced greater levels of fatigue (as measured by EMG signal amplitude and frequency) during bilateral ILEET intervention had greater reductions in resting SBP and MAP (Devereux et al, 2011). This suggested that a stimulus associated with exercise intensity and fatigue during ILEET may be responsible for RBP reductions following this type of exercise training. This requires further investigation within the context of this research thesis before any definitive conclusions can be drawn.

Continuing on from the work of Wiles et al (2010) and Devereux et al (2010b; 2011), Baross et al (2012) also demonstrated that the observed RBP reductions after bilateral ILEET could be extended to older participants. Middle aged males (aged 55 ± 5 years) performed 8 weeks of bilateral ILEET 3 times a week, with training sessions consisting of 4 repetitions of 2 minute isometric contractions. Baross et al (2012) also examined the impact of isometric exercise intensity on RBP reductions, with one group performing ILEET to 85% HR_{peak} whilst the other performed ILEET to 70% HR_{peak}. Results demonstrated that significant reductions in RBP were only observed in the group performing ILEET at 85% HR_{peak} (SBP -10.8 mmHg; MAP -4.7 mmHg), whilst the 70% HR_{peak} group experienced no significant reductions in RBP. This indicates that exercise intensity is an important factor in the cardiovascular adaptation to IET, with a specific threshold of intensity that must be exceeded in order to reduce RBP over an 8 week period.

As this review has demonstrated, it is now widely accepted that both IHET and ILEET interventions have the ability to induce significant RBP reductions in hypertensive and

normotensive populations. As such, the majority of the current research to date is designed to identify the physiological mechanism(s) that adapt in response to IET and subsequently may induce RBP adaptation (these possible mechanism(s) are discussed in section 1.4 of this thesis). Little attention has been given to identifying the physiological stimulus / stimuli induced by isometric exercise that may cause an adaptation in the physiological mechanism(s) responsible for RBP reduction following IET. Future work within this field should focus upon identifying this exercise induced physiological stimulus / stimuli so that future IET training studies can tailor IET protocols to ensure maximum exposure to this stimulus for optimal RBP reduction.

1.3 Identifying the physiological stimulus /stimuli for resting blood pressure reductions after isometric exercise training.

Currently it is not known as to what the physiological stimulus / stimuli may be for RBP reductions following IET. Tailoring IET protocols to maximise exposure to a physiological stimulus / stimuli would not only ensure safe and effective exercise prescription, but would also increase the opportunity for reductions in RBP to occur. Identification of the physiological stimulus / stimuli for RBP reductions after IET may also provide an insight into the physiological mechanism(s) that are responsible for RBP adaptation. As such, identification of the physiological stimulus / stimuli for RBP reductions after IET will be explored within the context of this current research thesis.

There is evidence to suggest that this physiological stimulus / stimuli may be closely related to IET intensity. In a study completed by Baross et al (2012), it was established that older participants who performed 8 weeks of ILEET at a higher exercise intensity of 85% HR_{peak} had a greater reduction in resting SBP post intervention, than those participants who performed ILEET at a lower exercise intensity of 70% HR_{peak}. Whilst the group performing ILEET to 85% HR_{peak} experienced a significant reduction in resting SBP (-10.8 ± 7.9 mmHg) and MAP (-4.7 ± 6.8 mmHg) following training, the group performing ILEET to a lower exercise intensity of 70% HR_{peak} experienced no significant reductions in RBP following exercise intervention. Furthermore, Devereux et al (2011) found a significant relationship between IET intensity when expressed relative to $\%EMG_{peak}$ and reductions in resting SBP following a 4 week ILEET program. Together this demonstrates that it may be likely that isometric exercise intensity over a given training duration is important in the magnitude of RBP reduction following IET. As such, isometric exercise intensity acts as an exercise training stimulus that in turn elicits a specific physiological response. The physiological

response observed may then act as the physiological stimulus / stimuli for RBP adaptation. Therefore it is feasible to suggest that the physiological stimulus may be greater at higher isometric exercise intensities, which consequently may induce a significantly greater RBP adaptation. Figure 1 demonstrates the link between an exercise training stimulus (which this current research thesis proposes is intensity), a physiological stimulus (to be investigated), a physiological mechanism and the final outcome of a RBP adaptation.

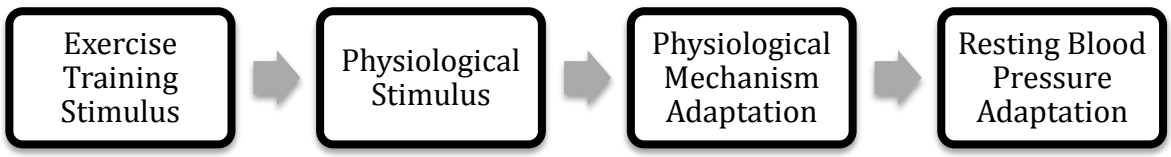

Figure 1. *Diagram to demonstrate the concept of a link between an initial exercise training stimulus (proposed to be exercise intensity) that elicits a physiological response, that in turn acts as a physiological stimulus / stimuli for an adaptation to a physiological mechanism that is responsible for resting blood pressure adaptation.*

Some authors have suggested that isometric exercise is uniquely defined by its effects upon blood flow (BF). At higher isometric exercise intensities it is evident that mechanical compression of blood vessels and intramuscular pressure is so great that BF to the exercising musculature is severely restricted (Barnes, 1980). This is reported to occur around 10-15 %MVC for static leg contraction (Gaffney et al, 1990; Saltin et al, 1981; Sjogaard et al, 1988). The reduced blood supply to the exercising musculature creates an inefficiency to meet the energy requirements (demand for oxygen) needed to sustain high intensity isometric exercise. As a result, acidic metabolites (which include an increase in muscle venous lactate, H^+ concentration, PCO_2, bradykinins and prostaglandins) begin to accumulate (Rowell & O'Leary, 1990). Together with the reduced ability to remove these acidic byproducts of static contraction, muscular fatigue rapidly occurs. Devereux et al (2010b) have indicated that the presence of 'fatigue' associated with higher intensities of isometric exercise may be important in determining the magnitude of RBP reductions after IET. This was based upon the finding that reductions in RBP after 4 weeks of ILEET training at 95% HR_{peak} in normotensive participants significantly correlated with markers of isometric exercise intensity when expressed relative to $\%EMG_{peak}$, and neuromuscular measures of fatigue from the EMG signal (EMG signal amplitude and frequency). Devereux et al (2010b) suggested that these markers of intensity may reflect the extent to which local muscle fatigue was induced, which in turn appears to be important in the reductions in RBP observed after ILEET. This implies that the physiological stimulus for RBP reductions after IET training may be closely related to the degree of fatigue that is induced at higher intensities of isometric exercise.

In summary, there is a small amount of preliminary evidence to suggest that the physiological stimulus / stimuli during IET for RBP adaptation may be closely associated with isometric exercise intensity. It is feasible to suggest that the stimulus / stimuli may be more pronounced at higher exercise intensities which induce a greater amount of fatigue. This may result in a greater magnitude of RBP adaptation. As such, future investigations might focus upon identifying the exact physiological response to higher intensities of fatiguing isometric exercise that could be considered as the physiological stimulus / stimuli for RBP adaptations following IET.

1.4 Previously proposed physiological mechanisms for resting blood pressure reductions following isometric exercise training.

The physiological mechanism(s) that adapt in response to a physiological stimulus / stimuli during IET, and are therefore responsible for the reductions in RBP observed following IET are yet to be fully identified. Inconsistencies in previous mechanistic investigations involving the type of participants utilised (i.e., hypertensive, normotensives, pre-hypertensives) have made it difficult to identify the specific mechanism(s) that may be responsible for RBP adaptation. It is well established that, in comparison to the physiology of an individual with normal RBP (normotensive), hypertension is associated with abnormalities in the renin-angiotensin system (Laragh et al, 1972; Navar et al, 2010), elevated sympathetic nervous system activity (Abboud, 1982; Anderson et al, 1989; Esler et al, 2010; Guzetti et al, 1988;, Mark, 1996), and endothelial dysfunction (Lind, 2000; Perticone et al, 2001; Versari et al, 2009). Due to the distinct difference in the physiology of a normotensive versus hypertensive participant, it is plausible to suggest that the physiological mechanism(s) that are responsible for a reduction in RBP following IET may also be different between these two participant populations. Thus, in the context of this research thesis, previous mechanisms that have previously been proposed as the physiological mechanism for IET induced reductions in RBP in hypertensive participants may not have the same degree of physiological influence on the RBP reductions noted in normotensive participants. The physiological mechanism(s) responsible for RBP adaptation following IET in normotensive participants remains to be established.

Wiley et al (1992) stated that the physiological mechanism(s) for RBP reductions following IET must involve adjustments in one or more of the components that determine resting MAP - cardiac output ($\dot{Q}$) or total peripheral resistance (TPR). Cardiac output is defined as the

volume of blood ejected from the left ventricle into the aorta each minute (Opie, 2004). The components that determine $\dot{Q}$ are stroke volume (SV) (volume of blood pumped from the left ventricle per beat) and heart rate (HR) (number of heart beats per minute), whereby $\dot{Q}$ = SV x HR (Opie, 2004). As the blood flows through the systemic vascular system, it is met by a certain amount of resistance to flow (Brzezinski, 1990). This resistance is termed total peripheral resistance (TPR) (Aletti et al, 2006; Brzezinski, 1990). Poiseuiles law states that TPR is directly proportional to blood viscosity and length of vessel, whilst being inversely proportional to the fourth power of the radius (Pfitzner, 1976). Greater viscosity of blood combined with an extended blood vessel length, and smaller radius of the blood vessel will result in an increase in resistance to BF, thus an increase in TPR, and RBP (Brzezinski, 1990; Weir & Sowers, 1988). Whilst blood viscosity and length of the vessel are mostly consistent, vessel radius can easily fluctuate, and therefore alter TPR (Brzezinski, 1990; Weir & Sowers, 1988). The components that determine $\dot{Q}$ and TPR have become the focus for mechanistic studies in an attempt to identify the physiological mechanism(s) responsible for reductions in RBP after IET.

Indeed the Millar et al (2014) commentary simplifies the mechanism(s) that may be responsible for adjustments in $\dot{Q}$ or TPR into cardiac adaptations, autonomic nervous system adaptations, vascular adaptations and oxidative stress. Each of these categories will be discussed extensively below in relation to the possible mechanism(s) responsible for RBP reductions after IET in normotensive participants.

1.4.1 Cardiac adaptations

Very few IET studies have directly measured $\dot{Q}$ changes after isometric exercise intervention. In the few studies that have estimated $\dot{Q}$ (Baross et al, 2012; Devereux et al, 2010b; Wiles et al, 2010) no significant changes in this parameter have been observed, despite significant reductions in RBP. Ostensibly this suggests that cardiac adaptations may not be the mechanism for the observed reductions in RBP after IET. Whilst Baross et al (2012) reported a decrease in resting HR by 5 beats·min^{-1} after middle aged men performed an 8 week bilateral ILEET intervention, no significant changes in $\dot{Q}$ were observed. Millar et al, (2014) suggested that the techniques used to estimate $\dot{Q}$ in these studies may have not been sensitive enough to detect small changes in $\dot{Q}$ if present, and therefore future studies should focus on utilising alternative methodologies to estimate $\dot{Q}$, such as Doppler ultrasound. Echocardiographic methods utilising Doppler ultrasound will be used in this thesis in an

attempt to determine whether adaptations in $\dot{Q}$ act as a physiological mechanism for RBP reductions following IET.

1.4.2 Autonomic nervous system adaptations

1.4.2.i Cardiac autonomic regulation

Measures such as heart rate variability (HRV) and blood pressure variability (BPV) can provide non-invasive assessment of changes to the regulation of HR and BP by autonomic inputs, and this may provide an insight into any changes in cardiac sympathetic and vagal modulation after IET intervention (Millar et al, 2013). Previous investigations have often utilised different participant groups (i.e. hypertensive or normotensives) and used different methods to assess cardiac autonomic regulation (frequency domain or non-linear methods), which has made it difficult to fully determine the influence of changes to autonomic nervous system input in the RBP reductions commonly observed after IET.

It is apparent from the existing literature that two HRV methods in particular have been utilised to explore the influence of cardiac autonomic regulation in the RBP reductions associated with IET. These two methods can be categorised into frequency domain and non-linear methods. Parati et al (1995) described frequency domain methods as subdividing the variability of BP and HR into different frequency components and to quantify the variance or "power" at each specific frequency. Specifically, the frequency components are divided into low frequency (0.04–0.15 Hz) and high frequency (0.15–0.4 Hz) which represent sympathetic and vagal activity respectively (Berntson et al, 1997), and therefore provide an insight into the balance between sympathetic and parasympathetic modulation. Non-linear methods of measuring BPV or HRV, such as sample entropy or power law exponent (Francesco et al, 2012), are based upon the reasoning that the mechanisms that determine BP and HR are complex and therefore measuring the variability of these cardiovascular systems will require a multidimensional process (Mansier et al, 1996; Miller et al, 2013b).

Several authors have utilised frequency domain methods to establish the role of cardiac autonomic regulation changes in the reductions in RBP following IET. The results have been conflicting. Wiles et al (2010) and Devereux et al (2010b) found that RBP reductions following bilateral ILEET in normotensives were not accompanied by statistically significant changes in frequency domain measures of HRV. In contrast, Taylor et al (2003) reported significant changes post IHET in hypertensive participants for SBP that were accompanied by

statistically significant changes in SBP BPV. Specifically a decrease in the low frequency component (which corresponds to sympathetically mediated activity [Taylor et al 2003]) was observed, with increases in the high frequency component (thought to reflect parasympathetic modulation of cardiac function [Taylor et al 2003]). Together these changes resulted in a corresponding decrease in the low frequency / high frequency ratio, which suggested IHET decreased sympathetic and enhanced parasympathetic modulation of BP at rest, leading to a reduction of RBP. As it is possible that the mechanism(s) responsible for RBP reductions after IET may differ between hypertensive and normotensive populations, it is unknown whether the same changes in BPV would occur in normotensive participants. Whilst the number of previous investigations are limited, evidence suggests that cardiac autonomic modulation adaptation as measured by frequency domain measures may not be the primary mechanism responsible for the reductions in RBP following IET for normotensive populations.

More recently, changes in cardiac autonomic modulation have been assessed by non-linear methods. Miller et al (2013) has utilised these methods to determine changes in HRV following IET. Results demonstrated that following 8 weeks of IHET in hypertensive participants, significant reductions in RBP had occurred (SBP -5 mmHg; MAP -3 mmHg) that coincided with statistically significant changes in HRV. The work of Miller et al (2013) indicated that improved cardiac autonomic modulation via improved sympathovagal balance may be one of the mechanisms responsible for reductions in RBP after IET. These findings are limited to hypertensive participants, and thus it is unknown as to whether similar results would be observed in normotensive population.

Overall, it is not fully established as to the role that cardiac autonomic function may play in the reductions in RBP after IET for normotensive participants. The inclusion of HRV measures within this thesis will attempt to establish whether cardiac autonomic function adaptation occurs in response to IET, and whether these possible adaptations may go some way in explaining the reductions in RBP commonly observed after this type of exercise intervention.

1.4.2.ii. Neural regulation of vascular tone

Vascular tone is the degree of constriction experienced by a blood vessel relative to its maximally dilated state (Klabunde, 2011). All arterial vessels undergo some form of smooth muscle contraction via a number of intrinsic factors (myogenic mechanisms, endothelial

factors, local hormones) and extrinsic factors (sympathetic nervous system influence) that determine the diameter and tone of the vessel, and subsequent resistance to flow (Klabunde, 2011). Specifically in relation to extrinsic factors, sympathetic neurohumoral influences such as circulating catecholamine's (adrenaline and noradrenaline), the renin-angiotensin aldosterone system and anti-diuretic hormone all act as vasoconstrictors, causing the smooth muscle within the blood vessel lumen walls to contract, reducing lumen diameter. Figure 2 demonstrates the structure of a typical artery and the location of smooth muscle within the arterial walls. An increase in vascular tone (via intrinsic or extrinsic factors) would directly act on the smooth muscle fibers located within the media component of a typical artery, causing a vasoconstriction response, which would reduce the diameter of the artery lumen and increase resistance to BF.

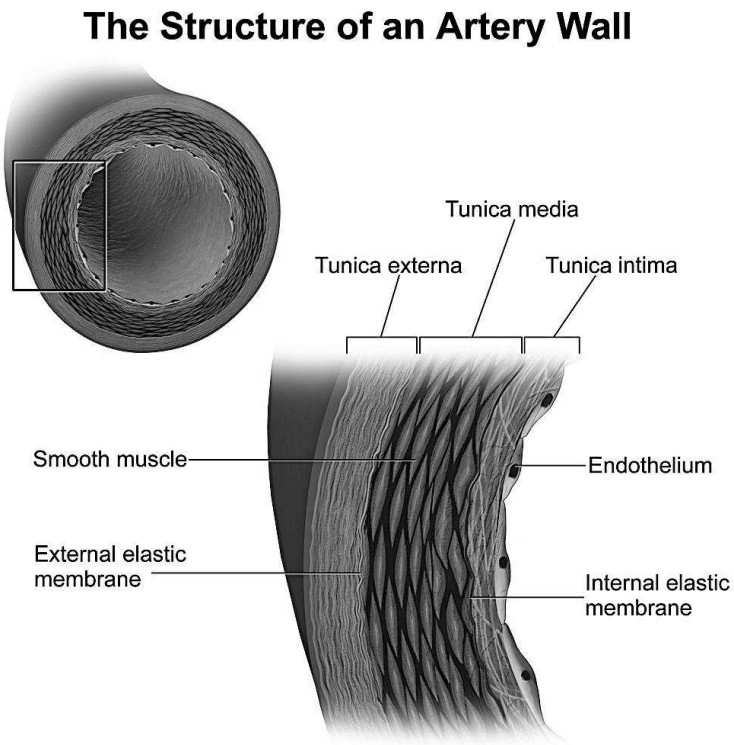

Figure 2. *The structure of a typical human artery, Image taken from: http://upload.wikimedia.org/wikipedia/commons/thumb/c/c8/Blausen_0055_ArteryWallStructure.png/1024px-Blausen_0055_ArteryWallStructure.png. With copyright permission.*

There is limited data exploring the role of peripheral sympathetic nervous activity or vascular tone as a mechanism for RBP reduction after IET (Miller et al 2013). Ray & Carrasco (2000) did explore the role of muscle sympathetic nervous activity (MSNA) (which is representative of sympathetic nervous system outflow) in RBP reductions after 5 weeks of IHET in normotensive participants. Whilst their results demonstrated small changes in resting DBP (-5

mmHg) and MAP (-4 mmHg), no significant changes in SBP were observed. Muscle sympathetic nervous activity did not significantly change after the isometric exercise intervention. This suggests that changes in sympathetic nervous outflow to skeletal muscle were not responsible for the observed reductions in resting DBP and MAP. As Miller et al (2013) identified, this study was only performed with a small sample size and in normotensive populations who have "normal" levels of sympathetic outflow. In contrast, hypertensive populations have an elevated MSNA (Abboud et al, 1982; Anderson et al, 1989; Esler et al, 2010; Guzzetti et al, 1988; Mark, 1996) that may have more potential to be corrected following this type of exercise training.

In addition, Miller et al (2013) also identified that Taylor et al (2003) observed a decrease in the low frequency spectrum of SBP BPV (thought to reflect sympathetic modulation of cardiac function, Taylor et al [2003]). As Miller et al (2013) proposed, in patients with hypertension where sympathetic outflow is increased, IET may reduce RBP through attenuations in peripheral sympathetic vasoconstrictor activity. Indeed this might help explain the RBP reductions and associated increase in femoral artery diameter (AD) and vascular conductance in middle aged males after ILEET intervention observed by Baross et al (2012).

In summary, whilst current evidence suggests that changes in the neural regulation of vascular tone are feasible, these are more likely to be a mechanism for reductions in RBP after IET in hypertensive populations, as this population specifically has an elevated sympathetic outflow that may be more likely to respond to IET (Miller et al, 2013). In contrast, whilst the supporting evidence is limited to just one study, neural regulation of vascular tone in normotensives does not seem to change in response to IET, and does therefore not appear to be a mechanism for RBP adaptation (Ray & Carrasco, 2000). This may in part be due to a "normal" sympathetic outflow that is less likely to respond to an IET stimulus if already functioning at an optimal level.

1.4.3 Oxidative stress

Peters et al (2006) have performed the only study to date that examines the role of oxidative stress in RBP reductions following IET based upon the observation that an increased oxidative stress is largely associated with hypertension. Peters et al (2006) observed significant reductions in RBP in hypertensive participants after six weeks of IHET that were accompanied by an improved ratio of resting whole blood glutathiane to oxidized glutathiane and reduced aerobic exercise induced reactive oxygen species (ROS) production. Miller et al

(2013) suggest that changes in oxidative stress may be mediated by increased availability of nitric oxide (NO), which acts as an antioxidant and anti-inflammatory molecule. Isometric exercise training has been associated with increased NO production at rest, as McGowan et al (2007b) demonstrated increased local conduit artery NO dependent vasodilation in hypertensives following 8 weeks of IHG intervention. Whilst the role of oxidative stress in RBP reductions after IET requires substantially greater investigation, it is unlikely that the same relationship between oxidative stress and RBP would be seen in normotensive populations since normotensives have lower levels of oxidative stress (Rodrigo et al, 2007).

1.4.4 Vascular adaptations

Currently, there is a popular focus within the existing literature investigating the role of vascular adaptations as a possible physiological mechanism for RBP reductions following IET. Indeed McGowan et al, (2006, 2007a&b) suggested that repeated exposure to a haemodynamic shear stress (SS) stimulus (defined as the frictional force of the BF against the endothelium, [Gonzales et al, 2009]) may intrinsically influence the vascular tone of an artery via the up regulation of the vasodilator NO. This may lead to improved endothelial function (improved ability of the artery to dilate in response to a SS stimulus) or conduit AD remodeling to a larger lumen diameter at rest. Locally this may reduce TPR, which consequently may lead to a reduction in RBP. Several studies have demonstrated that NO is involved in the central regulation of sympathetic tone (Guyenet, 2006; Pechanova, 2010; Togashi et al, 1992) which is important for RBP regulation. Indeed Togashi et al (1992) established that when L-NMA (a NO inhibitor) was administered to anaesthetized rats, significant increases in RBP were seen that coincided with increases in central sympathetic outflow. Thus it is apparent that whilst NO may play a role in lowering RBP via local vascular mechanisms (such as conduit artery remodeling and improved endothelial function), NO may also act in the central nervous system to reduce vascular sympathetic tone (Togashi et al, 1992). This may help to explain how a local vascular adaptation might induce a systemic RBP adaptation following IET. The following section in this review will discuss the plausibility of improved endothelial function and conduit AD remodeling as the mechanism for RBP reductions after IET.

1.4.4.i Improved endothelial function

The endothelium has emerged in recent years as the key regulator of vascular homeostasis (Deanfield et al, 2007). Lerman & Zeiher (2005) suggested that the endothelium is a monolayer of endothelial cells that line the lumen of the vascular bed, and is mechanically

and metabolically strategically located separating the vascular wall from the circulation and blood components. The endothelium is able to respond to physical and chemical signals by producing a wide range of factors that regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation and vessel wall inflammation (Deanfield et al, 2007). Within the context of this research thesis, the endothelium is particularly important for RBP control via the regulation of vascular tone. To regulate vascular tone the endothelium produces and releases several vasoactive molecules (endothelial NO synthase, bradykinin, adenosine, endothelial growth factor, serotonin, endothelin, angiotensin) that relax or constrict the vessel by acting upon the smooth muscle located within the media (Deanfield et al, 2007). Therefore a "healthy" endothelial function maintains a balance between vasoconstriction and vasodilation. This may contribute towards the management of a "normal" RBP via the regulation of TPR. In contrast, endothelial dysfunction as seen in hypertensive populations, (Lind et al, 2000; Perticone et al, 2001; Versari et al, 2009) is characterised by a shift in the balancing actions of the endothelium towards a reduced vasodilation (Endemann & Schiffrin, 2004), which in turn may increase TPR and therefore increase RBP. Thus it is apparent that maintaining a "healthy" endothelial function is a significant factor contributing towards optimal RBP regulation, and therefore an important component to consider as a possible mechanism responsible for RBP reductions following IET.

To date, very few studies have explored the role of improved endothelial function as a mechanism for RBP reductions following IET in normotensive populations. In the two studies that have, methodological inconsistences with regards to the techniques used to assess endothelial function and IET protocol make it difficult to fully quantify the influence of endothelial function adaptation towards RBP reductions. McGowan et al (2007a) were the first to examine whether improvements in endothelial function coincided with RBP reductions in normotensive participants after 8 weeks of IHET. McGowan et al (2007a) hypothesised that an elevated BP response during acute bouts of isometric exercise increased SS, and that repeated exposure to this SS stimulus during IET may have enhanced the bioactivity and/or bioavailability of NO at rest. Over time this may enhance the ability of the endothelium to respond to a resting SS stimulus and increase vasodilation, leading to a lower TPR, and consequently a lower RBP. Whilst statistically significant reductions in RBP were noted following the exercise intervention, no significant changes in endothelial function (as assessed by flow mediated dilation technique) following IHET occurred. Flow mediated dilation (FMD) is an assessment method of endothelial function that involves measuring changes in

conduit AD from baseline and following exposure to a SS stimulus induced by inflation and then deflation of a sphymomanometric arm cuff (Gokce, 2011). This technique elicits a reactive hyperemia (increase in BF) and artery vasodilation, which is predominately caused by endothelial derived vasoactive mediators (Gokce, 2011; Raitakari & Celermajer, 2000). Whilst FMD is viewed as the gold standard for endothelial function assessment (Gokce, 2011), many authors have argued that the technique carries many limitations, in that the application is technically challenging, requires expert training and there is a considerable lack of methodological standardisation between studies (Flammer et al, 2012; Gokce, 2011; Raitakari & Celermajer, 2000; Thijssen et al, 2011). With these limitations in mind, it would be unreasonable to dismiss the role of improvements in endothelial function as a mechanism for RBP reductions following IET in normotensive participants before further studies have been conducted. Several authors advise (Charakida et al, 2010; Harris et al, 2010; Thijssen et al 2011) that future studies need to standardise the FMD technique of measuring endothelial function, so that its true influence as a mechanism for RBP adaptation following IET can be identified.

One other research group has also explored whether improvements in endothelial function coincide with RBP reductions following IET. Badrov et al (2013) utilised the technique of forearm reactive hyperemia (a method used to assess resistance vessel endothelial function) to assess resistance vessel endothelial functional changes after 8 weeks of IHET in female normotensive participants. Whilst statistically significant reductions in resting SBP were observed in conjunction with a 52% increase in forearm reactive hyperemic BF, further analysis revealed that the resting SBP reductions had occurred by week 4 of IHET, whereas improvements in resistance vessel endothelial function occurred after 4 weeks. As a result, Badrov et al (2013) concluded that it was likely that improvements in resistance vessel endothelial function were not a contributing mechanism to RBP reductions following IET. As this study only measured resistance vessel endothelial function, it is unknown as to whether conduit artery improvements in endothelial function occurred, and whether these might have coincided with the time line of RBP reductions in these normotensive participants.

Another factor that should be taken into consideration when interpreting the findings of McGowan et al (2007a) and Badrov et al (2013) (in that endothelial function adaptation does not appear to be a mechanism for RBP reductions after IET) is the validity of the current techniques used to assess endothelial function. Deanfield et al (2007) suggested that current techniques do not reflect the dynamic biology of the endothelium, and in light of this, a panel

of several tests are needed to characterise the multiple factors of endothelium biology and therefore endothelium function. In addition, McGowan et al (2007a) acknowledge that the FMD technique assesses the ability of the endothelium to dilate in response to a maximal increase in SS stimulus (induced by cuff inflation and then deflation) and as such is not necessary representative of endothelial function at rest. As a reduction in RBP may potentially be influenced by an endothelial function adaptation at rest in response to a SS stimulus under resting conditions, assessing endothelial function in response to a maximal SS stimulus may not be representative of any resting adaptation that may have occurred. Consequently this may go some way to explaining why reductions in RBP observed following IHET in the study of McGowan et al (2007a) did not coincide with endothelial function adaptation. The same limitation can also be applied to the reactive hyperemia test as used by Badrov et al (2013) to assess resistance vessel endothelial function, as again endothelial function is measured in response to a maximal SS stimulus, and not assessed when the endothelium is in a resting state. Therefore it must be considered that the techniques utilised by McGowan et al (2007a) and Badrov et al (2013) to assess endothelial function may not have fully identified the role of endothelial function adaptation at rest as a mechanism for RBP reduction following IET in normotensives.

The current techniques for the assessment of endothelial function may also not be sensitive enough to detect small but physiologically significant changes in endothelial function that may occur in normotensive populations following IET. As normotensives typically have a "normal" endothelial function it could be speculated that any vascular adaptation that may occur will be minimal, so as to not disrupt vascular homeostasis. Indeed it seems sensible to suggest that more than one physiological mechanism may adapt in response to IET. In this instance, it is likely that each of these mechanisms change by a small fraction in an already "healthy" normotensive population, that in combination induce a significant reduction in RBP. Given the relationship between resting vascular endothelial dysfunction and hypertension (Versari et al, 2009; Perticone et al, 2001; Lind et al, 2000) a change in endothelial function may be one of these mechanisms. If these changes are small, then the current techniques for assessment of endothelial function may not be sensitive enough to detect these changes. As such this may help explain why McGowan et al (2007a) and Badrov et al (2013) did not observe changes in endothelial function that coincided with RBP reductions following IET.

Improvements in endothelial function may also only influence RBP regulation when local vascular adaptation occurs in larger conduit arteries (e.g. the femoral artery) with a large surface area, as opposed to smaller arteries with less surface area (such as the brachial artery). In order to adequately perfuse the large quadriceps musculature, the femoral artery is structurally wide and long. In comparison, the brachial artery is anatomically smaller, as the musculature that it perfuses is much smaller (the biceps and triceps). Theoretically small adaptations in endothelial function (that may occur in normotensive populations) specifically in the brachial artery following IHET, may not be significant enough in an artery with a smaller surface area to influence RBP regulation. In contrast, a small endothelial function adaptation in the much larger femoral artery may have a greater impact on RBP regulation, as the adaptation would occur over a greater artery surface area which would be likely to cause a greater reduction in TPR. Although this is largely speculative, several studies from the existing literature provide some supporting evidence for this theory. It is apparent from the existing literature that those studies exploring the role of brachial or forearm endothelial function adaptation as a mechanism for RBP reduction after IET have typically found no relationship between endothelial function adaptation and RBP adaptation for both normotensive (McGowan et al, 2007a; Badrov et al, 2013) and hypertensive (McGowan et al, 2007b) populations. The work of Baross et al (2012) demonstrated that significant reductions in RBP in pre-hypertensives coincided with significant vascular adaptation in the femoral artery following 8 weeks of bilateral ILEET. Whilst vascular adaptation was measured via changes in femoral resting AD, Tinken et al (2010) have previously established that structural changes in artery structure (diameter) supersede endothelial function adaptations. Whilst endothelial function was not directly measured in the study of Baross et al (2012), these findings may indicate that indeed a endothelial function adaptation may have occurred earlier on in the training program that consequently may have contributed towards RBP adaptation. As this adaptation occurred in a pre-hypertensive population it is currently unknown as to whether similar findings would occur in normotensive populations. Nevertheless, the work of Baross et al (2012) may provide preliminary evidence demonstrating the importance of endothelial adaptation location for endothelial function adaptation to be considered as a mechanism for RBP adaptation following IET. This remains to be investigated further, particularly in normotensive populations before any definitive conclusions can be made with regards to the role of endothelial function as a mechanism for IET induced RBP adaptation.

Taken together, the work of McGowan et al (2007a) and Badrov et al (2013) have made an initial attempt to provide some insight into the role of endothelial function adaptation as a

mechanism for RBP reduction following IET in normotensive populations. Whilst their findings suggest that endothelial function may not be a mechanism for RBP adaptation, the methods utilised to assess endothelial function may have had reduced validity due to limitations of these techniques. Thus it is not yet fully established as to whether endothelial function adaptation may act as a physiological mechanism for RBP reduction following IET. Until more sensitive techniques that specifically assess endothelial function under resting conditions are developed, future studies may not be able to ascertain the true role of endothelial function as a mechanism for RBP reduction after IET. As such, the exploration of structural adaptations to the vasculature may provide a greater insight in identifying the influence of vascular adaptations in RBP reductions commonly associated with IET.

1.4.4.ii Conduit artery diameter remodeling

Conduit AD remodeling has previously been proposed as a possible mechanism responsible for RBP reductions after IET by both McGowan et al (2007a&b) and Wiles et al (2010). This suggestion is based upon the notion that the artery may remodel to a larger diameter via increased availability and activity of NO at rest, thus lowering vascular resistance to BF (TPR), and therefore cause a reduction in RBP. Currently, it remains largely uninvestigated as a possible mechanism.

To date, there is a well established link between conduit AD remodeling and exercise training utilising other exercise modalities than IET. Unfortunately the influence of these conduit AD adaptations on changes in RBP were not identified (Rakobowchuk et al, 2005b). Rakobowchuk et al (2005b) demonstrated conduit brachial artery adaptation at rest after 12 weeks of whole body resistance exercise in young, healthy males. Significant AD increases were observed by the mid point of training (at 6 weeks), and these changes were still present by the end of the training protocol (12 weeks later). Aerobic and endurance exercise training studies demonstrate structural enlargement of conduit vessels following exercise training (Dienno et al 2001; Guyton and Hartley, 1985; Langille et al, 1989; Lloyd et al, 2001; Miyachi et al, 2001; Prior et al, 2003; Rodbard, 1975; Zamir, 1977). In addition to this, a number of research studies have demonstrated greater conduit AD remodeling in those athletically trained as compared to controls (Dinenno et al, 2001; Green et al, 2012; Haskell et al, 1993). Zeppilli et al (1995) found enlarged aortic, carotid and subclavian arteries in track cyclists and long distance runners as compared to control subjects after body surface area had been corrected for. Huonker et al (2003) & Schmidt - Trucksass et al (2000) also both found wider femoral arteries in cyclists, middle distance runners and triathletes than control subjects

or paraplegics. Specifically with regards to IET, Rowley et al (2011) demonstrated that squash players had larger brachial AD in their racket arms when compared to their non-racket arm. As gripping the squash racket requires prolonged isometric hand grip contraction, this study may provide evidence that repeated bouts of isometric exercise contraction may induce significantly greater resting AD adaptations. This remains to be validated by different isometric exercise protocols before conduit AD remodeling can be considered as the mechanism for reductions in RBP following IET.

Only one study has explored the role of resting AD changes as the mechanism for RBP reduction after IET. Baross et al (2012) noted a significant correlation between increases in common femoral AD and reductions in RBP after pre-hypertensive male participants performed 8 weeks of high intensity (85% HR_{peak}) bilateral ILEET. These vascular adaptations were confined to the vasculature of the trained limb, and suggested that local conduit AD remodeling may be a mechanism for RBP reductions following IET. Baross et al (2012) suggested that a likely explanation for the observed resting common femoral AD changes may be due to increased bioavailability and bioactivity of NO at rest, resulting from an increased SS stimulus during isometric exercise. Baross et al (2012) proposed that this explanation is largely speculative as there is little evidence to support SS induced adaptation in the common femoral artery. This may be attributed to a reduced ability in the common femoral artery to dilate in response to a SS stimulus (Walther et al, 2008). In comparison, other arteries such as the brachial artery appear to be more sensitive to a SS stimulus and induce a greater dilatory response (Walther et al, 2008). Furthermore, exercising SS was not measured in this study, therefore it is not possible to determine whether SS increased during isometric exercise to act as the physiological stimulus for femoral artery adaptations. In addition, Baross et al (2012) suggested that these AD changes may also have been caused by adaptations in sympathetic regulation of resting vascular tone. As no measure of the neural regulation of vascular tone was made, it remains to be established as to the influence increased sympathetic regulation of vascular tone may have on the conduit AD changes observed in this study. Nonetheless, the work of Baross et al (2012) gives plausibility to the consideration of conduit AD remodeling as a mechanism for RBP reductions following IET in pre-hypertensive populations. There are also a number of studies that have shown the structure of blood vessels is remodeled to a smaller lumen diameter in individuals with high BP (Epstein et al, 1994; Heagerty et al, 1993; Intengan & Schiffrin, 2001; Mulvany, 2002). This evidence supports the suggestion that a conduit artery remodeled to a larger resting diameter may be associated with a reduced RBP.

As a whole, it is not currently known as to whether conduit artery remodeling may be a mechanism for RBP changes following IET in normotensive population. In terms of a hypothesis for this thesis, it is expected that conduit AD remodeling may be a significant mechanism for RBP adaptation following IET. This thesis will utilise vascular ultrasound to assess any changes in conduit AD that may occur over an IET training period in an attempt to further the understanding surrounding the role of conduit AD remodeling as a possible mechanism for RBP reduction following IET in normotensive participants.

It is well established that conduit AD remodeling is mediated by a SS stimulus (Green et al, 2010; Kamiya & Togawa, 1980; Langille and O'Donnell, 1986; Tinken et al, 2010). Shear stress is a measure of the frictional force of BF against the endothelial surface of the arterial lumen (Padilla et al, 2008). Early work completed by Langille and O'Donnell (1986) demonstrated that a reduction in the BF stimulus in the common carotid artery of rabbits by 70% was able to induce a 21% decrease in AD within 2 weeks. Furthermore, when the endothelium of the artery was removed, the decrease in AD was abolished, despite a reduced flow stimulus. The work of Langille and O'Donnell (1986) demonstrated the relationship between a BF stimulus, the endothelium and an AD remodeling process. Furthermore, Kamiya & Togawa (1980) manipulated the level of BF and SS through the arteries of dogs, and established that greater levels of BF and SS induced greater resting AD's. In human studies, Tinken et al (2010) manipulated the level of SS during 8 weeks of rhythmic handgrip exercise by using cuff inflation to reduce SS levels to near zero in one arm, whilst the contralateral arm had no cuff inflation and thus experienced a exercise induced SS stimulus. Post exercise training results revealed significant increases in brachial artery dilatory response to ischemic exercise (a measure of AD remodeling to a maximal flow stimulus, [Green et al 2011]) in the arm exposed to an exercise induced SS stimulus, whilst there were no changes in brachial artery dilatory response to ischemic exercise in the cuffed arm that experienced a minimal SS stimulus during exercise training. Likewise, Green et al (2010) also found that when participants underwent 8 weeks of bilateral forearm heating with one arm cuffed to diminish SS and the contralateral arm uncuffed to undergo increased SS, microvascular function was only significantly enhanced post intervention in the uncuffed arm that experienced the increased SS stimulus. Overall, both animal and human studies have demonstrated the necessity and importance of a SS stimulus and the presence of an intact endothelium to induce AD adaptation.

It is also apparent that artery structural remodeling via an increased SS stimulus is dependent on endothelium derived NO. Nitric oxide is a potent vasodilator that plays a pivotal role in the maintenance of vascular tone and vascular reactivity (Moyna & Thompson, 2004). McGowan et al (2006, 2007a&b) suggested that repeated exposure to a SS stimulus could intrinsically influence the vascular tone of an artery via improvements in endothelial function or induce a remodeling process to a larger lumen diameter through increased bioavailability and bioactivity of NO. Nitric oxide is synthesized from the metabolism of L-arginine to L-citriuline by endothelial nitric oxide synthase (eNOS) (Niebauer & Cooke, 1996), that is released in response to SS induced dose dependent influx of calcium ions (Ca^{2+}) through cation channels in the endothelial cell (Johnson et al, 2011). Moyna & Thompson (2004) proposed that NO diffuses directly into the sub-endothelial space and vascular lumen where it activates smooth muscle cell guanylate cyclase, which relaxes smooth muscle. Tuttle et al (2001) established a significant correlation between eNOS expression and shear rate, whilst chronic exercise has been shown to increase up regulation of eNOS expression and subsequent NO production as a result of increases in endothelial SS (Sessa et al, 1994). Rudic et al (1998) demonstrated that when eNOS was disrupted in the carotid arteries of rats, the artery did not remodel to a larger lumen when exposed to an increased flow stimulus, whereas the rats with an undisrupted eNOS did experience AD remodeling. These studies together reinforce that artery remodeling is regulated by SS dependent eNOS and subsequent NO production. Green et al (2004) suggest that exercise training induced structural enlargement of conduit vessels may be an adaptive response, which acts to mitigate the increases in transmural pressure and wall stress brought about by repeated exercise bouts. This structural remodeling and consequent normalisation of shear removes the need for ongoing functional dilatation (Green et al 2004).

It is also suggested that the type of SS is important for artery structural remodeling. Johnson et al (2011) stated that there are two types of SS - laminar and oscillatory. Laminar BF typically produces antegrade (forward moving) SS, that is characterised by steady, undisturbed flow that creates a pulsatile SS along the endothelial cell surface, and enhances expression of anti-atherogenic molecules such as NO (Johnson et al, 2011). In contrast, high frequency oscillatory flow characterised by high levels of retrograde (backwards moving) SS with little net directional flow may be too fast for Ca^{2+} to influx into the endothelial cell (Johnson et al, 2011) and can increase the expression of pro-atherogenic genes and decrease anti-atherogenic genes (Thijssen et al, 2009a). Oscillatory SS characterised by high levels of retrograde shear is associated with increased oxidative stress via increased release of

intracellular superoxide radicals and NAPH oxidase activity (Johnson et al, 2011). Increased levels of oxidative stress is largely associated with endothelial dysfunction through decreased NO bioavailability (Higashi et al, 2009; Hunt et al, 2012; Johnson et al, 2011), and thus may not be able to provide the stimulus for NO dependent AD remodeling.

It remains to be established whether isometric exercise can induce sufficient increases in SS to mediate conduit AD remodeling. It is important that the SS response to isometric exercise be identified, as presence of an increased SS response may give plausibility to the possibility of conduit AD remodeling as a mechanism for RBP adaptation after IET. Evidence from studies exploring the BF response to isometric exercise have documented large increases in BF during contraction and in the immediate periods after (Gaffney et al, 1990; Hamann et al, 2004; Jensen et al, 1993; Osada et al, 2003; Sjogaard et al, 1988). As increases in BF are associated with increases in SS (Ando & Kamiya, 1993; Pyke et al, 2008; Topper & Gimbrone, 1999;), it is likely that SS may also increase in response to isometric contraction. The characteristics of this possible increase in SS (laminar versus oscillatory) are likely to determine whether a conduit AD remodeling process takes place (Johnson et al, 2011). Further investigation is required to establish whether isometric exercise has the ability to induce the correct haemodynamic conditions (i.e., increased SS characterized by laminar flow) to induce conduit AD remodeling.

Furthermore, consideration of conduit AD remodeling as a mechanism for RBP reductions after isometric exercise provides insight into the possible physiological stimulus for RBP adaptation. As section 1.3 of this current chapter identified, based on the work of Devereux et al (2010b) it is likely that the physiological stimulus for these IET induced cardiovascular adaptations is related to the intensity and level of fatigue induced during IET. It is suggested that this physiological stimulus is more prominent at higher levels of isometric exercise intensity to induce the greatest RBP adaptation. Previous research investigating the cardiovascular response to increasing intensities of isometric exercise have established that BP responds during isometric exercise in an intensity dependent manner, in that the greater the isometric exercise intensity, the greater the increase in BP from baseline values (Davies & Starkie, 1985; Ferguson & Brown, 1997; Friedman et al, 1992; Jandik et al, 1985; Petrofsky et al, 1975; Petrofsky et al, 1981; Smolander et al, 1998). This BP response is thought to occur to increase blood perfusion pressure and oxygen delivery to the exercising muscle that is rapidly fatiguing due to increased mechanical impedance to flow (and therefore a reduced oxygen supply) at higher intensities of isometric exercise (Hansen et al, 1993; Sadamoto et al,

1983). This is also supported by a number of studies utilising isometric handgrip exercise which have reported isometric intensity dependent increases in arm BF during isometric contraction (Hamann et al, 2004; Jensen et al, 1993; Osada et al, 2003). Since BF and SS are inter-related (Ando & Kamiya, 1993; Pyke et al, 2008; Topper & Gimbrone, 1999), intensity dependent increases in BF also bring about intensity dependent increase in SS. Therefore, it could be hypothesized that exposure to greater magnitudes of SS at higher isometric exercise intensities during training may bring about greater conduit AD remodeling adaptation, which may lead to greater reductions in RBP. Although largely theoretical, this may give support to the notion that a haemodynamic challenge (particularly SS) could be considered as the physiological stimulus for RBP reductions after IET. This remains to be investigated within the scope of this research thesis.

1.5 Chapter summary, research question and study aims.

It is evident that IET can significantly reduce RBP in both hypertensive and normotensive populations. Specifically, bilateral ILEET has demonstrated reductions of up to ~11 mmHg in SBP and up to ~3 mmHg in DBP (Baross et al, 2012; Devereux et al, 2010b; Wiles et al, 2010). It is currently not established as to what the physiological stimulus / stimuli may be to bring about this RBP reduction in normotensive participants. Although work completed by Devereux et al (2010b) suggests that this physiological stimulus may be more prominent at higher intensities of isometric exercise.

In order to help identify the physiological stimulus / stimuli for RBP adaptation to IET in normotensives, the possible mechanism that this physiological stimulus / stimuli may influence to bring about a reduction in RBP was examined. A critical review of the literature has identified that whilst a number of mechanisms have been investigated as contributing towards the RBP adaptations observed following IET, the results of Baross et al, (2012) suggested that the most likely mechanism relates to vascular adaptation, specifically that of conduit AD remodeling. Conduit AD remodeling is mediated by an increase in BF induced SS (predominately laminar in nature) acting upon the endothelium to increase release of the vasodilator NO that acts to relax smooth muscle within the artery walls, and thus dilate the artery at rest (Johnson et al, 2011; McGowan et al, 2006, 2007a&b; Moyna & Thompson, 2004). As this process is entirely dependent upon a combination of increased BF, SS, and the characteristics of the shear response, it would suggest that a haemodynamic stimulus may be the physiological stimulus for RBP reductions assuming conduit AD is the effector mechanism for this adaptation. Therefore the primary research question for this thesis is:

Do BF haemodynamics play a significant role in the RBP reductions commonly observed after IET in normotensives?

For BF haemodynamics to be considered as a primary physiological stimulus for RBP reductions after IET, it must first be identified whether there is a haemodynamic response to isometric exercise performed in normotensive participants. Furthermore, it must be established whether any haemodynamic response present is closely related to isometric exercise intensity, in line with the findings of Devereux et al (2010b). The characteristics of this haemodynamic response must be closely examined to identify whether this physiological response has the optimal behavior pattern (laminar flow vs. oscillatory) to up regulate the bioavailability and bioactivity of NO for a conduit AD remodeling process to occur, that subsequently may lead to a reduction in RBP following IET.

Therefore studies 2, 3 and 4 of this research thesis (chapters 3, 4 & 5) will explore whether a haemodynamic stimulus could be considered as an physiological stimulus for RBP adaptation following IET in normotensive participants. This will be achieved by establishing the BF (chapter 3), shear rate (chapter 4), and shear pattern (chapter 5) response to an acute bout of bilateral isometric leg extension exercise of increasing intensity (Study 1 in chapter 2 will explore the reliability of the techniques used to collect this haemodynamic data). Specifically, each of these haemodynamic variables will be measured during isometric leg extension contraction and in the immediate periods after contraction has ceased. By increasing isometric exercise intensity, it will be possible to identify whether this haemodynamic response is magnified at higher levels of isometric exercise intensity when oxygen delivery to the working muscle is reduced, and fatigue is present. The data gained from these acute response studies will help to determine whether high intensities of bilateral isometric leg extension exercise can induce a haemodynamic challenge in normotensive participants that could be considered as the physiological stimulus for RBP adaptation after IET for normotensive populations.

It will then be possible (chapter 6, study 5) to address this thesis' primary research question directly. This will be achieved by performing 8 weeks of bilateral ILEET to a specific isometric exercise intensity that induces either a high haemodynamic challenge or a low haemodynamic challenge in normotensive participants. Based upon the conclusions of pages 23–32, it is hypothesised that those participants that perform bilateral ILEET with a high

haemodynamic challenge may experience a greater reduction in RBP after IET via greater conduit AD adaptation caused by repeated exposure to an increased haemodynamic stimulus. In contrast, the group performing bilateral ILEET with a low haemodynamic challenge may experience less or no reduction in RBP, as the haemodynamic challenge is not a great enough stimulus to induce conduit AD adaptation, and subsequent RBP reduction. Therefore, the results of this study will fully establish the role of blood haemodynamics in the RBP adaptation to IET.

Furthermore, the investigations performed in chapter 6 may also provide an insight into the physiological mechanism(s) that adapt in response to IET, which consequently lead to a reduction in RBP in normotensive participants. In particular, this chapter will focus upon establishing any vascular changes (specifically conduit AD remodeling) that may occur over the 8 week training period, and consequently may coincide with RBP reduction. Changes in other non-invasive cardiovascular measures, such as TPR, $\dot{Q}$, HRV and systemic vascular adaptations pre to post bilateral ILEET will also be investigated. Together, these measures may provide further insight into the role that a number of physiological mechanism(s) may play in the reductions in RBP commonly associated with IET in normotensive participants.

Chapter 2

General Methods

Chapter 2: General Methods

This chapter details the general methods used in this thesis aimed at investigating the role of local blood haemodynamics in reductions in RBP following IET. In order to address the research questions raised, it was decided to undertake a series of laboratory based research studies. Figure 3 schematically demonstrates the sequence of studies designed for this thesis in an attempt to identify the possible physiological stimulus / stimuli and physiological mechanism(s) responsible for reductions in RBP after bilateral ILEET.

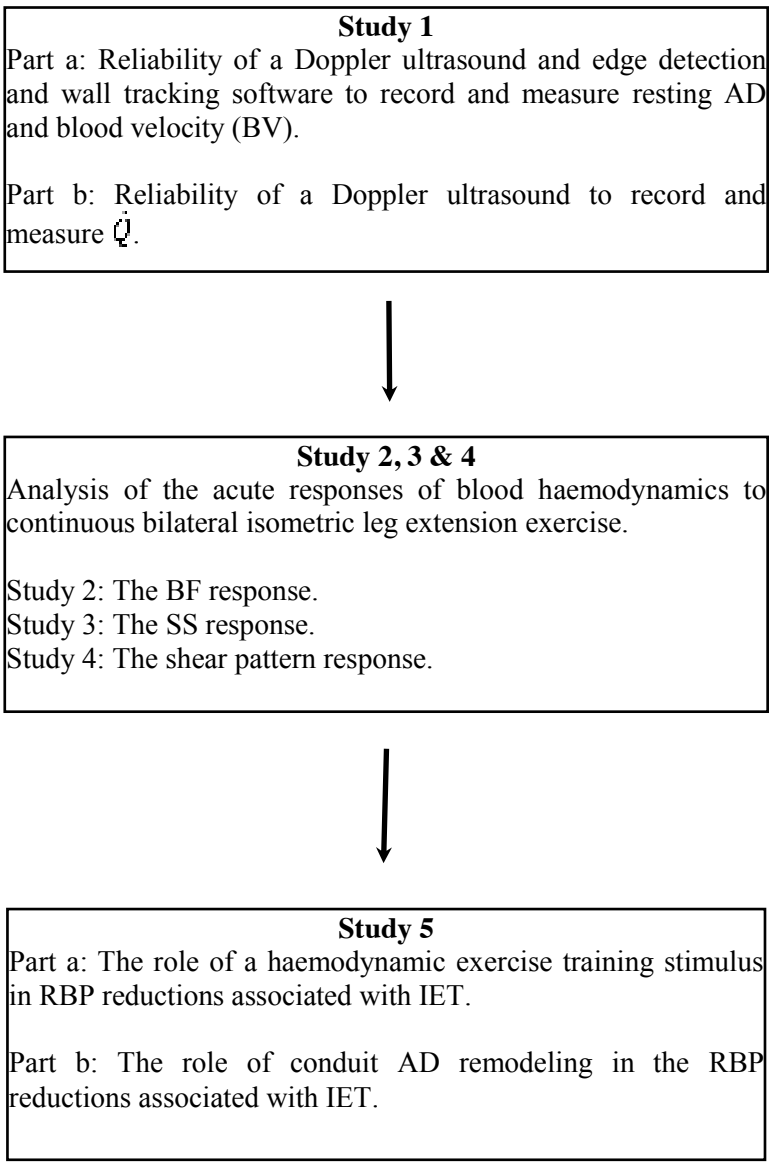

Figure 3. *Schematic of the design and process of studies within this thesis*.

As figure 3 demonstrates, this thesis uses a combination of descriptive and repeated measures study designs. Specifically, the reliability studies presented in this chapter & Chapters 3, 4 and 5 involve cross-sectional study design, whilst Chapter 6 involves a randomized controlled trial. This methodology chapter will document the processes, equipment and physiological measures taken, will evaluate the reliability of the measurements and estimate sample size to achieve each study design that contributed to the investigation of the wider research question. The organisation of this chapter is such that it is divided into sub-sections, part 2.1 - Ethical considerations (page 38), part 2.2 Participants and recruitment (page 38); part 2.3 - Instrumentation and procedures (page 39), part 2.4 - Reliability of measurement (page 63) and part 2.5 Sample size estimation of dependent variables (page 74). In addition, part 2.6 details a pilot study conducted to perform analysis of Doppler ultrasound BV data (page 75).

2.1 Ethical considerations

All testing was carried out at the Canterbury Christ Church University BASES accredited Laboratory in Canterbury, Kent. All studies and procedures were reviewed and approved by the University's Ethics Committee and adhered to the guidelines set by the 1964 Declaration of Helsinki.

2.2 Participants and recruitment.

Healthy active males (staff and students) (18-44 years old) were recruited from Canterbury Christ Church University for the studies included in this thesis. All participants were screened before participation using a personal and family heredity health questionnaire (see appendix 1, page 249). Participants were free from any cardiovascular (including hypertension), respiratory or metabolic disorders that may have affected their response to exercise performance. Applicants were not selected if they were currently taking medication that could affect their cardiovascular response to exercise. All participants were non - smokers and had no history of smoking. Blood donation has been shown to alter blood volume and blood chemistry (Bouchard et al, 1995), which could have repercussions on the cardiovascular measures made within this thesis. Applicants who regularly donated blood, or planned to do so during the course of the study were also not selected for participation.

Participation in the studies involved in this thesis was on an entirely voluntary basis, and included no monetary benefits, but did offer students additional experience of being involved in postgraduate level research. A number of participants volunteered for participation in multiple studies included in this thesis. In this instance a sufficient amount of time was left between each study for any physiological adaptations due to training effect (if any) that may have occurred in these participants to reverse (Skinner, 2005). All prospective participants were supplied with detailed information sheets for the study which they wished to participate in (information sheets used can be found in appendices 2-4, pages 252-260). Prior to their first laboratory visit, participants also met with the lead researcher to discuss the testing procedure in greater detail, and to answer any questions that the prospective participant may have. Once the participant had agreed to participate and had signed an informed consent (see appendix 5, page 263), they were then invited to the laboratory for a familiarisation session. At this first session, participants were required to complete the personal and family heredity health

questionnaire as well as a RBP assessment. The questionnaire was checked for any health contraindications, whilst assessment of RBP allowed determination of the participants RBP classification. The Hypertension guidelines (2011) produced by the NHS National Institute for Clinical Excellence (NICE) and British Hypertension Society (BHS) state that if SBP is measured at above 140 mmHg and DBP is measured at above 90 mmHg, then participants are deemed "hypertensive". If this circumstance arose during a participants RBP assessment, the lead researcher would document the reading in a written letter to the participants GP and request that this reading be confirmed and diagnosed by a qualified medical professional. Consequently, if a qualified medical professional deemed the participant as "hypertensive" the participant was not invited to partake in the study.

2.3 Instrumentation and procedures

This section of the methodology chapter documents the equipment and procedures used within the study design of this thesis, and also details in which specific study each piece of equipment was used.

2.3.1 Biodex System 3 isokinetic dynamometer (used in Chapters 3, 4, 5 & 6).

The dominant mode of exercise used in this thesis was isometric leg extension exercise. This was based upon previous work of Wiles et al (2010), Devereux et al (2010b) & Baross et al (2012) that also examined RBP reductions following IET, specifically using isometric leg extension exercise. A Biodex System 3 isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY) and Biodex advantage software for Windows XP (Microsoft Corporation, Redmond, Washington, USA) was used to perform all isometric leg extension exercise in this thesis. This specific dynamometer allows the user to contract their muscles isometrically during a pre-set joint angle and plane of motion, whilst also measuring and recording torque output. A 16 channel chart recorder (PowerLab, ADInstruments Ltd, Australia) was also connected to the Biodex advantage software to allow torque measurements to be synched with recorded electromyography (EMG) measurements during exercise. Drouin et al (2004) and Zawadzki et al (2010) have suggested that the Biodex System 3 isokinetic dynamometer demonstrates good trial-to-trial and day-to-day re-test reliability, as well as valid measurements for isometric torque and position. This therefore indicates that the Biodex System 3 isokinetic dynamometer is likely to provide reliable and valid torque measurements needed for the scope of this research study.

The set up of the Biodex System 3 isokinetic dynamometer to perform isometric leg extension exercise was mostly identical to the protocol used by Wiles et al (2010) and Devereux et al (2010b). Please refer to Figure 4 for a visual reference to the set up of the Biodex System 3 isokinetic dynamometer.

Attachment arm - This protocol involved modifying a standard hip attachment to create an attachment that allowed bilateral leg extension exercise to be performed. This was inserted into a knee attachment movement arm to allow the modified hip attachment to be positioned around the legs. The movement arm containing the modified hip attachment was secured to the participant's legs 1cm superior to the medial malleoli of the ankles with a large velcro strap that surrounded the lower half of the calf. Foam was also inserted into the space between the strap and the participant's legs to avoid discomfort for the participant when performing maximal voluntary leg extensions.

Participant's position - Participants sat in an slightly tilted position with 110 degrees flexion at the hip to allow access to the common femoral artery (refer to image 10 page 53) for ultrasound analysis during testing protocol. When there was no requirement to perform ultrasound analysis on the common femoral artery, participants were still required to maintain 110 degrees of flexion at the hip to standardise the exercise testing position. The Biodex seat was individually adjusted for each participant depending upon their physiological requirements. Chair depth was altered if needed so that each participant had their lower back supported by the lumbar rest of the chair, whilst their thighs were fully supported with the crease at the back of their knees tight against the end of the seat. The horizontal and vertical planes of the chair were also adjusted so that the lateral femoral condyle of the participant's right leg was aligned with the center of the dynamometer movement head. Each participants Biodex chair settings were recorded at their first session to ensure standardised participant position between future trials to be performed.

During exercise - Participants were instructed to keep their upper body relaxed to isolate contraction of the leg muscles, as increased stabilization from the upper body has been shown to increase leg extension force production (Magnusson et al, 1993; Mendler, 1976). Due to the slightly tilted seated position, the hips and chest muscles were firmly strapped to the Biodex chair to prevent participants lifting their

hips to aid maximal contraction of the legs with their hip flexor muscles. Participants were also instructed to breathe normally during exercise periods to avoid a valsalva manoeuver, which has been shown to cause acute increases in TPR and MAP (Porth et al, 1984).

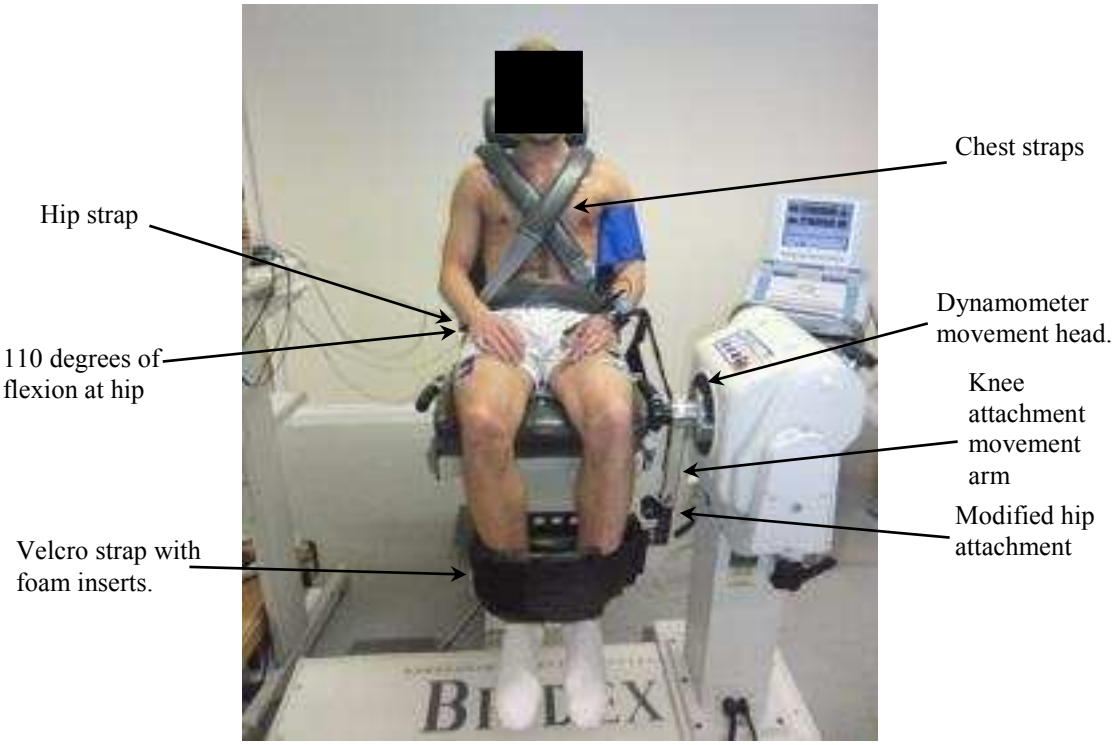

Figure 4. Biodex chair set up with modified hip attachment.

2.3.2 Electromyographic (EMG) recording (used in Chapters 3, 4, 5 & 6)

Isometric leg exercise utilised in Chapters 3, 4, 5 & 6 was performed to a $\%EMG_{peak}$ intensity. This was based upon the exercise protocols of Devereux et al (2010b), Wiles et al (2010) & Baross et al (2012) who examined RBP reductions when performing isometric leg exercise to a $\%EMG_{peak}$. In line with Wiles et al (2010), Devereux et al (2010b) & Baross et al (2012), surface EMG was recorded from the vastus lateralis muscle (figure 5) of both the left and right leg using a duel bio amplifier and 16 channel chart recording software (PowerLab, ADInstruments Ltd, Australia). The vastus lateralis muscle was chosen as a suitable muscle within the quadriceps group to record EMG from, as Alkner et al (2000) demonstrated a linear relationship between vastus lateralis EMG measurements and quadricep force production when performing leg extension exercise.

Electrodes and placement - Positive and negative electrodes (Sensor T ECG Pads, Ambu Inc, Maryland, USA) were used to record EMG from the vastus lateralis muscles of each leg. Electrodes were placed two thirds of the way along the line from the anterior spina iliaca superior to the lateral side of the patella in the direction of the muscle fibers (figure 6). An earth electrode was placed on the right olecranon at the proximal end of the ulna. SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles, www.seniam.org) was used to provide reference points for electrode placement and to provide skin preparation guidelines. Skin was first shaved to remove any hair from the electrode location, and then lightly abraised to remove any dead skin and to maximise skin-electrode conductivity. The area was cleaned with an alcohol wipe, and left to dry before the electrode was fitted. Once the electrodes had been placed, skin impedance values were checked, and were deemed acceptable if below 10kΩ (Hermens et al, 2000). If skin impedance was not acceptable, electrodes were removed and skin preparation procedures (i.e. shaving, abrasion and cleaning) were repeated. Fresh electrodes were then re-applied and a skin impedance test repeated. Skin preparation and skin impedance checks were completed prior to every exercise test performed.

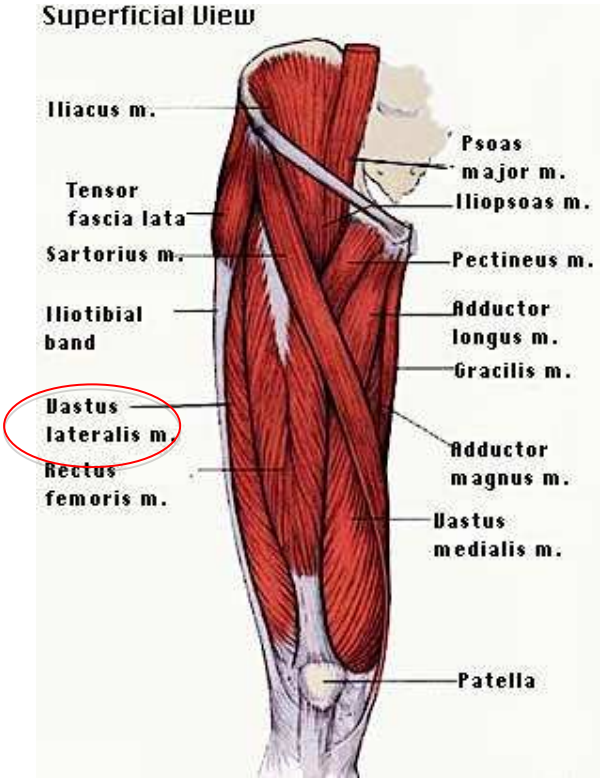

Figure 5. *Anterior view of the quadriceps muscles. Lucy.stanford.edu/img/ant_comp5.jpg. With copyright permission.*

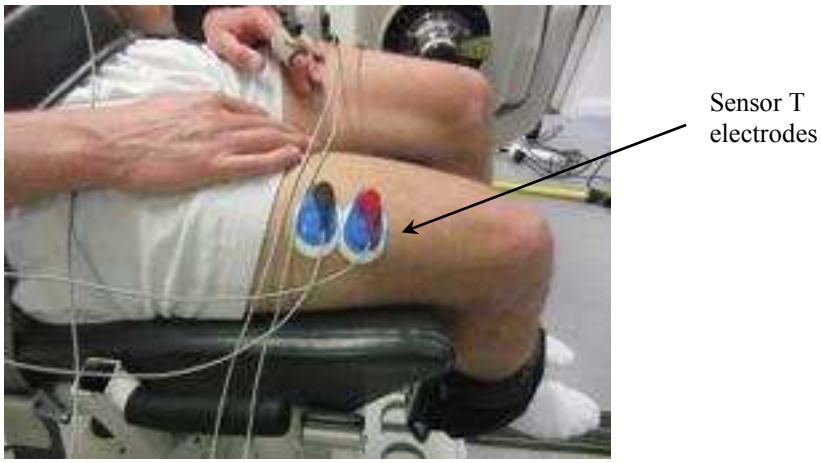

Figure 6. Electrode placement for EMG recording.

Computation of EMG - Chart 7 for Windows XP recording software (ADInstruments Ltd, Australia) was used to compute the root mean square of the raw EMG data from each leg. The EMG data was then smoothed at 1 second using high and low pass digital filters. EMG data from each leg was combined and averaged to provide one mean EMG value. These values were then used at a later stage to calculate $\%EMG_{peak}$ exercise intensities, for which isometric leg extension exercise was to be performed (Wiles et al, 2010). When performing leg extension isometric exercise, the EMG value was displayed in real time for each participant so that they could achieve the correct target $\%EMG_{peak}$ value in relation to leg extension contraction intensity in the studies documented in this thesis.

EMG amplitude and EMG frequency - EMG signal amplitude (EMGamp) and EMG signal frequency (EMGfreq) were used as a measure of fatigue during isometric leg extension exercise in Chapter 3 of this research thesis. The protocol used in this research thesis to record and compute EMGamp and EMGfreq was largely based on that used by Devereux et al (2010b), who also measured indices of fatigue during isometric leg extension exercise. Chart 7 for Windows XP recording software was used to record and analyse EMGamp and EMGfreq measures. EMG signal amplitude was calculated by subtracting the minimum signal voltage from the maximum signal voltage. In order to record and analyse EMGfreq, an additional channel was set up for cyclic frequency measurement of each vastus lateralis muscle. EMG signal amplitude and EMGfreq were averaged over a 5 second period continuously throughout exercise protocol. Therefore a 2 minute contraction period provided a total of 24 EMGamp and EMGfreq measurements. EMG amplitude and EMGfreq values from both legs were combined

and then averaged to provide one mean EMGamp value and one mean EMGfreq value for every 5 second period throughout exercise (Devereux et al, 2010b).

2.3.3 Electrocardiography (ECG) (used in Chapters 3 & 6)

Heart rate was measured via ECG using a 16 channel chart recorder (PowerLab, ADInstruments Ltd, Australia) with Chart 7 for Windows XP recording software (ADInstruments Ltd, Australia). Heart rate was recorded in Chapters 3 & 6 to represent the cardiovascular response to isometric exercise. Specifically, in Chapter 3 exercising HR was recorded in response to acute bouts of isometric exercise, where as resting HR was recorded and analysed in Chapter 6 to determine if there was any cardiovascular adaptation after an isometric exercise intervention. Electrocardiography involves recording the electrical activity of the heart. This electrical impulse is recorded as an ECG rhythm strip, which demonstrates the depolarization (contraction) and repolarization (relaxation) of the atria and ventricles (Hampton, 2013). However it should be noted that this ECG trace was used only to determine HR and HRV, and was not analysed for diagnostic purposes.

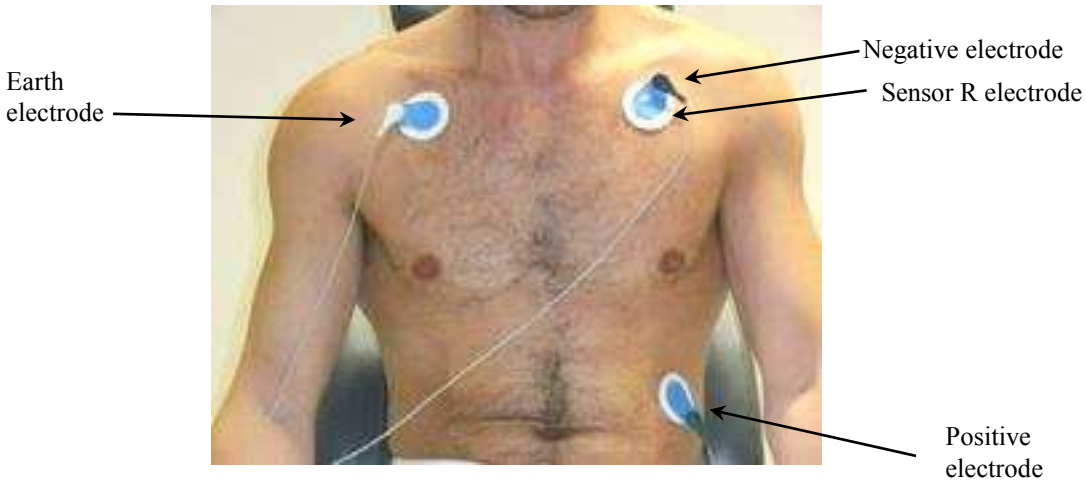

Figure 7. Three lead bipolar ECG placement

Electrodes and placement - Sensor R electrodes (Ambu Inc, Maryland, USA) were placed in a standard three lead bipolar arrangement, inferior to the right (earth) and left (negative) clavicle, midway between the conoid tubercle and costal tuberosity, and over the tenth rib (positive) on the left side (see figure 7). The skin was prepared prior to electrode placement by shaving the area to remove any hair, and any dead skin was removed using a light abrasive paper. The area was then cleaned with alcohol and left to

dry before the electrode was applied. Heart rate was continuously recorded at rest and during exercise.

2.3.4 Heart rate variability (HRV) (used in Chapter 6)

Heart rate variability is a measure of the moment to moment fluctuation in HR, by which the analysis of its spectral power components is deemed to provide information about the sympathetic and parasympathetic modulation of HR (Perkins et al, 2006). Heart rate variability was determined from the ECG data, and the methods used to collect this data are described in section 2.2.c of this methodology chapter. Heart rate variability was measured for 5 minutes after participants had undergone a 15 minute rest period. The Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology (1996) recommend that breathing must be paced to be able to clearly distinguish between high frequency and low frequency components of HRV. In line with this recommendation, participants were required to breath at a rate of 12 breaths . min^{-1} (0.2 Hz) using a metronome as a reference and completely refrained from talking during this measurement period.

Lab Chart software (ADInstruments Ltd, Australia) detects each QRS complex during the 5 minute ECG recording via R wave detection, and then converts this time domain data to frequency domains using Fast Fourier Transform (FFT) algorithm. The data segment size was set to 1024 points for FFT calculation (Perkins et al, 2006). The frequency domains measured were total power (TP), high frequency power (0.15 - 0.4 Hz) (HF), low frequency power (0.04 - 0.15 Hz) (LF), high frequency normalised units (HFnu), low frequency normalised units (LFnu) and the LF/HF ratio (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Each recorded ECG trace was manually scanned for ectopic beats, which were subsequently excluded from the HRV calculation.

2.3.5 Blood Pressure Measurement (used in Chapters 3 & 6)

Blood pressure was the primary outcome variable to be measured in this thesis, as the main aim of this work was to determine the possible mechanism(s) that lead to the RBP reductions consistently observed by Wiles et al (2010), Devereux et al (2010) & Baross et al (2012) following IET. Specifically, BP was measured using two different methods depending on the requirements of the study. Continuous exercising BP measurements (as seen in Chapter 3) were recorded using a Finometer (Finapres, TNO instruments,

Amsterdam, The Netherlands), whilst RBP was recorded using an automated BP monitor (Dinamap Pro 300 Critikon, GE Medical Systems, Slough, Berks, UK), as used in Chapter 4.

Finometer

The Finometer is a non- invasive device that utilises the Peñáz (1969) volume-clamp method and the Physiocal (Physiological calibration) criteria of Wesseling et al (1995) to provide continuous beat to beat measurements of finger BP, in an attempt to reconstruct intra-arterial brachial pressures. Please refer to Figure 8 for the set up of the Finometer. Despite possible physiological differences in brachial pressures as opposed to finger pressure, the Finometer utilises several correction methods (such as waveform filtering, level correction and level calibration) to permit the reconstruction of brachial pressure from the non-invasive finger arterial measurements (Guelen et al, 2003). Several studies have demonstrated the validity of the Finometer to reconstruct intra-arterial brachial pressure from finger arterial measurements (Bos et al, 1996; Guelen et al, 2003; Guelen et al, 2008; Parati et al 1989; Van Egmond et al, 1985). Consequently, the Finometer has demonstrated its reliability in detecting both small acute and longer term changes in BP measures (Schutte et al, 2003). An infrared photo - plethysmograph built in to the finger cuff detects changes in arterial diameter. An increase in BP, and thus an increase in AD is detected by a photodiode as an increase in light absorption, which causes a decrease in signal coming from the plethysmograph. When BP decreases and subsequent AD decreases, there is a decrease in light absorption and a subsequent increase in the plethysmograph signal. The Volume Clamp method of Peñáz (1969) increases / decreases the pressure in the inflatable air bladder within the finger cuff, in relation to the changing plethysmograph signal caused by varying arterial pressures within each cardiac cycle, thus aiming to keep AD at a constant set point. An expert system called Physiocal (Wesseling et al, 1995) uses a computer algorithm to define and maintain the correct point at which the artery is set. A servo controller system then compares a measured AD (via the light detector) against this set point. The difference between the two values is used to control the pneumatic proportional valve in the front unit that modulates air pressure generated by a air compressor and thus causes changes in finger cuff pressure to oppose the change in arterial diameter. For example, if during systole an increase in arterial diameter is detected, the finger cuff pressure is immediately increased by the servo controller system to prevent the diameter change. If during diastole, there is a decrease in arterial diameter then the servo controller will

decrease the amount of air pressure in the finger cuff to oppose the diameter change. As a result, AD is continuously kept at a constant set point (Finger Pressure Reference Guide, FMS Finapres Medical Systems BV).

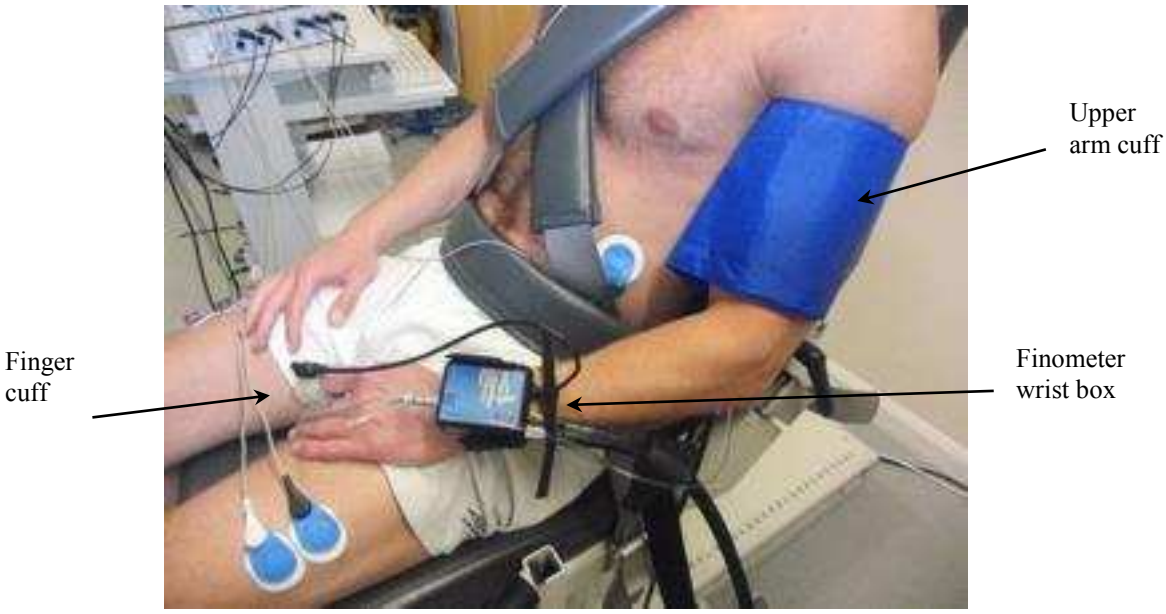

Figure 8. Finometer set up.

The Wesseling et al (1995) Physiocal criteria states that intra - arterial pressure will be equal to finger cuff pressure only if transmural pressure is zero. Transmural pressure is simply the pressure difference between the pressure on the inside of the artery and the pressure of the surrounding tissue. Therefore it is suggested that when transmural pressure is zero, the finger cuff pressure must equal intra - arterial pressure and therefore represent true BP measurement. For example, if during systole an increase in arterial diameter is detected, and finger cuff pressure is increased by the servo controller system to prevent this change in AD, and if transmural pressure is zero, then the increase in the finger cuff pressure must equal the increase in intra-arterial pressure, and thus SBP is known. Likewise, if a decrease in AD is detected, and pressure in the finger cuff is decreased to oppose the diameter change, then the pressure decrease in the finger cuff will equal the pressure decrease intra-arterially inline with Wesseling et al (1995) Physiocal criteria, and thus DBP will be known.

Systolic, DBP & MAP was recorded from the left arm and left index finger of the left hand using the correct sized finger cuff for each participants finger circumference

during exercise. All measurements made during each lab visit were made on the same arm and finger for consistency. Participants underwent a 15 minute rest period before BP recording took place. The Finometer was also connected to the 16 channel chart recording software (PowerLab, ADInstruments Ltd, Australia) so that real time BP could be displayed against ECG and EMG.

Automated blood pressure monitor

The Dinamap Pro 300 was used to measure SBP, DBP, and MAP at rest, in order to determine chronic changes in BP (utilised in Chapter 6), as used by Wiles et al (2010) and Devereux et al (2010b). This device uses the oscillometric technique first demonstrated by Marey in 1876, and measures BP by monitoring the pulsatile changes in pressure that are caused by the flow of blood through the brachial artery that is restricted by a pneumatic cuff placed around the participants upper left arm (Pickering et al, 2005). The Dinamap Pro Series 100-400 Monitor Operation Manual (Critikon, GE Medical Systems, UK) describes how the Dinamap Pro 300 is able to determine SBP and DBP using the oscillometric technique. The cuff is initially inflated to a pressure that completely occludes BF through the brachial artery. Pressure in the cuff is then decreased to deflate the cuff in a controlled and progressive manner. Systolic blood pressure is detected when the cuff pressure decreases to allow the flow of some blood through the partially occluded brachial artery. As a result, pressure oscillations increase in amplitude and are detected by the transducer. As the pressure in the cuff decreases further, the pressure oscillations reach maximum amplitude, which is the point at which MAP is measured. Diastolic blood pressure is determined when the pressure oscillations begin to decrease in amplitude. The values of SBP, DBP and MAP are then computed using an algorithm specific to the Dinamap Pro 300 (Dinamap Pro Series 100-400 Monitor Operation Manual, Critikon).

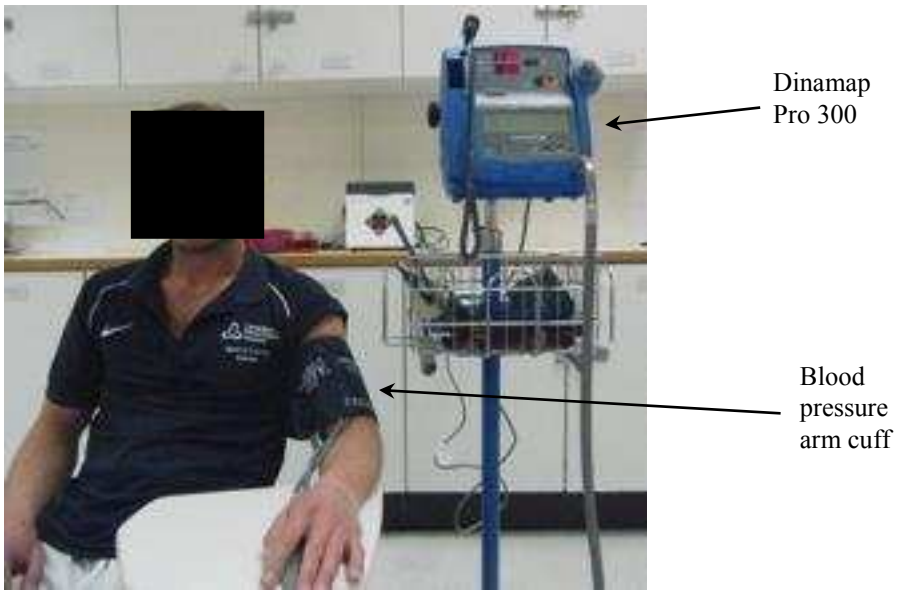

Figure 9. Dinamap Pro 300 set up.

In order to accurately measure BP using the Dinamap Pro 300, a BP cuff was placed on the participants upper left arm, approximately 1.5 cm above the antecubital fossa, roughly level with the participant's heart (please refer to figure 9). Arm circumference was initially measured to allow the selection of the appropriate sized cuff from a range of sizes (small, medium and large). All BP measurements were made following the American Heart Association BP Measurement Recommendations (Pickering et al, 2005). The participant was comfortably seated in a chair, with legs uncrossed, feet flat on the floor and the back and left arm supported. The upper left arm was free from restrictive clothing, whilst the cuff used encircled at least 80% of the arm circumference. Participants first underwent a 15 minute resting period before any measurements were made. The participants were instructed to relax as much as possible, and were asked not to talk throughout the resting period and measurement protocol. Three BP measurements were made, with at least 60 seconds between each measurement, as in accordance with Wiles et al (2010) & Devereux et al (2010b). When analysing BP data, the lowest of the three measurements (for each SBP, DBP and MAP) were used, as it has been previously suggested that initial measurements are often higher than subsequent measures, and thus are not reflective of actual BP (Katzel et al. 1995). Wiles et al (2010) and Devereux et al (2010b) also utilised this method of analysis.

The reliability of this device has previously been investigated in the unpublished doctoral thesis of Devereux (2010a) using a very similar participant cohort as to that used in this thesis. Inter-day reliability of the Dinamap Pro 300 was examined using young, healthy male adults. The coefficient of variation (CV%) between repeat trials was found to be 1.84 % (SBP), 2.60% (DBP) and 2.09% (MAP). These CV % values were subsequently used to calculate sample size for Chapter 6 in this research thesis. Sample size estimation can be viewed on page 74.

2.3.6 Total peripheral resistance (TPR) (used in Chapter 6)

Total peripheral resistance is the term for the resistance to flow as the blood moves through the vascular system. Poiseuiles' law stated that TPR is directly proportional to blood viscosity and length of vessel, whilst being inversely proportional to the forth power of the radius. Total peripheral resistance change was assessed before and after a isometric exercise intervention in Chapter 6. Total peripheral resistance can be calculated as:

[Mean arterial pressure (MAP) - central venous pressure (CVP)]/ Cardiac output ($\dot{Q}$)

As CVP is normally near 0 mmHg, the calculation can be simplified as:

$$TPR = MAP / \dot{Q}$$

(Devereux et al, 2010b)

The reliability of this calculation to determine TPR is discussed on page 72.

2.3.7 2-Dimensional (2D) ultrasound and Doppler ultrasound measurements (used in Chapters 3, 4, 5 & 6).

A 2D and Doppler ultrasound was used to perform vascular measurements of blood haemodynamics in the reliability studies presented in this current chapter, and in Chapters 3, 4, 5 & 6. This was also used to perform transthoracic echocardiographic measurements of the heart in the reliability studies presented in this current chapter and Chapter 6. Before each specific measurement can be described in detail, 2D ultrasound and Doppler ultrasound technology will first be introduced.

2 -Dimensional ultrasound

Ultrasound is simply high frequency sound waves that travel through a medium by causing local displacement of particles within that medium (Thrush & Hartshorne, 1999). A transducer applied to the skin's surface transmits ultrasound waves into the body using the piezoelectric effect. The piezoelectric effect is the method by which piezoelectric crystals in the transducer vibrate mechanically when a varying voltage is applied across them (Thrush & Hartshorne, 1999). The frequency of the voltage applied will determine the frequency at which the crystals will vibrate (Polak, 1992). When an appropriate coupling medium is used, i.e. ultrasound gel, the vibrations are transmitted into the human body. As the waves travel through the different mediums of the body (e.g. skin, tissue, blood, water), part of the ultrasound beam is reflected back towards the transducer. The transducer uses the piezoelectric effect to convert the returning ultrasound vibrations back into electrical signals. These signals are then analysed by the ultrasound machine and form the basis of a 2D ultrasound image.

High resolution B mode imaging (also known as 2D imaging) is a commonly used technique for visualising and measuring structures within the human body, as it is non-invasive, cost effective and is a reliable technique once the user has undergone training and had sufficient practical experience (Thijssen et al, 2011). In order to achieve the best user reliability possible, the user of the B mode imaging in this current research thesis (Jenna Smith) had undergone an Cardiovascular Ultrasound training course at Liverpool John Moores University, and had undertaken several months of practice scans before the testing protocol commenced. The CV% data presented in section 2.4 (page 63) represents the ability of this user to reliably use B mode imaging and Doppler ultrasound to reproduce AD, BV and $\dot{Q}$ measurements.

Doppler ultrasound

Pulsed-wave Doppler ultrasound is a common technique to asses moving structures within the human body, for example moving red blood cells. Doppler ultrasound works on the principles of the Doppler effect. When the ultrasound beam hits a stationary structure within the human body, it is reflected back at the same frequency. However, if the beam hits a moving structure, the frequency of the reflected beam differs from that originally transmitted. In terms of moving red blood cells, each red blood cell encounters more transmitted waves per second as it is traveling through a vessel. Each of these encountered waves are reflected back resulting in a greater number of reflected

waves per second, and thus a different frequency (waves per second) (Kandath et al, 1990).

The Doppler effect is used to analyse the velocity of moving blood within the human body. The frequency of the transmitted ultrasound is compared to the returning wave frequency. The difference between the two is termed the frequency shift. Polak (1992) states that frequency shift can be calculated as:

$$\Delta F = Fr - Ft = \frac{2Ft \times V \times \cos^\circ}{c}$$

(Polak, 1992)

Where by:

ΔF = frequency shift

Fr = frequency of reflected ultrasound beam

Ft = frequency of transmitted ultrasound beam

V = velocity of red blood cells

cos° = angle between the ultrasound beam and direction of BF

c = velocity of ultrasound in bodily tissues (fixed at 1540 ms/sec).

Polak (1992) explained that the Doppler frequency shift caused by the motion of the object is equal to the difference between the Ft and Fr. If cos° is known, and c is fixed, blood velocity (BV) can easily be calculated as V is proportional to ΔF:

$$V = \frac{\Delta F \times c}{2Ft \times \cos^\circ}$$

The inbuilt computer within the ultrasound machine analyses each frequency shift, and consequently calculates velocity. Velocity is then displayed on a spectral display in centimeters per second (cm/sec). Blood velocity with 0% error is obtained when the ultrasound beam is parallel to BF. As the angle of insonation increases, velocity becomes underestimated. The best 2D image is obtained when the structure is perpendicular to the ultrasound beam, therefore the operator must compromise an Doppler ultrasound beam that is as close as possible with the direction of BF (Polak, 1992). For measurement of BV in the vasculature, a compromise of an angle of less than 60° is deemed acceptable, with a minimal measurement error of 12% (Polak,

1992). For measurement of BV within the heart structures, an ultrasound beam of greater then 20° parallel to BF is unacceptable as this produces a 6% error in measurement (Kandath et al, 1990).

i. Haemodynamic measurements of the vasculature used in this investigation.

Artery diameter measurement (measured in the section 2.4.i, page 64 and Chapters 3, 4, 5 & 6).

A LOGIQ e (GE Healthcare, UK) ultrasound video imaging system combined with a linear transducer probe (8L - RS, GE Healthcare, UK) operating at an imaging frequency of 8 MHz was used to image AD. The reliability of the measurement of conduit AD using the LOGIQ e ultrasound and edge detection and wall tracking software can be found on page 64.

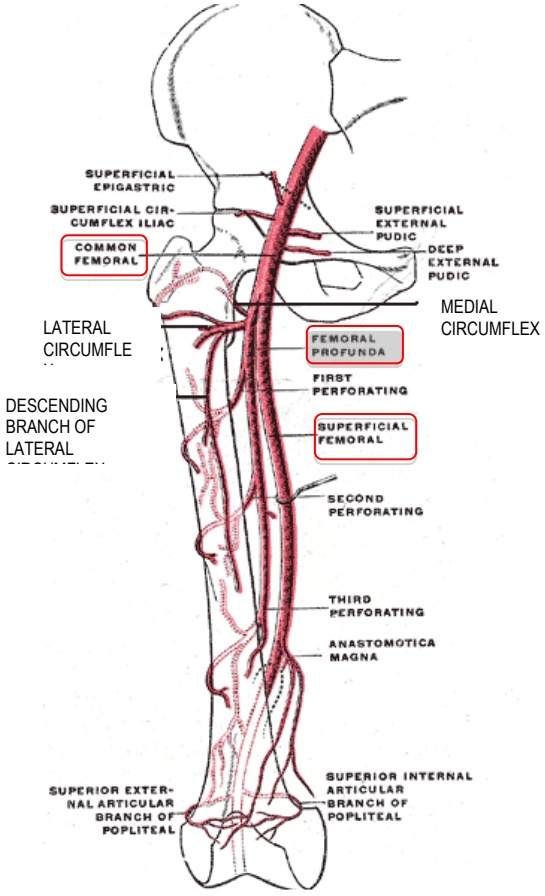

Figure 10. *Arterial anatomy of leg from an anterior view..*
http://en.wikipedia.org/wiki/Medial_circumflex_femoral_artery. With copyright permission.

Resting and exercising conduit AD was imaged within the research studies of this thesis. All resting conduit AD measurements were made after an initial 15 minute rest period. The common femoral (Chapters 3, 4, 5 & 6), superficial femoral (section 2.3.i)

and brachial artery (Chapter 6) were all imaged in this investigation. When imaging the common femoral artery, the probe was placed distal to the inguinal ligament and above the bifurcation into the superficial and profunda femoral branch (Radegran et al, 1997; Baross et al, 2012). The superficial artery was located distal to the bifurcation of the common, superficial and profunda arteries (Kooijman et al, 2008). Figure 10 shows a visual representation of the location of these arteries in reference to the anatomy of the leg. The brachial artery was also imaged by placing the probe over the brachial artery approximately 9 cm proximal to the medial epicondyle (Baross et al, 2012).

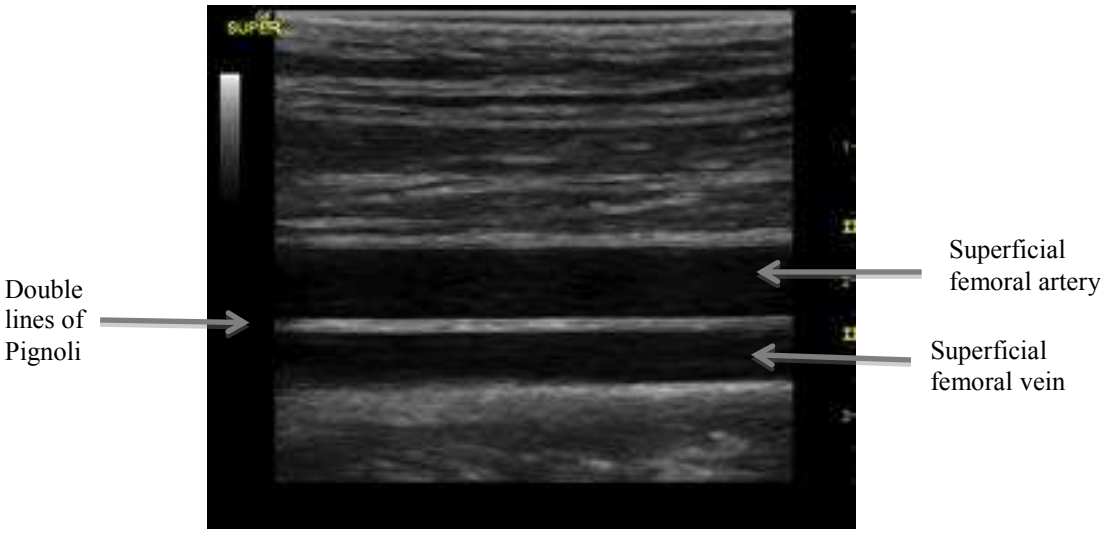

Figure 11. *Superficial femoral artery in the longitudinal axis*

When inter-day repeat measurements were required, several measures were taken to ensue that the artery was imaged and analysed in the same location for each examination. Medical permanent marker pens were used to mark the skin where the optimal probe location was. The probe was drawn around to leave a probe shaped trace on the skin, thus the exact location could be easily identified at every examination. Distance measurements between the probe and bony landmarks of the limbs in question were also made. For example, to locate the brachial artery probe position, the distance from the proximal end of the probe to the acromion process of the shoulder was measured and recorded, whilst the distance from the distal end of the probe to the medial epicondyle of the humerus was also measured and recorded. If necessary, the skin could then be marked up with the measurements to create an accurate location for the artery to be imaged. Within the ultrasound image itself, artifacts could also be identified that could be used as markers for the location of the probe. For example, when imaging the common femoral artery the femoral bifurcation was used as a point to

image the artery from as diameter measurements were always made at least 2-3 cm above the bifurcation (Shoemaker et al, 1996).

Resting artery diameters were imaged in the longitudinal plane (figure 11), with a perpendicular beam, which allows the best possible visual image for analysis using edge detection and wall tracking software (Thijssen et al, 2011). When imaging each artery, ultrasound settings and controls were adjusted to optimise the image with the aim of clearly identifying the double lines of Pignoli (Pignoli et al, 1986) (figure 11). The ultrasound probe was held constant whilst a 30 second video recording of the artery was made.

Edge detection and wall tracking software was used to analyse the conduit AD B mode video recordings. It is suggested that the use of edge detection and wall tracking software has a greater reproducibility of AD measurements as it eliminates significant observer error, thus increasing the precision of the diameter measurement (Woodman et al, 2001; Green et al, 2002). For example, Woodman et al (2001) found a mean intra-observer CV of 6.7 % when using the software to perform repeated measures of FMD, as opposed to manual methods that produced CV's of 24.8 % and 32.5%. A second benefit of the software is that it allows continuous artery measurement, therefore mean AD values encompass both systole and diastole components, and is representative of the AD through the whole cardiac cycle (Green et al, 2002).

To perform analysis using the edge detection and wall tracking software, B - mode video recordings of the artery were stored as audio video interleaves (AVI's) on the computer to which this software was installed. A region of interest (ROI) was selected on each artery video, encompassing a section of the artery to be analysed. The diameter was then calibrated against the image size on the computer by drawing a region between two points on the ultrasound image that were a known distance apart. A parallel-prong rake VI algorithm of 200- 400 parallel lines within the ROI with subsequent quadratic spline interpretation at 20-30 Hz was used to calculate the diameter (Green et al, 2002).

Blood velocity measurement (measured in the section 2.4.ii, page 66 and Chapters 3, 4, 5 & 6)

A LOGIQ e (GE Healthcare, UK) combined with a linear transducer probe (8L-RS, GE Healthcare, UK) operating at a variable frequency of 4 - 10 MHz was used to perform

pulsed-wave Doppler measures of conduit artery BV. Blood velocity measurements were taken from the common femoral artery and brachial artery.

All BV measurements were performed in duplex ultrasound mode. This allows the ultrasound to perform continuous real time imaging in B mode of AD, whilst also performing continuous pulsed-wave Doppler measurement of BV. In order to maintain optimal imaging of the AD at a perpendicular angle of 90 degrees in B mode, the LOGIQ e ultrasound system only allows a minimal Doppler beam to vessel angle of 70 degrees in relation to the direction of BF. It has been well established that a Doppler beam to vessel angle of less than 60 degrees has minimal associated measurement error (12%), as opposed to angles greater than 60 degrees which have increasing measurement error and a poorer quality of Doppler signal (a Doppler beam to vessel angle of 70 degrees has 19% measurement error), (Polak, 1992). However, the Doppler beam to vessel angle of 70 degrees was kept consistent in all the studies within this thesis, and it was deemed that the magnitude and change in BV were of more importance than the true absolute BV values. Blood velocity was also sampled over the width of the artery and therefore the width of the parabolic velocity profile, as Radegran (1997) has demonstrated that sampling BV just in the center of the artery results in an overestimation of BV of 22.6 ± 9.1 %.

Blood velocity was recorded continuously throughout resting and exercising protocol. Video recordings were stored in 30 second blocks. Before resting measurements were taken subjects were required to rest for 15 minutes. The inbuilt analysis system of the LOGIQ e was then used to analyse BV, as the edge detection and wall tracking software used to determine AD could not compute the spectral displays produced by the LOGIQ e. The reliability of the LOGIQ e to measure inter-day BV is presented on page 66.

Blood flow calculation (calculated in section 2.4.iii, page 68, Chapters 3 & 6)
Blood flow ($ml \cdot min^{-1}$) was calculated from AD and BV data using the following equation:

$$\text{Blood flow } (ml \cdot min^{-1}) = BV \times \text{cross-sectional area of artery} \times 60$$

(Gonzales et al 2008).

The reliability of this equation to calculate BF is discussed on page 68.

Shear stress calculation (calculated in section 2.4.iii, page 68, Chapters 4 & 6)
Shear stress in conduit arteries can be estimated as:

SS = blood viscosity x BV/ vessel diameter

(Pyke et al, 2005)

However, as blood viscosity was not directly measured in this thesis, shear rate (SR) (s^{-1}) was used as a surrogate measure (Pyke et al, 2005; Newcomer et al, 2008), and was estimated from the following equation:

Shear rate (s^{-1}) = 4(mean BV/ diameter)

(Padilla et al, 2010)

The reliability of this equation to calculate SR is discussed on page 68.

ii. Cardiac measurements used in this investigation. (used in section 2.4.iv, page 70, and Chapter 6).

Transthoracic echocardiography combined with Doppler was used to measure resting $\dot{Q}$ throughout this thesis. Orlando et al (1988) suggested that this method is the most functional way of measuring $\dot{Q}$ as it offers many advantages such as; the technique can be carried out safely and accurately, one unit may be used for many subjects, risk and discomfort are avoided, the technique is non-invasive and can be repeated as frequently as necessary. Transthoracic echocardiography is described as the "the standard practice of imaging the heart and the great vessels from the anterior surface of a patients chest using ultrasound" (p163, Oxborough, 2008). This is achieved via a 2D production of a gray scale image that is typically associated with ultrasound. Doppler can also complement the 2D echocardiographic image and provides quantitive data of the direction and velocity of BF throughout the cardiac cycle (Oxborough, 2008). Please refer to page 50 for a more detailed discussion of general ultrasound and Doppler theory.

Cardiac output

Cardiac output is simply defined as the volume of blood ejected from the left ventricle into the aorta each minute (Opie, 2004). Cardiac output can be calculated from:

$$\dot{Q} = SV \times HR$$

(Opie , 2004)

Where:

$\dot{Q}$ = cardiac output

SV = stroke volume

HR = heart rate

The use of Doppler echocardiography to determine $\dot{Q}$ using this method is generally considered reproducible. Coats (1990) reported that the coefficient of repeat measurement of $\dot{Q}$ using this technique is 5-8%. Short term variability (minutes to hours) varies from 4 - 10%, with long term variability (days to weeks) from 9 - 14%. The reliability of the investigator in this thesis and the LOGIQ e Doppler ultrasound to measure $\dot{Q}$ using transthoracic echocardiographic methods is presented in page 70.

In order to calculate SV using echocardiography, the following variables must be determined:

SV = cross-sectional area of aorta X velocity-time-integral (VTI)

(Ihlen et al, 1987)

Cross-sectional area of the aorta

A LOGIQ e ultrasound (GE Healthcare, UK) combined with a cardiac probe (3L-RS, GE Healthcare, UK) was used to visualise cardiac structures in B mode to provide a grey scale image. The LOGIQ e also had a ECG component built into the system, therefore HR was also measured via an ECG which was displayed on the ultrasound screen. A standardised three lead ECG set up was used, identical to that described on page 44.

After an initial 15 minutes rest period, participants were asked to lay in the left lateral decubital position. This position involves the participant laying on their left side which causes the heart to move closer to the chest wall, and therefore closer to the transducer. The participant was also asked to raise their left arm above their shoulder, supporting their head to create the biggest possible acoustic window possible to scan in (Oxborough, 2008). The probe was then placed between the 3rd and 4th intercostal space against the left side of the sternal border to provide a long axis, parasternal view of the heart, with the index point of the transducer facing towards the participants right shoulder. This provided a view of the aortic valve (see figure 12). Once the view was

optimised with the ultrasound controls, a cine video of the valve was recorded for the duration of 10 cardiac cycles.

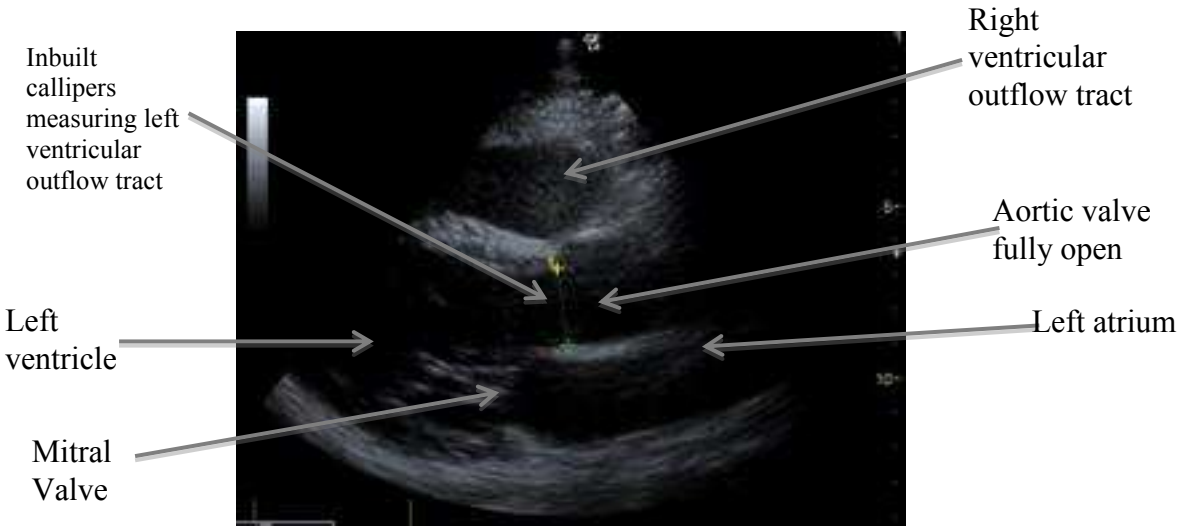

Figure 12. *Parasternal view of the aortic valve and diameter measurement of left ventricular outflow tract when participant in systole*.

At a later date, cross-sectional area of the aorta was measured from this stored cine video (see figure 12). The best three cardiac cycles with the clearest image were used for this measurement. The image was frozen when the participants corresponding ECG trace demonstrated that their ventricle was in systole, and therefore the aortic valve was fully open. At this point, the inbuilt system calipers of the LOGIQ e were used to measure the diameter of the left ventricular outflow tract (LVOT) inside edge to inside edge, immediately before the aortic valve. This measurement was repeated three times for each of the three chosen cardiac cycles. These values were then averaged to provide a mean systolic aortic diameter from all the measurements taken. This value was then inserted into the following equation to calculate LVOT cross-sectional diameter:

$$\text{cross-sectional area} = \prod(\text{radius})^2$$

(Lewis et al, 1984)

The resulting cross-sectional area measurement was then used to calculate $\dot{Q}$.

It should be noted that LVOT cross-sectional area was only measured and calculated during the participant's first data collection session of the associated studies in this thesis where $\dot{Q}$ has been determined. This value was then repeatedly used with the

varying velocity time integral values from each laboratory visit to calculate $\dot{Q}$ where required. This is because measurement of the LVOT cross-sectional area has been shown to demonstrate high user variability. As such it was deemed best to only measure this variable once to minimise user error in the $\dot{Q}$ calculation.

Velocity time integral (VTI)

The LOGIQ e (GE Healthcare, UK) was also used to perform 2D echocardiology to visualise an apical 5 chamber view of the heart. The transducer was placed between the 6th and 7th rib space in the mid axillia with the index pointing to the participants left shoulder whilst the participant was in the left lateral decubital position, with their left arm raised and positioned under their head (Oxborough, 2008). This gives an "upside down" view of the heart, with the ventricles on top of the image and the atria at the bottom. Velocity time integral (VTI) measurements were made immediately after aortic diameter measurements, therefore participants had already rested for 15 minutes. If aortic diameter had been previously measured at an earlier lab visit, participants still completed a 15 minute rest period before any VTI measurement was made.

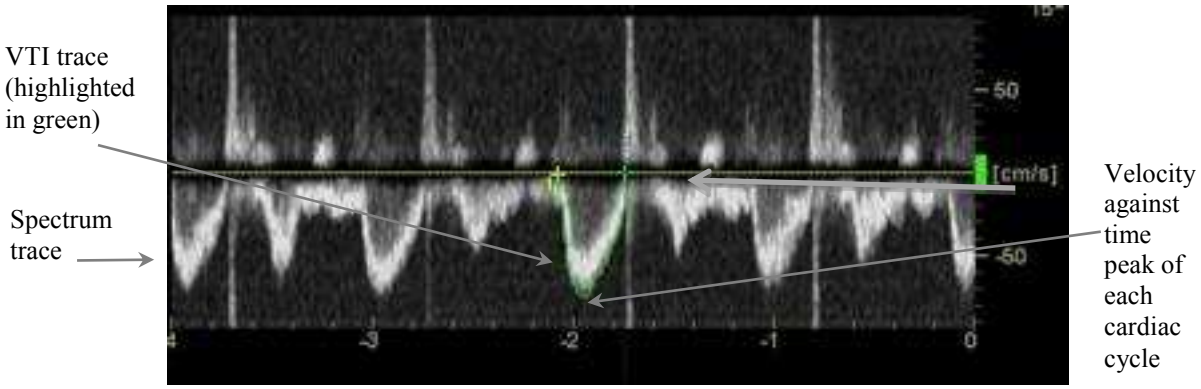

Figure 13. *VTI spectrum trace of aortic blood velocity with VTI measurement*.

Once the apical 4 chamber heart view had been located, and ultrasound controls were set to optimise the image, the transducer was manipulated to provide an apical 5 chamber view. Pulsed wave Doppler at a frequency of 2.2 MHz was then used to sample the velocity of blood moving from the left ventricle into the aortic opening. This was achieved by placing the sample gate in front of the aortic valve. Care was taken to ensure that the angle of the ultrasound Doppler beam to BF was kept below 20°. A

video cine loop was then recorded of the BV against a time axis spectrum trace (figure 13).

At a later time, the VTI was then measured using an inbuilt analysis system within the LOGIQ e, (figure 13). Velocity time integral is simply a representation of the area under the curve, or the area enclosed by the baseline and Doppler spectrum. Velocity time integral was measured from the 10 most clear velocity against time peaks from the spectrum trace. These values were then averaged to provide one mean VTI that was inserted into the $\dot{Q}$ calculation.

Isovolumic relaxation time (IVRT)

Isovolumic relaxation time (IVRT) was also measured from the Doppler velocity against time spectrum from the same 10 velocity against time peaks used in determining VTI, using the inbuilt analysis system of the LOGIQ e (figure 14). Isovolumic reaction time represents the time between the closure of the aortic valve to the onset of filling by the opening of the mitral valve (Thomas & Weyman, 1991). Changes in IVRT pre-post training are representative of an adaptation to diastolic function and therefore $\dot{Q}$ after an exercise intervention.

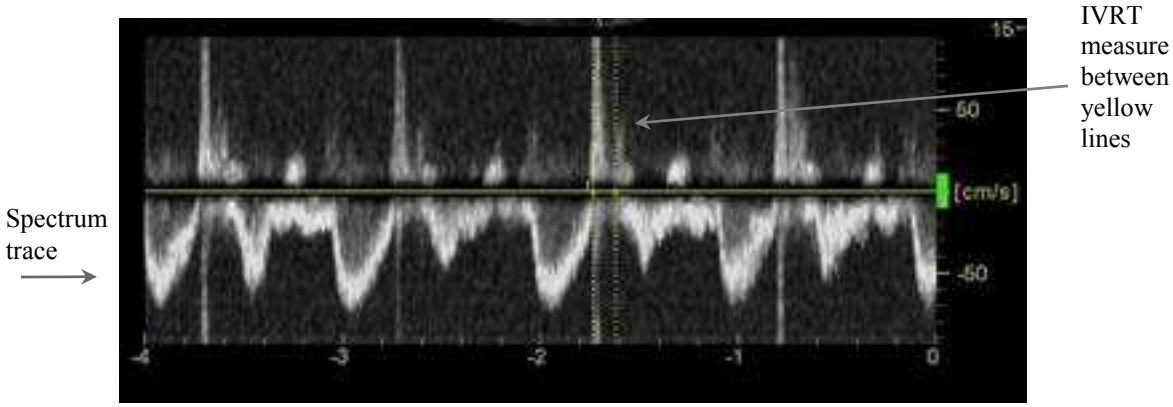

Figure 14. *Measurement of IVRT from VTI spectrum trace of aortic blood velocity.*

Left Ventricular Ejection Time (LVET)

Left ventricular ejection time (LVET) was also measured from the Doppler velocity against time spectrum trace from the same 10 velocity against time peaks used in determining VTI, using the inbuilt analysis system of the LOGIQ e (figure 15). Left ventricular ejection time is the time interval from the opening to the closure of the aortic valve, and is used as a measure of systolic function (Hirschfield et al, 1975).

Changes in LVET pre-post training are representative of an adaptation to systolic function and therefore $\dot{Q}$ after an exercise intervention.

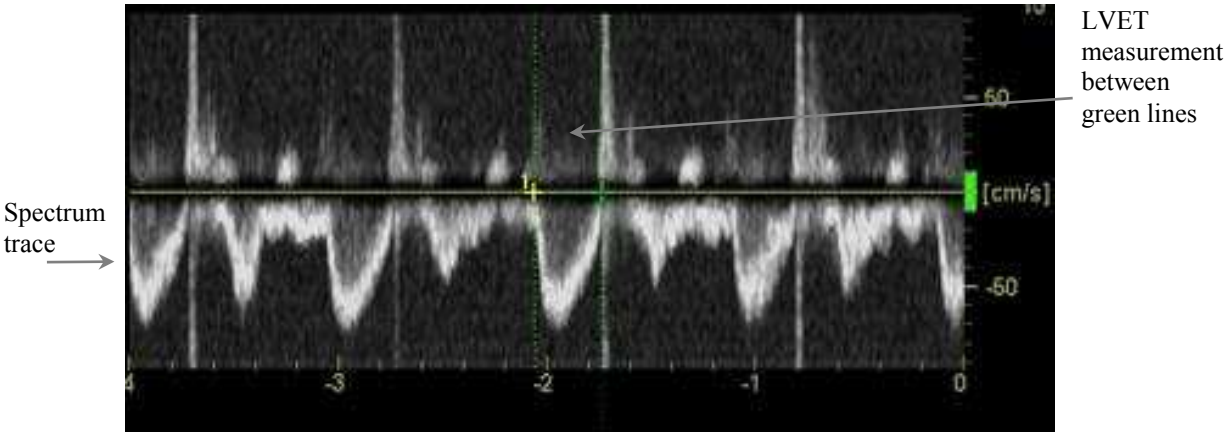

Figure 15. *LVET measurement from aortic blood velocity VTI spectrum trace*.

2.4 Reliability of Measurement and Sample Size Estimation of Dependent Variables.

In order to determine appropriate sample sizes for each study that will allow changes in the mean of the primary outcome variable in this research thesis to be identified, typical error of these variables was established. This was achieved through a series of studies involving an inter-day, repeated measures study design. Typical error was expressed as a percentage of the mean value of the dependent variable value. This is known as the coefficient of variation (CV), and is calculated by dividing the standard deviation of the data collected by the mean and multiplying it by 100 (Atkinson & Neville, 1998). A 95% confidence interval (CI) and corresponding confidence limits (CL) will also be calculated using the methods of Hopkins (2000). It is impossible to test the CV of the whole population for each variable, therefore a CI is calculated to demonstrate the likely true range of the population. Confidence limits are the values at the end of the CI. Sample size estimation will also be presented based on the methods of Hopkins (2000). When sample size for a crossover study design is required (such as that seen in Chapters 3,4 & 5 of this thesis), the number of participants is calculated as $n = 16s^2/d^2$, where n is sample size, s is the typical error and d is the likely change score. If the study design is experimental and contains a control group (such as that seen in Chapter 6 of this thesis), then $n=64s^2/d^2$ can be used to calculate sample size (Hopkins 2000).

2.4.1 Reproducibility of conduit artery resting diameter measurement and sample size estimation.

Methods:

Participants

Seven active male participants (age = 24 ± 5 yrs, height = 181.43 ± 6.7 cm, mass = 82.72 ± 7.9 kgs) volunteered to take part in this study. All participants were healthy, non smokers and free from medication. Each participant received an written explanation of the testing protocol and the nature of the measures to be taken. After participants provided written informed consent, and completed an exercise readiness questionnaire which was deemed acceptable, they were recruited into the study. Participants were required to visit the laboratory on 3 separate occasions, at the same time of day for each visit. Prior to each visit, participants were also instructed to fast for 4 hours before their visit, and refrain from drinking alcohol and caffeine for at least 12 hours prior to testing protocol. Participants were also asked to avoid strenuous exercise in the 24 hours prior to a laboratory visit. Adherence to these criteria were confirmed verbally prior to each testing session. Please refer to section 2.1 (page 38) for the ethical considerations that this study adhered to.

Experimental protocol

A LOGIQ e (GE Healthcare, UK) combined with a 8L - RS transducer (GE Healthcare, UK) was operated at a frequency of 8MHz to image the superficial femoral artery in each participant's right leg at rest. After an initial 15 minutes of rest in a supine position, the superficial artery was located, the image was optimised and a cine video loop was recorded. Edge detection and wall tracking software was then utilised to measure AD. Please refer to page 53 for full details of the method used to determine AD in this reproducibility study.

Data analysis

Data were assessed for normality. Data that did not meet a normality criteria ($P < 0.05$) was logarithmically transformed in order to meet the normality criteria. Mauchley's test of sphericity was used to determine whether the assumption of sphericity was met. A one way repeated measure analysis of variance (ANOVA) was then used to assess whether there was a significant difference between trials. If a significant difference was identified, a Bonferroni post hoc test was used to identify where that difference lay

within trials. The level of significance for all statistical tests was $P < 0.05$. Coefficient of variation was determined using the root mean square error (RMSE) of an ANOVA.

Results:

Artery diameter data was normally distributed. ANOVA results revealed that there was no statistically significant difference between trials as $P > 0.05$. The CV% for AD measurement was 4.63% (95% CI 3.32 - 7.64%). Sample size estimation for an experimental study design is presented in table 4.

Table 4*. Sample size needed to detect changes in artery diameter.*

Variable	CV (%)	Smallest detectable change (%)	n =
AD	4.63%	12.35 % (Baross et al, 2012)	9

Discussion:

After 7 participants performed 3 trials, the CV of measuring AD using the LOGIQ e ultrasound and edge detection and wall tracking software was 4.63 %, with the 95% CL suggesting that the populations true value is likely to fall between 3.32-7.64 %, 95% of the time. After estimating sample size, a minimum of 9 participants would be needed to ensure that any changes in mean AD as a result of an intervention were not obscured by the noise from typical measurement error.

Previous literature suggests that hydration status (Krause et al, 2001), variation in cardiac cycle, food intake and physical activity (Peiffer et al, 2007) may provide a source of biological error when measuring conduit AD. In addition mechanical error may have arisen from differences in probe placement, cursor placement on the obtained image, and subject limb placement (Peiffer et al, 2007). Despite these possible sources of biological and mechanical error, care was taken to reduce typical error by controlling these sources where possible in each study. As a result, it is apparent that the use of the LOGIQ e and edge detection and wall tracking software is a reproducible method to measure AD, as sample size is small enough to be realistic in terms of an experimental study with a power of 80 % and a 5% significance level, whilst still allowing for changes in AD to be detected without being concealed by typical error in the measurement.

2.4.2. Reproducibility of conduit artery resting blood velocity measurement and sample size estimation.

Methods:

Participants

Ten active male participants (age = 23 ± 6 yrs, height = 179.92 ± 4.54 cm, mass = 81.54 ± 9.59 kgs) volunteered to take part in this study. All participants were healthy, non smokers and free from medication. Each participant received an written explanation of the testing protocol and the nature of the measures to be taken. After participants provided written informed consent, and completed an exercise readiness questionnaire which was deemed acceptable, they were recruited into the study. Participants were asked to visit the laboratory on 5 separate occasions, at the same time of day for each visit. Prior to each visit, participants were also asked to fast for 4 hours before their visit, and refrain from drinking alcohol and caffeine for at least 12 hours prior to testing protocol. Adherence to these criteria were confirmed verbally prior to each testing session. Participants were also asked to avoid strenuous exercise in the 24 hours prior to a laboratory visit. Please refer to section 2.1 (page 38) for the ethical considerations that this study adhered to.

Experimental procedure

A LOGIQ e combined with a 8L - RS transducer was used to image the superficial femoral artery in each participant's right leg at rest. After an initial 15 minutes of rest in a supine position, the superficial artery was located, the image was optimised. Pulsed-wave Doppler using a 8L - RS transducer was used to measure the velocity of the blood moving through the vessel. Once Doppler controls had been optimised, a cine video loop was recorded. The inbuilt analysis system of the LOGIQ e was used to analyse each VTI trace for 10 cardiac cycles in each cine video loop. Please refer to page 55 for the full methodological details used to determine BV.

Data analysis

(Please refer to data analysis section on page 64).

Results:

Blood Velocity data was normally distributed. ANOVA results revealed that there was no significant difference between trials as $P > 0.05$. The CV% was 7.83% (95% CI 6.19

- 10.66%) for BV measurement. However, even with a small sample size the estimated BV change detectable is much smaller than changes previously noted in the existing literature which are approximately ~150 % (Shoemaker et al, 1996).

Discussion:

After 10 participants performed 5 trials, the CV for the measurement of BV at rest was 7.83%, with the CL suggesting that the populations true value is likely to fall between 6.19 - 10.66% 95% of the time. Due to the large changes in BV evident in pre-existing literature (~150%, Shoemaker et al, 1996), a small sample size is needed to be able to establish clear changes in the mean value of BV in a crossover study design without being hidden by typical error. This method is reproducible enough to detect changes in BV during exercise without being obscured by the typical error. However, in order to ensure this reliability, care was taken in each study to ensure participants followed a standard pre-testing protocol to reduce biological error, whilst the user gained experience to ensure optimal Doppler ultrasound quality to minimise noise from the measurement.

2.4.3. Reproducibility of blood flow and shear rate calculated variables and sample size estimation.

Methods:

Participants

(Please refer to participants section on page 64).

Experimental procedure

After an initial 15 minute rest period, BV and AD data were collected from the superficial femoral artery using the LOGIQ e (please refer to pages 53-56 for details regarding velocity and diameter measurements). Blood flow was then calculated from the following equation:

$$\text{Blood flow } (ml \cdot min^{-1}) = BV \times \text{cross-sectional area of artery} \times 60$$

(Gonzales et al 2008).

Shear rate was also calculated from the collected BV and AD data, and was calculated using the following equation:

$$\text{Shear rate } (s^{-1}) = 4(\text{Mean BV} / \text{diameter})$$

(Padilla et al, 2010)

Data analysis

(Please refer to data analysis section on page 64).

Results

Blood flow and SR data was normally distributed. ANOVA results revealed that there was no statistically significant difference between trials for both BF and SR variables, as $P > 0.05$. The CV% was 13.30 % (95% CI 9.54 - 21.95%) for the calculation of BF, whilst the CV% was 10.09% (95% CI 7.24 - 16.66%) for the calculation of SR. Due to the large changes in BF (~300%, Wray et al 2005) and SR (~250%, Gonzales et al, 2010) evident in pre-existing literature, a small sample size is needed to be able to establish clear changes in the mean value BF and SR without being obscured due to typical error.

Discussion

After 10 participants performed 3 trials, the CV for determining BF was 13.30%, with the 95% CL suggesting that the populations true value is likely to fall within 9.54 - 21.95%, 95% of the time. The CV for determining SR was 10.09%, with the 95% CL suggesting that the populations true value is likely to fall within 7.24 - 16.66%, 95% of the time. For both BF and SR variables, the required sample size to detect any possible changes in the mean without being obscured by typical error is very small. This is because the smallest detectable change in each of these variables is so large when performing exercise (BV ~ 300% Wray et al, 2005; SR ~250% Gonzales et al, 2010), that any changes observed are far greater then the typical error.

2.4.4. Reproducibility of cardiac output, isovolumic relaxation time & left ventricular ejection time and sample size estimation.

Methods:

Participants

(Please refer to participants section on page 66).

Experimental procedure

After an initial 15 minute resting period, transthoracic echocardiology using a LOGIQ e and 3L - RS transducer was used to image cardiac structures and record the velocity of blood moving through the heart and the aorta. Section 2.9.b on pages 57-62 describes in detail the protocol of measuring and analysing $\dot{Q}$, IVRT and LVET from 10 velocity against time peaks within the spectrum trace used in this reproducibility study.

Data analysis

(Please refer to data analysis section on page 64).

Results:

Cardiac Output, IVRT and LVET data was normally distributed. ANOVA results revealed that for $\dot{Q}$, IVRT and LVET, there was no statistically significant difference between trials as $P > 0.05$. The CV% for $\dot{Q}$ measurement was 9.56 % (95% CI 7.22 - 14.14%), the CV % for IVRT measurement was 13.14 % (95% CI 9.93 - 19.43%), whilst the CV% for LVET measurement was 5.02% (95% CI 3.79 - 7.42%). Sample size estimation for an experimental study design is presented in Table 5.

Table 5 *Sample size estimation for $\dot{Q}$ and IVRT. Please note that it has not been possible to find a reported smallest detectable change for LVET in the literature.*

Variable	Coefficient of Variation (%)	Smallest detectable change (%)	n =
$\dot{Q}$	9.56	2.91 % (Wiles, 2008a)	691
IVRT	13.14	7.94 % (Belardinelli et al, 1995)	174

Discussion:

After 10 participants performed 3 repeated trials, the CV % for $\dot{Q}$ measurement is 9.56 %, with the 95 % CL suggesting the populations true value is likely to fall within 7.22 - 14.14%, 95% of the time. It is apparent that this method of measuring $\dot{Q}$ is not precise enough to detect changes in $\dot{Q}$ using a realistic sample size. Therefore, it is accepted that $\dot{Q}$ measurement utilised in this research thesis is reproducible but lacks precision. Therefore the measurement of $\dot{Q}$ will be subject to type II error which may result in a failure to detect a significant change in $\dot{Q}$ that is actually present.

The CV% for measurement of IVRT is 13.14%, with the 95% CL suggesting that the populations true value will fall between 9.93 - 19.43%, 95% of the time. Similar to $\dot{Q}$, it is estimated (using Hopkins, 2000) that a large, unrealistic sample size (>174) is needed to be able to detect changes in IVRT that are not effected by type II error. The CV% for measurement of LVET is 5.02%, with the 95% CL suggesting that the populations true value will fall between 3.79 - 7.42%, 95% of the time. As it as not possible to find a reported smallest detectable change in this variable within existing literature, it is not possible to perform Hopkins (2000) sample size estimation.

Transthoracic echocardiology does provide a reproducible method for measurement of $\dot{Q}$, IVRT and LVET, yet the physiological changes in these variables are so small that very large sample sizes are needed to be able to detect systematic changes in each measurement. These large sample sizes required are outside the scope of this current research study, and run the risk of a type II error. However, measures of $\dot{Q}$, IVRT and LVET will still be made in this research thesis, as these measures may provide some insight into any physiological adaptations that may occur after exercise intervention, and could also provide data for any future meta-analysis.

2.4.5. Reproducibility of resting total peripheral resistance (TPR) calculation and sample size estimation.

Methods

Participants

Eight active male participants (age = 23 ± 6 yrs, height = 182.. 91 ± 5.74 cm, mass = 83.72 ± 8.13 kgs) volunteered to take part in this study. All participants were healthy, non smokers and free from medication. Each participant received an written explanation of the testing protocol and the nature of the measures to be taken. After participants provided written informed consent, and completed an exercise readiness questionnaire which was deemed acceptable, they were recruited into the study. Participants were asked to visit the laboratory on 3 separate occasions, at the same time of day for each visit. Prior to each visit, participants were also asked to fast for 4 hours before their visit, and refrain from drinking alcohol and caffeine for at least 12 hours prior to testing protocol. Adherence to these criteria were confirmed verbally prior to each testing session. Participants were also asked to avoid strenuous exercise in the 24 hours prior to a laboratory visit. Please refer to section 2.1 (page 38) for the ethical considerations that this study adhered to.

Experimental procedure

Total peripheral resistance was calculated from the following equation:

$$TPR = MAP / \dot{Q}$$

(Devereux et al, 2010b)

After an initial 15 minutes rest period, MAP and $\dot{Q}$ were determined in each participant. Cardiac output measures were determined by echocardiographic methods (discussed in detail on page 57). Mean arterial pressure was measured using an automated BP monitor (Dinamap Pro 300). Details of this methodology can be found on page 48.

Data analysis

(Please refer to data analysis section on page 64).

Results:

Total peripheral resistance data was normally distributed. ANOVA results revealed that there was no statistically significant difference between trials for TPR, as $P > 0.05$. The CV% for TPR calculation 17.09 % (95% CI 12.51 - 26.95%). Sample size estimation for an experimental study design is presented in Table 6.

Table 6. *Sample size estimation for TPR measurement.*

Variable	Coefficient of variation (%)	Smallest detectable change (%)	n =
TPR	17.09	5.49% (Wiles, 2008a)	621

Discussion:

After 8 participants performed 3 repeated trials, the CV % for calculation of TPR was 17.09%, with the 95% CL suggesting that the populations true value is likely to fall within 12.51 - 26.95%, 95% of the time. It is apparent from this large confidence interval that the calculation of estimating TPR is not very precise. This is due to the use of $\dot{Q}$ values in the calculation for TPR, as section 2.4.IV, page 70 has demonstrated, the measurement of $\dot{Q}$ in itself is not very precise as a large sample size is needed to detect any real change. Sample size estimation (Using the methods of Hopkins 2000) suggests that > 621 participants will be needed to detect true changes in TPR after exercise intervention that are not subject to type II error. This large sample size required is unrealistic for the current scope of this study. The measurement of TPR will still be made as the collected data may provide an insight into the physiological adaptations after exercise intervention, although results must be interpreted cautiously. In addition, collected data could be used for any future meta-analysis.

2.5 Overall sample size estimation.

Utilising the methods of Hopkins (2000), reliability data that has either been previously collected in this thesis (presented in pages 63-75), or collected within the active research group of the Sport and Exercise Science department at Canterbury Christ Church University, has been combined with data on the likely changes in each of the parameters derived from work within the department and from published literature, for a study at 0.8 (80%) power and a 0.05 (5%) significance level. Sample size was calculated as:

-Sample size for Chapters 3, 4 & 5 crossover study design (exercising values):

For a crossover study at 80% power and a 5% significance level, sample size was calculated as > 7 participants.

-Sample size for Chapter 6 experimental study design (resting values):

For an experimental study at 80% power and a 5% significance level, sample size was calculated at 36 participants. This is great enough to detect the likely change in the main primary outcome variable of this study (RBP) and the secondary outcome variable, resting AD.

2.6 Establishing a Sampling time frame for blood velocity and artery diameter measurements from a cine video loop.

2.6.1. Introduction:

When using the LOGIQ e ultrasound to record real time AD and BV data, cine video loops were created to record the data in 30 second blocks throughout resting and exercise protocols. However a number of problems presented themselves when using this method of recording the data:

a. By recording testing protocol continuously (separated into 30 second blocks), AD and BV data was present for every cardiac cycle throughout an exercise test. To contexualise this, a discontinuous incremental isometric exercise test (DIIET) (as used in Chapters 3, 4, 5 & 6) could last between 7-63 minutes depending on the peak exercise stage each individual participant reached in the test. Each participant performed 3 trials. This produced very large amounts of ultrasound data to analyse. Whilst the edge detection and wall tracking software is able to analyse AD from the B mode ultrasound image, it is not compatible with the velocity against time spectrum trace of the LOGIG e, and therefore the inbuilt analysis system of the LOGIQ e had to be used instead to determine BV values. Whilst the inbuilt software of the LOGIQ e is automated, it only measures velocity one peak at a time. Therefore manual input was required to scroll through each cine video loop to analyse each velocity peak one at a time. Whilst this would give a very detailed insight into the acute BV response to isometric exercise, it was decided that attempting to analyse this amount of data would not be realistic within the time frame allocated to complete this research thesis. It was proposed that a more suitable method of analysing this data be found instead.

b. There was substantial movement of the common femoral artery between the contraction and relaxation phases of the DIIET protocol (as used in Chapters 3, 4, 5 & 6). This was due to the contraction/relaxation of the quadricep muscle group to perform the desired leg extension exercise. Between each of these stages, slight adjustments to the location of imaging and ultrasound controls often had to made to ensure an optimal image throughout testing protocol. This often resulted in a time delay of 10-20 seconds in the transition between exercise and rest stages.

It is important for the purpose of this thesis that AD and BV be measured from the same time points to ensure accurate BF and SR values. Therefore it was proposed that in order to deal with the issues presented, and to ensure AD and BV sample points were matched, that a sample block of time should be established that would allow the analysis of a small section of the 30 second cine video to reflect the "bigger picture" of the whole 30 seconds.

As there was the issue of optimising the image between transition phases of the exercise protocol in the first 10-20 seconds, it was proposed that the time frame of 20-30 seconds of each 30 second block may be an ideal sample point for blood haemodynamic data. The data presented below documents the ability of this smaller sample block (at 20-30 seconds of each 30 second block) to represent the haemodynamic data collected from a whole 30 second block.

2.6.2 Methods:

Participants

Five healthy, normotensive males (age = 22 ± 2 yrs; height = 180.16 ± 4.17 cm; mass = 77.71 ± 11.11 kg) volunteered to participate in this study. Prior to testing, all participants received a written explanation of the procedures to be used, along with the potential risks of participating in the study. Once participants provided written informed consent, they were required to complete an exercise readiness questionnaire. All participants fasted for 4 hours, abstained from caffeine and alcohol for at least 12 hrs prior to start of testing procedures (Jauregui - Renaud et al, 2001), and maintained normal physical activity and diet throughout the length of the study. Adherence to these criteria were confirmed verbally prior to each testing session. All participants completed identical familiarisation sessions prior to data collection. Please refer to section 2.1 (page 38) for the ethical considerations that this study adhered to.

Isometric exercise:

All isometric exercise was conducted using a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY). Full details of the procedure used to perform bilateral isometric leg extension exercise can be found on page 39 of this chapter.

Maximal voluntary contraction and EMG_{peak}:

Maximal voluntary contraction and EMG_{peak} were determined prior to each DIIET. Participants performed 3 maximal effort static double leg extensions, with a 90 degree angle at the knee, for 2 seconds. Each contraction was separated by 2 minutes of rest. EMG_{peak} was derived from the MVC producing the highest torque value, and was established from the mean of the EMG activity recorded 0.25 seconds immediately prior to maximum torque (Wiles et al, 2008b). This EMG_{peak} was then used to create $\%EMG_{peak}$ target incremental values for the subsequent DIIET.

Discontinuous incremental isometric exercise test (DIIET):

Participants underwent 2 DIIET tests using double leg extension exercise on separate occasions (at least 48 hrs apart). The first test performed was used as a familiarisation session, whereas the following test was used for data collection. Participants performed static double leg extension exercise in an incremental nature for 2 minute time intervals, each separated by 5 minutes of rest. Participants started at $10\%EMG_{peak}$, with intensity increasing in $5\%EMG_{peak}$ increments until volitional fatigue was reached or participants could no longer maintain their %EMG target value within ± 5% (Wiles et al, 2010). Figure 16 provides a visual representation of this protocol. All tests were performed at approximately the same time of day (± 1hr).

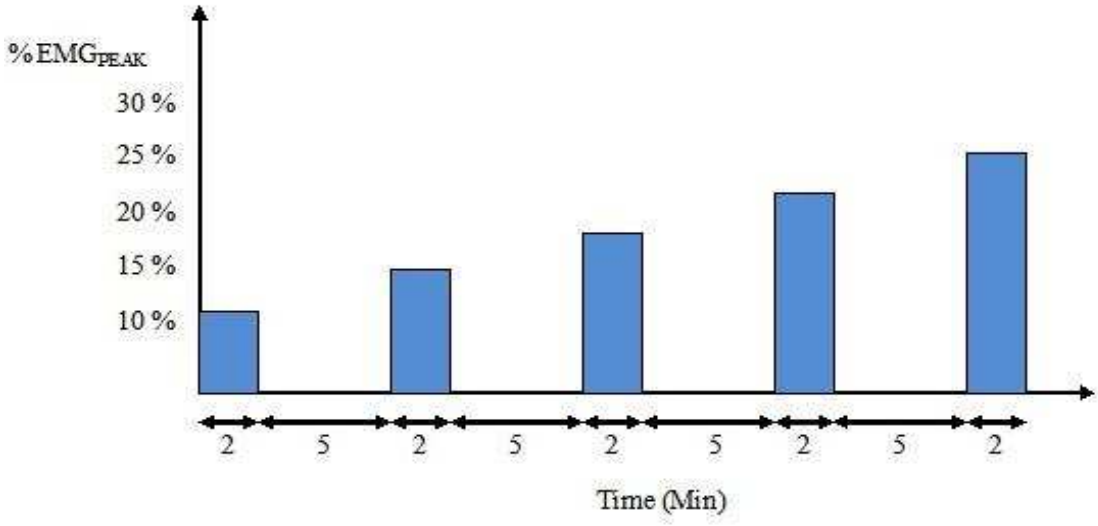

***Figure 16**. Discontinuous Incremental Isometric Exercise Test. Participants performed static bilateral leg extension exercise at increments of 5% EMGpeak, starting at 10% EMGpeak until volitional fatigue was reached. Each exercise stage lasted for 2 mins (represented by the bars) and was separated by 5 min rest periods.*

Electromyography (EMG) recording:

Surface EMG measurements were taken from the vastus lateralis muscle of both legs using a dual bio amplifier and a 16 channel chart recorder (Powerlab, ADInstruments Ltd, Australia) during exercise. This muscle has previously been shown to exhibit a linear relationship between force production and EMG when performing isometric leg extension exercise (Alkner et al, 2000). The root mean square of the raw EMG signal was computed using chart recording software (LabChart 7, ADInstruments Ltd, Australia) and was smoothed at 1s using a high and low pass digital filter. EMG from each vastus lateralis muscle was combined and averaged to give one value, and synchronised with the force data from the Biodex System 3 pro isokinetic dynamometer. In order to record EMG from each vastus lateralis, electrodes (Sensor T ECG pads, Ambu Inc, Maryland, USA) were placed two-thirds along the line from the anterior spina iliaca superior to the lateral side of the patella in the direction of the muscle fibres (Surface Electromyography for the Non-Invasive assessment of Muscles, http://www.seniam.org). Please refer to page 41 for full methodological details relating to the use of EMG.

Blood velocity measurement:

A 8 MHz multifrequency wide band linear array probe (8L-RS GE healthcare,) and a ultrasound machine (LOGIQ e, GE healthcare) were used to image the common femoral artery below the inguinal ligament, 2-3 cm above its bi-furcation into the superficial and profunda femoral branches (Radegran et al, 1997; Baross et al 2012). Once an optimal image was obtained, the probe was held constant and ultrasonic parameters were adjusted to enhance the longitudinal B-mode image of the lumen-arterial interface. Pulsed-wave Doppler velocity profiles of the common femoral artery were obtained throughout testing protocol using the LOGIQ e at an insonation angle of < 70 degrees. The LOGIQ e was used in duplex mode to continuously record BV and AD in 30 second blocks throughout exercise and rest. Exercise components consisted of four 30 second blocks as contraction time was 2 minutes, whereas post-exercise data was comprised of ten 30 second blocks, as each rest period was 5 minutes in duration. The inbuilt analysis software of the LOGIQ e was then used to analyse BV values from each 30 second cine video loop. Please refer to page 55 for full methodological details relating to the collection of BV data.

Data analysis:

Mean blood velocity values for the first 20 seconds of a 30 second block were compared to the mean BV values for the last 10 seconds of a 30 second block throughout each stage of an DIIET test. Stages were separated into isometric contraction stages and post-exercise stages. All data was logarithmically transformed, and was assessed for normality. All data conformed with parametric assumptions as $P > 0.05$ (Field, 2000). Correlation and regression analysis was used to determine the correlation coefficient and standard error of the estimate value for each participant. A level of $P < 0.05$ was set as the threshold for statistical significance.

2.6.3 Results:

All data was normally distributed as $P > 0.05$. Table 7 reports the mean correlation coefficients and standard error of the estimate values for the participants, during isometric contraction, and post-isometric contraction.

***Table** 7. Mean correlation coefficient and standard error of the estimate during and post-isometric exercise.*

	Contraction				Post - exercise			
	R	P	R^2	SEE (68%)	R	P	R^2	SEE (68%)
Mean	0.815	0.00	0.843	7.459	0.831	0.00	0.789	7.091
S.D	0.137	0.00	0.043	3.479	0.134	0.00	0.144	1.814

2.6.4. Discussion

From the data presented in table 7, it can be seen that there is a highly significant ($P < 0.01$) positive, strong relationship ($R^2 > 0.789$) between BV values measured in the last 10 seconds of a 30 second block compared to BV values measured in the first 20 seconds of a 30 second block in both contraction and post-exercise phases. R^2 values demonstrate that the BV data collected in the last 10 seconds of a 30 second block has a > 79 % chance of representing the BV data in the first 20 seconds of a 30 second block. The standard error of the estimate demonstrates that there is low variance around the line of best fit of these predicted scores in the regression calculation. These results suggest that sampling the last 10 seconds of a 30 second BV block is acceptably representative of a whole 30 second block.

Preamble to Chapters 3, 4 and 5

Preamble to Chapters 3, 4 and 5

It is well established that IET induces significant reductions in RBP in both normotensive and hypertensive populations (Howden et al, 2002; McGowan et al, 2007a; McGowan et al, 2007b; Miller et al, 2007; Miller et al, 2008; Ray & Carrasco, 2000; Taylor et al, 2003; Wiley et al, 1992). Specifically bilateral ILEET has been shown to induce reductions in resting SBP (~11 mmHg), DBP (~3 mmHg), and MAP (~5 mmHg) (Baross et al, 2012; Devereux et al, 2010b; Wiles et al, 2010), in healthy young to middle aged normotensive males.

Whilst previous studies have focused upon identifying the physiological mechanism(s) that may be responsible for RBP adaptation to IET, no attempt has been made to identify the physiological stimulus that may initiate these adaptations. Evidence suggests that the physiological stimulus may be closely related to exercise intensity during IET. Devereux et al (2011) established that reductions in resting SBP (-4.9 mmHg) after 4 weeks of bilateral ILEET correlated with exercise intensity when expressed relative to peak ($\%EMG_{PEAK}$), and indices of fatigue (EMG signal amplitude and frequency). Those participants that trained to a higher $\%EMG_{PEAK}$ and induced greater levels of fatigue experienced greater reductions in resting SBP. This suggests that reductions in RBP following IET may be closely related to the intensity that IET is performed at, and the subsequent fatigue that is induced over a set duration. The resultant physiological stimulus likely to be potent at higher intensities of isometric exercise that is able to stimulate a physiological adaptation in the mechanism responsible for this reduction in RBP remains to be identified.

It is plausible to suggest that exploring the response of local blood haemodynamics in response to acute isometric exercise may be able to provide an insight into how the exercise training stimulus relates to the physiological stimulus necessary to induce greater BP reductions. Furthermore it is important to establish whether any haemodynamic response observed is linked to isometric exercise intensity and fatigue before BF haemodynamics can be considered as a physiological stimulus for RBP adaptation following IET.

Moreover, for a local blood haemodynamic response to be considered as a

physiological stimulus, it must be able to induce change in a physiological mechanism that subsequently influences RBP. It has been shown previously that an increased BF and SS stimulus is associated with adaptations to the vasculature, both functionally and structurally (Green et al, 2010; Langille & O'Donnell, 1986; Kamiya et al, 1980; Tinken et al, 2010). Conduit AD remodeling may be a contributing mechanism for RBP reduction following IET. Whilst an increased BF and SS response is the stimulus for this remodeling process, it is specifically the physical characteristics of the SS response that mediates any structural adaptation (Green et al, 2009; Johnson & Wallace, 2012).

Therefore the studies presented in Chapters 3, 4 and 5 of this thesis aim to investigate the nature and characteristics of the haemodynamic response in the exercising limbs to acute isometric bilateral leg exercise of increasing $\%EMG_{PEAK}$ exercise intensity. Specifically BF, SR and shear stress pattern (retrograde shear rate, antegrade shear rate and oscillatory shear index) in the exercising limbs at each isometric exercise intensity during exercise and in the immediate rest period following the cessation of exercise will be defined. After establishing the response of these variables to acute isometric leg exercise of increasing intensity in an incremental nature, the question as to whether local blood haemodynamics could be considered as the physiological stimulus for the greater reductions in RBP typically seen following higher intensity IET will be discussed. A definition of the haemodynamic response to acute bouts of bilateral ILEET may provide further insight into the role that conduit AD remodeling may have as a physiological mechanism for RBP adaptation following IET.

Chapter 3

Study 2: Local Blood Flow Response to Acute Isometric Leg Exercise

Chapter 3: Local Blood Flow Response To Acute Isometric Leg Exercise

3.1 Introduction

The characteristic cardiovascular response to isometric leg exercise is an immediate increase in HR, followed by an increase in $\dot{Q}$ which in turn results in an increase in MAP (Gaffney et al, 1990; Kagaya & Homma, 1997). Taylor et al (1988) suggested that the observed cardiovascular response to static exercise is mediated by 2 primary signals; the first being central command which involves the activation of central cardiovascular controllers in the brain to stimulate parallel activation of the autonomic and motor nervous systems, whilst the second signal originates in the exercising muscle and is transmitted via group III and group IV muscle afferent fibers excited by mechanical and chemical stimuli to provide feedback to the central nervous system regarding the contractile and metabolic states of the exercising muscle. In the presence of muscle ischemia, these two signals are designed to increase MAP to elevate muscle BF, perfusion pressure and oxygen delivery, and thus offset a mechanical impedance to BF produced by a sustained rise in intramuscular pressure (Hansen et al, 1993) typical during isometric exercise (Sadamoto et al, 1983).

As would be expected, an increase in BF to about twice the resting level has been documented during isometric exercise (Kiens et al, 1989) in the presence of increased intramuscular pressure. This increased BF response appears to be increasingly exercise intensity dependent during isometric arm exercise (Hamann et al, 2004; Jensen et al, 1993; Osada et al, 2003), but not during isometric leg exercise (Gaffney et al, 1990; Sjogaard et al, 1988). For example, Gaffney et al (1990) found greater leg BF at 15%MVC than at 25 and 50%MVC during static leg extension exercise, and Sjogaard et al (1988) found that BF during contraction decreased with increasing exercise intensity. These findings may be due to the fact that isometric leg extension exercise in both these studies was held to fatigue, resulting in lower intensity contractions held for a longer duration than those performed at high intensities, which in turn may have influenced BF values. Figure 17 displays the BF results taken from Sjogaard et al (1988), and demonstrates a clear interaction between exercise intensity and exercise duration on BF during contraction. Gaffney et al (1990) and Sjogaard et al (1988) present BF values in their absolute value. Indeed presenting this data relative to the time duration that each exercise intensity was sustained for may reveal a linear relationship between exercise intensity and exercising BF values during isometric leg extension exercise.

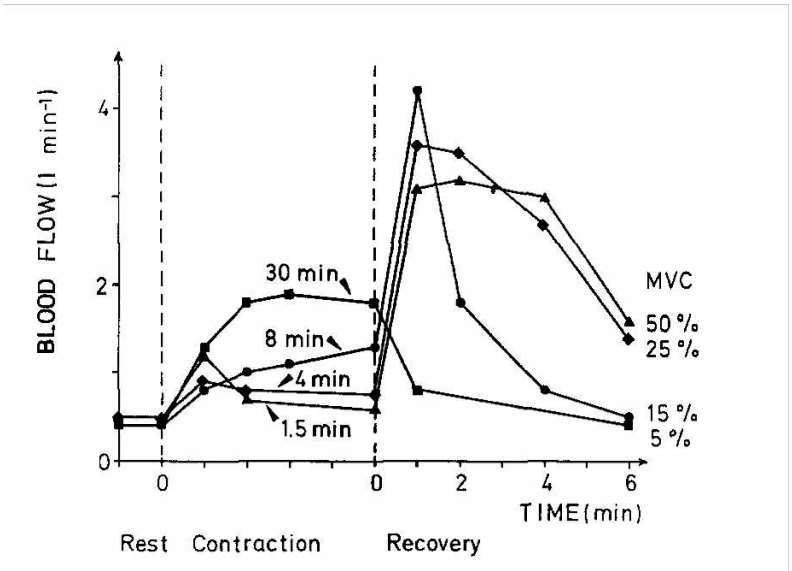

Figure 17*. Figure referenced from Sjogaard et al (1988). Blood flow through the knee extensors during 5, 15, 25 and 50% MVC and in the following recovery periods. The time scale during contraction is different for the four contraction forces, and the length of contraction time is shown by the respective numbers on each graph.*

There is limited research exploring leg BF during graded intensity static leg exercise. Hansen et al (1993) and Gaffney et al (1990) propose that muscle BF during static exercise is the net result of several events; perfusion pressure, mechanical obstruction to flow via intramuscular pressure, and the net balance between local metabolite vasodilation and centrally mediated sympathetic vasoconstriction. When intramuscular pressure overrides increased local metabolic vasodilation and increased peripheral sympathetic vasoconstriction to increase muscle perfusion pressure, BF is markedly reduced and therefore oxygen supply becomes insufficient. This is reported to be around 10-15% MVC for static leg contraction (Gaffney et al, 1990; Saltin et al, 1981; Sjogaard et al, 1988), which may go some way as to explaining why Gaffney et al (1990) and Sjogaard et al (1988) observed greater leg BF at lower exercise intensities, as BF may have been occluded after 15% MVC. This remains to be fully investigated during isometric leg exercise studies examining the BF response.

It is also apparent that the significant increases in BF seen during isometric exercise continue after the exercise has stopped. Large increases in BF were observed immediately post-isometric arm exercise (Osada et al, 2003), and also post-isometric leg exercise (Endo et al, 1994; Gaffney et al, 1990; Kiens et al, 1989). These increases in BF occur despite a drop in BP and HR to near pre-exercise resting values (Gaffney et al, 1990). Similar to the BF response observed during isometric contraction, the observed increases in BF post-isometric

contraction (termed post-exercise hyperemia) also appear to be exercise intensity dependent in the arm (Kagaya & Homma, 1997; Osada et al, 2003). It is yet to be established as to whether the same exercise intensity dependent response is also observed for the leg, since there are a limited number of studies that have explored the post-exercise BF response in the leg arteries after isometric leg exercise. The results of the two studies that have (Gaffney et al, 1990; Sjogaard et al, 1988), must be interpreted with caution as leg extension exercise was held to fatigue, and therefore the post-exercise BF response would not only be significantly influenced by exercise intensity, but also the maximal contraction duration. Both Gaffney et al (1990) & Sjogaard et al (1988) demonstrated that a greater post-exercise BF response was observed following lower intensities of isometric exercise (25% MVC; 15% MVC respectively) than that observed following the greatest intensity of isometric exercise (50% MVC). Greater exercising BF values at the lower exercise intensities (15% MVC & 25% MVC) may have remained increased for the immediate periods after contraction until BP and HR values returned back to near resting levels. This in combination with the influence of the accumulation of fatigue inducing metabolites may have mediated the greater post-exercise BF response seen at 15% MVC and 25% MVC in comparison to 50% MVC. Previous studies have attributed post-exercise hyperemia predominantly to the recovery from acidosis created during isometric contraction that results in the release of local vasodilatory ions and metabolites (Bangsbo & Hellsten, 1998; Gaffney et al, 1990). Reduced sympathetic nervous activity upon contraction cessation that promotes parasympathetic vasodilation has also been suggested to play a role in the post-exercise hyperemia response (Bangsbo & Hellsten, 1998). Parasympathetic vasodilation may relax vascular tone, which in turn may reduce the resistance to flow (TPR).

Previous research utilising isometric bilateral leg extension exercise has prescribed exercise intensity using $\%EMG_{peak}$ (Baross et al, 2012; Devereux et al, 2010; Wiles et al, 2010). This is in contrast to the majority of other studies which have predominately used %MVC to examine the BF responses during and post-isometric exercise, (Barnes, 1980; Gaffney et al, 1990; Hamann et al, 2004; Hansen et al, 1993; Humpreys & Lind, 1963; Jensen et al, 1993; Kiens et al, 1989; Lind & McNicol, 1967; Osada et al, 2003; Saltin et al 1981; Sjogaard et al, 1988). This study will also prescribe isometric bilateral leg extension exercise using $\%EMG_{peak}$. Wiles et al (2010) proposed using this method of exercise prescription based upon the fact that working to a constant EMG produces a more stable cardiovascular response which plateaus within 2 minutes, as opposed to a continued rise when using %MVC. Wiles et al (2010) believed that this made it possible to identify more precisely the level of the

cardiovascular response, which helps to identify the physiological stimulus that may be responsible for BP reductions after IET. This method is deemed more appropriate for the current study since it should produce a more consistent haemodynamic stimulus, allowing a more precise investigation of its potential role in IET induced reductions in RBP. As far as the author is aware no study using this exercise intensity prescription method has explored the BF responses during and immediately after isometric contraction. Furthermore this study will be the first of its kind to examine the BF response during and post-isometric bilateral leg extension exercise. This study will utilise Doppler ultrasound to analyse real time BV and AD to determine BF.

The primary aim of this investigation is to examine the magnitude of quadricep BF response to graded isometric bilateral leg extension exercise held at a constant $\%EMG_{peak}$, during contraction and immediately after contraction. Establishing the presence and characteristics of this response during and following an acute bout of isometric exercise at different exercise intensities will help to determine whether the BF haemodynamic response to isometric leg exercise can be considered as a possible stimulus for BP reductions after IET.

The second aim of this study will be to document the BP and HR responses to an acute bout of isometric leg exercise at different exercise intensities to establish the contribution of central command and local muscle afferent fiber feedback to the cardiovascular response during this type of muscle contraction.

Lastly, electromyography indices of fatigue (EMGamp and EMGfreq) will also be monitored from the quadriceps muscle during static leg exercise in this study. Changes in EMG indices of fatigue are commonly used as an index to measure neuromuscular fatigue during exercise (Viitasalo & Komi, 1977; Moritani et al, 1986) (please refer to chapter 2, methodology section 2.3.2 for greater detail regarding the use of EMG indices of fatigue). Therefore the third aim of this study will be to examine these indices to establish the level of fatigue (if any) induced by the leg exercise in this study, and to determine how this fatigue influences the cardiovascular and haemodynamic responses to isometric leg exercise.

3.2 Methodology

3.2.1 Participants

Twelve healthy, normotensive males (age = 22 ± 2 yrs; height = 180.16 ± 4.17 cm; mass = 77.71 ± 11.11 kg) volunteered to participate in this study. Prior to testing, all participants received a written explanation of the procedures to be used, along with the potential risks of participating in the study. Once participants provided written informed consent, they were required to complete an exercise readiness questionnaire that can be viewed in appendix 1 on page 249. All participants fasted for 4 hours, abstained from caffeine and alcohol for at least 12 hrs prior to the start of testing procedures (Jauregui - Renaud et al, 2001), and maintained normal physical activity and diet throughout the length of the study. All participants completed two familiarisation sessions prior to data collection. Please refer to section 2.1 (chapter 2, page 38) for the ethical considerations that this study adhered to.

3.2.2 Equipment and procedures

Isometric exercise

All isometric exercise was conducted using a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY). Full details of the procedure used to perform bilateral isometric leg extension exercise can be found on page 39 of the Methodology chapter (Chapter 2). Isometric exercise intensity was expressed relative to each individual's maximum intensity reached during the DIIET, and is therefore known as relative exercise intensity (REI).

Maximal voluntary contraction (MVC) and EMG_{peak}

To establish each participants isometric exercise workload, MVC and EMG_{peak} were established. Full details of determining MVC and EMG_{peak} can be found on page 77 in Chapter 2, methodology).

Discontinuous incremental isometric exercise test (DIIET)

Participants underwent the DIIET test on 5 separate occasions (at least 48 hrs apart) performing double leg isometric extension exercise. Sessions 1 and 2 were familiarisation sessions, whilst the remaining 3 sessions were used for data collection. Full details of the protocol used to perform the DIIET test can be found on page 77 of the methodology chapter (Chapter 2). Figure 16 on page 77 also provides a visual representation of the DIIET test. For

the purpose of data analysis, exercise intensity at each stage within the DIIET was expressed relative to the maximum $\%EMG_{peak}$ achieved during the test (%REI).

Measurements taken during the discontinuous incremental isometric exercise test:

Electromyography (EMG) recording

Electromyography was recorded for the purpose of prescribing isometric exercise intensity in the DIIET. Electromyography was recorded from the vastus lateralis muscle of both the left and right leg using a duel bio amplifier and 16 channel chart recording software (PowerLab, ADInstruments Ltd, Australia). Please refer to section 2.3.2 on page 41 of the methodology chapter (Chapter 2) for full details regarding the use of EMG recording.

For the purpose of data analysis, each stage of the DIIET test performed to a set $\%EMG_{peak}$ target value was expressed relative to the maximum $\%EMG_{peak}$ stage that each participant achieved, known as percentage relative exercise intensity (%REI). Expression of isometric exercise intensity in this manner allows a direct comparison of haemodynamic and cardiovascular variables between participants.

Blood flow haemodynamics

Blood flow haemodynamics were measured for the purpose of identifying and defining the BF response during and immediately after a DIIET test. A 8 MHz multifrequency wide band linear array probe (8L-RS, GE Healthcare, UK) and a ultrasound machine (LOGIQ e, GE Healthcare, UK) were used to image the common femoral artery of the left leg below the inguinal ligament, 2-3 cm above its bi-furcation into the superficial and profunda femoral branches (Radegran et al 1997; Baross et al, 2012). Once an optimal image was obtained, the probe was held constant and ultrasonic parameters were adjusted to enhance the longitudinal B-mode image of the lumen-arterial interface. Doppler velocity profiles of the common femoral artery were obtained throughout testing protocol using the LOGIQ e at an insonation angle of < 70 degrees. The LOGIQ e was used in duplex mode to continuously record BV and AD in 30 second blocks throughout exercise and rest. Please refer to page 53 for specific details concerning the collection of BV and AD data. Exercise components consisted of four 30 second blocks as contraction time was 2 minutes, whereas post-exercise data was comprised of ten 30 second blocks as each rest period was 5 minutes in duration.

Blood velocity ($cm \cdot sec^{-1}$)

Blood velocity data was recorded for the purpose of calculating BF. Chapter 2, page 55 provides greater detail concerning the procedure used to measure BV. Blood velocity measures were analysed from the last 10 seconds of each 30 second recording of data, as this has been shown to be representative of the mean velocity over a 30 second period (refer to section 2.6 on page 75 in Chapter 2).

Peak systolic antegrade blood velocity (Vmax) (defined as the highest velocity measured in Doppler spectrum of a cardiac cycle, Thijssen et al, 2009a) and peak retrograde blood velocity (Vmin) (defined as the lowest velocity in the Doppler spectrum of a cardiac cycle, Thijssen et al 2009a) were analysed from the velocity spectrum using the in-built analysis software of the LOGIQ e ultrasound. Mean blood velocity (Vmean) was then calculated by averaging Vmax and Vmin to represent the average velocity of the Doppler spectrum across the entire cardiac cycle. Utilising both the antegrade and retrograde component of BV is more representative of the true nature of the BV profile, as it allows expression of the BV across the whole cardiac cycle (Green et al, 2005).

Mean blood velocity values from each cardiac cycle in a block were then averaged to give a single Vmean value for the last 10 seconds in each 30 sec video block. Mean blood velocity raw values were then used to calculate BF.

Artery diameter (cm)

Artery diameter was recorded during and immediately after each incremental stage in the DIIET for the purpose of calculating BF. Chapter 2, page 53 provides greater detail concerning the procedure used to measure AD. Edge detection and wall tracking software was used to analyse common femoral AD after testing protocol as it eliminates investigator bias (Woodman et al, 2001). Artery diameter was also measured during the last 10 seconds of each 30 second block. Analysis of AD using the edge detection and wall tracking software was used to determine a single mean diameter value for each block that encompassed both the systolic and diastolic pressure influence on diameter measurements. Artery diameter measurements were then used to calculate BF.

Blood flow ($ml \cdot min^{-1}$)

Blood flow for the last 10 seconds in each 30 second block of video during exercise and post-exercise was calculated from AD and Vmean using the following equation:

π (radius) x Vmean x 60.

(Gonzales et al, 2008)

Blood flow was then represented in the following ways to best demonstrate its response to isometric bilateral leg exercise:

1. Mean blood flow ($ml \cdot min^{-1}$). This is the average BF calculated from either the 2 minute contraction period (MBF), or the 5 minute post-exercise period (PE-MBF).
2. Peak blood flow ($ml \cdot min^{-1}$). This is the peak BF calculated from either the 2 minute contraction period (PBF), or the 5 minute post-exercise period (PE-PBF)
3. Change in blood flow (%). This is the percentage change in BF from exercise to post-exercise (ΔBF). The ΔBF is calculated from the last 10 seconds of the last 30 second block of exercise and the last 10 seconds of the first 30 second block during post-exercise.

Heart rate & blood pressure

Heart rate and BP were recorded throughout the DIIET to provide insight into the relationship between incremental isometric exercise intensity and the level of the cardiovascular response. Heart rate was recorded at rest and continuously during exercise via a three lead bipolar ECG arrangement using sensor R ECG electrodes (Ambu Inc, USA), on a 16 channel chart recorder sampled at a frequency of 1000 Hz (Powerlab, ADInstruments Ltd, Australia). Section 2.2.c on page 44 of the Methodology chapter (Chapter 2) provides greater detail regarding the use of ECG. Systolic blood pressure, DBP and MAP was measured using a Finometer (Finapres, TNO Instruments, Amsterdam, The Netherlands), at rest and continuously during exercise. Section 2.3.3 on page 45 of the Methodology chapter (Chapter 2) explains in detail the procedure used to record BP using the Finometer. Resting HR and BP was recorded for 5 minutes after a 15 minute resting period.

Indices of fatigue (EMG amplitude and EMG frequency)

EMG amplitude and EMGfreq were recorded and analysed using LabChart 7 Software (ADInstruments Ltd, Australia) during exercise phases in the incremental test to determine the relationship between level of fatigue in each vastus lateralis muscle and increasing exercise intensity. An identical protocol to that used by Devereux et al (2011) was utilised to record and analyse EMGamp and EMGfreq. EMG amplitude was calculated by subtracting the minimal signal voltage from the maximum signal voltage (maximum mV - minimum mV)

(Devereux et al, 2011). EMG frequency was analysed by utilising a separate channel, set up from cyclic frequency measurement (Devereux et al, 2011). Throughout the contraction period, EMGamp and EMGfreq was collected from the vastus lateralis of both legs, then combined and averaged to give one EMGamp value and one EMGfreq value. Values were averaged into 5 second blocks across the 2 minutes of isometric contraction duration. Page 41 of the methodology chapter (Chapter 2) provides more detail regarding the procedure used to record EMGamp and EMGfreq.

3.2.3 Data analysis

All data was assessed for normality. If parametric assumptions were not met, data was logarithmically transformed (Field, 2000). Correlation coefficients with repeated observations were used to assess linear dependence between REI and the primary outcome variables for this study (MBF, PBF, PE-MBF, PE-PBF & ΔBF), and between REI and the secondary outcome variables (SBP, DBP, MAP, HR, EMGamp & EMGfreq). A level of $P < 0.05$ was set as the threshold for statistical significance across all statistical tests performed.

3.3 Results

3.3.1 Blood flow

Of the 12 participants, it was not possible to obtain clear and accurate Vmin values in BV spectrum traces throughout the DIIET in 2 participants. As a result, a Vmean that represented both systolic and diastolic velocity components in the cardiac cycle could not be established, and consequently a true mean BF that represented all stages of the cardiac cycle could not be calculated for these 2 participants. Results from the remaining 10 participants demonstrated that BF measured from the common femoral artery increased from baseline values of 116 ± 49 $ml \cdot min^{-1}$ to peak values of up to 302 ± 65 $ml \cdot min^{-1}$ during the DIIET, and up to values of 419 ± 82 $ml \cdot min^{-1}$ in the 5 minute post-exercise period.

All BF variables met parametric assumptions. Statistically significant correlation coefficients with repeated observations results are revealed in table 8. The relationship between REI and BF variables can also be viewed in Figures 18 – 20.

Table 8 *Correlation coefficient analysis results for blood flow variables versus relative exercise intensity*.

Variable	r value	P value
MBF	0.669	< 0.01
PBF	0.610	< 0.01
PE-MBF	0.742	< 0.01
PE-PBF	0.660	< 0.01
ΔBF	0.574	< 0.01

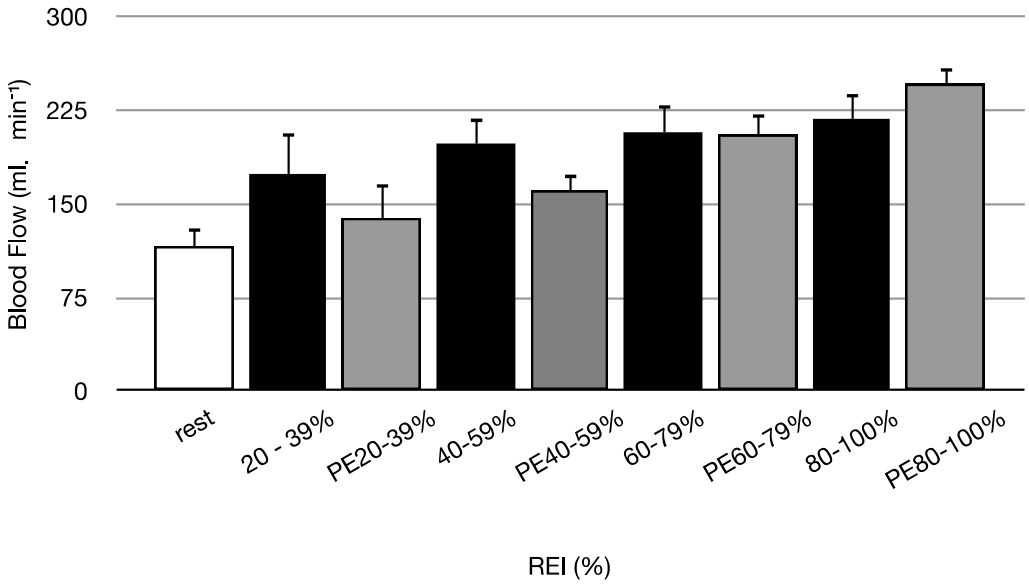

Figure 18. *Mean blood flow (ml·min^{-1}) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting mean blood flow is represented by white bars, whilst MBF is represented by black bars, and PE-MBF is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and MBF (r = 0.669, P < 0.01) and PE-MBF (r = 0.742, P < 0.01).*

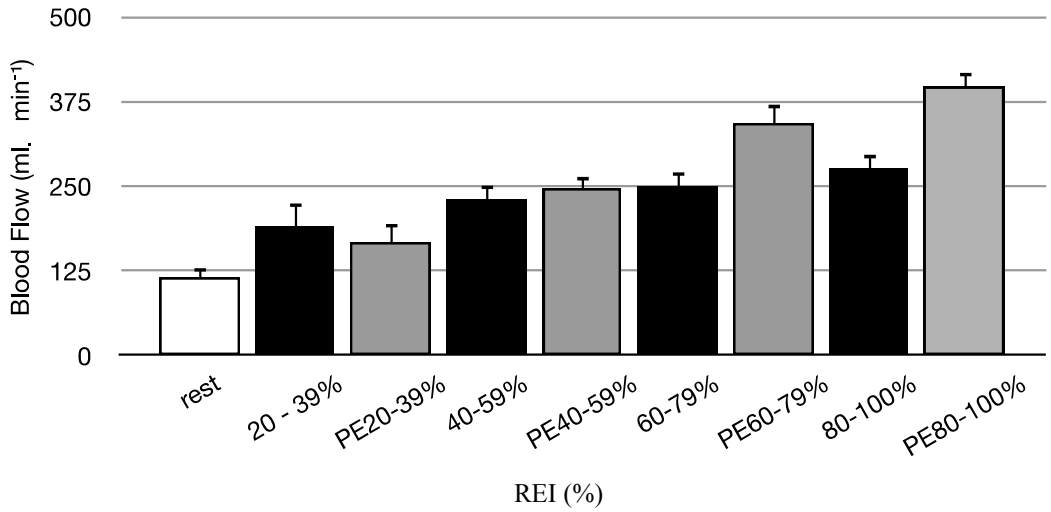

Figure 19 *Peak blood flow (ml·min^{-1}) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting peak blood flow is represented by a white bar, whilst PBF is represented by black bars and PE-PBF is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and PBF (r = 0.610, P < 0.01) and PE-PBF (r = 0.660, P < 0.01).*

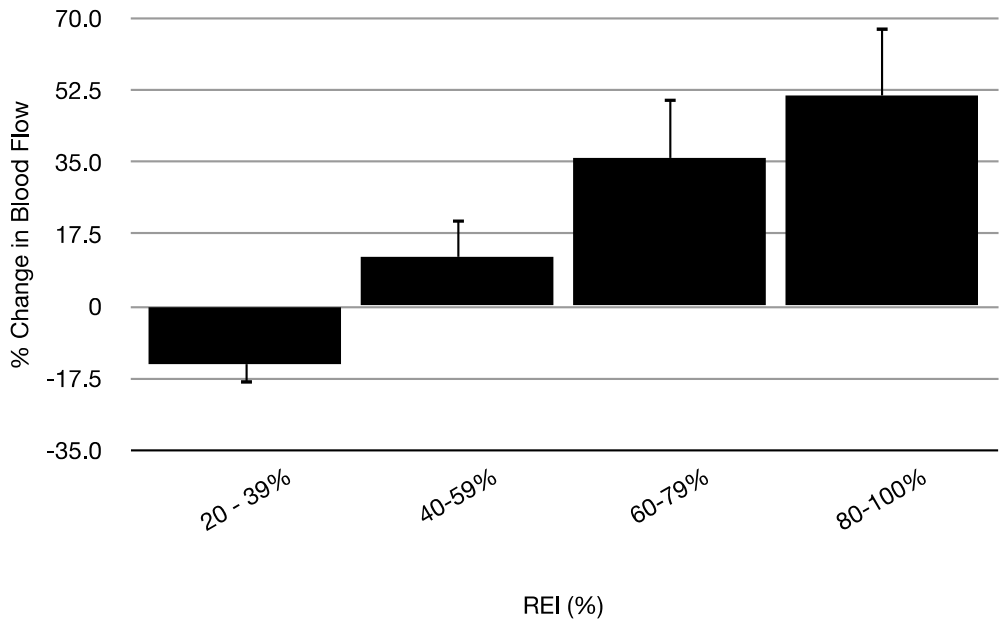

Figure 20. *Delta change in blood flow (ΔBF) from contraction to post-exercise and standard error. Correlation co-efficient analysis revealed a significant relationship between REI (%) and delta change in blood flow (r=0.574, P< 0.01).*

3.3.2 Systemic response

Exercising SBP, DBP, MAP and HR responses during the DIIET were analysed in all 12 participants. Results demonstrate that SBP, DBP, MAP and HR all increased above baseline levels during exercise.

Normality assumptions were met for SBP, DBP, MAP & HR variables (as $P > 0.05$). Statistically significant correlation co-efficient results are revealed in table 9. The relationship between REI and cardiovascular variables can also be viewed in Figure 21.

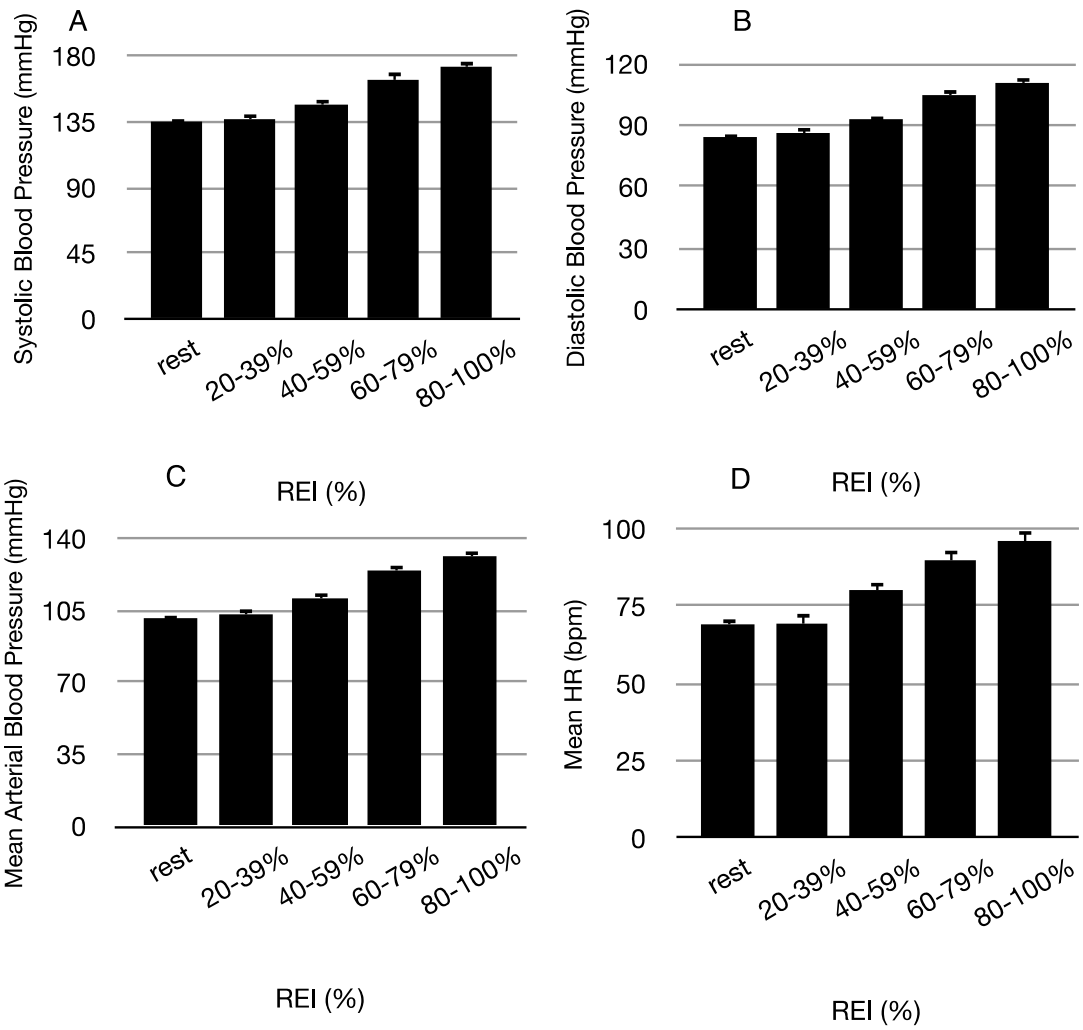

Figure 21 *Systemic response to an discontinuous incremental isometric double leg exercise test of increasing REI (%) with standard error. Correlation co-efficient analysis revealed that exercising SBP (Bar chart A), DBP (Bar Chart B), MAP (Bar chart C) and HR (Bar chart D) all increased in relation to REI (%) ($r = 0.918$, $P < 0.01$; $r = 0.927$, $P < 0.01$; $r = 0.927$, $P < 0.01$; $r = 0.671$, $P < 0.01$).*

3.3.3 Neuromuscular response

Indices of fatigue (EMGamp and EMGfreq) were also analysed in all 12 participants during a DIIET. Normality assumptions were met for EMGamp and EMGfreq variables ($P > 0.05$). Statistically significant correlation coefficient results are revealed in table 9. The relationship between REI and neuromuscular variables can also be viewed in Figure 22.

Table 9 *Correlation coefficient results for cardiovascular and neuromuscular variables versus relative exercise intensity.*

Variable	r value	P value
SBP	0.918	< 0.01
DBP	0.927	< 0.01
MAP	0.927	< 0.01
HR	0.671	< 0.01
EMGamp	0.955	< 0.01
EMGfreq	-0.797	< 0.01

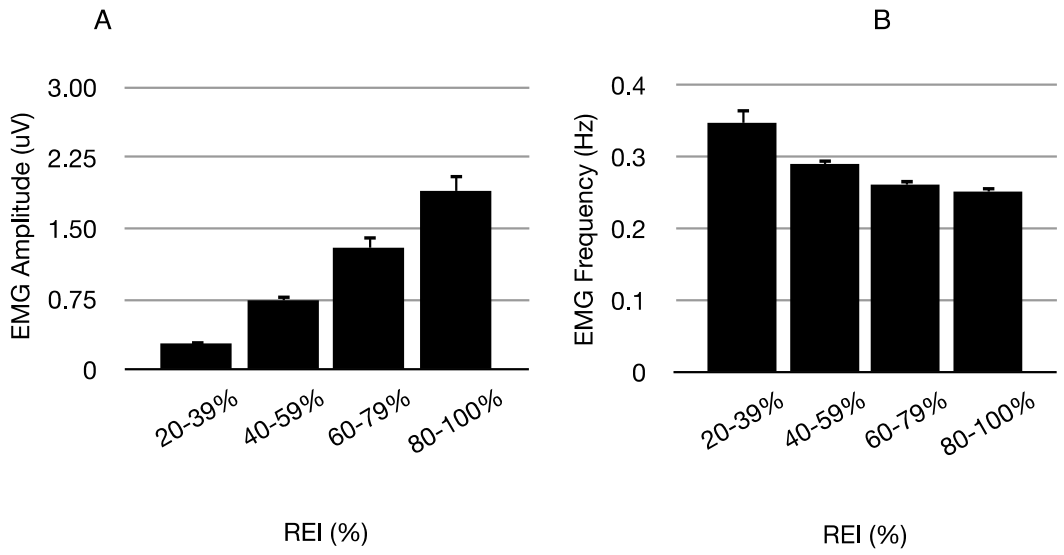

Figure 22 *EMGamp and EMGfreq response to an discontinuous incremental isometric double leg exercise test of increasing REI (%) with standard error. Correlation co-efficient analysis revealed significant correlations between REI (%) and EMGamp (Bar chart A) and EMGfreq (Bar chart B), ($r = 0.955$, $P < 0.01$; $r = -0.797$, $P < 0.01$).*

3.4 Discussion

The primary aim of this study was to determine the local BF response to sustained isometric bilateral leg exercise of graded exercise intensity, specifically focusing on BF during and immediately after isometric contraction. This was in order to assess whether BF haemodynamics could be considered as a physiological stimulus for RBP adaptation following IET. This study was unique in that it was the first of its kind to examine the local BF response during and post-isometric bilateral leg extension exercise, that was graded in intensity to $\%EMG_{peak}$. Exercising BP, HR and EMG indices of fatigue were also observed as secondary measures. The results of this study demonstrate that local BF to the quadricep muscles during (MBF & PBF) and after (PE-MBF & PE-PBF) isometric bilateral leg exercise, as well as ΔBF are greatly influenced by increasing isometric exercise intensity (REI) during sustained bilateral leg extension exercise. Exercising SBP, DBP, MAP, HR, EMGamp and EMGfreq were also largely influenced by increasing isometric bilateral leg extension exercise intensity (REI).

Blood flow during isometric incremental bilateral leg extension exercise

The BF supplying the quadriceps muscle group, as measured in the left leg common femoral artery, increased above resting levels during all exercise intensities, reaching a peak value of 302 ± 65 $ml \cdot min^{-1}$ as demonstrated in figure 19. Exercising BF values were highly correlated with REI (%) for both MBF and PBF, suggesting a linear relationship between exercise intensity and exercising quadricep BF during a DIIET.

Only 2 studies have previously examined the local BF response to graded isometric leg extension exercise. Gaffney et al (1990) found that BF measured in the common femoral artery using the thermodilution technique during unilateral isometric leg extension exercise did not increase linearly with exercise intensity, as BF during 15% MVC reached 1.76 ± 0.45 $L \cdot min^{-1}$, whilst 25% MVC and 50% MVC BF values only reached 0.90 ± 0.32 and 1.04 ± 0.42 $L \cdot min^{-1}$. In addition, whilst Sjogaard et al (1988) did not report exact BF values, it is clear that BF during contraction decreased with each increasing intensity from 5% MVC, 15% MVC, 25% MVC, & 50% MVC. Figure 17 (page 85) demonstrates this response. Since in both of these studies, contractions were independently held to fatigue, it is likely that a interaction of exercise intensity and contraction duration may have created a greater BF response at lower exercise intensities, as they were maintained for a longer duration to the point of fatigue.

Together these two previous studies suggest that muscle BF in the leg becomes insufficient above 10-15% MVC when static contractions of the quadricep muscles are performed.

The results of this current study are not totally consistent with that of Gaffney et al (1990) and Sjogaard et al (1988) as local BF to the exercising muscle (whether expressed as MBF or PBF) did continue to increase linearly with exercise intensity, and did not become reduced or impeded at higher exercise intensities. It must be taken into consideration that the exercise protocols utilised by Gaffney et al (1990) and Sjogaard et al (1988) are very different to the exercise protocol utilised in this current study. Gaffney et al (1990) and Sjogaard et al (1988) performed isometric leg extension contractions at increasing exercise intensities on independent occasions, and furthermore these contractions were held to fatigue. In the exercise protocol of this current study, participants performed a DIIET, where contractions of increasing intensity were performed in a continuous nature (separated by 5 minutes rest), and maintained for 2 minute bouts until participants reached volitional fatigue or could no longer sustain ± 5% of their EMG_{peak} target value for that particular intensity. Therefore participants performing this discontinuous test did not reach maximal fatigue until the final stage of their incremental test. Furthermore, torque data recorded during exercise periods in this current study suggests that participants were producing an average mean % MVC of 28.25 ± 7.27 when they worked at their individualised peak $\%EMG_{peak}$ intensity during the DIIET. This demonstrates that for this current study, BF continued to increase past 10 - 15% MVC, which has previously been described as the threshold before muscle leg BF becomes insufficient during static leg exercise (Gaffney et al, 1990; Sjogaard et al, 1988). The significant differences between these 2 exercise protocols with regards to the interaction between exercise intensity and duration of contraction may go some way as to explaining the differences observed in the relationship between exercising BF and increasing exercise intensity between the work of Gaffney et al (1990) & Sjogaard et al (1988) and this current study. It is also suggested that the differences in the prescription of exercise intensity (%MVC vs $\%EMG_{peak}$) might offer further explanation as to the dissimilarity of these studies in regards to the relationship between exercising local BF and increasing exercise intensity.

By performing isometric leg extension exercise to a prescribed $\%EMG_{peak}$ as opposed to %MVC, the cardiovascular and consequent local BF response is likely to be altered. It is well established that isometric contraction is characterised by high levels of intramuscular pressure and mechanical compression of contracting muscle fibers (Petrofsky & Lind, 1975). Intramuscular pressure is defined as the hydrostatic fluid pressure within a muscle (Sejersted

et al, 1984). Unlike dynamic muscular contractions, where the rhythmic nature of the exercise releases the intramuscular pressure during muscle relaxation periods, intramuscular pressure is held continuously for the duration of the isometric contraction (Crenshaw et al, 1997; Sadamoto et al, 1983; Sejersted et al, 1984; Sjogaard et al, 2004). During static contractions that work to a constant %MVC, the main focus is to maintain a required level of force production. At exercise intensities where BF is impeded, and thus oxygen delivery to the working muscle reduced, muscle fibers start to fatigue. As a result, the electrical activity of the muscle increases to recruit higher threshold motor units to prevent a decrease in force output (Edwards & Lippold, 1956). It is well documented that EMG (a measure of muscle sympathetic activation of motor unit recruitment) increases during force constant isometric contractions (Crenshaw et al, 1997; Kouzaki et al, 2002; Sadamoto et al, 1983; Sjogaard et al, 2004). Coinciding with this, a continuous rise in intramuscular pressure throughout contraction has also been observed during isometric leg extension exercise maintained for 2 minutes (Sadamoto et al, 1983) and during isometric leg extension at 25% MVC sustained to fatigue (Crenshaw et al, 1997). Both Sadamoto et al (1983) and Crenshaw et al (1997) suggest that this continuous rise in intramuscular pressure may be attributable to an continuous increase in motor unit activity (as measured by EMG) during force constant conditions, which may increase local muscle fiber tension and / or the number of muscle fibers recruited (Sergested et al, 1984). Additionally, a continuous increase in intramuscular pressure during force constant isometric contraction may also be as a result of the accumulation of intramuscular water due to increased capillary perfusion pressure and the number of perfused capillaries caused by an increase in BP and BF during isometric contraction (Sadamoto et al, 1983; Crenshaw et al, 1997). Crenshaw et al (1997) are of the opinion that this would favor an efflux of fluid from the vascular space into the interstitium, resulting in extravascular fluid accumulation. Crenshaw et al (1997) highlighted that this increase in intramuscular pressure during force constant conditions is dependent upon intensity of the isometric contraction. This is based upon the observation that during sustained isometric leg extension exercise to fatigue performed at 70% MVC, no rise in intramuscular pressure was observed. Crenshaw et al (1997) suggested that during 70% MVC isometric contraction, mechanical compression of the blood vessels may prevent extravascular fluid accumulation, thus preventing an increase in intramuscular pressure.

In contrast, when a static contraction is held to a %EMG such as that used in this current study, the main aim is to maintain the required level of electrical activity during a contraction. When muscle fibers start to fatigue, the electrical activity of the muscle will not increase as it

is required to remain constant to meet the set prescribed exercise intensity %EMG_{peak} target. As a result motor unit activity and subsequent recruitment of greater numbers of muscle fibers will not increase (as typically occurs in force constant isometric conditions [Sergested et al, 1984]), resulting in a more consistent and continuous intramuscular pressure (Sadamoto et al, 1983). Indeed Sadamoto et al (1983) has demonstrated that when isometric leg extension exercise was sustained for 2 minutes, EMG and intramuscular pressure remained constant, whilst force output decreased. Sejersted et al (1984) also found similar results when isometric leg extension exercise contraction was performed to a constant intramuscular pressure, and a 30% decrease in force output was noted. As such, these findings indicate that when holding isometric contraction to a constant level of EMG activity, higher threshold motor units are not recruited and force output drops off leaving only the fatigue resistant motor units to maintain the contraction (Schibye et al, 1981).

This concept is demonstrated in a study completed by Schibye et al (1981), where participants performed static leg extension exercise in 2 conditions. The first condition required participants to hold contraction at 20% MVC (force development condition), whereas in the second condition participants were required to hold the level of EMG activity constant. During the EMG constant condition, force output decreased from 20 % to 12 %. In the force constant condition, the level of EMG doubled. In both conditions there was a significant increase in MAP, however the rise in MAP in the EMG constant condition was smaller and increased at a steadier rate when compared to the force constant condition. Schibye et al (1981) suggested that a higher MAP was observed in the force constant condition as intramuscular pressure was higher and BF was restricted.

The variation in the physiological conditions when performing isometric contraction to a constant force or constant EMG may be responsible for the differences observed between the BF responses of this study and that of Gaffney et al (1990) and Sjogaard et al (1988). By prescribing static leg extension exercise to a constant force, Gaffney et al (1990) and Sjogaard et al (1988) may have induced a muscular contraction where intramuscular tension continued to increase throughout the exercise duration as greater numbers of motor units were recruited to maintain a desired level of force production (Sejersted et al, 1984). Increases in BP and BF would have been initially mediated by central command upon immediate start of contraction, and then by the pressor reflex as fatigue started to develop. This action in itself would increase intramuscular pressure further as an increase in capillary perfusion pressure in combination with an increase in the number of capillaries perfused would cause greater

extravascular fluid accumulation (Sadamoto et al, 1983; Crenshaw et al, 1997). The increase in intramuscular pressure will ultimately reach a critical level whereby compression of intramuscular blood vessels occurs (Crenshaw et al, 1997). Intramuscular pressure may have become so great at higher contraction intensities above 15% MVC, that the immediate central command response and the following pressor reflex response may not have been able to increase perfusion pressure to a great enough extent to overcome an increasing intramuscular pressure induced compression of blood vessels, thus hindering the BF supply to the quadriceps muscle. As this current study prescribed exercise intensity using $\%EMG_{peak}$ it is plausible to suggest that as intramuscular tension remained constant and did not increase during each contraction duration, central command and the pressure reflex response were able to increase BP to a level that ensured muscle BF perfusion throughout all exercise intensities.

The results of this study demonstrate a linear relationship between MBF and PBF during isometric bilateral leg extension exercise and REI. Gaffney et al (1990) suggested that local BF during static exercise is determined by several factors - perfusion pressure, degree of obstruction to flow (as a result of mechanical compression of blood vessels from static nature of isometric contraction, as well as intramuscular compression of blood vessels as a result of increases in intramuscular pressure), and the net balance between centrally mediated vasoconstriction and local metabolite vasodilation. Each of these factors will be discussed below in relation to the current findings.

Perfusion pressure is determined by the pressure of the blood in the cardiovascular system, also known as BP. Exercising SBP, DBP, MAP as well as HR all increased relative to exercise intensity in this current study. Gandevia & Hobbs (1990) suggested that the cardiovascular response to static exercise (such as the one seen in this current study, figure 21, page 96) is determined by a combination of central command and the muscle chemoreflex response. Central command involves descending signals from higher brain centers that centrally mediate the cardiovascular system (Williamson et al, 2006). Central command is responsible for the BF perfusion pressure in the initial stages of isometric contraction (Rowell & O'Leary, 1990). Blood pressure is a product of $\dot{Q}$ multiplied by vascular resistance. During isometric exercise, an increase in BP is attributed solely to an increase in $\dot{Q}$, which is created by a central command mediated increase in HR via parasympathetic vagal nerve activity withdrawal (Bezucha et al, 1982; Goodwin et al, 1972; Martin et al, 1974; Shoemaker et al, 2007). Vascular resistance does not typically change during static contraction (Martin et al, 1974; Shoemaker et al, 2007). However, Rowland & Fernhall (2007) have contested the lack

of contribution of vascular resistance to the BP response during static contraction, since when analysing BF and BP on a beat to beat basis (as opposed to a average value over time), vascular conductance was shown to increase. Rowland & Fernhall (2007) suggested that this response is consistent with the expected mechanical compression of the blood vessels by the nature of static muscular contraction, and that this revised model demonstrates that the acute rise in BP is accounted for by the increase in vascular resistance rather than just solely a rise in $\dot{Q}$ as suggested in traditional models. It seems possible that both $\dot{Q}$ and vascular resistance may contribute to the initial pressure response during isometric exercise. Bezucha et al (1982) documented a 19% increase in $\dot{Q}$ that occurred during isometric leg extension exercise at 30% MVC, in conjunction with an 8% increase in vascular resistance, thus suggesting a combined effort whereby $\dot{Q}$ may be slightly more dominant.

It is apparent that the level of central command is related to the number of motor units activated in a static contraction, in that the greater the number of motor units recruited, the greater the central command response (Smolander et al, 1998; Mitchell et al, 1983; Mitchell et al, 1980; Seals et al, 1983). Taylor et al (1988) noted that EMG activity during rhythmic hand grip exercise was strongly correlated with the HR and BP response, thus concluding that the relationship between motor unit activation and the cardiovascular response is "consistent with the idea that central command exerts its influence via parallel activation of motor and autonomic nervous systems" (page 36). Since the participants in this study performed exercise to a set level of EMG, it is very unlikely (based upon the size principle) that additional motor units became recruited throughout the duration of each contraction within the DIIET. Therefore intramuscular pressure was held at a set level for each exercise intensity stage (Sadamoto et al, 1983). It is likely that the level of central command was consistent for each exercise intensity level, in relation to the number of motor units recruited at that particular intensity. This is further supported as some studies have used the level of EMG as an indirect index for the level of central command during exercise (Taylor et al, 1988; Schibye et al, 1981). As exercise intensity increased throughout the DIIET, a greater number of motor units would have been recruited to maintain the desired level of EMG for that particular intensity stage. This would then imply that the level of central command would have also increased, leading to a greater centrally mediated BP response, as seen in the exercise intensity dependent BP response of this study. This may elevate perfusion pressure, and consequently contribute to the observed increase in BF during isometric leg extension exercise as REI increases.

Central command sets the basic patterns of effector activity at the onset of static exercise (Rowell & O'Leary, 1990), after which this activity is then modulated by a peripheral mechanism that consists of a reflex pathway originating in the contracting muscle (Seals et al, 1983). When intramuscular pressure is high and an oxygenated blood supply becomes reduced such that it can no longer meet the metabolic demands of the exercise, metabolites begin to accumulate as fatigue occurs (Lind & McNicol, 1967; Rowell & O'Leary, 1990). In response to the increasing acidity within the contracting muscle due to the accumulation of metabolites, (which include an increase in muscle venous lactate, H^+ concentration, PCO_2, bradykinins and prostaglandins; Rowell & O'Leary, 1990), type IV muscle afferents provide feedback to higher brain centres regarding the metabolic conditions of the working muscle (Coote et al, 1971). In response, a metaboreflex is activated resulting in a exercise pressor reflex response (Alam & Smirk, 1937; McCloskey & Mitchell, 1972; Rowell O'Leary 1990). This pressor reflex invokes a vasodilatory response to the arteries surrounding the contracting muscle combined with an increase in BP by increasing peripheral sympathetic vasoconstriction (Gandevia & Hobbs, 1990) to increase perfusion pressure and to maintain BF to the exercising muscle so that the delivery of O_2 and washout of metabolites is preserved.

Coote et al (1971) noted a relationship between tension developed in the contracting muscle, the size of the pressor response and the extent of the local dilation of resistance vessels in these muscles. As a result, Coote et al (1971) concluded that the local hyperemic BF response and the reflex pressor response must be initiated by the same chemical stimulus within the muscle. Similarly, Osada et al (2003) noted that when handgrip exercise was performed with arterial occlusion (AO) and the exercise became ischemic, a higher BF was observed in comparison to when the handgrip exercise was performed without AO and was classed as non-ischemic. Osada et al (2003) suggested that during the non-ischemic exercise, BF was not arrested as there was no limit to the washout of vasodilatory metabolites. Therefore the higher BF observed in the ischemic exercise was due to an increased production of vasodilatory metabolites that may have been caused by limited O_2 utilisation in the working skeletal muscle as it worked in anaerobic conditions (Osada et al 2003). It could be suggested that the same relationship occurred in this study. As exercise intensity increases (over the course of the DIIET), the greater the increase in muscle tension and the greater the increase in intramuscular pressure, the greater the pressor response (due to increased metabolite accumulation). This is further supported by the observed isometric exercise intensity dependent response of EMG indices of fatigue (increases in EMGamp and decreases in

EMGfreq) in this study, suggesting that as the DIIET progresses in terms of exercise intensity, the test becomes more fatiguing. This may have led to a greater dilation of resistance vessels within these muscles at higher isometric leg extension exercise intensities at the later stages of the DIIET, leading to a greater BF response.

Hamann et al (2004) suggested that conducted vasodilation and / or FMD are likely to be essential for coordinating the response of the skeletal muscle bed to increase BF during static exercise. The neurotransmitter acetylcholine triggers hyperpolarization which is conducted along the endothelial cells in the resistance vessels, resulting in endothelial cell to cell communication that induces a conducted vasodilation response (Segal & Kurjiaka, 1995; Welsh & Segal, 1997). Segal & Jacobs (2001) demonstrated that when this conduction pathway was damaged in hamsters during rhythmic exercise, the increase in BF was reduced by half when compared to exercising BF when the conduction pathway was intact. Flow mediated dilatation relies on the ability of endothelial cells to respond to an increased BF induced SS on the endothelium to release dilating factors that relax smooth muscle cells, resulting in vasodilation and normalisation of SS (Segal& Kurjiaka, 1995). A number of dilating substances have been attributed to SS induced endothelial mediated dilation including NO, prostacyclin, adenosine and endothelium derived hyperpolarization factor (Radegran, 2003). The influence of these dilating factors on the haemodynamic response to isometric exercise will be discussed in greater detail in Chapter 4.

Activation of the pressor reflex and excitation of type III muscle afferents that are activated through metabolic stimuli initiate a sympathetic vasoconstriction response in non-exercising vasculature to raise BP during isometric exercise contraction (Rowell & O'Leary, 1990). During 2 minutes of static handgrip exercise at 40% MVC, Shoemaker et al (2007) observed a decrease in portal vein diameter, indicating splanchnic vasoconstriction. Shoemaker et al (2007) concluded that the rise in sympathetically mediated vasoconstriction during fatiguing isometric exercise was needed to direct BF towards the heart so total BF towards the exercising muscle could increase. Likewise, Gaffney et al (1990) noted an substantial decrease in resting limb BF during isometric leg exercise despite an augmented BP, suggesting significant sympathetic vasoconstrictor activity had occurred to direct blood towards the exercising muscle. The muscle sympathetic nerve activity (MSNA) response also appears to be exercise intensity dependent. Rowell & O'Leary (1990) demonstrated that there was a strong relationship present between increases in MSNA, BP and EMG during fatiguing handgrip contractions of 25 and 30% MVC, with the rate of the increase of all variables greatest at 30% MVC. This would suggest that at the higher EMG exercise intensity stages of

the DIIET used in this current study, MSNA could have been increased, inducing greater vasoconstriction in the relaxing vasculature, thus increasing BP and therefore BF to the contracting quadriceps muscles. It is possible that the combination and balance of local resistance vessel vasodilation and sympathetic vasoconstriction in the resting vasculature as a result of the pressor reflex response may have contributed to the linear relationship between BF and REI documented in this study.

This study documented increases in MBF and PBF during a incremental DIIET that were exercise intensity dependent. In summary, it appears that as Gaffney et al (1990) suggested, a combination of perfusion pressure, intramuscular pressure and the net balance between central vasoconstrictor activity and local vasodilation may mediate the BF response to static leg exercise. As this exercise was performed to set levels of $\%EMG_{peak}$, it is likely that the level of intramuscular pressure would have been consistent at each stage of exercise intensity within the DIIET. It is reasonable to suggest that the level of central command and the strength of pressor reflex response at each exercise intensity was able to create a great enough perfusion pressure to overcome any mechanical compression, and ensure a continued and increased blood supply at each REI.

Blood flow immediately post incremental isometric bilateral leg extension exercise

This study also examined the BF response in the quadriceps muscle group immediately after isometric bilateral leg extension exercise during the DIIET. Post-exercise BF measured in the left leg common femoral artery increased above resting levels during all exercise intensities, reaching a peak value of 419 ± 82 $ml \cdot min^{-1}$ (as demonstrated in figure 19). Post-exercise BF was highly correlated with REI for both PE-MBF and PE-PBF, suggesting a positive linear relationship between exercise intensity and post-exercise quadricep BF (as measured in a 5 minute rest period) after isometric bilateral leg extension exercise stages sustained for 2 minutes.

The linear relationship between exercise intensity and the magnitude of the post-exercise BF response to bilateral isometric leg exercise in this current study appears to be consistent with the findings of other studies that have explored the post-exercise BF response to isometric contraction (Kagaya & Homma, 1997; Osada et al, 2003; Walloe & Wesche, 1988). Specifically in relation to leg extension exercise, Gaffney et al (1990) noted an exercise intensity dependent BF response in the rest periods after isometric leg extension exercise at 15, 25 & 50% MVC held to exhaustion. Post-exercise BF peaked to values significantly

greater than that seen in this current study (3500 $ml \cdot min^{-1}$ vs 419 $ml \cdot min^{-1}$). In this current study however, participants individually worked at a much lower peak exercise intensity, which equates to ~28% MVC in comparison to that of Gaffney et al (1990) of 50% MVC. In addition, the exercise stages in the DIIET utilised in this current study were held for 2 minutes in duration (with exception to the final stage where fatigue ceased contraction before the end of the 2 minute bout), where as the exercise bouts in the study of Gaffney et al (1990) were held to fatigue. As previously discussed, it is also clear that contraction BF in the study of Gaffney et al (1990) reached higher peak BF values than that seen during contraction in this current study. This may have contributed to a higher BF post-exercise in the study of Gaffney et al (1990) in comparison to this current study.

The increase in BF after isometric contraction occurs despite decreases in BP and HR towards baseline levels when exercise has ceased (Gaffney et al, 1990). This suggests that the mechanisms that lead to this observed increase in BF post-exercise are not centrally mediated. A number of mechanisms have been suggested that cause this augmented BF response post-isometric exercise. These include the mechanical obstruction to BF created during isometric contraction, reduced sympathetic nervous activity in the recovery from exercise and the magnitude of the O_2 debt during isometric contraction (Taylor et al, 1988). Each of these mechanisms will now be discussed in relation to the results of the current study.

Taylor et al (1988) proposed that the magnitude of post-exercise BF is reflective of the magnitude of the O_2 debt and subsequent level of the stimulus for the pressor reflex response to exercise (metabolite accumulation). Osada et al (2003) found that after 2 minutes of isometric handgrip at 30% MVC versus 2 minutes of AO, the post-exercise BF response returned to baseline much quicker in the AO condition, indicating that a substance or metabolite produced during the exercise condition prolonged post-exercise BF. Furthermore, the post-exercise BF response was much greater after ischemic handgrip exercise than that seen after non-ischemic handgrip exercise. Osada et al (2003) concluded that the greater BF response after ischemic handgrip exercise was probably due to the large influence of accumulated vasodilatory metabolites that are not washed out of the working muscle due to the reduced blood supply. A number of vasodilatory metabolites have been suggested as being responsible for the increase in BF post-exercise. These include potassium, lactate, adenosine, NO and prostaglandins (Clifford & Hellsten, 2004). This reasoning is further supported by Sjogaard et al (1986) after no increase in BF was observed after static leg extension exercise at 5% MVC, which indicated an optimal regulated capillary BF to provide an adequate blood supply to the working quadricep muscle. A number of other studies also support this view, in

that locally released metabolites play a dominant role in the post-exercise BF response (Bangsbo & Hellsten, 1998; Gaffney et al, 1990; Kagaya & Homma, 1997; Walloe & Wesche, 1988).

It is plausible to suggest that the intensity dependent post-exercise BF response to isometric leg exercise seen in this current study could be related to a greater level of BF perfusion in an attempt to ensure greater O_2 availability during each progressive stage of exercise intensity over the course of the DIIET. At each incremental $\%EMG_{peak}$ exercise stage an increased number of motor units would have been recruited, corresponding with the increase in EMG activity (Kouzaki et al, 2002). This may have resulted in the occurrence of mechanical compression due to the nature of static contraction, and a rise in intramuscular pressure. As a result, blood supply may have been increasingly inadequate to washout metabolites, resulting in metabolite accumulation as the intensity stages of the incremental test progressed. Upon release of contraction these dilatory metabolites may have been released into the circulation, eliciting a post-exercise dilatory response that increased BF post-exercise. Furthermore, several other studies have also related the level of EMG during static contraction to the magnitude of the post-exercise BF response (Taylor et al, 1988; Lind et al 1981).

This study also demonstrated that BF during static leg extension exercise stages continued to increase throughout contraction in relation to incremental exercise intensity stages during the DIIET, and appeared not to become occluded at any point. This would suggest that increases in post-exercise BF still occurred despite an adequate perfusion BF during contraction in the common femoral artery to remove the majority of accumulated metabolites. A critical point of this study is that during leg extension exercise the main conduit artery feeding the quadricep muscle group is the profunda femoral artery (also known as the deep femoral artery), which branches off the common femoral artery (Wray et al, 2005) (please refer to page 53 for anatomical diagram). Due to this vessels deep location within the leg musculature, imaging the profunda vessel during leg extension exercise is very difficult, and it is not possible to obtain clear BV spectrum traces. Imaging the common femoral artery instead was seen as a viable alternative as it feeds the profunda femoral artery. It may be possible that the blood supply further downstream in the profunda femoral artery was in fact occluded or significantly reduced by mechanical compression of muscle fibers, in which case a build-up of metabolites would have occurred in the quadriceps muscle. Upon release of contraction, these vasodilatory metabolites may have induced vasodilation and therefore increased BF further downstream in the profunda femoral artery. As Segal & Kurjiaka (1995) suggested, FMD in downstream vessels will increase BF in upstream vessels. This downstream FMD

response may be responsible for the increase in BF that was observed in the upstream common femoral artery. However, this is theoretical as downstream BF in the profunda femoral artery during and post static leg extension exercise was not measured and hence the haemodynamic response for this vessel during this type of activity is yet to be determined.

This study documented an increase in PE-PBF and PE-MBF immediately after isometric bilateral leg exercise that was exercise intensity dependent during the DIIET. Although this is speculative, it is plausible to suggest that during isometric leg extension exercise, quadricep muscle BF may become significantly reduced downstream in the profunda femoral artery, resulting in metabolite production with limited washout. Release of these vasodilatory metabolites upon cessation of contraction may induce downstream vasodilation that initiates an upstream FMD response in the common femoral artery. This response appears to be mediated by the magnitude of the O_2 debt induced (and therefore isometric exercise intensity) during isometric contraction and the subsequent magnitude of vasodilatory metabolite accumulation.

Change in blood flow from exercise to rest

Delta blood flow was significantly positively correlated with REI. This linear relationship demonstrates that the BF immediately post-exercise must have been greater then the BF seen during isometric leg exercise (302 ± 65 $ml \cdot min^{-1}$ PBF during exercise vs 419 ± 82 $ml \cdot min^{-1}$ PBF post-exercise) for the correlation to be positive, and that the difference between the contraction and post-exercise BF became more pronounced as exercise intensity increased at each incremental stage during the DIIET.

There is a distinct lack of research exploring the change in BF from contraction to relaxation after isometric exercise, however the few studies that have compared post-exercise BF to baseline BF note a distinct increase. Gaffney et al (1990) is one such study that has reported BF values both for during isometric leg extension contraction and post-isometric leg extension contraction at 15, 25 and 50% MVC. Whilst Gaffney et al (1990) did not directly assess the change in BF, it can be calculated that there is an statistically non-significant relationship between change in BF and exercise intensity, as ΔBF showed a 42% increase at 15% MVC, a 288% increase at 25% MVC and a 188% increase at 50% MVC.

The discussions of this current study have proposed that a combination of BF control mechanisms from both exercise and non-exercise periods may contribute to the observed increase in BF from contraction to post-exercise. Perfusion flow during contraction is

increased to overcome the level of intramuscular pressure and degree of mechanical compression (Rowell, 1993). This is achieved by substantial increases in BP (Goodwin et al, 1972; Petrofsky & Lind, 1975; Smolander et al, 1998). When isometric contraction ceases and the muscle relaxes, BP returns back to near baseline values. However, the return of BP back to near resting values is not an immediate response, but one of a gradual nature. Immediately after the end of isometric contraction BP is still reasonably elevated. The elevated BP response in the early stages of the post-exercise period may go some way as to explaining the higher post-exercise BF values. This increased BF response in the immediate post-exercise period may also provide a SS stimulus for the endothelium in the femoral artery to release vasodilatory metabolites (Pyke et al, 2008; Pohl et al, 1986). If FMD is also occurring downstream in the profunda femoral artery as previously hypothesised, it could be suggested that this would result in an increase in BF upstream in the common femoral artery (Segal & Kurjiaka, 1995), promoting further vasodilation to normalise the SS stimulus. It is therefore speculated that BF is increased in the post-exercise period due to the presence of a contraction mediated increased perfusion BF (until exercise BP subsides back to near baseline values), combined with a downstream FMD response in the profunda femoral artery that induces increased BF upstream in the common femoral artery.

These results also demonstrate that the magnitude of the change in BF became more pronounced as exercise intensity increased. The greater the exercise intensity, the greater the change in BF from contraction to post-exercise. This pattern of BF change could be attributed to a larger FMD stimulus downstream in the profunda femoral artery as exercise intensity increases. Although, it may be possible at higher exercise intensities intramuscular pressure and mechanical compression of the vasculature surrounding the quadricep muscles is greatly augmented as the number of muscle fibers recruited increases to meet the increasing demand of the exercise. This may then result in greater dilatory metabolite accumulation, so that when contraction is released these metabolites induce a large FMD response that elevates post-exercise BF. At higher exercise intensities, this possible post-exercise FMD response is more dominant than the input of contraction mediated increased perfusion BF on the post-exercise BF response, such that at higher exercise intensities the increased difference in BF from contraction to post-exercise becomes much more pronounced.

Conclusion

Whilst it is evident that the cardiovascular response and degree of muscular fatigue increases with isometric leg extension exercise intensity, it is also evident that isometric bilateral leg extension exercise of increasing intensity induces intensity dependent increases in common

femoral artery BF. Previous work suggests that this BF response is determined by the influence of central and local control mechanisms to increase perfusion pressure to overcome the mechanical pressure created during isometric contraction. Blood flow also increases in an exercise intensity dependent manner in the 5 minutes post-exercise period, and increases to values greater than that seen during isometric leg extension exercise. It is suggested that this is due to an elevated perfusion pressure overlapping from the contraction period that took time to subside, combined with the release of intramuscular pressure and mechanical occlusion downstream in the quadricep vasculature (profunda artery) that resulted in a possible FMD response that increased BF upstream in the common femoral artery. However as BF and FMD in the profunda artery were not directly measured in this study, this remains speculative.

It is plausible to suggest that the peak response of BF post-exercise at high intensities of isometric exercise may provide a physiological stimulus that if produced on several occasions during a bout of isometric exercise, may provide a stimulus for vascular adaptation that could lead to a reduction in RBP. Whilst this remains to be investigated in Chapter 6 of this research thesis, the specific characteristics of this increased BF response require further investigation before any definite conclusions can be drawn as to whether a haemodynamic stimulus could be considered as the physiological stimulus for RBP reductions following IET.

Chapter 4

Study 3: The Local Shear Rate Response During and Post Isometric Leg Exercise.

Chapter 4: The Local Shear Rate Response During and Post Isometric Leg Exercise.

4.1 Introduction

It is well established that increases in BF are associated with increases in SS (Ando & Kamiya, 1993; Pyke et al, 2008; Topper & Gimbrone, 1999). Shear stress is a characteristic measure of BF and is defined as "the frictional force of the blood against the endothelium" (Gonzales et al, 2009). It is proposed that increases in SS stimulate endothelial cells to release endothelial factors that cause endothelial dependent vasodilation (Pohl et al, 1986). As a result, the endothelium dilates and the SS is normalised (Dimmeler & Zeiher 2003; Koller & Kaley, 1990; Neibauer et al, 1996; Zarins et al, 1987). It has been suggested that repetitive bouts of increases in SS over time may improve endothelial function and induce arterial adaption (Tinken et al, 2010). It is hypothesised that this may be a mechanism for the RBP reductions commonly observed following an appropriate period of IET. Chapter 3 observed marked increases in BF during and post-isometric leg exercise. It is suggested that an elevated SS response would also be expected (Pyke et al, 2008). Therefore, this current study aims to identify and define the SS response to isometric leg exercise. This will address in part the wider research question regarding the physiological stimulus responsible for RBP reductions following IET.

Many studies have documented an increase in SR - a valid measure of SS, (Newcomer et al, 2008) from baseline during exercise, that is also exercise intensity dependent (Gonzales et al 2009; Tanaka et al, 2006; Wray et al 2005). The majority of these studies have utilised dynamic exercise. There is little research that has investigated the SS response to isometric exercise. In light of this, it is not surprising to find that it has not been established as to whether the SS response to isometric leg exercise is also exercise intensity dependent. Dynamic leg extension exercise has documented that SR increases linearly with workload (Wray et al, 2005) and can increase up to values of 300 s^{-1} in healthy males (Gonzales et al, 2009). As Pyke et al (2008) suggested, there is a clear relationship between BF and SS, in that increases in BF are accompanied by increases in SS. Thus it could be hypothesised that since isometric leg exercise induced intensity dependent increases in BF that were observed in Chapter 3, intensity dependent increases in SS may also be seen in this current study.

It is also feasible to propose that an elevated SS response would be expected post isometric leg exercise, particularly as BF was significantly elevated post-exercise in Chapter 3. In

relation to this, very few studies have explored the SS response after exercise, and those that have primarily focused on dynamic exercise. The common finding between these studies is that conduit arteries continue to be exposed to SS following an exercise bout (Johnson & Wallace, 2012; Padilla et al, 2008). Indeed Padilla et al (2008) found that brachial artery SS post dynamic walking exercise was greater than that seen after moderate and low intensity walking. Recent work by Johnson & Wallace (2012) found a greater post-exercise SR response in the brachial artery after high intensity running when compared to lower exercise intensities. Evidence from these studies suggests a possible exercise intensity dependent post-exercise SS response after dynamic exercise. However the post-exercise SS response to isometric leg exercise remains unknown.

The primary aim of this study is therefore to examine the SR response in the common femoral artery during and immediately following bilateral leg extension exercise. This study will utilise Doppler ultrasound to analyse real time BV and AD, thus allowing the SR response to be calculated using the methods of Padilla et al (2010), Newcomer et al (2008) & Thijssen et al (2009a). By utilising the DIIET (Wiles et al, 2008b), it will also be possible to determine if the SR response during and post-isometric leg extension exercise is exercise intensity dependent.

The secondary aim of this study will be to analyse the vasodilatory response of the conduit common femoral artery to an increased SR response (if present) during and post-isometric leg exercise. Shear stress is thought to aid exercise induced vasodilation through the release of vasodilatory substances from the vessel endothelium (Davies, 1995; Newcomer et al, 2011; Wray et al, 2005). The majority of studies in this area have explored the FMD response to dynamic exercise, whilst very few have studied isometric exercise. Padilla et al (2006) did examine the FMD response to static handgrip exercise of 10% MVC, and found no FMD response. In this case, it was suggested that the SS exerted on the endothelium was not sufficient enough to substantially stimulate the endothelium to generate a response. This would imply that there may be a minimum intensity threshold during isometric exercise to stimulate an endothelial FMD response. This study will explore this notion by establishing the vasodilatory response during and post-isometric leg exercise and whether any vasodilatory response observed is exercise intensity dependent.

Therefore the current study is the first if its kind to explore the SR response during and after a range of isometric leg extension exercise intensities, and to examine the SR response to

isometric exercise prescribed using constant EMG. It is suggested that an effective means of achieving the study aim is to replicate previous isometric bilateral leg extension exercise studies that have prescribed exercise intensity based upon $\%EMG_{peak}$ (Baross et al, 2012; Devereux et al, 2010b; Wiles et al, 2010). As already discussed in Chapter 3 of this thesis, Wiles et al (2010) suggests that using this method of exercise prescription makes it possible to maintain more precisely the level of the cardiovascular response (HR has been shown to plateau within 2 minutes when performing exercise to a set level of EMG), and therefore help to identify any physiological stimulus that may be responsible for BP reductions after IET. Thus, the acute SR response will be explored in this study to determine whether SS could be considered as the stimulus responsible for BP reductions after IET.

4.2 **Methodology**

4.2.1 Participants:

Please see page 88 of Chapter 3 for participant details.

4.2.2 Equipment and procedures:

- Isometric exercise

All isometric exercise was conducted using a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY). Full details of the procedure used to perform bilateral isometric leg extension exercise can be found on page 39 of the Methodology chapter (Chapter 2). Isometric exercise intensity was expressed relative to each individual's maximum intensity reached during the DIIET, and is therefore known as relative exercise intensity (REI).

- Maximal voluntary contraction (MVC) and EMG_{peak}

To establish each participants isometric exercise workload, MVC and EMG_{peak} were established. Full details of determining MVC and EMG_{peak} can be found on page 77 in Chapter 2, methodology).

- Discontinuous incremental isometric exercise test

Participants underwent the DIIET on 5 separate occasions (at least 48 hrs apart), performing double leg extension exercise. Sessions 1 and 2 were familiarisation sessions, whilst the remaining 3 sessions were used for data collection. Full details of the protocol used to perform the DIIET can be found on page 77 of the Methodology chapter (Chapter 2). Figure 16 on page 77 also provides a visual representation of the DIIET.

Measurements taken during the discontinuous incremental double leg isometric exercise test:

- Electromyography (EMG) recording

Electromyography was recorded for the purpose of prescribing isometric exercise intensity in the DIIET. EMG was recorded from the vastus lateralis muscle of both the left and right leg using a duel bio amplifier and 16 channel chart recording software (PowerLab, ADInstruments Ltd, Australia). Please refer to section 2.3.2 on page 41 of the methodology chapter (Chapter 2) for full details regarding the use of EMG recording.

For the purpose of data analysis, each stage of the DIIET performed to a set $\%EMG_{peak}$ target value was expressed relative to the maximum $\%EMG_{peak}$ stage that each participant achieved, known as percent relative exercise intensity (%REI). Expression of isometric exercise intensity in this manner allows a direct comparison of haemodynamic and cardiovascular variables between participants.

- *Shear stress / shear rate (s^{-1})*

Shear stress in conduit arteries can be estimated as:

Shear stress = blood viscosity x blood velocity / vessel diameter

(Pyke et al, 2005)

As blood viscosity was not directly measured in this study, SR (s^{-1}) was used as a surrogate measure (Pyke et al, 2005; Newcomer et al, 2008), and was estimated using the following equation:

Shear rate (SR) = 4*(blood velocity / vessel diameter)

(Newcomer et al, 2008)

In order for SR values to be calculated, it was necessary to record real time BV and AD during and immediately after a DIIET. A 8 MHz multifrequency wide band linear array probe (8L-RS, GE Healthcare, UK) and an ultrasound machine (LOGIQ e, GE Healthcare, UK) were used to image the common femoral artery of the left leg below the inguinal ligament, 2-3 cm above its bi-furcation into the superficial and profunda femoral branches (Radegran et al 1997; Baross et al, 2012). Once an optimal image was obtained, the probe was held constant and ultrasonic parameters were adjusted to enhance the longitudinal B-mode image of the lumen - arterial interface. Doppler velocity profiles of the common femoral artery were obtained throughout testing protocol using the LOGIQ e at an insonation angle of < 70 degrees. The LOGIQ e was used in duplex mode to continuously record BV and AD in 30 second blocks throughout exercise and rest. Exercise components consisted of four 30 second blocks as contraction time was 2 minutes, whereas post-exercise data was comprised of ten 30 second blocks as each rest period was 5 minutes in duration.

Blood velocity ($cm \cdot sec^{-1}$): page 90 of Chapter 3 details the protocol used to measure BV in this current study. Velocity mean (Vmean) values were then used to calculate SR.

Artery diameter (cm): Page 90 of Chapter 3 details the protocol used to measure AD in this current study. Artery diameter measurements were then used to calculate SR.

Shear rate for the last 10 seconds in each 30 second block of video during exercise and post-exercise was calculated from AD and Vmean using the equation documented above. Shear rate was then represented in the following ways to best demonstrate its response to isometric bilateral leg exercise:

1. Mean SR (s^{-1}). This is the average SR calculated from either the 2 minute contraction period (MSR), or the 5 minute post-exercise period (PE-MSR).
2. Peak SR (s^{-1}). This is the peak SR calculated from either the 2 minute contraction period (PSR), or the 5 minute post-exercise period (PE-PSR)
3. Change in SR (%). This is the % change in SR from exercise to post-exercise (ΔSR). The ΔSR is calculated from the last 10 seconds of the last 30 second block of exercise and the last 10 seconds of the first 30 second block during post-exercise.

- *Artery diameter response*

In order to establish the possible effects of SS on the endothelium and subsequent vasodilatory response, AD was recorded continuously in the common femoral artery during and post-exercise using the methods described on page 90 of Chapter 3. Artery diameter was then represented in the following ways to best demonstrate its response to isometric bilateral leg exercise:

1. Artery Diameter Mean (cm). This is the average AD measured either during a 2 minute contraction period (MAD), or during the 5 minute post-exercise rest period (PE-MAD).
2. Artery Diameter Peak (cm). This is the peak AD measured either during a 2 minute contraction period (PAD), or during the 5 minute post-exercise period (PE-PAD).
3. Change in Artery Diameter (%). This is the % change in AD from exercise to post-exercise (ΔAD). The ΔAD is calculated from the last 10 seconds of the last 30 second block of exercise and the last 10 seconds of the first 30 second block during post-exercise.

4.2.3 Data Analysis

All data was assessed for normality. If parametric assumptions were not met, data was logarithmically transformed (Field, 2000). Correlation coefficients with repeated observations were used to assess linear dependence between REI and the primary outcome variables for this study (MSR, PSR, PE-MSR, PE-PSR & ΔSR), and between REI and the secondary

outcome variables (MAD, PAD, PE-MAD, PE-PAD, ΔAD). An alpha level of $P < 0.05$ was set as the threshold for statistical significance across all statistical tests performed.

4.3 **Results**

4.3.1 Shear rate response

Of the 12 participants, it was not possible to obtain clear and accurate Vmin values in 2 participants throughout the DIIET. As a result, a Vmean that represented both systolic and diastolic velocity components in the cardiac cycle could not be established, and consequently a true mean SR that represented all stages of the cardiac cycle could not be calculated for these 2 participants. Results from the remaining 10 participants demonstrated that SR measured from the common femoral artery increased from baseline values of 12.1 ± 5.3 s^{-1} to peak values of up to 28.0 ± 10.1 s^{-1} during the DIIET; and up to values of 40.8 ± 5.9 s^{-1} in the 5 minute post-exercise period.

All SR variables met parametric assumptions. Statistically significant correlation coefficients with repeated observations results are revealed in Table 10. The relationship between REI and SR variables can also be viewed in Figures 23 – 25.

Table 10*. Correlation coefficient results for shear rate variables versus relative exercise intensity.*

Variable	r value	P value
MSR	0.726	< 0.01
PSR	0.668	< 0.01
PE-MSR	0.839	< 0.01
PE-PSR	0.868	< 0.01
ΔSR	0.511	< 0.01

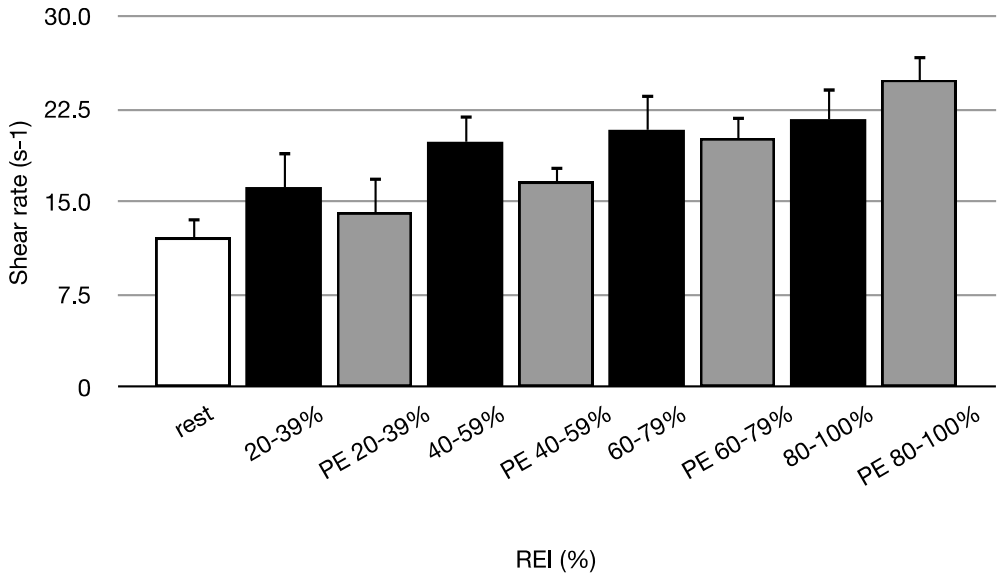

Figure 23 *Mean shear rate (s^{-1}) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting mean shear rate is represented by white bars, whilst MSR is represented by black bars, and PE-MSR is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and MSR (r = 0.726, P < 0.01) and PE-MSR (r = 0.839, P < 0.01).*

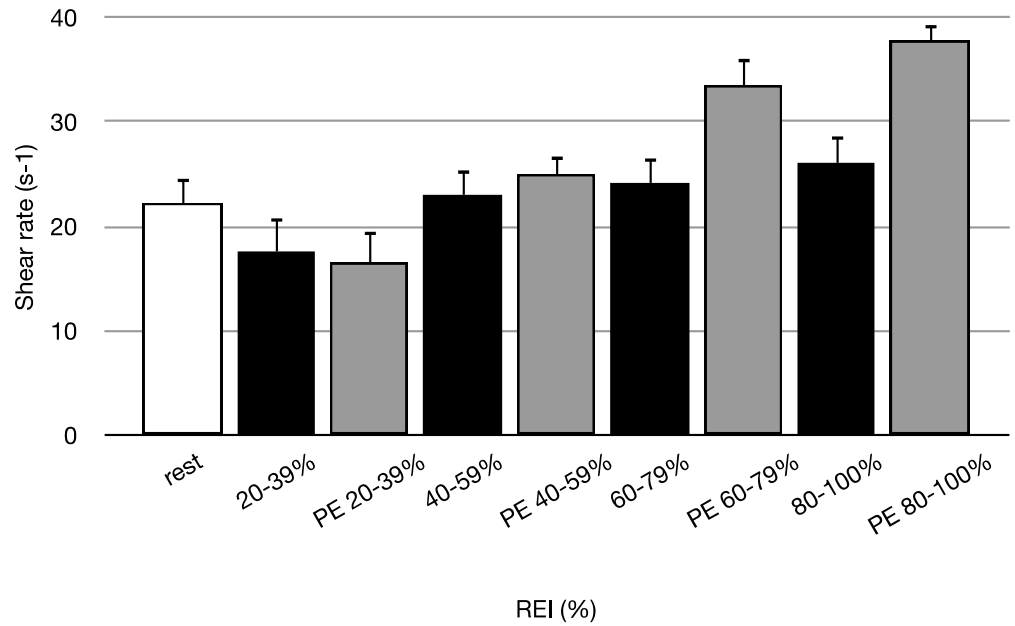

Figure 24 *Peak shear rate (s^{-1}) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting peak shear rate is represented by white bars, whilst PSR is represented by black bars, and PE-PSR is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and PSR (r = 0.668, P < 0.01) and PE-PSR (r = 0.868, P < 0.01).*

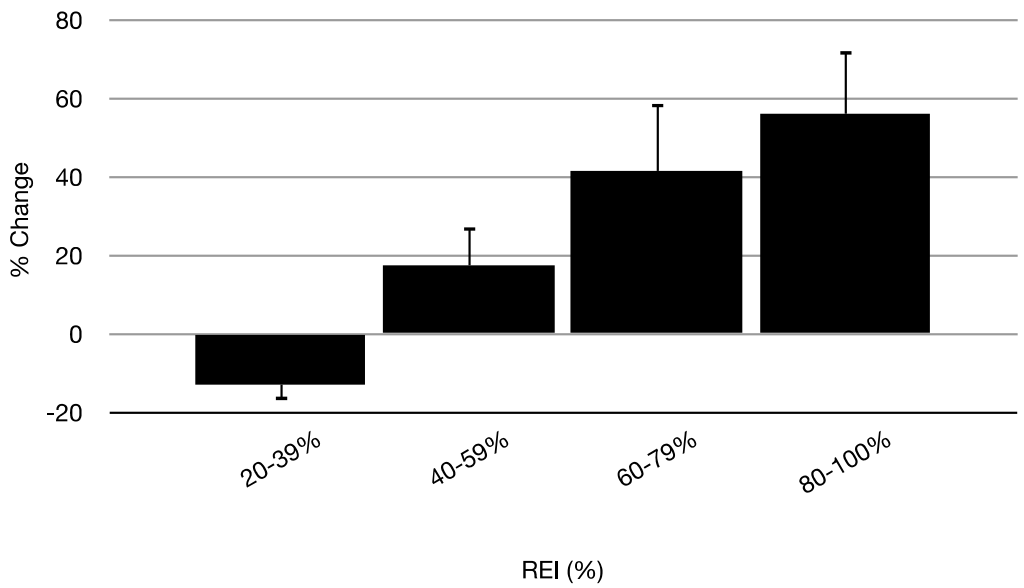

Figure 25 *Delta change in shear rate from contraction to post-exercise and standard error. Correlation co-efficient analysis revealed a significant relationship between REI (%) and ΔSR ($r = 0.511$, $P < 0.01$).*

4.3.2 Artery Diameter Response

Artery diameter variables met parametric assumptions. Correlation coefficients with repeated observations analysis revealed that MAD, PAD, PE-MAD, PE-PAD and ΔAD were not significantly correlated with REI (%) as $P > 0.05$. Figures 26, 27 and 28 documents MAD, PAD, PE-MAD, PE-PAD and ΔAD at each REI during the DIIET.

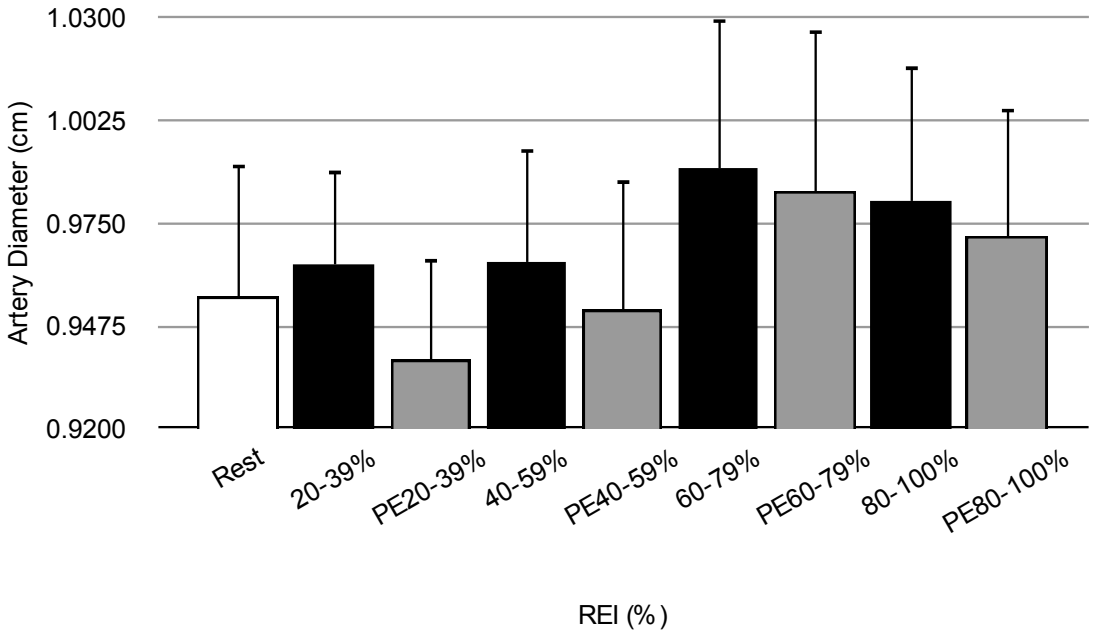

Figure 26 *Mean artery diameter (cm) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting mean artery diameter is represented by white bars, whilst MAD is represented by black bars, and PE-MAD is represented by grey bars. Correlation co-efficient analysis revealed no significant correlations between REI and MAD and PE-MAD as $P > 0.05$.*

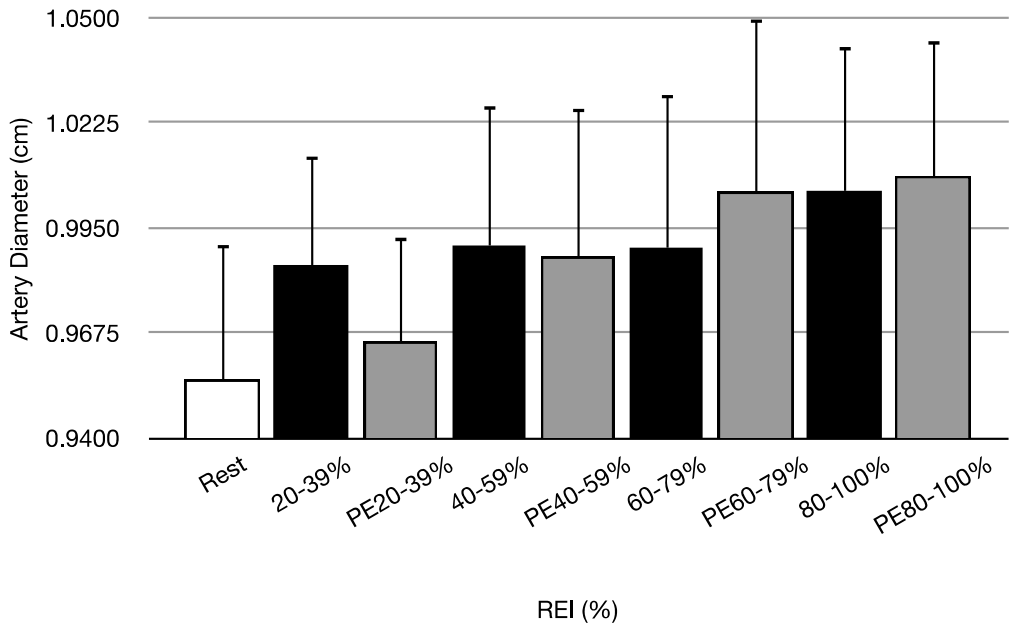

Figure 27 *Peak artery diameter (cm) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting peak artery diameter is represented by white bars, whilst PAD is represented by black bars, and PE-PAD is represented by grey bars. Correlation co-efficient analysis revealed no significant correlations between REI and PAD and PE-PAD as P > 0.05.*

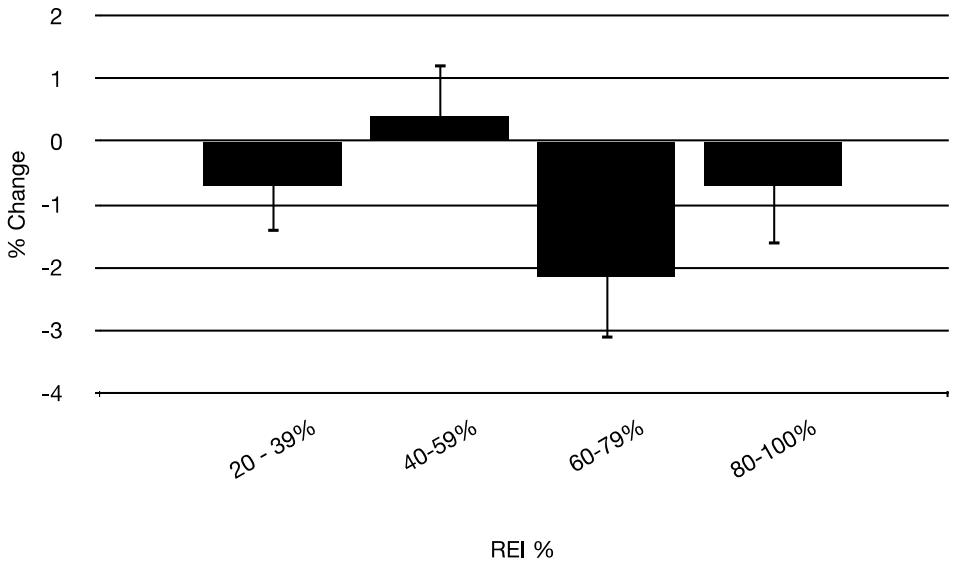

Figure 28 *Delta change in artery diameter from contraction to post-exercise and standard error. Correlation co-efficient analysis revealed no significant relationship between REI (%) and ΔAD (P > 0.05).*

4. 4 **Discussion**

The primary aim of this study was to determine the local SS response to sustained isometric bilateral leg exercise of graded exercise intensity. This study specifically focused on the SR response during and post-isometric leg extension contraction. This study was unique in that it was the first of its kind to examine the SR response to graded isometric leg exercise, both during and after exercise. The common femoral AD response during and after isometric leg extension exercise was also observed as a secondary measure. The results of this study demonstrate that SR measured in the common femoral artery during and after isometric bilateral leg extension exercise is largely influenced by increasing isometric exercise intensity. Despite marked increases in exercise and post-exercise SR at each REI, the common femoral AD response during and after exercise did not correlate with increasing isometric exercise intensity.

Shear stress during isometric bilateral leg extension exercise

For the purpose of this investigation SR was used as an estimation of SS (Pyke et al, 2005; Newcomer et al, 2008). Shear rate measured in the common femoral artery increased above resting levels during all exercise intensities, reaching a peak exercising value of $28.0 \pm 10.1 s^{1}$. Exercising SR values were highly correlated with REI for both MSR and PSR, suggesting a linear relationship between exercise intensity and common femoral artery SR during isometric bilateral leg extension exercise sustained for 2 minutes. Therefore the greater the intensity of the isometric leg exercise performed, the greater the exercising SS response in the local common femoral artery.

Whilst there is limited research that has explored the SS response to isometric exercise, other studies utilising dynamic exercise also document that exercising SS increases relative to exercise intensity (Gonzales et al, 2009; Tanaka et al, 2006; Thijssen et al, 2009a; Wray et al, 2005). Specifically in relation to dynamic leg extension exercise, both Gonzales et al (2009) and Wray et al (2005) documented peak exercising SR values of $> 200\ s^{-1}$ in the common femoral artery. In comparison, it is evident from the results of this study that isometric leg extension exercise induces much lower peak values of SR ($28.0 \pm 10.1\ s^{-1}$) than that seen during dynamic leg exercise.

Due to the fact that SS is a characteristic measure of BF (Gonzales et al, 2009), it is likely that increases in BF (as observed in Chapter 3) would be responsible for the increases in SR

observed in this study. As previously suggested, a combination of perfusion pressure, intramuscular pressure and the net balance between centrally mediated vasoconstrictor activity and local vasodilation may have mediated the exercise intensity dependent BF response to isometric leg exercise, which may consequently determine the exercise intensity dependent SR response seen in this study.

Gaenzer et al (2001) believed that exercise induced increases in BF causes endothelial SS that is sensed and transduced by the vessel endothelium, resulting in vasodilation being proportional to the increase in SS. This vasodilation response is thought to be mediated by the release of a number of SS induced substrates from the endothelium including NO, prostacyclin, endothelin, endothelial derived hyperpolarizing factor and adenosine (Joyner & Dietz, 1997; Koller & Kaley, 1990; Wilson & Kappor, 1993). Research studies have provided conflicting results with regards to identifying which vasodilatory substance released from the endothelium primarily mediate a conduit artery vasodilatory response. The most recent consensus is that when a SS stimulus is initiated there is a sequential recruitment of vasodilators, where one vasoactive compound may initially be recruited, but as the SS stimulus continues other vasoactive compounds may take over as the primary vasodilator (Clifford & Hellsten, 2004; Pyke et al, 2005). The results of the current study indicate that common femoral AD during isometric exercise was not dependent on the exercise intensity. This result seems rather surprising since SR responded in an exercise intensity dependent manner, and as Gaenzer et al (2001) suggested, SS mediates a vasodilation response that is proportional to increases in SS. It appears that in this study the increases in SS have not stimulated an acute vasodilatory response in the common femoral artery.

Whilst the common femoral artery is the main conduit vessel for blood delivery to the lower extremity vasculature during exercise (Wray et al, 2005), a number of previous studies indicate that this vessel does not dilate during exercise in response to a increased flow / shear stimulus. For example, Gonzales et al (2009), Radegran et al (1997), Radegran et al (2000) and Wray et al (2005) all observed little or no change in common femoral AD during dynamic leg extension exercise. This is in stark contrast to a very reactive brachial artery that demonstrates a well established relationship between a SS stimulus during exercise and a vasodilation response (Wray et al, 2005; Shoemaker et al, 1997). Thijssen et al (2008) explains that conduit artery size affects the FMD response, in that as arteries become smaller they possess more smooth muscle relative to elastic laminae and therefore have enlarged wall to lumen ratios when compared to larger vessels. Those vessels with larger ratios typically

exhibit a hyper-responsiveness to vasodilatory substances activated by a SS stimulus (Thijssen et al, 2008). This may provide an explanation as to why increases in SS in this current study were not related to increases in common femoral AD during exercise. Indeed evidence suggests the common femoral AD is large enough to have a relatively small wall to lumen ratio, therefore suggesting that the artery has a reduced responsiveness to shear stimuli and as a result does not dilate in an exercise intensity dependent manner (Radegran et al, 1997; Radegran et al, 2000; Wray et al, 2005).

There is also evidence to suggest that different types of shear pattern have a different impact on the endothelial release of the vasodilator NO. Green et al (2005) demonstrated that an oscillatory shear pattern in the brachial artery during leg exercise led to the release of NO from the endothelium, as opposed to forearm exercise that induced primarily an antegrade flow in the brachial artery that was not associated with release of NO from the endothelium. Despite the fact that the shear pattern in the common femoral artery during isometric leg extension exercise has not currently been established, it is possible that the leg extension exercise used in this study produced a shear pattern that did not result in the release of NO from the endothelium, and therefore would not be able to initiate a FMD response. Whilst this might provide an alternative explanation as to why the common femoral artery did not demonstrate vasodilation in response to exercise intensity dependent increases in SS, since the shear pattern was not directly assessed in this study, this remains speculative.

Finally, it could be suggested that the SR stimulus produced during isometric leg extension exercise used in this study, although elevated above baseline levels, was not sufficiently large enough to stimulate the release of vasodilatory substances from the endothelium. For example Wray et al (2005) demonstrated that a peak SR of ~300 s^{-1} only induced a peak dilatory response of ~0.05cm in the brachial artery during dynamic handgrip exercise. The peak SR measured in this study was just 28.0 ± 10.1 s^{-1}. It is also noteworthy that Wray et al (2005) recorded peak SR values of ~300 s^{-1} in the common femoral artery that failed to induce changes in AD, confirming that the common femoral artery is largely unresponsive to a SS stimulus.

In summary, it is apparent that bilateral isometric leg extension exercise induces increases in SS that are largely dependent upon isometric exercise intensity. As SS is a characteristic measure of BF (Gonzales et al, 2009), it is suggested that blood perfusion pressure, intramuscular pressure in the contracting muscle and the balance between centrally controlled

vasoconstrictor activity and locally controlled vasodilation determines exercising BF, which in turn determines the SS response. However the results obtained indicate that the increase in SS created during isometric leg extension exercise is not a sufficient stimulus to cause endothelial dependent dilation in the common femoral artery. Evidence suggests that this may be due to the large lumen diameter of the common femoral artery, which makes the artery endothelium unresponsive to shear.

Shear stress post isometric bilateral leg extension exercise

This study also established the SR response post-exercise to increasing intensities of bilateral isometric leg exercise. Post-exercise SR measured in the common femoral artery increased above resting levels during all exercise intensities, reaching a peak value of 40.8 ± 5.9 s^{-1}. Post-exercise SR was highly correlated with REI for both PE-MSR and PE-PSR, thus demonstrating a linear relationship between isometric bilateral leg exercise intensity sustained for 2 minutes and post-isometric exercise SR. Post-exercise common femoral AD, expressed as PE-MAD or PE-PAD once again was not significantly related to REI.

No study has directly explored the post-exercise SR response after an acute bout of isometric exercise, however Padilla et al (2008) and Johnson & Wallace (2012) both reported marked increases in post-exercise SR following dynamic exercise. These observed increases in post-exercise SR were also exercise intensity dependent, in that the higher the exercise intensity the greater the post-exercise SR response. For example, Padilla et al (2008) found immediately after a bout of walking exercise, the highest SS values were recorded after high intensity walking (~11 dynes / cm^2), compared to medium and low intensity walking (~9.5 dynes/cm^2; ~8.5 dynes/cm^2 respectively). Similarly Johnson & Wallace (2012) recorded peak mean post-exercise SR values of ~70 s^{-1} in the brachial artery after high intensity running exercise, as opposed to moderate intensity running exercise where peak mean post-exercise SR values reached ~50 s^{-1} in the brachial artery. Whilst the current study is unique in that it is the first study of its kind to examine the post-exercise SR response to incremental isometric leg exercise, it is apparent that isometric leg exercise demonstrates a similar post-exercise intensity dependent SR response to that seen after dynamic exercise, despite the relatively lower peak SR values.

It is likely that the increases in post-exercise SS are attributed to an elevated BF after isometric exercise. As Chapter 3 demonstrated, post-exercise BF also responded in an

intensity dependent manner, which again would determine the exercise intensity response of post-exercise SS. As was suggested in Chapter 3, a combination of the magnitude of the O_2 debt and consequent metabolite accumulation during exercise (Taylor et al, 1988), along with a possible downstream increase in BF upon mechanical release of the contraction may have increased the upstream post-exercise BF induced SS response. Please refer to Chapter 3, p106 for greater explanation surrounding the post-exercise BF response.

It is clear from the results of this study that the magnitude of post-exercise SR did not influence the common artery to vasodilate in an intensity dependent manner. This again reiterates the lack of responsiveness of the common femoral artery to a SS stimulus. Wray et al (2005) observed a dilatory response in the smaller downstream profunda branch of the femoral artery to a SS stimulus. In Chapter 3 it was suggested that it was likely that the downstream profunda artery (which directly supplies the quadricep with blood) was likely to be largely affected by mechanical compression from isometric contraction, and in combination with an increase in intramuscular pressure, may lead to a possible reduction of BF and subsequent increased metabolite accumulation. When the contraction was released, increased BF as a result of the release of mechanical hindrance to flow combined with the large influence of vasodilatory metabolites (which may have induced a FMD response) would have markedly increased BF and SS at this downstream level. It is evident that this response is dependent upon the intensity of the isometric contraction. Segal & Kurjiaka (1995) suggested that downstream increases in BF may also lead to increases in BF further upstream. This response may therefore also induce increases in SS in the upstream common femoral artery as exercise intensity increases at each new contraction within the DIIET. However as the common femoral artery appears to be unresponsive to a SS stimulus, it is unlikely that the increases observed in SS upstream as exercise intensity increases would induce an intensity graded vasodilatory response in the common femoral artery.

In summary, it is apparent that SS remains elevated after bilateral leg extension exercise in the post-exercise rest periods. It is suggested that this is a result of an elevated post-exercise BF due to an overlapping of high perfusion BF from exercising periods combined with a strong increase in downstream BF upon the release of mechanical pressure at the cessation of isometric contraction. Furthermore, post-exercise SR follows the same pattern as post-exercise BF, in that the post-exercise SR response is exercise intensity dependent. However, similar to those results observed during exercise, the increased post-exercise SS response does

not stimulate endothelium dependent vasodilation in the common femoral artery. This reiterates the inability of this artery to respond to increased shear.

Change in shear stress from exercise to rest.

The ΔSR from the end of contraction to the post-exercise period was also assessed at each exercise intensity throughout the DIIET. Results demonstrated a positive relationship between REI and ΔSR. This indicates that as isometric exercise intensity increases, SR after exercise is greater than the SR during contraction. Furthermore, it is also apparent that the difference in SR from contraction to post-exercise becomes augmented as exercise intensity increases.

It is not possible to compare the ΔSR data from the current study with change data from other studies since this study appears to be the first of its kind to study the SR change from exercise to post-exercise. In comparison to the BF response in Chapter 3, BF data also demonstrated a similar relationship, in that increases in exercise intensity induced a greater post-exercise BF response whilst the percentage change in BF increased relative to exercise intensity. Therefore, since SS is a characteristic measure of BF (Gonzales et al, 2009), it would be expected that the response of SS in this study would follow a similar pattern of that seen in the BF response to isometric leg exercise. It is plausible to suggest that the determinants of BF would also influence the SS response to isometric exercise. It is apparent from Chapter 3 that the observed relationship between BF change and REI was possibly due to an exercise intensity dependent large flow stimulus as a result of the release of mechanically occluded contraction BF that was fueled by increased perfusion pressure, combined with post-exercise downstream FMD that increased upstream BF (Segal & Kurjiaka, 1995). It could be suggested that these same mechanisms also influenced the magnitude of the change in SS response to isometric leg exercise.

Conclusion

It is apparent that isometric bilateral leg extension exercise of increasing intensity induces intensity dependent increases in common femoral artery SR. This observed increase in SR is dependent upon the BF perfusion response to isometric bilateral leg exercise (seen in Chapter 3). Shear rate also increases in an intensity dependent manner in the post-exercise rest periods after isometric contraction. Again, these exercise intensity dependent increases in BF post isometric exercise are assumed to be responsible for the exercise intensity dependent increases in post-exercise SR observed. Despite increases in SR both during exercise and in

the immediate time periods after, common femoral AD did not respond in an exercise intensity dependent manner, thus suggesting that this magnitude of SR may not be an appropriate stimulus to induce endothelium dependent dilation in the common femoral artery.

The results of this study do confirm that SR is elevated during and after acute isometric leg exercise. Specifically, it is apparent from correlation coefficient analysis that despite relatively small values, peak post-exercise SR could be considered as a possible physiological stimulus for RBP reductions after IET. Thus it would be plausible to suggest that over a training bout of isometric leg extension exercise, the endothelium of the common femoral artery would be continuously exposed to a increased SS stimulus immediately following exercise bouts. Whilst it appears that the endothelium in the common femoral artery does not respond in an acute fashion to an increased shear stimulus, the release of vasodilatory substances from the endothelium was not directly assessed in this study. As such the release of vasodilatory substances cannot be ruled out in response to the increase in SS, even though the common femoral artery remained unresponsive to these substances and failed to dilate. The repetitive activation of this pathway over time (i.e. in training) could potentially still lead to vascular adaption. This remains to be investigated later in this thesis. Furthermore, vasodilatory substances could have been carried downstream and influenced the profunda femoral artery to induce an endothelial dependent vasodilatory response. Wray et al (2005) has previously demonstrated the responsiveness of the profunda femoral artery to dilate in response to a SS stimulus. It may be that the increases in SS, specifically that observed immediately after exercise, have the potential to induce vascular adaptation further downstream that may lead to RBP reduction if this exercise was to be repeated in a training bout. The exact characteristics of the SS response must firstly be examined before any definitive conclusions can be established.

Chapter 5

Study 4: The Local Shear Pattern Response During and After Isometric Leg Exercise.

Chapter 5: The Local Shear Pattern Response During and After Isometric Leg Exercise

5.1 Introduction

It is apparent from Chapters 3 and 4 that increases in BF during and after isometric leg exercise is associated with increases in SS. In relation to RBP, a consistent low SS stimulus promotes a proatherogenic state of the endothelium (Chappell et al, 1998), which is associated with hypertension (Malek et al 1999). In contrast, a pulsatile SS (within a physiological range >15 dynes.cm^2) may encourage the release of vasodilatory substances (such as NO and prostacyclin) to regulate vascular tone (Gonzales et al, 2008; Green et al, 2005). Theoretically the outcome of this would be a reduction in RBP.

It is widely accepted that the characteristics of an increased SS stimulus (also known as shear pattern) define any modifications to the function and structure of the vascular endothelium (Green, 2009; Johnson & Wallace, 2012). The 3 SS characteristics to be investigated in the current study are antegrade shear (ASR), retrograde shear (RSR) and oscillatory shear (OSI). Antegrade shear is SS travelling in a forward direction though the vasculature, whilst RSR is SS travelling in a reverse direction through the vasculature (Gonzales et al, 2008). Oscillatory SS relates to the pattern of fluctuations between ASR and RSR (Gonzales et al, 2008). As such, the OSI can be used to define the degree of oscillation. The values of OSI range from 0 - 0.5, where a value of 0.0 relates to linear SR (little/no fluctuations between ASR and RSR components) through the cardiac cycle, whilst a value of 0.5 corresponds to a SR characterised by large oscillations (significant fluctuations between ASR and RSR components) throughout the cardiac cycle (Padilla et al, 2010).

Haliwill & Minson (2010) suggested that the origin of ASR and RSR could be explained by the Starling Resistor theory. This theory assumes a downstream resistance (in a blood vessel) has a critical pressure which decreases under vasodilatory influence and increases under vasoconstrictor influence. If arterial blood upstream is flowing at a pressure above this critical pressure, then the BF will overcome this downstream resistance and flow will travel in a forward (antegrade) direction through the vasculature. In contrast, if upstream BF is at a lower pressure to the critical pressure created by the downstream resistor, upstream BF will not be able to overcome this resistance, and as a result will travel backwards (retrogradely), (Haliwill & Minson, 2010).

Shear stress specifically characterised by high levels of RSR is thought to: increase endothelin-1 expression (Ziegler et al 1998); increase adhesion molecules (Chappell et al, 1998; Himberg et al 2007); enhance the release of superoxide (McNally et al 2003) and expression of reactive oxygen species producing enzymes (NADPH oxidase) (De Keulenaer et al 1998; Hwang et al 2003); decrease endothelial NO synthase expression (De Keulenaer et al 1998; Hwang et al 2003); and can decrease FMD in healthy subjects (Thijssen et al 2009a). Thus a shear pattern specifically characterised by predominately high levels of RSR is normally associated with adverse vascular responses / adaptations. Indeed, Tinken et al (2009) established that augmented RSR during handgrip exercise reduced endothelial function in a dose dependent manner, whilst Thijssen et al (2009a) induced a dose dependent increase in RSR by using cuff inflation, and again found FMD was impaired in a dose dependent manner. In contrast ASR appears to significantly influence FMD (Tinken et al, 2009), and is associated with improved vascular response / adaptation. However, Green et al (2005) found that increases in BF during handgrip exercise that was predominately characterised by ASR (with very little RSR influence) were not NO mediated, and therefore unlikely to induce a NO mediated vascular response /adaptation. Green et al (2005) implied that indeed a SS stimulus that is characterised by both ASR and RSR, and therefore oscillatory in nature may present a greater stimulus for NO mediated vascular adaptation, as a repetitive drawing of flow across the surface of the endothelium may increase the upregulation of NO. Thus in the context of the wider research aims of this thesis, it may be speculated an OSI pattern characterised by both ASR and RSR during isometric leg exercise may provide a physiological stimulus for vascular adaptation and subsequent RBP reductions following IET.

At this point in time however, there is limited research exploring the shear pattern to sustained isometric contractions. Whilst Chapter 4 of this research thesis established that there is a SS response to isometric leg exercise that is exercise intensity dependent, it is also necessary to identify the exact shear pattern during isometric leg exercise in order to inform the wider research aims of this thesis. Therefore the first aim of this study is to elucidate the shear pattern to isometric leg extension exercise of increasing exercise intensity.

The second aim of this study is to define the shear pattern in the immediate rest periods after isometric leg exercise of increasing intensity. Whilst no previous study has directly examined the post-exercise shear pattern after isometric exercise, one study has established the post-exercise shear pattern after high intensity running. Johnson & Wallace (2012) established that ASR peaked in the immediate period after exercise, with the highest values observed after the greatest intensity of running. In contrast, RSR and the OSI were lowest in the immediate

period after exercise. This provides clear evidence that a shear pattern stimulus may continue even after exercise is finished. In relation to the wider research aims of this thesis it is important to define the shear pattern response immediately following isometric leg exercise.

This study will use a DIIET to establish the shear pattern in the common femoral artery during and post-isometric leg extension exercise of increasing exercise intensity. Ultrasound Doppler techniques will be utilised to measure and calculate the shear pattern response during and after this method of exercise.

5.2 Methodology

5.2.1 Participants

Please see page 88 of Chapter 3 for participant details.

5.2.2 Equipment and procedures:

- *Isometric exercise*

All isometric exercise was conducted using a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY). Full details of the procedure used to perform bilateral isometric leg extension exercise can be found on page 39 of the Methodology chapter (Chapter 2). Isometric exercise intensity was expressed relative to each individual's maximum intensity reached during the DIIET, and is therefore known as relative exercise intensity (REI).

- *Maximal voluntary contraction (MVC) and EMG_{peak}*

Full details of determining MVC and EMG_{peak} can be found on page 77 in the Methodology chapter (Chapter 2).

- *Discontinuous incremental isometric exercise test*

Participants underwent the DIIET on 5 separate occasions (at least 48 hrs apart), performing double leg extension exercise. Sessions 1 and 2 were familiarisation sessions, whilst the remaining 3 sessions were used for data collection Full details of the protocol used to perform the DIIET can be found on page 77 of the Methodology chapter (Chapter 2). Figure 16 on page 77 also provides a visual representation of the DIIET.

Measurements taken during the discontinuous incremental isometric exercise test:

- *Electromyography (EMG) recording*

EMG was recorded for the purpose of prescribing isometric exercise intensity in the DIIET. EMG was recorded from the vastus lateralis muscle of both the left and right leg using a duel bio amplifier and 16 channel chart recording software (PowerLab, ADInstruments Ltd, Australia). Please refer to section 2.3.2 on page 41 of the methodology chapter (Chapter 2) for full details regarding the use of EMG recording.

For the purpose of data analysis, each stage of the DIEET performed to a set $\%EMG_{peak}$ target value was expressed relative to the maximum $\%EMG_{peak}$ stage that each participant achieved, known as percent relative exercise intensity (%REI). Expression of isometric exercise intensity in this manner allows a direct comparison of haemodynamic and cardiovascular variables between participants.

- *Antegrade and retrograde shear stress / shear rate (s^{-1})*

As blood viscosity was not directly measured in this study, SR (s^{-1}) was used as a surrogate measure of SS (Pyke et al, 2005; Newcomer et al, 2008). Antegrade shear rate and RSR were determined from the left leg common femoral artery during and post a DIIET. Antegrade shear represents forward flow, whilst RSR results from a reversal of flow (Young et al, 2010). Therefore, ASR and RSR were was estimated using the following equation:

Antegrade shear rate (ASR) = 4*(Vmax / vessel diameter)
Retrograde shear rate (RSR) = 4*(Vmin / vessel diameter)
(Padilla et al, 2010)

In order for ASR & RSR values to be calculated, it was necessary to record real time BV and AD during and immediately after a DIIET. A 8 MHz multi-frequency wide band linear array probe (8L-RS, GE Healthcare, UK) and a ultrasound machine (LOGIQ e, GE Healthcare, UK) were used to image the common femoral artery of the left leg below the inguinal ligament, 2-3 cm above its bi-furcation into the superficial and profunda femoral branches (Radegran et al 1997; Baross et al, 2012). Once an optimal image was obtained, the probe was held constant and ultrasonic parameters were adjusted to enhance the longitudinal B-mode image of the lumen - arterial interface. Doppler velocity profiles of the common femoral artery were obtained throughout testing protocol using the LOGIQ e at an insonation angle of < 70 degrees. The LOGIQ e was used in duplex mode to continuously record BV and AD in 30 second blocks throughout exercise and rest. Exercise components consisted of four 30 second blocks as contraction time was 2 minutes, where as post-exercise data was comprised of ten 30 second blocks as each rest period was 5 minutes in duration.

Blood Velocity ($cm \cdot sec^{-1}$): Page 90 of Chapter 3 details the protocol used to measure BV in this current study. Peak systolic antegrade blood velocity (Vmax) (defined as the highest velocity measured in Doppler spectrum of a cardiac cycle, (Thijssen et al, 2009a) and peak retrograde blood velocity (Vmin) (defined as the lowest velocity in the Doppler spectrum of a

cardiac cycle, (Thijssen et al 2009a) were analysed from the velocity spectrum using the in-built analysis software of the LOGIQ e ultrasound. Peak systolic antegrade velocity and Vmin values from each cardiac cycle in a block were then averaged to give a single Vmax and Vmin value for the last 10 seconds in each 30 sec video block. Peak systolic antegrade velocity and Vmin values were then used to calculate ASR and RSR.

Artery Diameter (cm): Page 90 of Chapter 3 details the protocol used to measure AD in this current study. Artery diameter measurements were then used to calculate ASR and RSR.

Antegrade shear rate and RSR for the last 10 seconds in each 30 second block of video during exercise and post-exercise were calculated from AD and Vmax or Vmin using the equations documented on page 137.

Antegrade shear rate was then represented in the following ways to best demonstrate its response to isometric bilateral leg exercise:

1. Mean ASR (s^{-1}). This is the average ASR calculated from either the 2 minute contraction period (MASR), or the 5 minute post-exercise period (PE-MASR).
2. Peak ASR (s^{-1}). This is the peak ASR calculated from either the 2 minute contraction period (PASR), or the 5 minute post-exercise period (PE-PASR)
3. Change in ASR (%). This is the % change in ASR from exercise to post-exercise (ΔASR). The ΔASR is calculated from the last 10 seconds of the last 30 second block of exercise and the last 10 seconds of the first 30 second block during post-exercise.

Retrograde shear rate was then represented in the following ways to best demonstrate its response to isometric bilateral leg exercise:

1. Mean RSR (s^{-1}). This is the average RSR calculated from either the 2 minute contraction period (MRSR), or the 5 minute post-exercise period (PE-MRSR).
2. Peak RSR (s^{-1}). This is the peak RSR calculated from either the 2 minute contraction period (PRSR), or the 5 minute post-exercise period (PE-PRSR)
3. Change in RSR (%). This is the % change in RSR from exercise to post-exercise (ΔRSR). The ΔRSR is calculated from the last 10 seconds of the last 30 second block of exercise and the last 10 seconds of the first 30 second block during post-exercise.

- *Oscillatory Shear Index*

Oscillatory shear index in the common femoral artery was also determined during the DIIET. The values of OSI range from 0 - 0.5, where a value of 0.0 relates to unidirectional SR through the cardiac cycle, whilst a value of 0.5 corresponds to an oscillational SR through a cardiac cycle (Padilla et al, 2010). Oscillatory shear index was calculated using the following equation:

OSI = ((retrograde shear rate / antegrade shear rate) + retrograde shear rate)

(Padilla et al, 2010)

Oscillatory shear index was calculated for the last 10 seconds of each 30 second block throughout exercise and resting periods during the DIIET using ASR and RSR values. Oscillatory shear index was then represented in the following ways to best demonstrate its response to isometric bilateral leg exercise:

1. Mean OSI. This is the average OSI measured either during a 2 minute contraction period (MOSI), or during the 5 minute post-exercise rest period (PE-MOSI).
2. Change in OSI (%). This is the % change in OSI from exercise to post-exercise (ΔOSI). The ΔOSI is calculated from the last 10 seconds of the last 30 second block of exercise and the last 10 seconds of the first 30 second block during post-exercise.

5.2.3 Data Analysis:

All data was assessed for normality. If parametric assumptions were not met, data was logarithmically transformed (Field, 2000). Correlation coefficients with repeated observations were used to assess linear dependence between REI and the shear pattern study variables (MASR, PASR, PE-MASR, PE-PASR & ΔASR; MRSR, PRSR, PE-MRSR, PE-PRSR & ΔRSR; MOSI, PE-MOSI & ΔOSI). An alpha level of $P < 0.05$ was set as the threshold for statistical significance across all statistical tests performed.

5.3 Results

5.3.1 Antegrade Shear Rate Response

Results demonstrated that ASR measured in the common femoral artery increased from baseline values of 37.3 ± 10.4 s^{-1} to peak values of up to 72.6 ± 16.6 s^{-1} during the DIIET, and up to values of 98.4 ± 13.6 s^{-1} in the 5 minute post-exercise period.

Antegrade shear rate variables met parametric assumptions. Statistically significant correlation coefficient with repeated observation results are revealed in table 11. The relationship between REI and ASR variables can also be viewed in Figures 29 – 31.

Table 11. *Correlation coefficient results for antegrade shear rate variables versus relative exercise intensity.*

Variable	r value	P value
MASR	0.859	< 0.01
PASR	0.893	< 0.01
PE-MASR	0.895	< 0.01
PE-PASR	0.899	< 0.01
ΔASR	0.640	< 0.01

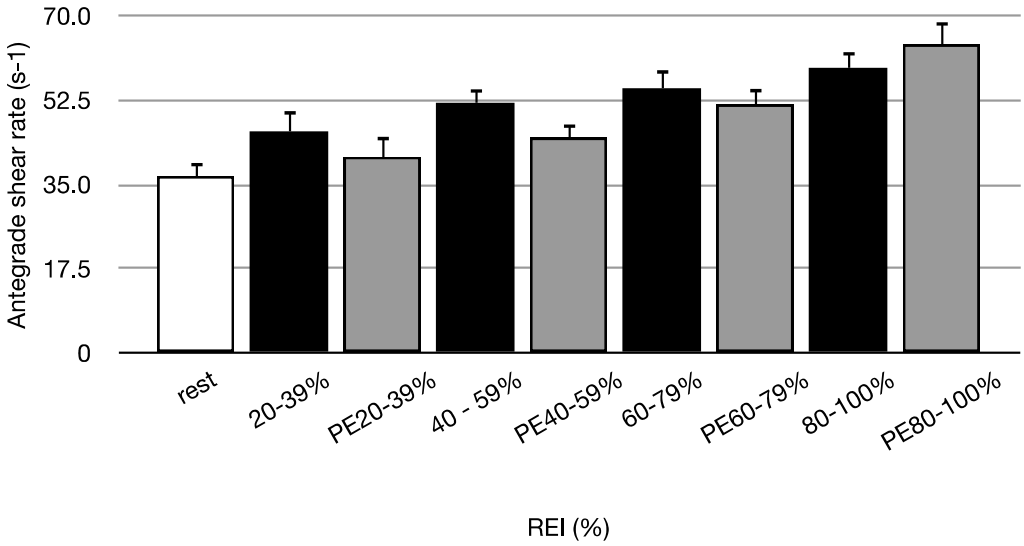

Figure 29. *Mean antegrade shear rate (s^{-1}) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting mean antegrade shear rate is represented by white bars, whilst MASR is represented by black bars, and PE-MASR is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and MASR and PE-MASR (r = 0.859, P <0.01; r = 0.895, P<0.01).*

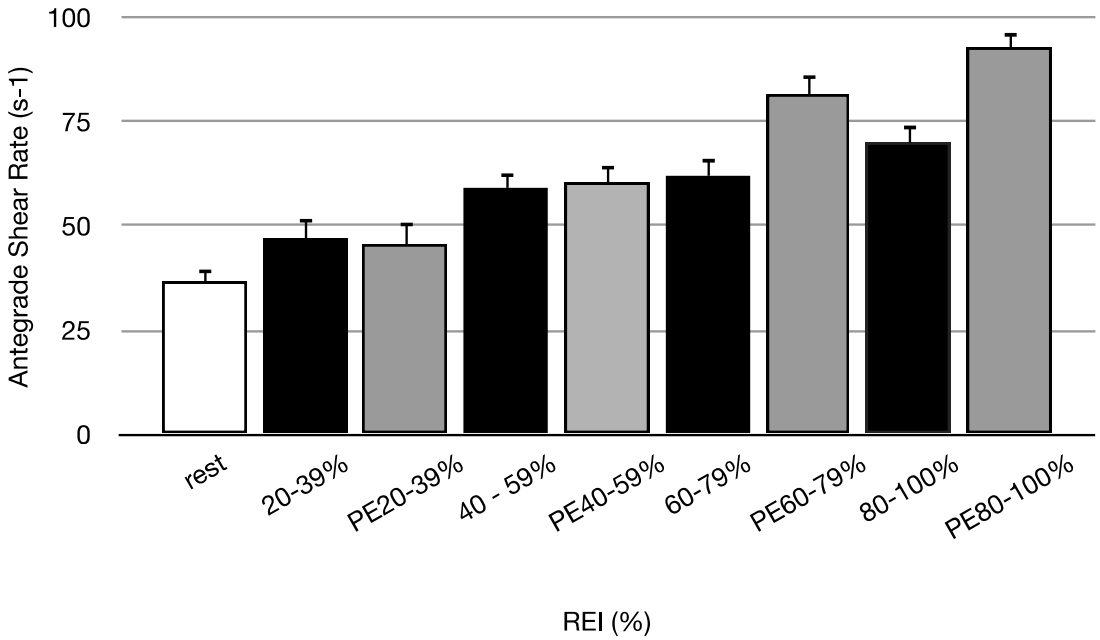

Figure 30. *Peak antegrade shear rate (s^{-1}) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting antegrade shear rate is represented by white bars, whilst PASR is represented by black bars, and PE-PASR is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and PASR and PE-PASR (r = 0.859, P <0.01; r = 0.895, P<0.01).*

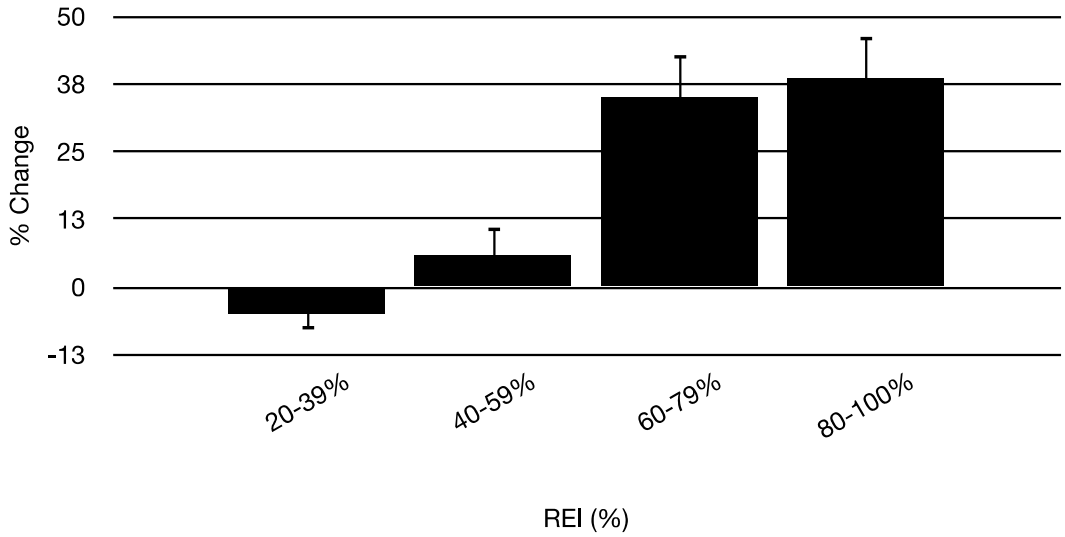

Figure 31 *Delta change in antegrade shear rate from contraction to post-exercise and standard error. Correlation co-efficient analysis revealed a significant relationship between REI (%) and ΔASR ($r = 0.640$, $P < 0.01$).*

5.3.2 Retrograde shear rate response

It was not possible to obtain clear and accurate Vmin values in two participants throughout the DIIET. As a result, RSR could not be calculated for these 2 participants. Results from the remaining 10 participants demonstrated that RSR measured from the common femoral artery increased from baseline values of -13.1 ± 6.2 s^{-1} to peak values of up to -21.5 ± 11.0 s^{-1} during the DIIET, and up to values of -19.3 ± 5.7 s^{-1} in the five minute post-exercise period.

All RSR variables met parametric assumptions. Statistically significant correlation coefficients with repeated observations results are revealed in table 12. The relationship between REI and RSR variables can also be viewed in Figures 32 – 34.

Table 12. *Correlation coefficients for retrograde shear rate variables versus relative exercise intensity.*

Variable	r value	P value
MRSR	-0.546	< 0.01
PRSR	-0.527	< 0.01
PE-MRSR	-0.427	< 0.01
PE- PRSR	-0.647	< 0.01

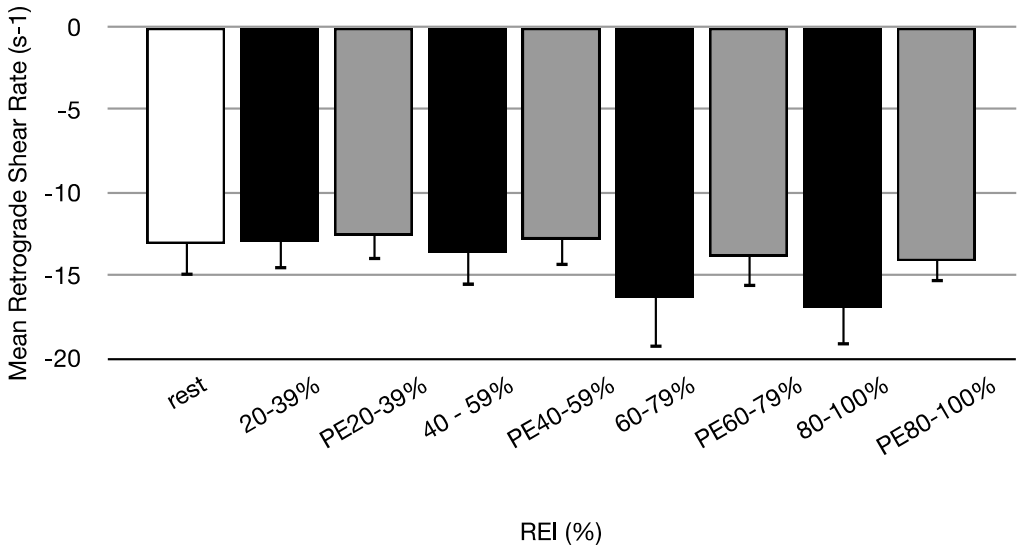

Figure 32 *Mean retrograde shear rate (s^{-1}) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting mean retrograde shear rate is represented by white bars, whilst MRSR is represented by black bars, and PE-MRSR is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and MRSR and PE-MRSR (r = -0.546, P <0.01; r = -0.527, P<0.01).*

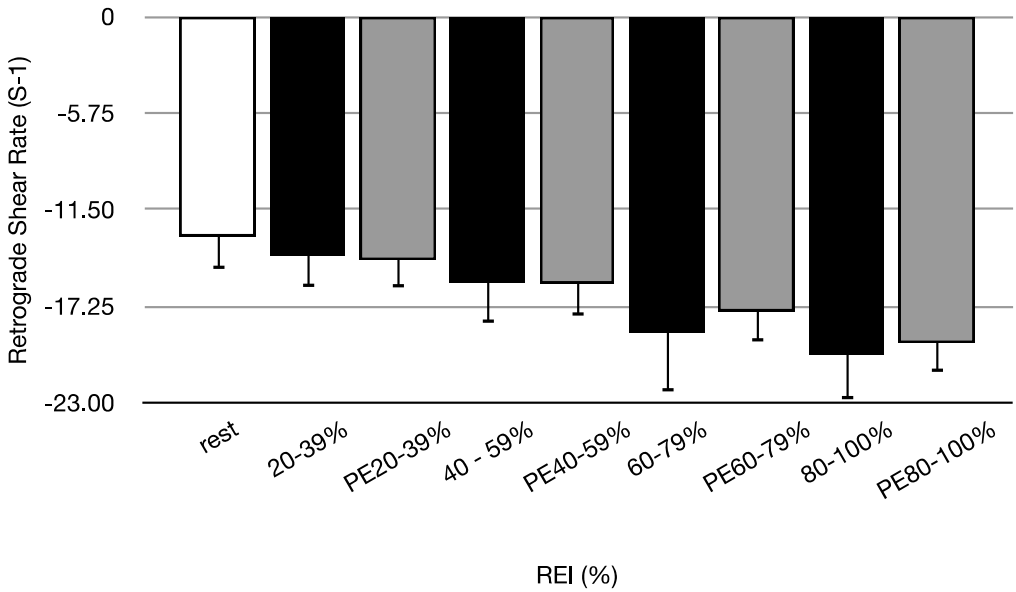

Figure 33 *Peak retrograde shear rate (s^{-1}) and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting retrograde shear rate is represented by white bars, whilst PRSR is represented by black bars, and PE-PRSR is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and PRSR and PE-PRSR (r = -0.427, P <0.01; r = -0.647, P<0.01).*

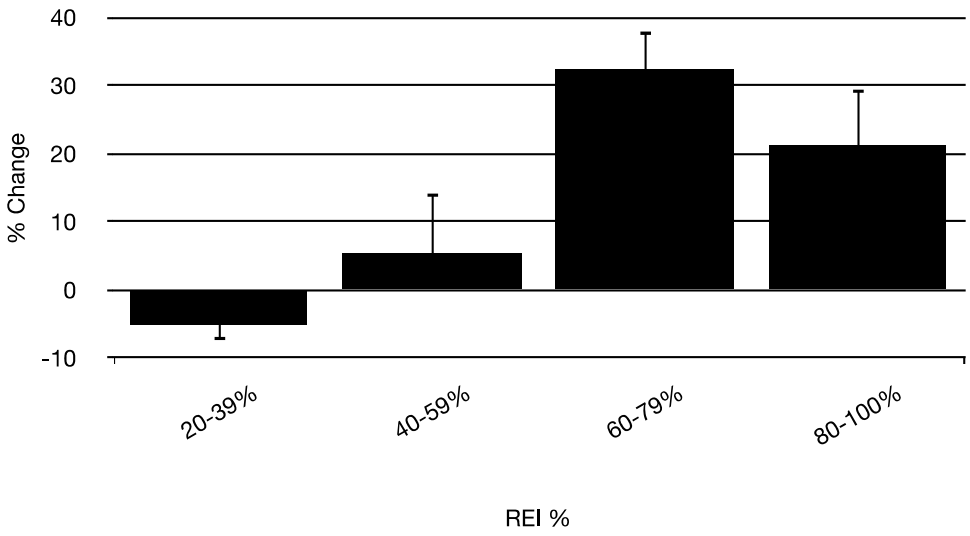

Figure 34 *Delta change in retrograde shear rate from contraction to post-exercise and standard error. Correlation co-efficient analysis revealed no significant relationship between REI (%) and ΔRSR as P > 0.05.*

5.3.3 Oscillatory shear index response

It was not possible to obtain clear and accurate Vmin values for two participants from the BV spectrum traces throughout the DIIET. As a result, RSR, and consequently OSI could not be calculated for these 2 participants. Results from the remaining 10 participants demonstrated that OSI measured from the common femoral artery decreased from baseline values of 0.26 ± 0.09 to values of 0.23 ± 0.13 during the DIIET, and up to values of 0.20 ± 0.06 in the 5 minute post-exercise period, thus demonstrating a more laminar oscillatory shear response as exercise intensity increased.

Oscillatory shear index variables met parametric assumptions. Statistically significant correlation coefficient with repeated observations results are revealed in table 13. Figures 35 & 36 also demonstrate the relationship between REI and OSI.

Table 13 *Correlation coefficient results for oscillatory shear index variables versus relative exercise intensity.*

Variable	r value	P value
PE - MOSI	-0.404	< 0.05

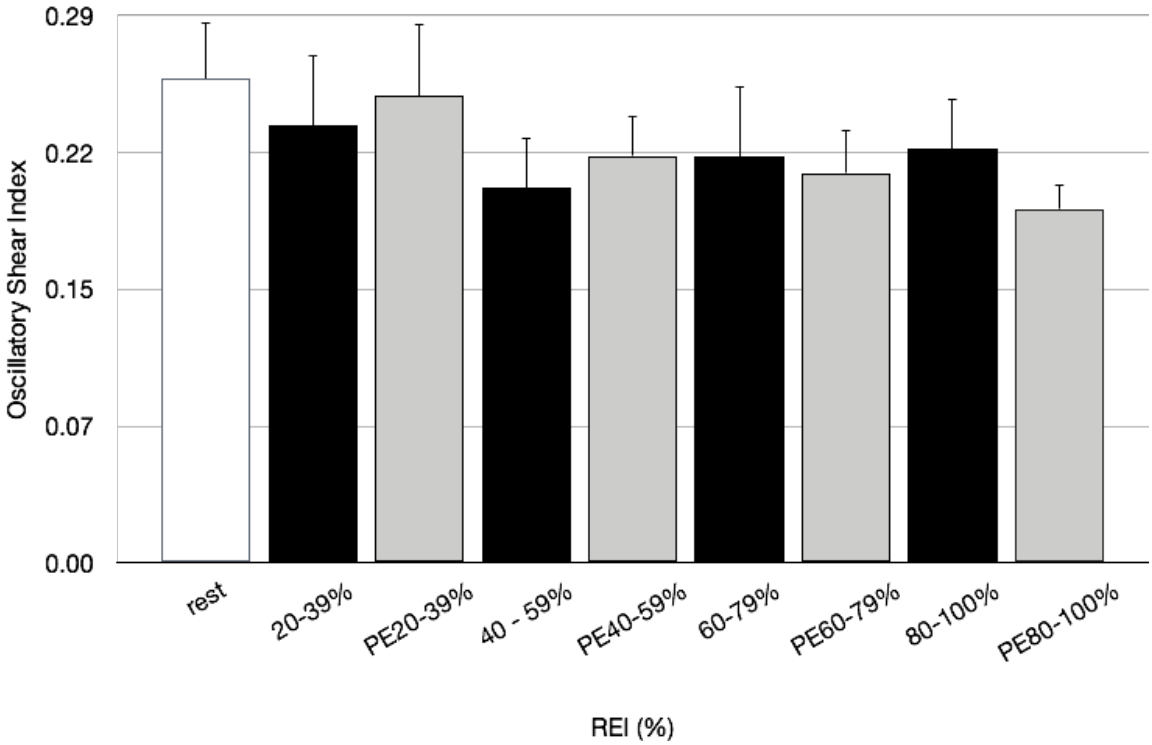

Figure 35 *Mean OSI and standard error during a discontinuous incremental isometric double leg exercise test of increasing REI (%). Resting OSI is represented by white bars, whilst MOSI is represented by black bars, and PE-MOSI is represented by grey bars. Correlation co-efficient analysis revealed significant correlations between REI and PE - MOSI ($r = -0.404$, $P < 0.05$), whilst there were no significant correlation between MOSI and REI as $P > 0.05$.*

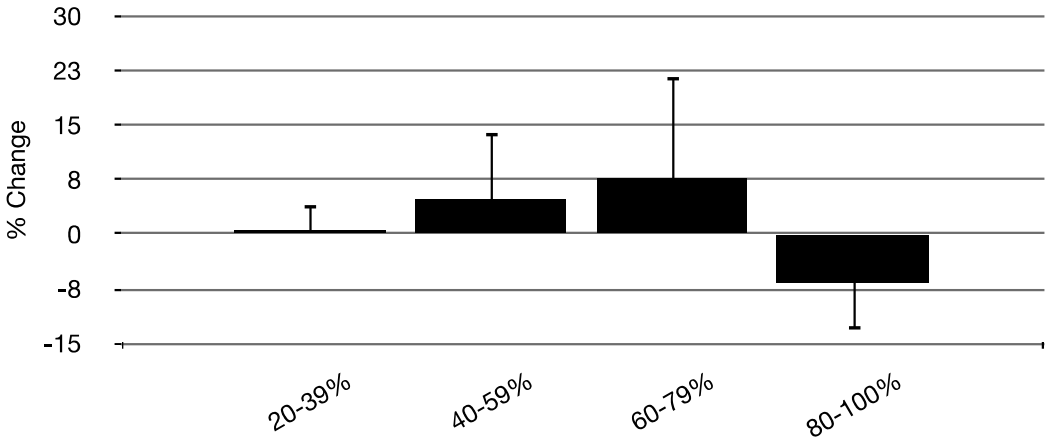

Figure 36 *delta change in OSI from contraction to post-exercise and standard error. Correlation co-efficient analysis revealed no significant relationship between REI (%) and ΔOSI as $P > 0.05$.*

5.4 Discussion

The aim of this study was to establish the shear pattern during and post-isometric leg exercise of increasing exercise intensity. This study is unique in that it is the first of its kind to measure the shear pattern to isometric leg exercise, encompassing measurements during both exercise and post-exercise. The results of this study reveal that during contraction, OSI was not significantly correlated with exercise intensity, however ASR and RSR both increased as exercise intensity increased. Post-exercise analysis revealed that as exercise intensity increased, OSI decreased, which suggests that as exercise intensity increased, post-exercise shear became less oscillatory and more uni-directional. Furthermore, both ASR and RSR post-exercise were significantly correlated to isometric exercise intensity.

Shear pattern during isometric bilateral leg extension exercise.

Research studies to date have typically presented an oscillatory shear pattern that is characterised by both ASR and RSR during dynamic exercise (Green et al, 2005; Tinken et al, 2009, Thijssen et al, 2008). Differences exist between the behavior of BF during dynamic exercise when compared to isometric contraction, in that increases in BF occur during the intermittent relaxation phases of dynamic exercise, since during contraction BF is limited or occluded due to augmented intramuscular pressure (Laaksonen et al, 2003). As Chapter 3 has demonstrated isometric exercise performed to a constant EMG and therefore constant intramuscular pressure (Sadamoto et al, 1983) experiences significant increases in BF during both contraction and relaxation periods. In light of this it was previously unknown as to what the shear pattern during isometric exercise would be. The results of the current study demonstrate MASR, MRSR, PASR and PRSR increased above resting levels in response to bilateral isometric leg extension exercise. Furthermore the increases observed in MASR, MRSR, PASR and PRSR during contraction were exercise intensity dependent. This study is the first to demonstrate statistically significant linear relationships between contraction ASR and RSR and relative isometric leg exercise intensity.

In terms of understanding the pattern of SR documented during IET, it has been suggested that increases in SBP are the driving force for increases in ASR. For example, Thijssen et al (2009a) found that dynamic leg extension exercise induced SBP driven changes in ASR that increased significantly with each exercise intensity, whilst no change in RSR was observed. As the results of Chapter 3 demonstrate, bilateral isometric leg extension exercise increased exercising SBP to mean values of 172.79 mmHg (figure 21). It was suggested that these

observed increases in BP were the result of a combination of centrally mediated increases in $\dot{Q}$ and reflex mediated vasoconstriction in the periphery vasculature to maximise perfusion pressure and increase BF supply to the working muscle vasculature that may have been mechanically occluded by high levels of intramuscular pressure. As intramuscular pressure increased with increasing exercise intensity, perfusion pressure was also required to increase proportionally to meet the demands of the exercise, which resulted in an intensity dependent SBP response. Similar to dynamic exercise, increases in SBP during isometric exercise would also lead to increases in BF induced SR (supported by the results presented in Chapter 4), which is characterised by increasing levels of ASR as isometric leg extension exercise intensity increases.

The generation of an exercise intensity dependent ASR response in the contraction periods of the DIIET can be explained by the Starling Resistor theory (Haliwill & Minson, 2010). During the isometric leg extension exercise performed incrementally in this current study, it is likely that a rise in sympathetic vasoconstrictor activity would increase the critical pressure of the resistance, leading to a higher pressure threshold that upstream BF would be required to overcome (Haliwill & Minson, 2010). If upstream pressure is not great enough to overcome the downstream resistance pressure, greater amounts of retrograde flow / shear will be produced. Vasodilation would lower the resistor critical pressure (Haliwill & Minson, 2010), and if upstream BF pressure was great enough to overcome this pressure, more antegrade flow / shear would occur as flow travels through the resistor vessel. This may go some way to explain in part why ASR increases linearly with REI, as local vasodilation during isometric leg exercise contraction would theoretically lower the resistor critical pressure. If upstream BP was greater than this critical pressure, antegrade flow would occur.

It was also observed that RSR also increased in relation to exercise intensity in the current study. The generation of retrograde flow / shear is attributed to the local compression of intramuscular vessels during muscle contraction (Barcrost and Dornhorst 1949, Sadamoto et al 1983). As a result, retrograde flow / shear is found to increase with muscle tension development (Green et al 2005, Lutjemier et al, 2005), as intramuscular pressure increases. Gonzales et al (2008) also demonstrated that when performing dynamic leg extension exercise, contractions of a faster rate produced more retrograde flow in the common femoral artery than slower contractions performed at the same level of contractile work. Gonzales et al (2008) speculated that fast contractions induced a greater intramuscular pressure than that during slow rate contractions leading to greater retrograde flow. No research study has studied the RSR response to increasing intensities of static isometric exercise. However isometric

exercise is characterised by its nature to induce high intramuscular pressure via mechanical compression of blood vessels (Rowland & Fernhall, 2007). Therefore it is plausible to suggest that as isometric leg extension exercise intensity increased, greater levels of intramuscular pressure were created during the contraction, resulting in a corresponding increase in RSR as BF increases to ensure adequate muscle perfusion.

Despite an observed exercise intensity dependent response from both ASR and RSR in this study, OSI was not significantly correlated with exercise intensity. Furthermore, isometric exercise contraction induced a more laminar BF through the common femoral artery, and therefore experienced less fluctuations in ASR and RSR as exercise intensity increased throughout the DIIET. Green et al (2005) also found that during handgrip exercise, brachial artery BF was more laminar and largely composed of ASR with little RSR. This was measured in contrast to cycling performance, which induced an ASR response as well as a significantly higher RSR response than that seen during handgrip exercise. This resulted in a greater oscillatory behaviour of shear in the brachial artery during cycling exercise than that seen during handgrip exercise. Green et al (2005) suggested that the differences in the shear characteristics between handgrip exercise and cycling may be due to a reduced downstream pressure in arm resistance vessels during forearm exercise in comparison to that seen during cycling. A reduced downstream pressure during handgrip exercise may have made it easier for an upstream arterial driving pressure (BP) to overcome any downstream resistance, and therefore a strong forward moving antegrade flow / shear response occurred.

Green et al (2005) also found that NO inhibition did not reduce BF in the largely antegrade handgrip exercise, whereas NO inhibition did reduce BF in cycling exercise. In response to this finding, Green et al (2005) implied that a stress stimulus that is characterised by both ASR and RSR, and therefore oscillatory in nature (such as that seen during cycling exercise) may indeed present a greater stimulus for NO mediated vascular adaptation, as a repetitive drawing of flow across the surface of the endothelium may increase the up regulation of NO. This implies that BF characterised predominately by ASR (such as that seen during the handgrip exercise in Green et al, 2005) may not provide as great of a stimulus for up regulation of NO bioavailability. Whilst this current study demonstrates a decrease in the oscillatory behaviour of SS as exercise intensity increases, BF was always characterised both by ASR and RSR components. In line with Green et al (2005), it may be possible that antegrade flow / shear became more dominant during isometric leg exercise due to increased perfusion pressure from a combination of local vasodilatation and increased central command

influence as isometric exercise intensity increased at each stage of the DIIET. As a result, this may have reduced the oscillatory behaviour of this increased BF / SS stimulus at higher intensities of isometric exercise. However as the degree of intramuscular pressure increases in conjunction with incremental increases in constant EMG at each stage of the DIIET, increases in retrograde flow / shear were also apparent as exercise intensity increased. The production of RSR would have ensured that the SS response remained in part oscillatory, and that as exercise intensity increased BF still continued to move in both a forward and backwards nature. Whilst this behaviour was not related to increasing exercise intensity, the oscillations in BF / SS may still have provided some basis for endothelial NO production (Hutcheson & Griffith, 1991) at higher intensities of isometric leg extension exercise.

Padilla et al (2010) have proposed that increased sympathetic nervous activity associated increases in conduit artery retrograde flow / shear patterns via increases in downstream vascular resistance may be overcome by elevations in BP. Padilla et al (2010) suggest that BP increases as a result of increased sympathetic nervous activity may alter the pressure gradient to one that favors decreases in retrograde flow / shear, a theory that would tend to be supported by the current results. Whilst the current study indicates a positive relationship between exercise intensity and RSR during isometric leg extension exercise, it could be suggested that the typical increases in BP associated with isometric exercise may have restricted RSR. This in turn would reduce the oscillatory behavior of shear as seen in the current study.

In summary, bilateral isometric leg exercise of increasing exercise intensity induces intensity dependent increases in both ASR and RSR that appears to be the result of a strong SBP influence to increase blood perfusion and increased intramuscular pressure from the static muscular contraction. Based upon current published work in this area, the observation that OSI did not increase with REI, despite significant exercise intensity dependent increases in ASR and RSR was unexpected. This would suggest that there could be other factors that play a role in determining the oscillatory response to isometric exercise. Padilla et al (2010) suggests that these possible other factors could include SV, HR, vessel compliance, reflex pathways and redistribution of regional BF.

Shear pattern post isometric bilateral leg extension exercise

The current study has demonstrated that post-exercise ASR and RSR respond in an exercise intensity dependent manner to increasing intensities of isometric exercise. Oscillatory shear index decreased below baseline levels post-exercise, also demonstrating an exercise intensity

dependent response. The current study is the first of its kind to report linear relationships between increases in post-exercise ASR and relative isometric leg exercise intensity, post-exercise increases in RSR and relative isometric leg exercise intensity and decreases in post-exercise OSI and relative isometric leg exercise intensity.

Only one other study has examined the shear pattern response after exercise. Johnson & Wallace (2012) found that high intensity running induced greater levels of ASR measured in the post-exercise period, compared to lower intensities. Despite the distinct differences between aerobic and isometric exercise the results of the current study are in agreement with those of Johnson & Wallace (2012), and also demonstrated an intensity dependent increase in post-exercise ASR.

Since there is very little research exploring the shear pattern post-exercise, any discussion must be largely based upon speculation. Based upon available evidence from Chapter 3 of this thesis, it could be suggested that the observed intensity dependent increases in ASR post-isometric contraction are the result of a post-exercise raised BP (until it returns to near resting levels) and an increase in upstream BF induced by downstream FMD. Indeed Thijssen et al (2009a) found that increases in ASR were associated with increases in SBP during dynamic leg extension exercise. Although this was determined during exercise, it has been established in Chapter 3 that BP stays elevated in the immediate periods following isometric leg exercise, and then gradually returns to resting levels over time. It is probable that this BP increase in the immediate post-exercise period is the result of the need to increase perfusion pressure during exercise, and that BP remains elevated after exercise as it is not physically possible to suddenly drop BP to resting levels. Rather it must decrease progressively (Tortora & Grabowski, 2002). Due to the fact that BP is increased to increase perfusion pressure, an increase in BF would also be observed which would go some way to explaining the increase in ASR post-exercise. Furthermore, as exercise intensity increases it is likely that BP would increase to greater levels during exercise to increase perfusion pressure of BF. When the contraction is released, an exercise intensity dependent increase in BF and ASR is observed. Evidence also suggests that when isometric contraction is released, downstream vascular resistance is decreased, resulting in a FMD response in the profunda branch of the femoral artery that leads to an increase in upstream BF, with a corresponding increase in ASR. As exercise intensity increases, the influence of increased levels of vasodilatory metabolites combined with the influence of increased perfusion BF (created during contraction that continues into the early immediate post-exercise period) may lead to an increased FMD

response especially at higher exercise intensities. This in turn would result in greater BF and consequently greater ASR post-exercise.

Retrograde shear rate measured post-exercise also increased with exercise intensity in this study. This is not a surprising finding since the generation of retrograde flow / shear is attributed to the local compression of intramuscular vessels during muscle contraction (Barcrost and Dornhorst 1949, Sadamoto et al 1983), which has also been shown to remain increased above pre-contraction levels following isometric contraction (Crenshaw et al, 1997). Therefore an exercise intensity dependent response in post-exercise intramuscular pressure (possibly as a result of increased extravascular fluid accumulation during exercise, Crenshaw et al, 1997) may go some way as to explaining why RSR also increased in an intensity dependent manner in the rest periods following IET. Post-exercise OSI decreased in relation to REI in this study. As exercise intensity increased, the post-exercise oscillatory shear became more laminar and uni-directional than that seen at baseline measures. This would suggest that the forward flowing increase in ASR was more dominant than the backward flowing RSR during these post-exercise rest periods.

In summary, it is apparent that the shear stimulus created during isometric exercise also continues in the post-exercise rest period. The current results suggest that the response of the shear pattern post-exercise is largely exercise intensity dependent. This response may be determined by the continued elevated BF seen after exercise as a result of an increased FMD response post-exercise and an increased perfusion pressure created during exercise that continues in part during resting periods. Increased ASR largely dominates the post-exercise shear pattern response and possibly overrides any RSR response in the immediate periods post-exercise. As a result, the OSI response post-exercise becomes more laminar as exercise intensity increases. However this said, RSR is present post-exercise, and increases relative to exercise intensity. Therefore is speculated that this repetitive drawing of BF back and forth across the endothelium in the periods immediately after isomeric exercise may provide a greater stimulus for NO upregulation (Green et al, 2005). In light of this, it is therefore possible that at higher intensities of isometric leg exercise, a potential haemodynamic stimulus may be present for vascular adaptation.

Change in Shear Pattern

The results of this study demonstrated that ΔASR increased with REI, suggesting that as exercise intensity increased the post-exercise ASR response was greater than that seen during contraction. Furthermore, the difference between contraction ASR and post-exercise ASR

becomes more pronounced as exercise intensity increases. In contrast, both the ΔRSR and ΔOSI response are not related to increasing REI.

No other study has explored the change in ASR from contraction to post-exercise, thus it is not possible to compare these results directly to existing literature. However, these results are consistent with those seen in the preceding Chapters 3 & 4, in that post-exercise BF and post-exercise SR are also significantly elevated to a greater extent than the BF and SR response during contraction. It could be expected that ASR would also demonstrate a similar response as ASR represents forward flow during systole. It is possible that flow remains elevated in this immediate post-exercise period as a result of an increased perfusion pressure from contraction gradually returning to baseline. In conjunction with this, post-exercise flow in the upstream common femoral artery may also be enhanced by a downstream FMD response in the profunda femoral artery, which again may lead to an increased ASR post-exercise.

Delta RSR and ΔOSI did not correlate with increasing isometric exercise intensity. It has been established that RSR both during contraction and in the rest periods after, is generated as a result of increasing intramuscular pressures during isometric contraction. As the level of intramuscular pressure correlates with increasing EMG exercise intensity (Sadamoto et al, 1983; Crenshaw et al, 1997), there must be other physiological mechanisms that contribute to the RSR response during and after isometric contraction that have not been identified in this study, and as such do not relate to increasing isometric exercise intensity. Oscillatory shear index during exercise also did not correlate with increasing exercise intensity, therefore it is unlikely that the ΔOSI response would correlate with isometric exercise intensity. This may be due to mechanisms other than ASR and RSR influencing OSI during exercise, whereas the post-exercise OSI response appears to be determined by the post-exercise ASR and RSR behavior.

Conclusion

It is apparent that isometric bilateral leg extension exercise of increasing intensity induces intensity dependent increases in common femoral artery ASR and RSR. It is suggested that this large increase in ASR is due to the large increase in SBP and consequent increase in forward flow to maintain perfusion pressure during exercise, whilst RSR appears to be generated by high levels of intramuscular pressure during isometric contraction. However, despite the observed changes in both ASR and RSR, OSI did not significantly correlate with

REI. This suggests that factors other than ASR and RSR may influence the OSI response during exercise.

Furthermore, isometric bilateral leg extension exercise of increasing intensity also induced intensity dependent increases in common femoral artery ASR and RSR after contraction, in the immediate rest periods post-exercise. It is likely that the ASR response was due to an elevated perfusion pressure that had overlapped from the contraction period, combined with a large increase in upstream BF due to a downstream FMD response. It is possible that an increase in retrograde flow was seen at this time as it represented vascular tone returning to baseline values. It is apparent that the ASR response was largely dominant as OSI decreased relative to exercise intensity, and thus the post-exercise shear pattern became more laminar as exercise intensity increased. However, this post-exercise response did not at any point become entirely laminar as RSR was still present.

It is apparent from the findings of this current chapter that the haemodynamic response to acute bouts of isometric exercise does display shear characteristics that are associated with potential vascular adaptations, which may be the mechanism for RBP reduction following IET. The next section of this thesis (page 156) reflects on these findings, and the results of Chapters 3 and 4 together as a whole to decide the plausibility of the haemodynamic response to acute isometric exercise as a stimulus for the RBP adaptations associated with IET.

Reflection On The Findings Presented In Chapters 3, 4 & 5.

Reflection On The Findings Presented In Chapters 3, 4 & 5.

In relation to the overarching research aim of this thesis, which was to determine the role of BF haemodynamics in reductions in RBP after IET, Chapters 3, 4 & 5 were part of a series of investigations to explore whether the haemodynamic response to isometric exercise could be considered as a viable physiological stimulus for RBP adaptation. The findings from Chapters 3, 4 & 5 did indeed demonstrate that there is a marked BF, SR and shear pattern response during exercise and in the immediate periods following exercise. This marked haemodynamic response was exercise intensity dependent for BF, SR, ASR, RSR & OSI variables both during and post-exercise (OSI only post-exercise). An interesting finding from these results was that the BF, SR & ASR response was significantly increased in the post-exercise periods when compared to the values seen during exercise. This may be suggestive of a greater haemodynamic stimulus present in the post-exercise periods as opposed to the haemodynamic stimulus created during isometric contraction.

Whilst the data that has been collected in Chapters 3, 4 & 5 is supportive of the ability of isometric exercise to produce a haemodynamic stimulus (particularly in the post-exercise periods), the concept of the haemodynamic stimulus presented in these Chapters is largely dependent upon the assumption that BF, SR and shear pattern are inter-related. These Chapters therefore speculate that isometric exercise would not produce a successful haemodynamic stimulus for RBP reductions unless a specific sequence occurs. This sequence starts with an increase in BF that consequently leads to an increase in SR and produces the correct shear pattern necessary for adaptation. In order to validate the concept of the haemodynamic stimulus presented from Chapters 3, 4 and 5, additional correlation analyses has been performed between BF, SR and shear pattern variables to establish whether these variables are indeed inter-related to one another. Before the findings of these correlation analyses are discussed, please note that all physiological variables met parametric assumptions as $P > 0.05$ when normality tests were performed.

-The relationship between BF and SR.

Chapter 4 established that there are exercise intensity dependent increases in SR both during and in the immediate periods after isometric exercise. Gonzales et al (2009)

states that SS is a characteristic measure of BF, therefore it is likely that the increases in BF seen in Chapter 3 would be responsible for the increases in SR seen in Chapter 4. The correlation findings do indeed confirm this relationship, as there are statistically significant correlations present between BF and SR during and in the immediate periods following acute isometric exercise. Table 14 displays the statistical findings of these correlations. These findings validate the hypothesis that increases in SR seen both during and immediately after isometric exercise of increasing exercise intensity (in Chapter 4) can be explained by the increases in BF presented in Chapter 3.

Table 14. *Statistical results from correlation analyses between BF and SR variables*.

Variables	r value	P value
MBF & MSR	0.823	< 0.01
PBF & PSR	0.787	< 0.01
PE-MBF & PE-MSR	0.722	< 0.01
PE-PBF & PE-PSR	0.727	< 0.01

MBF mean blood flow during contraction; *PBF* peak blood flow during contraction; *PE-MBF* post-exercise mean blood flow; *PE-PBF* post-exercise peak blood flow; *MSR* mean shear rate during contraction; *PSR* peak shear rate during contraction; *PE-MSR* post-exercise mean shear rate; *PE-PSR* post-exercise peak shear rate.

-The relationship between SR and shear pattern.

Chapter 5 hypothesised that although increases in SR were seen in Chapter 4, specific characteristics of the shear response would need to be present to induce up regulation of NO (Gonzales et al, 2008; Green et al, 2005), that may cause a vascular adaptation that consequently could lead to a RBP reduction following IET. The characteristics examined included ASR, RSR and OSI. As these variables are direct measures of different components of SR, it was assumed in Chapter 5 that changes in these variables in response to isometric exercise would be inter-related with changes in SR.

Correlation findings have established that there are statistically significant relationships present between SR and ASR, RSR and OSI variables during and in the immediate periods after acute isometric exercise (with the exception of PE-MSR & PE-MRSR). Table 15 documents the statistical findings from these correlations. These findings confirm that the shear pattern response to acute bouts of isometric exercise (as seen in Chapter 5) is indeed related to the SR response seen in Chapter 4.

Table 15. *Statistical results from the correlation analyses between SR and shear pattern variables*.

Variables	r value	P value
MSR & MASR	0.889	< 0.01
PSR & PASR	0.868	< 0.01
PE-MSR & PE-MASR	0.912	< 0.01
PE-PSR & PE-PASR	0.962	< 0.01
MSR & MRSR	- 0.348	< 0.01
PSR & PRSR	- 0.392	< 0.01
PE-PSR & PE-PRSR	- 0.309	< 0.05
MSR & MOSI	- 0.736	< 0.01
PE-MSR & PE-MOSI	- 0.503	< 0.01

MSR mean shear rate during contraction; *PSR* peak shear rate during contraction; *PE-MSR* post-exercise mean shear rate; *PE-PSR* post-exercise peak shear rate; *MASR* mean antegrade shear rate during contraction; *PASR* peak antegrade shear rate during contraction; *PE-MASR* post-exercise mean antegrade shear rate; *PE-PASR* post-exercise peak antegrade shear rate; *MRSR* mean retrograde shear rate during contraction; *PRSR* peak retrograde shear rate during contraction; *PE-MRSR* post-exercise mean retrograde shear rate; *PE-PRSR* post-exercise peak retrograde shear rate; *MOSI* mean oscillatory shear index during contraction; *PE-MOSI* post-exercise mean oscillatory shear index.

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-The relationship between SR and common femoral AD.

Although the relationship between SR and common femoral AD is not directly relevant to this reflection in terms of establishing the relationship between BF, SR and shear pattern variables for the validity of the haemodynamic stimulus, Chapter 4 identified a surprising result that correlational analysis may help address. It was evident in Chapter 4 that changes in common femoral AD had no relationship with increasing exercise intensity both during and immediately after acute isometric exercise. This was surprising as SR, which is thought to mediate a vasodilatory response in conduit arteries via the release of vasodilatory substances (Wray et al, 2005; Davis et al, 1995; Newcomer et al, 2011), did increase with increasing exercise intensity, both during and following isometric exercise. It was originally hypothesised that these increases in SR would cause a vasodilatory response in the common femoral artery and that this response would become more prominent as exercise intensity increased, and therefore SR increased. The correlation analysis results revealed that there is no statistically significant relationship between SR and common femoral AD changes during and after an acute bout of isometric leg extension

exercise as presented in Chapter 4. This finding supports the discussions of Chapter 4 that the common femoral artery may be unresponsive to an increased SS stimulus.

-Final reflections.

The findings from the correlational analyses performed in this reflection do support the presumptions made in Chapters 3, 4 and 5, that BF, SR and shear pattern are inter-related, and together form a haemodynamic challenge in response to acute isometric exercise contraction and in the rest periods immediately following exercise. It is suggested in this review that the post-exercise haemodynamic response may present a more successful stimulus for a physiological adaptation that may lead to a reduction in RBP, as it is of a greater magnitude than the haemodynamic response seen during isometric contraction. Furthermore, it is apparent from the findings in Chapter 5 that the post-exercise haemodynamic response is characterised by both antegrade and retrograde shear rate, which Green et al (2005) suggests is likely to favour the up regulation of NO. It is hypothesised in this thesis that the up regulation of NO may induce a vascular adaptation that may be a mechanism for RBP reductions following IET. With the findings of Chapters 3, 4 & 5 supported by the correlation analyses performed in this reflection, it is plausible to suggest that acute bouts of isometric leg exercise performed at higher exercise intensities induces a post-exercise BF response that could be considered as a physiological stimulus for RBP adaptation. This response is characterised by an increase in SR, and has an oscillatory shear pattern that whilst laminar in nature, still includes both increases in ASR and RSR, which may act as a successful haemodynamic stimulus for RBP reductions following a bout of IET. This remains to be investigated in the succeeding chapters of this thesis.

Chapter 6

Study 5: The Role Of A Local Post Exercise Haemodynamic Stimulus In Cardiovascular Adaptations To Isometric Exercise Training.

Chapter 6: The Role Of A Local Post Exercise Haemodynamic Stimulus In Cardiovascular Adaptations To Isometric Exercise Training.

6.1 Introduction

It is now well accepted that appropriately prescribed IET successfully reduce RBP for both normotensive (Badrov et al, 2013; Baross et al, 2012; Devereux et al, 2010b; Howden et al, 2002; Ray & Carrasco, 2000; Wiles et al, 2010) and hypertensive (McGowan et al, 2007b; McGowan et al, 2006; Millar et al, 2007; Peters et al, 2006; Taylor et al, 2003; Wiley et al, 1992) individuals. Despite the fact that it has been established that IET can induce reductions in resting SBP of up to ~20 mmHg (Taylor et al, 2003) and reductions in resting DBP of up to ~15 mmHg (Wiley et al, 1992), the exact nature of the physiological stimulus and / or mechanism(s) responsible for these RBP adaptations has yet to be elucidated.

In an attempt to provide greater insight into this fundamental research question, preceding work in Chapters 3, 4 and 5 explored the possibility of whether a haemodynamic stimulus could be considered as the exercise training stimulus for RBP reductions after IET. Furthermore, the work of Devereux et al (2011) suggested in the first instance that the physiological stimulus may be strongly related to the acute program variable of exercise intensity. Their work suggested that the greater the isometric exercise intensity (and degree of fatigue induced), the more prominent the exercise training stimulus which may subsequently lead to greater RBP adaptation following IET. In support of this suggestion, the findings presented in Chapters 3, 4 and 5 demonstrate a marked post-exercise haemodynamic response to acute bouts of isometric leg extension exercise that was significantly correlated with increasing exercise intensity. Thus, at higher fatiguing isometric exercise intensities, the haemodynamic response was more pronounced than that seen at lower isometric exercise intensities. Based upon these findings, it is suggested that a post-exercise haemodynamic response could be considered as an exercise induced physiological stimulus for RBP reduction after IET. The role of a haemodynamic stimulus in relation to the magnitude of RBP adaptations is currently unestablished, and remains to be investigated in this current chapter.

Several attempts have also been made to identify the resultant physiological mechanism(s) responsible for RBP reductions after IET. Wiley et al (1992) stated very

early on that this mechanism must involve adjustments in one or both of the components that determine RBP - $\dot{Q}$ and / or TPR. Since there is little evidence supporting the adaptation of $\dot{Q}$ to IET (Baross et al, 2012; Devereux et al, 2010b; Wiles et al, 2010), it seems more likely that alterations in TPR serve as the physiological mechanism underlying the RBP reductions commonly observed following IET. Indeed a number of homeostatic systems that determine TPR have been explored as the possible mechanism, including autonomic nervous system adaptations (Millar et al, 2013; Ray & Carrasco, 2000; Taylor et al, 2003), regulation of oxidative stress (Peters et al, 2006), and vascular adaptations (Badrov et al, 2013; Baross et al, 2012; McGowan et al, 2007a; McGowan et al, 2007b). The results from these investigations remain equivocal (not least due to differences in the BP status of participants), and as such the mechanisms responsible for the observed reductions in RBP after IET in normotensive participants remains unidentified.

Whilst definite findings linking changes in TPR with reductions in RBP following IET have not been forthcoming, preliminary evidence presented by Baross et al (2012) supports conduit AD remodeling as a possible mechanism for RBP reductions after IET. Older pre-hypertensive participants (resting SBP ~138 mmHg) performed 8 weeks of ILEET at 85% HR_{PEAK}, with significant reductions in resting SBP (~11 mmHg) and MAP (~5 mmHg) noted compared to baseline after an 8 weeks IET intervention. Results showed that the RBP reductions were significantly correlated with increases in common femoral AD. This suggested that a local conduit AD remodeling process may have occurred as a result of 8 weeks of IET, which in turn may have led to a reduction in TPR, resulting in a reduction in RBP. Since this investigation did not measure TPR, it remains to be established how a conduit artery remodeling process may influence RBP reductions.

The presence of a statistically significant haemodynamic stimulus post-isometric exercise (as demonstrated in Chapters 3, 4 & 5) provides additional support for the notion that conduit AD remodeling may be a possible mechanism for RBP reduction after IET. It is well established that haemodynamic SS mediates conduit AD remodeling in conduit arteries (Green et al, 2012; Green et al, 2010; Kamiya & Togawa, 1980; Langille & O'Donnell, 1986; Tinken et al, 2010). It is further suggested that AD remodeling is regulated by SS dependent endothelial gene expression (eNOS) and subsequent NO production (Sessa et al, 1994; Tuttle et al, 2001), that leads to the

remodeling of a larger diameter to normalise to an increased SS created during exercise bouts (Green et al, 2004). It has now been established (Chapter 4 and 5) that there is a large local post-exercise haemodynamic SS response present at high intensity isometric leg extension exercise, that is characteristic of a shear pattern that has previously been demonstrated to induce conduit AD remodeling (Green et al, 2010; Kamiya & Togawa, 1980; Langille & O'Donnell, 1986; Tinken et al, 2010). Based upon this evidence it is hypothesised that repeated exposure to this post-exercise haemodynamic stimulus during an appropriate IET protocol may remodel the artery to a larger diameter, which in turn may reduce TPR and subsequently cause a possible reduction in RBP.

The objective of this study is to try and identify the role of a haemodynamic stimulus in RBP reductions after ILEET in normotensive participants. This will be achieved by performing 8 weeks of bilateral ILEET (based upon the protocol established by Wiles et al, 2010), working to an intensity that elicits either a high post-exercise haemodynamic challenge, or a low post-exercise haemodynamic challenge. The CON group will receive no IET haemodynamic challenge. It is hypothesised that exposure to a high haemodynamic challenge will lead to greater RBP adaptation, then that seen with a low or no haemodynamic challenge. The results of this experiment should help to establish the role of post-exercise haemodynamics as the physiological stimulus for RBP reductions following IET.

In addition to the previous aim, SS mediated conduit AD remodeling will be investigated as a mechanism for reductions in RBP after IET. This study will investigate the relationship between any changes in AD that may occur locally in the common femoral artery and systemically in the brachial artery, which correspond with RBP adaptation over the exercise training period. However it is acknowledged that this is largely dependent on the assumption that a reduction in RBP will occur following the 8 week IET.

In order to provide further insight into the possible mechanism(s) responsible for any RBP reductions that may occur following IET, other non-invasive measures of cardiovascular function (in addition to conduit AD adaptation) will also be examined. These measures include $\dot{Q}$, HR, IVRT, LVET, HRV, and TPR. As previously discussed, it is a physiological fact that RBP adaptation must be attributed to changes in $\dot{Q}$ and/or TPR (Wiley et al, 1992), and therefore measurement of these cardiovascular variables

over the 8 week training period may help to identify whether reductions in RBP commonly seen in normotensive participants after IET are more closely related to cardiac adaptation or an adaptation within the vasculature. The measurement of common femoral AD and brachial AD may provide further support for changes in TPR as a mechanism for RBP reduction following IET. In addition, if this study identifies that $\dot{Q}$ may be a physiological mechanism for RBP reduction, then the examination of IVRT and LVET will help to identify whether $\dot{Q}$ adaptation occurred as a result of changes to diastolic (via IVRT measures) or systolic (via LVET measures) function. Lastly measures of HRV will also be assessed to determine the influence of the autonomic nervous system on RBP adaptation following IET, which consequently may have occurred as a result of changes in cardiac sympathetic and vagal modulation (Millar et al, 2013).

6.2 Methodology

6.2.1 Participants:

Thirty-six healthy, normotensive males (aged 24 ± 6 years, height 180 ± 5.71 cm, and body mass 75.70 ± 11.11 kgs) volunteered to participate in this study. Prior to testing, all participants received a written explanation of the procedures to be used, along with the potential risks of participating in the study. Once participants had provided written informed consent (in line with the regulations set out by the Declaration of Helsinki, 1964), they were required to complete an exercise readiness questionnaire (see appendix 1 page 250). Prior to data collection sessions, all participants fasted for 4 hours, abstained from caffeine and alcohol for at least 12 hours prior to the start of testing procedures (Jauregui - Renaud et al, 2001), and maintained normal physical activity and diet throughout the length of the study. This was confirmed verbally prior to the start of each session. All participants initially completed 2 familiarisation sessions (which included DIIET, measurement of baseline variables and bilateral ILEET sessions) prior to the commencement of resting data collection sessions and IET intervention.

Participants were randomly assigned to one of three groups (using the website www.random.org), which were high shear stress (HI), low shear stress (LO), or control group (CON).

6.2.2 Equipment and experimental procedure:

Sequentially the protocol can be broken down into 3 sections which include; prior to the training intervention, the training intervention, and data collection. Each of these sections will be presented below.

Prior to the training intervention:

-MVC and EMG_{peak} determination.

MVC and subsequent EMG_{peak} were determined prior to training intervention so that an DIIET could be performed. Participants underwent 3 MVC determination tests on 3 separate occasions. EMG_{peak} was determined from the session that induced the highest MVC. Please refer to page 77 in the methodology chapter (Chapter 2) for details regarding the determination of MVC and EMG_{peak}.

-Initial discontinuous incremental isometric exercise test

Participants underwent the DIIET on 3 separate occasions (at least 48 hours apart) performing double leg extension exercise, prior to the training intervention. This test was performed to identify the individualised exercise intensity that IET would be performed at during the exercise intervention for each participant. Full details of the protocol used to perform the DIIET can be found on page 77 of the methodology chapter (Chapter 2). Figure 16 on page 77 also displays the DIIET protocol.

During each DIIET, post-exercise BF, SR, ASR, RSR and OSI were recorded using the methods identified in Chapter 3 (BF page 88); Chapter 4 (SR page 116); and Chapter 5 (ASR, RSR & OSI page 136). This data was recorded to ascertain whether or not the peak post-exercise response of these haemodynamic variables may be the physiological stimulus to induce RBP reductions after IET.

The DIIET along with the measurement of post-exercise peak haemodynamic variables was then repeated after 4 weeks of training intervention. At this point in time, exercise training intensity was re-prescribed so as to adhere to the general training principle of progressive overload, helping to ensure that an appropriate level of overload (Hellebrandt, 1958) was maintained throughout the 8 week training period.

The training intervention:

-Exercise training protocol

Participants were required to perform bilateral leg extension exercise 3 times a week for 8 weeks. Each training session consisted of 4 leg extension isometric contractions performed for 2 minutes each. Determination of exercise intensity for isometric exercise is discussed below.

-Determination of exercise intensity for each group.

High (HI) and low (LO) groups were required to perform isometric exercise at an established exercise intensity throughout the duration of the training intervention. The control (CON) group was not required to participate in the exercise training intervention.

Exercise intensity for the HI group was based upon the individual specific exercise intensity during the initial DIIET that produced the greatest peak post-exercise BF, SR, ASR, RSR and OSI response. The HI group participants were then required to perform the training intervention at this identified individualised exercise intensity associated with their greatest post-exercise peak haemodynamic stimulus.

The LO group performed the training intervention at the intensity identified during the initial DIIET that produced the lowest peak post-exercise BF, SR, ASR, RSR and OSI response.

This exercise prescription method was based on each participant's individual response during the initial DIIET. For example, participant 1 in the HI group may have produced the greatest peak post-exercise haemodynamic stimulus at 20% EMG_{peak}, where as participant 2 of the HI group may have produced their greatest peak post-exercise haemodynamic stimulus at 35% EMG_{peak}. Therefore participant 1 would perform their training intervention at 20 %EMG_{peak}, whilst participant 2 would perform their training intervention at 35% EMG_{peak}.

Data collection:

Physiological data was collected to establish the effects of 8 weeks of bilateral ILEET on adaptations to the following variables measured at rest: RBP; HR; HRV; $\dot{Q}$; TPR; AD; IVRT and LVET. These measures were collected at week 0, after 4 weeks and after 8 weeks of exercise intervention:

- *Resting blood pressure*

Resting blood pressure measures were recorded to establish the effects of 8 weeks of IET on RBP adaptation. As such, RBP measures were taken at 3 key stages: before the training intervention commenced (at week 0), at the mid-point of training intervention (after 4 weeks) and after training intervention (after 8 weeks). Resting SBP, DBP and MAP were measured using an automated BP monitor after an initial 15 minute resting period, during which the participant refrained from moving or talking. For details please refer to page 45 of the methodology chapter (chapter 2) which documents the equipment and protocol used to measure RBP throughout the investigation.

- *Resting heart rate*

Resting HR was used as a measure of cardiac adaptation to IET. Three lead Electrocardiography was used to record resting HR in this study. Page 44 of the methodology chapter (chapter 2) documents the equipment and protocol used to measure resting HR. After an initial resting period of 15 minutes, 5 minutes of HR data was collected to establish the participants resting HR. Participants refrained from moving or talking during this data collection period.

- Resting heart rate variability

Resting HRV measures provided data regarding the adaptation of cardiac sympathetic and vagal modulation following IET. These measures included TP, HF, LF, HFnu, LFnu and LF/HF ratio. These HRV measures were used as a measure of autonomic function in this current study. Page 45 of the methodology chapter (Chapter 2) documents the equipment and protocol used to measure HRV measures. Heart rate variability measures were analysed from a 5 minute ECG recording, collected as described above. In addition to refraining from moving or talking during this measurement period, participants were required to standardise their breathing to 12 breaths $\cdot$ min^{-1} using a metronome as a reference.

- *Total peripheral resistance*

Total peripheral resistance was assessed to determine whether TPR adaptation occurs in response to 8 weeks of bilateral ILEET. Page 50 of the methodology chapter (chapter 2) documents how resting TPR was calculated in this study. Specifically TPR was calculated from RBP data and resting $\dot{Q}$ data collected after an initial 15 minute resting period at each data collection session.

- *Artery Diameter*

Resting common femoral and brachial AD's were assessed to determine whether 8 weeks of bilateral ILEET could cause physiological structural adaptation in these arteries. Page 53 of the methodology chapter (Chapter 2) documents the equipment and protocol used to measure resting AD of the common femoral artery and brachial artery in this study. Page 53 provides an anatomical diagram to demonstrate the location of the common femoral artery within the leg. The brachial artery is the main conduit artery

located in the upper arm, where it divides at the elbow to become the radial and ulna arteries. Two - dimensional ultrasound was used to image the diameter of these arteries at rest at each data collection session. This data was collected with the participant laying supine, and after an initial 15 minute rest period.

- *Cardiac Output*

Cardiac output measures at rest were assessed to determine the influence that IET may have on $\dot{Q}$ adaptation. Page 57 of the methodology chapter (Chapter 2) documents the equipment and protocol used to measure resting $\dot{Q}$ in this current study. Cardiac output was calculated from resting stroke volume (SV) and resting HR data. Resting ECG data was used to identify resting HR, and Doppler echocardiography was used to determine each participants SV. This data was not collected until after an initial 15 minutes resting period.

- *Isovolumic relaxation time*

Isovolumic reaction time was utilised as a measure of cardiac diastolic function adaptation to IET. Page 61 of the methodology chapter (Chapter 2) documents the equipment and protocol used to measure resting IVRT in this study. Isovolumic relaxation time is a measure of the efficiency of diastolic filling within the left ventricle of the heart that would partly determine $\dot{Q}$, and was identified using Doppler echocardiography methods.

- *Left ventricular ejection time*

In addition, LVET was also used as a measure of cardiac systolic function adaptation to IET. Page 62 of the methodology chapter (Chapter 2) documents the equipment and protocol used to measure resting IVRT in this study. Left ventricular ejection time is a measure of the ability of the left ventricle to empty during systole, which also would partly determine $\dot{Q}$ values. Again, this measure was identified using Doppler echocardiology methods.

6.2.3 Data analysis

All data were checked for assumptions of normality. Where these assumptions were not met the data was logarithmically transformed.

Repeated measures mixed model ANOVA was primarily used to assess any significant differences in RBP change (SBP, DBP, MAP) over the 8 week intervention period between HI, LO and CON groups. This data was used to establish the effectiveness of a haemodynamic stimulus for RBP reductions after IET.

ANOVA with a group * time interaction was used to assess any significant differences in RBP change (SBP, DBP, MAP) over the 8 week intervention period within each exercise training group (HI, LO, CON).

Correlation coefficients were then used to explore the relationships between changes in RBP and other physiological variables in order to provide insight into the physiological mechanisms associated with any changes in RBP. These variables included HR, HRV (TP, HF, LF, HFnu, LFnu, LF/HF), TPR, AD, $\dot{Q}$, IVRT & LVET.

In addition, the consistency of the haemodynamic challenge that both the HI and LO group performed IET at throughout the 8 week training period was also assessed using a repeated measures ANOVA.

The alpha level of significance for all tests was set at $P < 0.05$.

6.3 Results

6.3.1 Resting blood pressure change

Results revealed that when participants performed 8 weeks of bilateral ILEET to either a high post-exercise haemodynamic stimulus (HI group), a low post-exercise haemodynamic stimulus (LO group), or performed no bilateral ILEET (CON), there were no significant differences observed between groups for resting SBP, DBP or MAP pre-post exercise intervention. This result suggests that training to a personalised post-exercise haemodynamic challenge does not provide an appropriate exercise training stimulus to reduce RBP via IET. Table 16 demonstrates group mean values at the start and end of IET.

Table 16*. Group mean values (± SD) for systolic (SBP), diastolic (DBP) and mean arterial (MAP) pressure pre - post exercise training. * = significant difference to pre scores, at p < 0.05*

Condition	BP component	Pre (mmHg)	Post (mmHg)	BP Change (mmHg)
HI	SBP	117 ± 6	114 ± 4	-3
	DBP	69 ± 5	68 ± 6	-1
	MAP	85 ± 3	83 ± 4	-2
LO	SBP	116 ± 6	114 ± 8	-2
	DBP	70 ± 8	67 ± 6	-3
	MAP	85 ± 7	83 ± 6	-2
CON	SBP	113 ± 10	113 ± 11	0
	DBP	66 ± 3	67 ± 6	+1
	MAP	82 ± 4	83 ± 7	+1

When an ANOVA with a group * time interaction was performed to account for the mid training point measures taken at week 4, the results revealed statistically significant changes in MAP for the HI group mid - post exercise training (-2 ± 5 mmHg), MAP for the LO group pre - mid exercise training (-4 ± 6 mmHg) and DBP for the LO group pre

- mid, mid - post and pre - post exercise training (-5 ± 8 mmHg; -2 ± 3 mmHg; -3 ± 8 mmHg respectively). Figures 37, 38 and 39 illustrate these within group RBP changes.

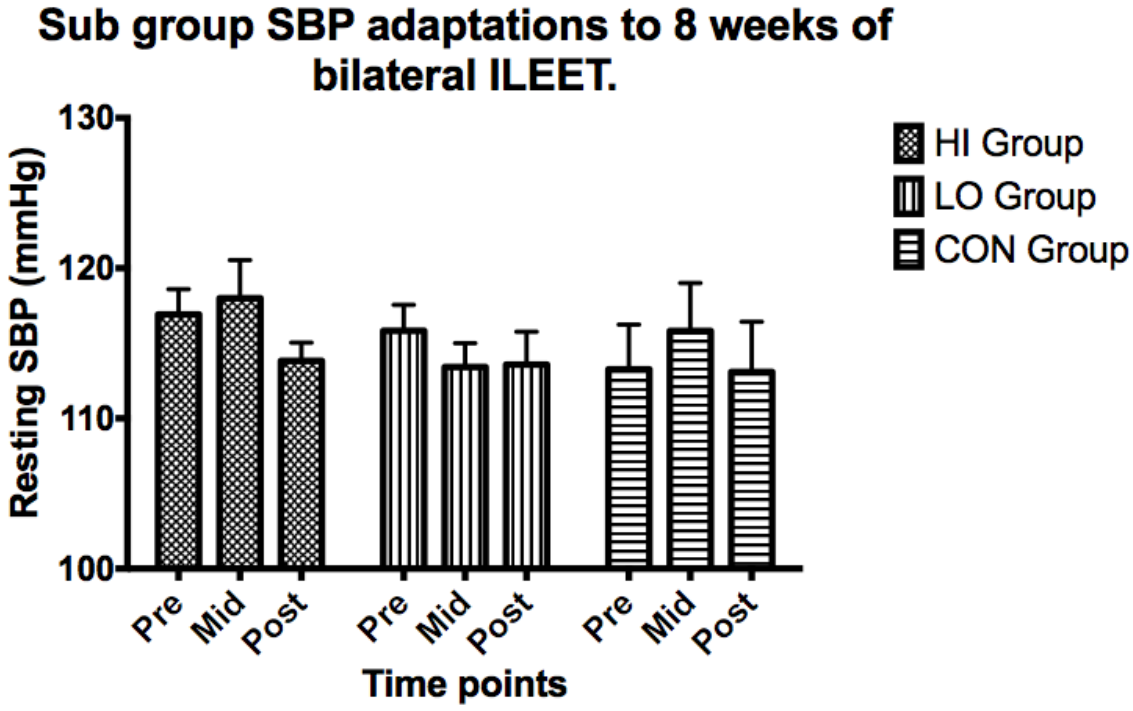

Figure 37*. Within group SBP adaptations to 8 weeks of bilateral ILEET. * denotes a significant difference, at P < 0.05.*

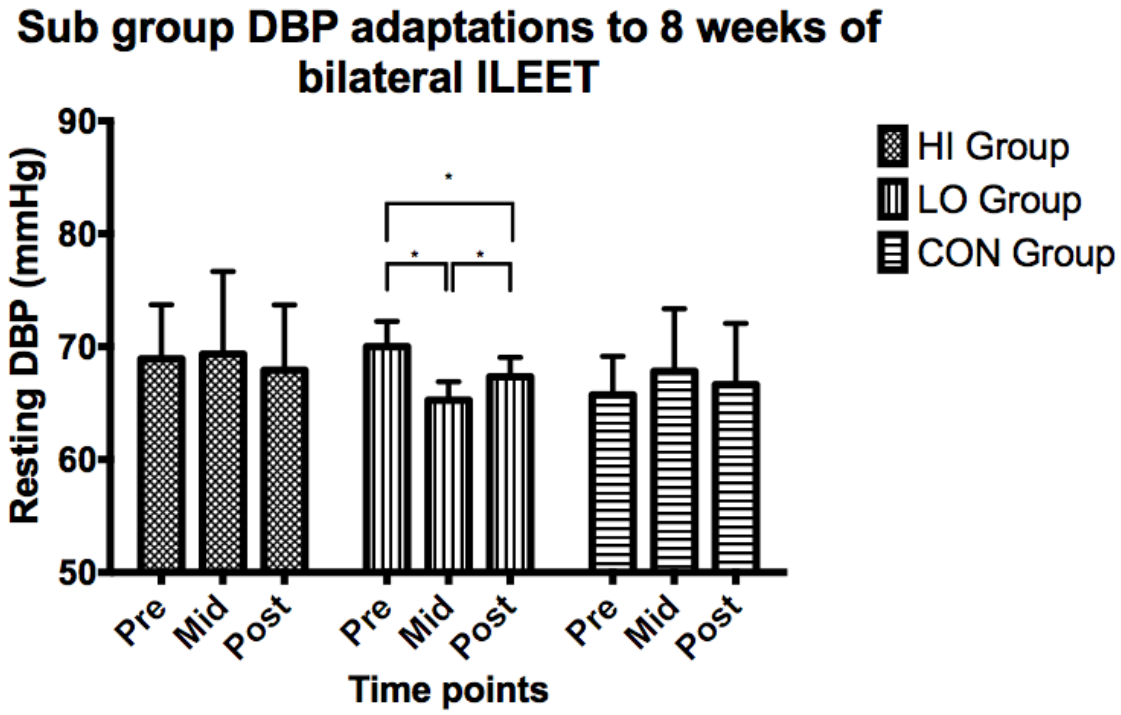

***Figure 38**. Within group DBP adaptations to 8 weeks of bilateral ILEET. * denotes a significant difference, at P < 0.05.*

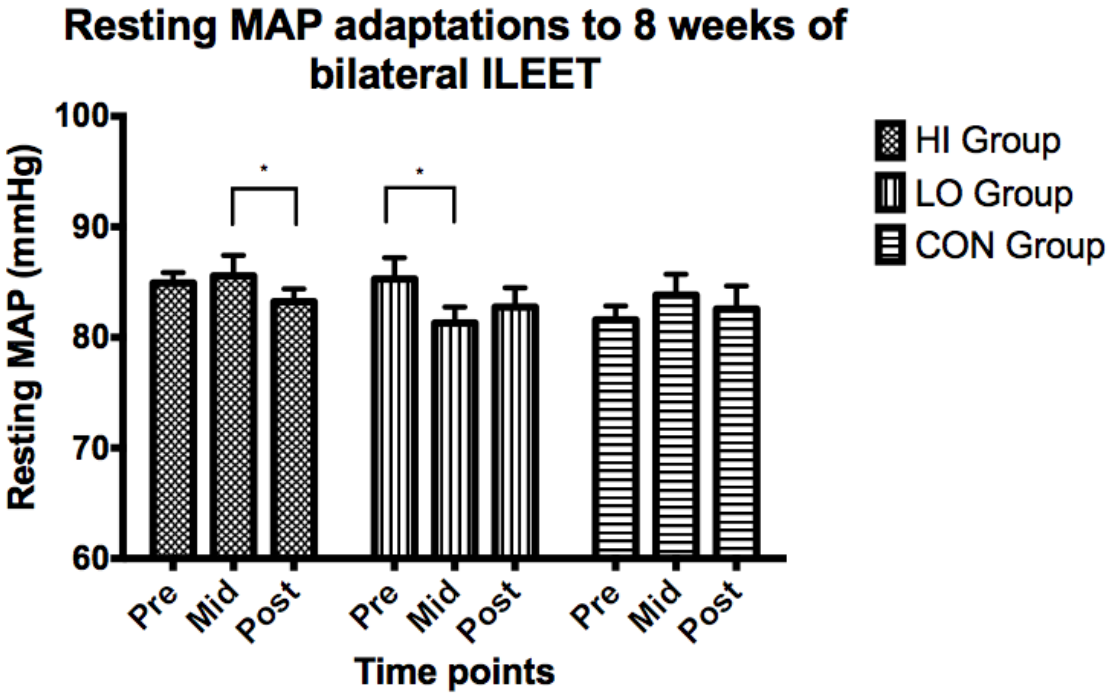

***Figure 39**. Within group MAP adaptations to 8 weeks of bilateral ILEET. * denotes a significant difference, at P < 0.05.*

6.3.2 Correlation results

There were no statistically significant correlations between RBP changes (DBP and MAP) and changes in the non-invasive resting cardiovascular measures, including common femoral AD, brachial AD, TPR, HR, HRV (TP, HF, LF, HFnu, LFnu, LF/HF), $\dot{Q}$, IVET and LVET as $P > 0.05$. Tables 17 & 18 document the group mean values for these resting cardiovascular measures collected at week 0, 4 and 8 of ILEET in the HI and LO groups. The CON groups resting cardiovascular data can be viewed in Appendix 7 on page 266.

Table 17. *HI Group mean values for resting cardiovascular variables at pre- training, mid-training and post-training time points. Changes in these resting cardiovascular variables over the exercise training period did not correlate with statistically significant HI group MAP changes.*

Resting cardiovascular variable	Pre-training point (0 weeks)	Mid-training point (4 weeks)	Post-training point (8 weeks)
Common femoral AD (cm)	0.92 ± 0.12	0.90 ± 0.10	0.94 ± 0.11
Brachial AD (cm)	0.40 ± 0.06	0.38 ± 0.04	0.40 ± 0.08
TPR	17.09 ± 2.85	19.04 ± 3.86	16. 31 ± 2.43
HR (beats $\cdot min^{-1}$)	65 ± 10	61 ± 10	67 ± 10
TP (ms^2)	8862 ± 6073	14444± 8250	14725 ± 8250
HF (ms^2)	2242 ± 2713	3875 ± 3571	5042 ± 3571
LF (ms^2)	3203 ± 2187	4794 ± 4165	5451 ± 4165
HFnu	30.02 ± 17.34	35.85 ± 14.74	30.79 ± 14.74
LFnu	63.50 ± 21.86	57.90 ± 18.79	65.11 ± 18.79
LF/HF	3.09 ± 2.15	3.13 ± 4.87	3.12 ± 4.87
$\dot{Q}$ ($L \cdot min^{-1}$)	5.14 ± 0.90	4.73 ± 0.82	5.31 ± 0.84
IVRT (msec)	57.6 ± 17.1	62.8 ± 18.5	57.4 ± 9.4
LVET (msec)	303.1 ± 16.9	305.1 ± 18.8	304.4 ± 14.8

AD artery diameter, *TPR* total peripheral resistance, *HR* heart rate, *TP* total power, *HF* high frequency, *LF* low frequency, *HFnu* high frequency normalized units, *LFnu* low frequency normalized units, *LF*/*HF* Low frequency / high frequency ratio, $\dot{Q}$ Cardiac output, *IVRT* Isovolumic relaxation time, *LVET* left ventricular ejection time.

Table 18. *LO Group mean values for resting cardiovascular variables at pre- training, mid-training and post-training time points. Changes in these resting cardiovascular variables over the exercise training period did not correlate with statistically significant LO group changes in MAP and DBP.*

Resting cardiovascular variable	Pre-training point (0 weeks)	Mid-training point (4 weeks)	Post-training point (8 weeks)
Common femoral AD (cm)	0.87 ± 0.07	0.80 ± 0.10	0.82 ± 0.09
Brachial AD (cm)	0.36 ± 0.04	0.37 ± 0.03	0.37 ± 0.04
TPR	15.64 ± 3.33	15.13 ± 3.67	14.61 ± 2.88
HR (beats $\cdot min^{-1}$)	70 ± 14	68 ± 10	71 ± 11
TP (ms^2)	24860 ± 40685	19357 ± 26007	25553± 24043
HF (ms^2)	11662 ± 24104	8078 ± 13840	6793 ± 6956
LF (ms^2)	5011 ± 6899	4550 ± 5642	4726 ± 3316
HFnu	33.35 ± 18.79	40.64 ± 13.27	37.93 ± 13.90
LFnu	52.13 ± 30.03	47.94 ± 21.26	42.82 ± 23.75
LF/HF	2.79 ± 3.23	1.57 ± 1.53	2.07 ± 3.55
$\dot{Q}$ ($L \cdot min^{-1}$)	5.66 ± 1.0	5.74 ± 1.28	5.96 ± 1.18
IVRT (msec)	56.8 ± 7.8	66.3 ± 18.8	58.6 ± 10.5
LVET (msec)	288.7 ± 15.2	302.2 ± 22.0	288.1 ± 16.0

AD artery diameter, *TPR* total peripheral resistance, *HR* heart rate, *TP* total power, *HF* high frequency, *LF* low frequency, *HFnu* high frequency normalized units, *LFnu* low frequency normalized units, *LF*/*HF* Low frequency / high frequency ratio, $\dot{Q}$ Cardiac output, *IVRT* isovolumic relaxation time, *LVET* left ventricular ejection time.

6.3.3 Reliability of the haemodynamic stimulus

The consistency of the haemodynamic challenge that both the HI and LO group performed IET at throughout the 8 week intervention was also assessed. In the initial DIIET, individualised $\%EMG_{peak}$ exercise intensity was determined based on the post-exercise haemodynamic response observed. This time point is known as baseline. At week 2 and week 6 of exercise training the haemodynamic response to a training session was also recorded to allow the consistency of the haemodynamic challenge to be assessed.

All haemodynamic data during baseline, week 2 and week 6 met parametric assumptions. The ANOVA results revealed that for all post-exercise haemodynamic variables (BF, SR, ASR, RSR and OSI), significant differences were observed at baseline, week 2 and week 6 between the HI and LO groups. Thus it is apparent the HI group consistently performed IET with a greater haemodynamic challenge than the LO group throughout the training period.

ANOVA results with a time interaction also revealed that for BF and RSR there was no significant difference between baseline, week 2 and week 6 of exercise training. For SR and ASR variables, significant differences were observed from baseline to week 2 and baseline to week 6, indicating that there was a slight decrease in the magnitude of the haemodynamic challenge from baseline. It is also evident that OSI significantly increased from baseline to week 6, suggesting that towards the end of the training period, the haemodynamic challenge became more oscillatory in nature. This pattern of change over the training period for all haemodynamic variables was the same for both the HI and LO exercise training groups. Table 19 details the specific haemodynamic challenge that both the HI and LO group performed IET at for baseline, week 2 and week 6 time points.

***Table 19**. The haemodynamic challenge for both HI and LO groups at baseline, week 2 and week 6 of exercise training.*
** = Significant difference from baseline as $P < 0.05$*
† = Significant difference from week 2 as $P < 0.05$.

Haemodynamic variable	**HI group (mean ± SD)**			**LO group (mean ± SD)**		
	Baseline	Week 2	Week 6	Baseline	Week 2	Week 6
Blood flow ($ml \cdot min^{-1}$)	380.27 ± 109.93	391.66 ± 204.89	381.19 ± 147.16	183.86 ± 73.66	153.41 ± 37.24	125.05 ± 39.22
Mean shear rate (s^{-1})	37.95 ± 12.18	31.72 ± 18.01*****	33.26 ± 14.87*****	20.88 ± 7.83	15.60 ± 5.03*****	12.90 ± 4.86*****
Antegrade shear (s^{-1})	107.27 ± 25.65	94.64 ± 33.63*****	93.33 ± 29.56*****	64.61 ± 16.59	53.69 ± 11.63*****	49.42 ± 12.66*****
Retrograde shear (s^{-1})	-31.37 ± 13.06	-31.19 ± 8.86	-26.82 ± 4.35	-22.85 ± 4.02	-22.49 ± 3.39	-23.63 ± 5.42
Oscillatory shear index	0.22 ± 0.06	0.26 ± 0.08	0.23 ± 0.06*****	0.27 ± 0.06	0.30 ± 0.04	0.33 ± 0.04*****

6.3.4 Trained vs. controls

Further statistical analysis revealed that if participants are separated into those who trained (regardless of their original training group) versus those who did not perform training intervention (i.e. the CON group), significant reductions of 2 mmHg in resting SBP and MAP are also apparent in the trained participants.

6.4 Discussion

The primary aim of this study was to determine whether a post-exercise haemodynamic stimulus might be the exercise training stimulus responsible for RBP reductions typically seen after IET. To determine the effects of a post-exercise haemodynamic stimulus on the magnitude of any RBP reductions, 2 exercise groups performed isometric leg extension exercise at an individualised intensity equating to either a high or low post-exercise haemodynamic challenge. The results of the current study demonstrated no statistically significant difference in the magnitude of SBP, DBP or MAP change pre-post 8 weeks of bilateral ILEET between groups (HI, LO and CON). This suggests that performing bilateral ILEET to an individualised post-exercise haemodynamic challenge does not provide the necessary physiological stimulus to induce reductions in RBP after IET for young normotensive males.

To corroborate this interpretation, further analysis of the individualised post-exercise haemodynamic challenge revealed that the HI group consistently performed IET with a greater post-exercise haemodynamic challenge than the LO exercise training group. For both exercise training groups there was a slight decrease in magnitude for some of the haemodynamic variables within this haemodynamic stimulus from the initial prescription of individualised isometric exercise intensity (based upon the post-exercise haemodynamic response) during the DIIET, and isometric training sessions measured at week 2 and week 6. Specifically, MSR and ASR haemodynamic variables decreased, whilst no significant difference between baseline and training bouts were observed for MBF and RSR. This was the same for both the HI and LO exercise training groups. The decrease in magnitude of MSR and ASR occurred only in the time between the DIIET and performing IET, as opposed to during the 8 week exercise training bout. This indicates that both exercise groups trained with a consistent within group (i.e. high or low) post-exercise haemodynamic challenge. It is also evident that there is an increase in OSI measured from the baseline incremental and week 6 of IET for both the HI and LO exercise groups. This implies that the haemodynamic shear stimulus became more oscillatory toward the end of 8 weeks of exercise training, likely due to a decrease in ASR whilst RSR stayed consistent.

It may be suggested that the apparent decrease in magnitude of MSR and ASR from baseline incremental to exercise training may go some way as to explaining why the HI group did not experience a reduction in RBP that was statistically significantly different from that observed

in the LO training group. It was initially hypothesised that IET may induce structural enlargement of the common femoral artery and/ or profunda femoral artery as a result of an adaptive response to normalise the repetitive increases in wall SS (MSR, ASR, RSR) induced during each IET session, and therefore remove the need for ongoing functional dilation via the release of endothelial factors (Dimmeler & Zeiher, 2003; Green et al, 2004; Koller & Kaley, 1991; Niebauer & Cooke, 1996; Pohl et al, 1986; Zarins et al, 1987). As MSR and ASR measured during IET at week 2 and 6 were significantly lower than the MSR and ASR values measured from the intensity that induced the greatest heamodynamic response during a DIIET at baseline (see table 19, page 179), it may be possible that this repeated heamodynamic stimulus did not consistently overload the endothelium throughout the 8 week training period to cause a continued adaptation. At the start of the exercise training programme it is plausible that the SS stimulus (MSR and ASR) would have been of a great enough magnitude to induce an adaptive response that acted to normalise the SS stimulus. However, once this level of SS was normalised, it is likely that an increased SS stimulus (in relation to MSR and ASR) would have been required to overload the normalisation and continue this adaptive response. As the MSR and ASR was reduced in magnitude from the initial baseline incremental test and did not demonstrate a significant increase between weeks 2 and 6 of exercise training in the HI group, this may have reduced the likelihood of a conduit AD remodeling process in the HI exercise training group. This in turn may explain why this group did not experience a significantly greater reduction in RBP in comparison to the LO exercise training group. Furthermore, this may highlight the importance of MSR and ASR rather than MBF and RSR as a means of determining a haemodynamic stimulus for RBP adaptation following IET.

Since this is the first study of its kind to examine the role of a haemodynamic stimulus in RBP reductions after IET, it is not possible to directly compare the findings of this current study to that of others. However, it is possible to comparatively evaluate the results of the current study in relation to other exercise training studies that have demonstrated reductions in RBP following IET may be related to improvements in vascular structure or function. This is based upon the fact that any vascular adaptations are strongly dependent upon a haemodynamic SS stimulus being present (Clarkson et al, 1999; Green et al, 2010; Green et al, 2004; Kamiya & Togawa, 1980; Langille & O'Donnell, 1986; McGowan et al, 2006; Niebauer & Cooke, 1996; Tinken et al, 2010). It must be acknowledged that these studies did not measure the presence of a haemodynamic SS stimulus during IET, and therefore any vascular adaptations that

occurred as a result of IET are based on an assumption that a SS stimulus was present during exercise training. As such further comparison must be interpreted with care.

In support of the findings of the current investigation, recent work by Badrov et al (2013) suggests that a direct haemodynamic stimulus may not be the primary physiological stimulus for RBP reductions following IET. Badrov et al (2013) explored the role of endothelial function in RBP reductions in normotensive female participants after 8 weeks of IET. Their results demonstrated that whilst both improvements in RBP and endothelial function were apparent, RBP reductions occurred before week 4 of the exercise training period, where as improvements in endothelial function occurred after this point. As previous evidence has established, improvements in endothelial function are dependent upon a SS stimulus (Clarkson et al, 1999; Green et al, 2004; McGowan et al, 2006; Niebauer & Cooke, 1996). However, as SS mediated endothelial function improvements occurred after a significant reduction in RBP in the exercise training period, it is likely that the haemodynamic SS stimulus was not the primary physiological stimulus for RBP reductions after IET in these normotensive participants. This finding tends to support the results of this current study.

Baross et al (2012) however indicated that a haemodynamic stimulus may be the physiological stimulus for RBP reductions after IET in pre-hypertensive older participants (resting SBP ~138 mmHg). Baross et al (2012) established that reductions in RBP after 8 weeks of bilateral ILEET training at 85% HR_{peak} were significantly correlated with increases in common femoral artery lumen diameter, suggesting that conduit AD remodeling is the mechanism for RBP reductions following IET. As Baross et al (2012) suggested, it is likely that the AD adaptation came about via repeated exposure to a haemodynamic SS stimulus created during IET bouts. Although, it should be acknowledged that Baross et al (2012) did not establish whether a SS stimulus was present, and therefore it is not possible to directly identify whether an increased haemodynamic SS stimulus was responsible for the associated conduit AD remodeling. Nonetheless, as previous evidence suggests, this remodeling process was probably dependent on a SS stimulus (Green et al, 2010; Langille & O'Donnell, 1986; Kamiya & Togawa, 1980; Tinken et al, 2010). Indirectly it is possible that a haemodynamic SS stimulus may have be been the physiological stimulus for RBP adaptations in the study of Baross et al (2012).

One important factor to be taken into consideration when interpreting the work of Baross et al (2012) and the findings of this current study is in relation to the exercise training stimulus

used to prescribe IET. Baross et al (2012) set ILEET training intensity at 85% HR_{peak} based upon previous work of Wiles et al (2010), who demonstrated this exercise intensity was successful at eliciting a reduction in RBP. As such, Baross et al (2012) then found that changes in common femoral AD accompanied this reduction in RBP, which implied a haemodynamic stimulus may have been present at this 85% HR_{peak} exercise training intensity. The current study set exercise training intensity at an individualised $\%EMG_{peak}$ associated with a specific level of haemodynamic stimulus. Whilst significant reductions in RBP were observed when all the participants that performed the ILEET programme (regardless of training group) were compared to those participants in the CON group that did not perform ILEET, performing ILEET to a individualised haemodynamic challenge using the high/low criteria employed in this investigation does not identify a specific exercise intensity that can consistently effect RBP adaptation. It is apparent that prescribing isometric exercise intensity to 85% HR_{peak} may be more successful at inducing a possible specific haemodynamic stimulus for RBP reductions after IET than that seen when performing IET to an individualised $\%EMG_{peak}$. This may be attributable to the specific characteristics or the magnitude of a haemodynamic stimulus at 85% HR_{peak} that are more consistent for a RBP adaptation than that seen when performing IET to an individualised $\%EMG_{peak.}$ However as the haemodynamic response to performing isometric exercise to a 85% HR_{peak} has not been assessed, it remains unidentified as to what these characteristics and / or magnitude may be.

The results of this current study also highlight that in some participants, performing IET to a $\%EMG_{peak}$ as low as $10\%EMG_{peak}$ was sufficient enough to induce a haemodynamic challenge that led to RBP adaptation. In contrast, some participants performed IET to 35% EMG_{peak} which was not sufficient enough to induce an appropriate haemodynamic challenge for RBP reduction. This demonstrates that each participant may respond to an individual level of haemodynamic stimulus that differs between each participant. It may therefore be the case that some participants are more sensitive to the haemodynamic challenge and may therefore be more likely to adapt in response to a lower level of haemodynamic challenge. In these particular participants, vascular adaptation may occur in response to a lower level of haemodynamic challenge, which in turn may induce a RBP reduction. This may go some way as to explaining why some participants in the LO group experienced RBP reductions following IET despite performing IET to a low haemodynamic stimulus. Likewise, not all participants in the HI group experienced a RBP reduction, despite performing IET with a high haemodynamic challenge. This implies that these participants may be less sensitive to a

haemodynamic challenge and therefore require a much greater haemodynamic challenge (or another stimulus yet to be identified) to induce vascular adaptation for RBP reduction than that induced in this current study. Unless the specific level of haemodynamic stimulus required for vascular adaptation is identified for each individual participant, performing IET to either a high or low haemodynamic stimulus is not a successful method to determine the appropriate $\%EMG_{peak}$ exercise intensity for IET to consistently induce significant RBP adaption.

One possible theory to explain the difference in findings between this current study and Badrov et al (2013), and that of Baross et al (2012) may relate to the baseline BP classification of the participants utilised in these studies. It is well established that hypertension is associated with a degree of endothelial dysfunction, in that the endothelium demonstrates a reduced responsiveness to dilate in the presence of potent vasodilators (Iiyama et al, 1996; Perticone et al, 2001; Taddei et al, 2000). Several studies have demonstrated that participants with endothelial dysfunction respond well to repeated exposure to a SS stimulus, with significant improvements in endothelial function often observed (Clarkson et al, 1999; Edwards et al, 2004; Hambrecht et al, 1998; Higashi et al, 1999; Linke et al, 2001; McGowan et al, 2004). In contrast, normotensive, healthy participants (who have a normal endothelial function) tend to not experience improvements in endothelial function after exercise training (Green et al, 2012; Maiorana et al, 2001b; McGowan et al, 2007a; Rakobowchuck et al, 2005b). This may have implications for the relative success of an haemodynamic stimulus (as opposed to previous used methods of determining IET intensity), as Green et al (2004) and Maiorana et al (2003) suggest that it may be difficult to enhance normal vascular function in healthy subjects, and therefore it may be harder for a haemodynamic stimulus to induce a vascular adaptation that may lead to a reduction in RBP. The pre-hypertensive participants utilised by Baross et al (2012) may have had a degree of endothelial dysfunction which responded well to a haemodynamic exercise training stimulus, leading to RBP reductions. In contrast, the participants utilised in this current study and that of Badrov et al (2013) were healthy normotensives, and therefore probably had normal endothelial function. This may explain their reduced responsiveness to a haemodynamic exercise training stimulus for RBP reductions. Furthermore, this also suggests that different exercise training stimuli may be responsible for the reductions in RBP after IET in hypertensive populations versus normotensive populations.

The second part of the current study investigated the role of conduit AD remodeling as the mechanism for RBP reductions following IET. It is reasonably well established that SS mediates conduit AD remodeling (Green et al, 2012; Green et al, 2010; Kamiya & Togawa, 1980; Tinken et al, 2010) via upregulation of the potent vasodilator NO (Sessa et al, 1994; Rudic et al, 1998). Based on the observation that acute bouts of isometric exercise can induce a significantly elevated post-exercise BF and SS response (see Chapters 3, 4 and 5), it was hypothesised that repeated exposure to a large post-exercise haemodynamic stimulus during IET may lead to the upregulation of NO synthase and subsequent NO production at rest. This may cause a remodeling process that widens the diameter of conduit arteries, which may reduce TPR, and consequently lower RBP following IET. Despite this hypothesis, local conduit AD changes in the common femoral artery did not correlate well with significant sub-group changes in resting DBP and MAP that were observed within the HI and LO groups of this study. This suggests that local conduit AD remodeling is not the primary mechanism for RBP reductions after IET in young normotensive male participants.

Only one other study has examined the role of local conduit artery remodeling in RBP reductions after IET. Baross et al (2012) observed significant reductions in resting SBP, DBP and MAP in middle aged men that performed 8 weeks of an bilateral ILEET program at 85% HR_{peak}. Changes in common femoral AD at rest also correlated with these RBP reductions, leading Baross et al (2012) to suggest that possible SS mediated, local AD changes may be responsible for the BP adaptations observed. It is apparent that the results of this current study are not in agreement with the findings of Baross et al (2012), in that significant RBP changes within the HI and LO groups of this current study were not correlated to changes in common femoral AD. However, if the participants of this current research study had a reduced responsiveness to a haemodynamic SS stimulus due to a structurally normal functioning vasculature, they may also be less likely to experience conduit AD remodeling. This may go some way as to explaining why local conduit AD remodeling appears to not play a role in the RBP reductions observed after IET. This would also suggest that the RBP reductions that normotensive and pre-hypertensive / hypertensive populations experience after IET may be governed by different mechanisms.

This current study also explored the role that non - invasive cardiovascular measures (such as $\dot{Q}$, IVRT, LVET, TPR, HRV, systemic conduit AD remodeling) may have in the reductions in RBP associated with IET. The results of this current study suggest that these non - invasive

cardiovascular measures do not contribute to the within group DBP and MAP reductions observed in the HI and LO groups. This is a relatively unexpected finding as Wiley et al (1992) state that reductions in RBP must come about via adjustments in the components that determine BP - $\dot{Q}$ and TPR. It is possible that since the methods used to record changes in some of these variables were affected by high variability, these particular methods may not have been precise enough to identify small physiological changes in these variables using the sample size utilised in this current study. Therefore these measures were subject to type II error, which may have resulted in the failure to detect true significant changes within these variables over the 8 week training period, producing instead a non-statistically significant result. These non-statistically significant results must be interpreted with care.

Despite the findings of this current study, it should be acknowledged that whilst no significantly different changes in RBP were observed between the HI, LO and CON groups of this study, analysis of the participants that performed bilateral ILEET (trainers) versus the CON group revealed that a number of trainers had a significant reduction in resting SBP and MAP of ~ 2mmHg, whereas no significant reduction in RBP was observed in the CON group. Whilst it is recognised that the CON group had fewer participants than the group performing bilateral ILEET, this result supports the efficacy of the bilateral ILEET program utilised in this current study to induce significant reductions in RBP, similar to the results established by that of Wiles et al (2010), Devereux et al (2010b) and Baross et al (2012).

Conclusion

The findings presented in this current chapter have shown that whilst bilateral ILEET may be successful at inducing RBP changes in normotensive populations, performing bilateral ILEET using an individualised post-exercise haemodynamic challenge to determine IET intensity does not identify an intensity for optimal RBP adaptation. This suggests that a high post-exercise haemodynamic challenge at higher intensities of isometric exercise may not be a primary physiological stimulus for RBP adaptation following IET. However it must be considered that the use of an individualised $\%EMG_{peak}$ exercise intensity and a slight decrease in the haemodynamic challenge over the training period may have influenced the effectiveness of the post-exercise haemodynamic challenge to elicit a greater magnitude RBP reduction at higher isometric exercise intensities.

In addition the results of this investigation strongly suggest that conduit AD remodeling is not the primary mechanism for RBP reductions after IET. This may in part be due to a lack of responsiveness to a haemodynamic stimulus in healthy normotensive populations. Furthermore, non-invasive cardiovascular variables such as $\dot{Q}$, IVRT, LVET, TPR, HRV and systemic AD remodeling appear to not play a role in the observed sub group RBP reductions observed in the HI and LO groups of this study. The methods used to measure these variables should first be evaluated in terms of their sensitivity to detect small but significant changes that may occur in normotensive participants before these variables can be dismissed as contributing mechanism(s) to the RBP reductions after IET.

Chapter 7

General Discussion

Chapter 7: General Discussion

7.1 General Discussion

This research thesis has reported data related to the haemodynamic challenge present during isometric exercise that may induce cardiovascular adaptation in response to IET. The data presented has been arranged into Chapters 3, 4, 5 and 6. Chapters 3, 4 and 5 specifically explored the local blood haemodynamic response to acute isometric leg exercise. The purpose of this was to understand the haemodynamic response in greater depth, and to establish whether the BF (Chapter 3), SR (Chapter 4) and shear pattern (Chapter 5) response to isometric exercise might be considered as a haemodynamic stimulus for RBP adaptation following IET. Based upon results that supported this contention, Chapter 6 of this research thesis then explored the influence of the local blood haemodynamic challenge on cardiovascular adaptations to IET, in particular RBP adaptation. This data was collected with the aim of investigating the following primary research question:

Does a haemodynamic challenge in response to isometric exercise play a significant role in the blood pressure reductions commonly observed after isometric exercise training?

The main findings of the studies included in this thesis that addressed the primary research question were:

1. There is a significant relationship between the acute BF response in the common femoral artery during and immediately after a single bout of isometric bilateral leg extension exercise of increasing exercise intensity. The post-isometric exercise BF response was of greater magnitude when compared to the BF during exercise at higher exercise intensities.
2. There is a significant relationship between the acute SR response in the common femoral artery during and immediately following a single bout of isometric bilateral leg extension exercise and increasing exercise intensity. This increase in SR did not correspond with increased common femoral artery vasodilation. It is also apparent the magnitude of the SR response was greater immediately following isometric leg extension exercise, as opposed to during exercise contraction as exercise intensity increases.
3. There is a significant relationship between the acute ASR and RSR response in the common femoral artery during and immediately following a single bout of isometric

bilateral leg extension exercise and increasing exercise intensity. Only ASR demonstrated a greater magnitude response post-exercise as exercise intensity increased. In addition, OSI in the common femoral artery only correlated with increasing exercise intensity immediately following isometric bilateral leg extension exercise.

4. The haemodynamic response to high intensities of acute isometric exercise may provide an effective challenge to the cardiovascular system, that upon repeated exposure via IET, may induce cardiovascular adaptation.
5. Performing IET to a high post-isometric exercise haemodynamic challenge does not induce a significantly greater adaptation in RBP from that seen when performing IET to a low post-isometric exercise haemodynamic stimulus or CON group. This suggests that the post-isometric exercise haemodynamic challenge may not be the primary stimulus responsible for inducing adaptations in physiological mechanisms that lead to a reduction in RBP following IET.
6. Common femoral AD changes did not correlate with sub-group BP changes suggesting that common femoral AD adaptation is not the mechanism for BP reductions after IET.
7. Other non-invasive cardiovascular measures, including systemic AD changes, TPR, $\dot{Q}$, IVRT, LVET and HRV did not correlate with sub-group BP changes.

7.2 According to the findings of studies 2, 3 and 4 (presented in Chapters 3, 4 and 5), could the haemodynamic response to acute bouts of isometric exercise be considered as a physiological stimulus for resting blood pressure reductions after isometric exercise training?

The work of Devereux et al (2010b) identified that normotensive participants who trained at higher isometric exercise intensities, and therefore experienced greater levels of fatigue, correlated well with greater reductions in RBP than those participants that trained at a lower isometric exercise intensity and therefore experienced less levels of fatigue. This suggested that isometric exercise intensity may be an important training stimulus for BP reductions following IET. However the physiological stimulus that may be associated with increased isometric exercise intensity for greater magnitude RBP reduction remains to be identified. This physiological stimulus must be more prominent at higher intensities of isometric exercise and be related to the magnitude of fatigue experienced. The findings presented in Chapters 3, 4 and 5 of this thesis demonstrate

that the haemodynamic challenge present at high intensities of acute isometric bilateral leg extension exercise could feasibly be considered as the physiological stimulus for RBP reductions after IET. Several observations from Chapters 3, 4 and 5 of this thesis support this conclusion, and will each be summarised below.

The main finding from the results of Chapters 3, 4 and 5 was that the haemodynamic response to an acute bout of isometric exercise was exercise intensity dependent, such that as exercise intensity increased during the DIIET, the greater the BF, SR and shear pattern response during and immediately after isometric bilateral leg extension exercise. Coinciding with this, indices of fatigue (EMGamp and EMGfreq) also increased relative to exercise intensity, demonstrating that the higher the intensity of isometric exercise, the greater the level of fatigue experienced. As previously discussed, it is likely that the physiological stimulus for greater RBP adaptation to IET may be more prominent at higher isometric exercise intensities which induce greater levels of fatigue (a theory based upon the work of Devereux et al, 2010b). It is therefore suggested that isometric exercise induced haemodynamics could be considered as this physiological stimulus, as the magnitude of the haemodynamic challenge is greatest at higher intensities of isometric bilateral leg extension exercise that also induces greater levels of fatigue.

Specifically Chapter 3 demonstrated that BF measured in the common femoral artery during an acute bout of isometric bilateral leg exercise increased relative to exercise intensity. It is suggested that the increase in BF observed came about via an increase in central command and the muscle chemoreflex response (Gandevia & Hobbs, 1990). Indeed previous evidence suggests that the level of central command experienced reflects the level of motor unit activation (Mitchell et al, 1983; Smolander et al, 1988). It is not surprising that the central command mediated increase in BF that occurs via a $\dot{Q}$ modulated increase in BP (Bezucha et al, 1982; Goodwin et al, 1972, Martin et al, 1974; Shoemaker et al, 2007) demonstrates an exercise intensity dependent response, since the number of motor units recruited increases at each exercise intensity to meet the higher target $\%EMG_{peak}$ (Schibye et al, 1981; Taylor et al, 1988;). As fatigue and consequently metabolite accumulation occur at higher exercise intensities, type IV muscle afferents communicate the metabolic conditions of the working muscle to higher centers of the brain, which in turn initiates an exercise pressor reflex response (Alam & Smirk, 1937; Asmussen & Nielsen, 1964; Coote et al, 1971; McCloskey & Mitchell, 1972; Rowell & O'Leary, 1990). As isometric exercise intensity and level of fatigue increased in this

study (study 2, Chapter 3), it is suggested that the pressor reflex response may have increased BF accordingly via a local vasodilatory response combined with increased peripheral sympathetic vasoconstriction in an attempt to maintain BF to the exercising muscle, so that the delivery of O_2 and the washout of metabolites was maintained (Gandevia & Hobbs, 1990). If the level of fatigue experienced at higher exercise intensities determines the magnitude of the BF response to isometric exercise, it seems plausible to suggest that a BF haemodynamic challenge at higher isometric exercise intensities could be considered as the physiological stimulus for RBP reduction after IET.

Chapter 3 also established that the magnitude of the immediate post-exercise BF response was determined by isometric exercise intensity. Taylor et al (1988) suggested that the magnitude of post-exercise BF is reflective of the magnitude of the O_2 debt and subsequent metabolite accumulation stimulus for a pressor reflex response. At higher levels of fatiguing isometric exercise, the degree of intramuscular pressure is increased, which may result in an inadequate blood supply to the exercising muscle to wash out accumulating metabolites (Lind & McNicol, 1967; Rowell & O'Leary, 1990). Upon the release of contraction, these dilatory metabolites may be released into the circulation, eliciting a post-exercise dilatory response, which consequently increases post-exercise BF.

A important point to note is that common femoral artery BF continued to increase during exercise, implying that at no point did the artery blood supply become reduced due to a restrictive increase in intramuscular pressure as a result of the intensity of static contraction. However it should be taken into consideration that the haemodynamic measures taken in this study were recorded upstream from the site of actual mechanical compression induced by the isometric bilateral leg extension exercise, which may help to explain why this was the case. Ideally haemodynamic measurements would have been taken from the profunda femoral artery, as this is located further downstream and directly supplies the quadriceps musculature with a blood supply (Wray et al, 2005). Due to its deep location within the quadriceps musculature, it is not possible to visualise the artery during isometric bilateral leg extension exercise. As the common femoral artery directly feeds blood to the profunda femoral artery (Wray et al, 2005), recording the haemodynamic response at the upstream common femoral artery level may provide some insight into the haemodynamic response at the downstream profunda level.

Recording the haemodynamic response in the common femoral artery may also give insight into the ability of the cardiovascular system to increase perfusion flow in response to the increasing isometric exercise intensity during a DIIET. However this does not establish whether this increased perfusion flow is successful at overcoming the increased intramuscular pressure brought about by isometric contraction at the profunda artery level. The effect that increasing levels of intramuscular pressure (as a result of performing isometric exercise to a %EMG) may have on the ability of the cardiovascular response to supply an efficient oxygenated blood supply to the exercising muscle remains to be established. Chapter 3 also documented that indices of fatigue (EMGamp and EMGfreq) increased relative to isometric exercise intensity. This would imply that as exercise intensity increases, there must be some mismatch between O_2 demand and O_2 supply, suggesting that increased perfusion pressure may not be able to overcome higher levels of intramuscular pressure entirely. Subsequent metabolite accumulation may occur, which upon release of contraction may influence the magnitude of the post-exercise BF response. It is suggested that the level of fatigue created during high intensities of isometric exercise also determines the magnitude of the immediate post-exercise BF response, which as such, may also be considered as the physiological stimulus for RBP reduction after IET.

An interesting observation from Chapter 3 is that whilst it is likely that the level of fatigue experienced during high intensity isometric exercise may influence both the magnitude of the BF response during exercise and in the immediate periods after, the magnitude of the post-exercise BF response is greater than that seen during isometric exercise. This was reflected in theΔBF data, which demonstrated that as isometric exercise intensity increased, the percentage change in BF from contraction to the immediate periods post-contraction also increased. From a theoretical perspective this is rather unpredicted since it would be expected that the exercise period would create the greatest BF response to meet the immediate demand of fatigue, as opposed to post-exercise where no immediate fatigue stimulus is present. This finding may indicate the effectiveness of vasodilatory metabolites and a subsequent downstream vasodilation facilitating an increased BF response post-exercise. As discussed in the previous paragraph, it is probable that increased intramuscular pressure may have resulted in metabolite accumulation (Lind & McNicol, 1967; Rowell et al, 1990). Release of isometric contraction may have released these metabolites into the blood supply and induced a vasodilatory response within the vasculature. As Chapter 4 demonstrates

however, an exercise intensity dependent dilatory response did not occur in the common femoral artery in response to an increased flow stimulus post-exercise. It is more probable that a vasodilation response may have occurred at the downstream profunda level as this artery would be directly affected by the mechanical compression of the quadriceps musculature, and thus may be the direct site that vasodilatory metabolites would be released into the blood supply as opposed to the upstream common femoral artery level. As proposed by Segal & Kurjiaka (1995), a dilatory response downstream may promote an increase in BF upstream, as resistance to flow would be reduced. This may go some way as to explaining why a greater post-exercise BF was observed post-exercise in the common femoral artery. In addition, the increased BF post-exercise from the common femoral artery may also influence a vasodilatory response downstream in the profunda artery as a result of an increased SS mediated vasodilation response (Corretti et al, 2002; Muller et al, 1997; Niebauer et al, 1996; Pyke & Tschakovsky, 2005; Silber et al, 2001; Thijssen et al, 2011).

The results of Chapter 4 demonstrate that SR post-exercise also increased relative to isometric exercise intensity, and is of greater magnitude in the post-exercise period as opposed to the SR during exercise (as demonstrated by ΔSR data). Increased SS is thought to stimulate endothelial cells to release vasodilators such as NO, prostacyclin, endothelin and adenosine (Joyner & Dietz, 1997; Koller & Kaley, 1990; Pohl et al, 1986; Wilson & Kapoor, 1993), which may subsequently result in endothelial dependent vasodilation with the aim of normalising the increased SS stimulus (Dimmeler & Zeiher, 2003; Koller & Kaley, 1991; Niebauer & Cooke, 1996; Zarins et al, 1987). The magnitude of SS post-exercise may also be enhanced by the contribution of increased perfusion BF from the exercise bout to the post-exercise BF response. As the results presented in Chapter 3 demonstrate, the observed increase in BP produced during exercise continues into the post-exercise period. This may contribute to higher post-exercise BF values, which in turn may provide a greater SS stimulus for a post-exercise endothelial dependent vasodilatation response to occur in the downstream profunda artery. It seems possible that higher intensity isometric exercise may also be responsible for the greater magnitude post-exercise SR response observed in this study. This finding adds further support to the hypothesis that a haemodynamic challenge may be considered as the physiological stimulus for RBP reductions after IET.

When viewed together as a whole, the results of Chapter 3 demonstrate that the BF response during and immediately after isometric exercise is consistently elevated as isometric exercise intensity and consequently fatigue increase. Whilst this result provided support for the contention that the haemodynamic challenge may be considered as the physiological stimulus for RBP reduction following an appropriate period of IET, Chapters 4 and 5 examined whether the characteristics of this haemodynamic stimulus had the potential to be able to induce a possible cardiovascular adaptation to IET that may have led to a reduction in RBP. Specifically a haemodynamic stimulus is associated with adaptations to the arterial system, which include improvements in endothelial function and AD remodeling (Green et al, 2010; Langille & O'Donnell, 1986; Kamiya & Togawa, 1980; Tinken et al, 2010). However the characteristics of this haemodynamic stimulus must be specific for an adaptation process to take place (Johnson et al, 2011). Therefore studies 3 ad 4 (chapters 4 and 5) explored whether acute isometric exercise can produce the necessary haemodynamic characteristics for a potential vascular adaptation to occur, that may subsequently also lead to a reduction in RBP. Specifically the SR, ASR, RSR and OSI response to isometric exercise were assessed before a definitive conclusion could be established as to whether the haemodynamic response to an acute bout of isometric exercise could be considered as the physiological stimulus for RBP reductions following IET.

Chapter 4 demonstrated that the SR response to an acute bout of isometric bilateral leg extension exercise and immediately following exercise was also exercise intensity dependent. The magnitude of the post-exercise SR response was also greater than that seen during contraction at all isometric exercise intensities (as reflected in ΔSR data). However, despite these observed increases in exercising and post-exercise SR, the magnitude of this response was less than that seen during dynamic exercise (Gonzales et al, 2009; Johnson & Wallace, 2012; Wray et al, 2005). It is likely that the nature of the repetitive short contractions during dynamic exercise act as a pump, which in turn may create a larger BF and SS response (Casey & Hart, 2008; Laughlin, 1987).

Chapter 5 also established that the observed SR response to an acute bout of isometric leg extension exercise is characterised by both increases in ASR and RSR. Specifically, the magnitude of exercise ASR and RSR; and the post-exercise ASR, post-exercise RSR and post-exercise OSI were significantly correlated with increasing isometric exercise intensity. The change in ASR from contraction to post-exercise was also

significantly correlated with increasing exercise intensity, suggesting that the magnitude of the post-exercise ASR response was greater in the immediate periods post-exercise. This significant finding was not observed for RSR and OSI variables.

Typically vascular adaptations are thought to be mediated by the repeated exposure to a SS stimulus, that enhances eNOS expression and increases NO availability at rest (Green et al, 2012; Green et al, 2010; Kamiya & Togawa, 1980; Tinken et al, 2010) with the aim of increasing AD to normalise the increased SS stimulus (Dimmeler & Zeiher, 2003; Koller & Kaley, 1991; Niebauer & Cooke, 1996; Zarins et al, 1987). Previous research by Green et al (2005) suggested that a SS stimulus characterised by both ASR and RSR (and therefore oscillatory in nature) may present a greater stimulus for NO up regulation, and subsequent vascular adaptation. As the results of Chapter 5 demonstrate, isometric bilateral leg extension exercise is characterised by exercise intensity dependent increases in ASR and RSR, both during exercise and immediately after. These observed increases in ASR are thought to be SBP mediated (Thijssen et al, 2009a). Increases in RSR are likely attributable to increases in intramuscular pressure in line with muscle tension development during exercise (Barcrost and Dornhorst, 1949; Green et al, 2005; Lutjemier et al, 2005; Sadamoto et al, 1983), and continue to remain elevated in the post-exercise period due to the accumulation of extravascular fluid (Crenshaw et al, 1997). This shear pattern response during and immediately after isometric contraction may provide an effective stimulus for the up regulation of NO, and subsequent vascular adaptation, which in turn could elicit a reduction in RBP following IET.

Whilst both the ASR and RSR response increased relative to isometric exercise intensity during and immediately after isometric exercise, OSI did not demonstrate the same response. The results of Chapter 5 demonstrated that the OSI response during isometric exercise did not correlate with increases in isometric exercise intensity. This was a surprising finding given the observed exercise intensity dependent increases in both RSR and ASR during exercise. Oscillatory shear index measured immediately post-exercise did however correlate with increasing isometric exercise intensity, but upon further analysis of the data it was identified that as exercise intensity increased, the oscillatory nature of the SS stimulus became more laminar. This response may be due to the dominance of the ASR component as a result of increased perfusion pressure from exercise contraction that continues into the post-exercise periods (to then decrease

gradually). Nonetheless, there still is a RSR component to the flow, which is thought to be attributable to an elevated intramuscular pressure post-contraction as a result of extravascular fluid accumulation during exercise (Crenshaw et al, 1997). As post-exercise OSI increased relative to isometric exercise intensity, it is plausible to suggest that this post-exercise OSI may provide the correct characteristics to a haemodynamic stimulus that promotes the expression of eNOS and production of NO. Thus it is considered that this post-exercise haemodynamic challenge at higher isometric exercise intensities may be the exercise training stimulus for RBP reductions after IET.

In summary, Chapters 3, 4 and 5 aimed to investigate and establish the nature and characteristics of the local haemodynamic response to acute isometric bilateral leg exercise of increasing $\%EMG_{peak}$ exercise intensity, specifically examining BF, SR and the shear pattern (ASR, RSR and OSI) during and post-exercise. This was completed as a prerequisite to the wider research aim of this thesis to examine the role of a possible haemodynamic stimulus induced by a bout of high intensity IET that may be the physiological stimulus for RBP reduction following IET. Based upon the premise established by Devereux et al (2011), that the greater the $\%EMG_{peak}$ isometric exercise intensity during training and therefore the greater level of fatigue induced, the greater the RBP reduction post intervention; it is plausible to suggest that the stimulus for RBP reduction whilst performing IET may be at its greatest when the exercise is performed at its highest possible intensity and inducing the greatest level of fatigue. Therefore the haemodynamic response to acute isometric exercise in this chapter was examined relative to exercise intensity with the aim of identifying a possible haemodynamic stimulus that could be investigated further as a potential mechanism causing RBP reduction following IET. It is clear from the results presented that a number of the haemodynamic variables measured in studies 2, 3 and 4 were strongly correlated with increasing REI and therefore fatigue. In particular, it appears from the correlation coefficient analysis that the peak response of haemodynamic variables post-exercise could be considered as a possible stimulus for BP reductions after a bout of IET (Appendix 6 page 265). Furthermore the post-exercise haemodynamic response is of a greater magnitude than that seen at baseline or during contraction. It could therefore be suggested that at higher exercise intensities the increased post-exercise peak BF response, which is characterised by an increase in SR, and has a shear pattern that includes an increase in both ASR and RSR, but is more laminar than pulsatile, may provide a stimulus for RBP reductions after a bout of IET. A critical point to note is that

the increase in SR during post-exercise periods did not influence the common femoral AD response to isometric leg exercise. It has ben established that the common femoral artery is largely unresponsive to a shear stimulus due to its large diameter (Wray et al, 2005), and it may be that the increased shear stimulus in this study may have a more direct effect on the vasculature downstream in the profunda artery, which is more responsive to a shear stimulus (Wray et al, 2005). The findings of studies 2, 3 and 4 suggest that a post-exercise haemodynamic stimulus may be considered as the physiological stimulus to induce cardiovascular adaptation that may lead to RBP reductions following IET.

7.3 According to the findings of study 5 (Chapter 6), is the post-exercise haemodynamic challenge the physiological stimulus for blood pressure reductions after isometric exercise training?

Based on the findings of studies 2, 3 and 4, it was hypothesised that the post-exercise haemodynamic challenge seen at higher intensities of isometric exercise may act as a physiological stimulus for RBP reductions following IET. To examine this hypothesis 36 participants performed 8 weeks of bilateral ILEET to a specific individualised post-exercise haemodynamic challenge. One group of participants performed high intensity bilateral ILEET to provide exposure to a high magnitude post-exercise haemodynamic challenge (HI group). A second group performed low intensity bilateral ILEET to provide exposure to a low magnitude post-exercise haemodynamic challenge (LO group). A third group acted as control, and did not perform bilateral ILEET and therefore were exposed to no exercise induced haemodynamic challenge (CON group). It was hypothesised that the HI group performing high intensity ILEET with a high magnitude post-exercise haemodynamic challenge should experience the greatest reductions in RBP compared to the CON group. The results of this study (as presented in Chapter 6) demonstrated that there was no significant difference in the blood pressure change between the HI, LO and CON groups. This suggests that a post-exercise haemodynamic challenge present at high intensity isometric exercise may not be the physiological stimulus for reductions in RBP commonly observed after IET.

This finding was somewhat unexpected since previous work completed by Baross et al (2012) indicates that a haemodynamic stimulus may be the exercise training stimulus for RBP reductions in pre-hypertensive participants. Whilst Baross et al (2012) did not

directly determine the presence of a haemodynamic stimulus during isometric leg extension exercise, an adaptation to common femoral AD occurred only in the high intensity exercise training group (85%HR_{peak}), versus the low intensity exercise training group (75%HR_{peak}). These changes in femoral AD also correlated with RBP reductions for the high intensity group, suggesting that conduit AD remodeling may be an important mechanism involved in this RBP adaptation seen following high intensity IET. Baross et al (2012) proposed that it was likely that repeated exposure to an increased haemodynamic SS challenge via high intensity IET may have mediated this adaptation, which consequently caused a reduction in RBP as observed in the highly trained group. This is based upon evidence that suggests repeated bouts of SS across the vascular endothelium induces improvements in both vascular function and structure (Clarkson et al, 1999; Green et al, 2004; Green et al, 2010; Kamiya & Togawa, 1980; Langille & O'Donnell, 1986; McGowan et al, 2006; Niebauer & Cooke, 1996; Tinken et al, 2010). However, as identified in this current study, exposure to a increased haemodynamic challenge during high intensity IET does not appear to be the exercise training stimulus for RBP reductions in normotensives, as no significant differences in the change in RBP after IET was observed between groups, regardless of the level of haemodynamic challenge participants were exposed to. Whilst it is possible that the reductions in RBP that occur after IET in pre-hypertensive and normotensive participants may be mediated by different physiological stimuli, it is also plausible to suggest that the changes in AD observed by Baross et al (2012) in the highly trained group may be as a result of an adaptation to resting vascular tone via modulation of sympathetic tone (Taylor et al, 2003). In this instance, it is unlikely that a haemodynamic challenge would have acted as the physiological stimulus to initiate sympathetic modulation adaptation. Therefore it is possible that a haemodynamic challenge may not be the physiological stimulus for RBP reductions after IET.

There is one key difference between this current study and that of Baross et al (2012) that may explain why a haemodynamic challenge in this current study is not effective as a physiological stimulus for RBP reductions. The participants in the study of Baross et al (2012) performed IET to 85% $HR_{peak,}$ which has previously been proven as a successful exercise intensity for inducing RBP reductions following IET (Wiles et al, 2010). Baross et al (2012) then observed that a conduit AD remodeling process had taken place than coincided with RBP adaptations, thus implying that a haemodynamic stimulus may have been induced by performing IET at 85% HR_{peak}. This current study

performed IET to an individualised %EMG_{peak} that theoretically remained constant for 8 weeks. It is unknown as to what %EMG_{peak} exercise intensity is successful for RBP reductions following IET. It is plausible to suggest that this current study did not perform IET at the correct exercise intensity to induce the necessary haemodynamic stimulus for RBP adaptation. This may be in relation to the magnitude or the characteristics of the haemodynamic stimulus. Since Baross et al (2012) did not measure the haemodynamic stimulus at 85% $HR_{peak,}$ it is currently unknown as to what the specific characteristics or magnitude of a haemodynamic stimulus for optimal RBP reduction may be.

It could be suggested that the characteristics of the haemodynamic challenge that the participants in the high trained group of this current study were repeatedly exposed to was not of an optimal nature to enhance eNOS expression and NO bioavailability at rest for a vascular adaptation that may consequently induce RBP adaptations following IET. A haemodynamic stimulus that is oscillatory in nature and characterised by both ASR and RSR may present a greater stimulus for the up regulation of NO due to the repeated drawing of BF across the endothelium (Green et al, 2005; Gonzales et al, 2009; Gonzales et al, 2008; Hutcheson & Griffith, 1991). It was established in study 3 and 4 of this current thesis that the post-exercise haemodynamic challenge was characterised by an increased SR, with a shear pattern that includes an increase in both ASR and RSR. As exercise intensity increased, the oscillatory behavior of this shear pattern became more laminar in nature. This may have been attributable to the large dominance of antegrade forward flowing shear resulting from increased exercise perfusion pressure and a probable downstream FMD response in the profunda artery (study 4 demonstrates this post-exercise response). Whilst the continued presence of RSR would have ensured that the post-exercise response was in part oscillatory, the increased presence of laminar flow may have affected the ability of this stimulus to influence optimal eNOS expression and production of NO (Green et al, 2005; Gonzales et al, 2009; Hutcheson & Griffith, 1991). Indeed Green et al (2005) demonstrated that during handgrip exercise (characterised by predominately laminar antegrade flow) NO inhibition did not reduce BF, thus suggesting that antegrade flow may not provide a stimulus for up regulation of NO. As such, this may have reduced the potential for a vascular adaptation to occur in the HI group of this current study, which in turn may go some way to explaining the failure of a high haemodynamic challenge to induce a RBP change in the HI group of this study that is significantly different from the LO and CON groups.

Whilst the work of Green et al (2005) supports a shear stimulus characterised by both ASR and RSR for the up regulation of NO, Tinken et al (2009) suggests that a high ASR is more favorable of the up regulation of NO. This is based upon the observation that a high ASR in the brachial artery during handgrip exercise significantly improved FMD when compared to a RSR influence (Tinken et al, 2009). Flow mediated dilation is based on the principle that increased flow induced SS stimulates the endothelium to release NO, leading to a dilatory response (Segal & Kurjiaka, 1995). It is evident that the ASR shear influence (as observed in study 4). However since there was no significant difference for BP change between the training groups of this study, it is suggested that a haemodynamic stimulus characterised by a large influence of ASR may not be an effective IET stimulus to induce RBP reduction.

Thijssen et al (2009a) identified that when the oscillatory behavior of flow in the brachial artery is characterised by high levels of RSR, FMD is significantly impaired in a dose dependent manner. This would suggest that high levels of RSR are detrimental to the expression of eNOS and availability of NO. The high post-exercise haemodynamic challenge in this current study was characterised by a lower influence of RSR. As there was no significant differences in BP change between the HI, LO and CON groups of this study, it could be suggested that a low influence of RSR is not an effective characteristic to induce specifically RBP change in the HI group after IET. In contrast, Gonzales et al (2008) demonstrated that increased RSR decreased vascular resistance in the common femoral artery, possibly due to the influence of the RSR on the oscillatory behavior of BF. Therefore it is plausible to suggest that the RSR component of the high post-exercise haemodynamic stimulus of this current study may not have been of great enough strength to directly influence the up regulation of eNOS and NO bioavailability for subsequent BP adaptation to IET via vascular function / structure modifications. As the specific level of RSR for vascular adaptation has not been identified, it is unknown as to whether the RSR experienced by the HI group of this training study was sufficient enough to induce a vascular adaptation. This said, it is apparent that a post-exercise haemodynamic challenge characterised by a low influence of RSR at high intensity bilateral ILEET may not the physiological stimulus for RBP adaptation.

A further explanation that could provide insight into why the post-exercise haemodynamic challenge utilised in this current study did not induce a statistically significant RBP change between the groups of this training study may be related to the

magnitude of the haemodynamic challenge that the participants were exposed to. It may be possible that the post-exercise haemodynamic challenge was not of a large enough magnitude to elicit enhanced eNOS expression and subsequent NO availability that would be required for a common femoral artery adaptation, and thus a consistent RBP reduction following IET in all participants of the HI group. As previously discussed, it is evident that vascular adaptation is SS mediated (Clarkson et al, 1999; Green et al, 2010; Green et al, 2004; Kamiya & Togawa, 1980; Langille & O'Donnell, 1986; McGowan et al, 2006; Niebauer & Cooke, 1996; Tinken et al, 2010). It has not yet been established as to the minimum level of SR required to induce vascular adaptation. Whilst Tinken et al, (2010) is one of the few studies to have explored the role of exercise induced SR mediated AD remodeling, the magnitude of the SR stimulus participants underwent during rhythmic handgrip exercise training was not reported. Tuttle et al (2001) reports that values of ~4000 s^{-1} were used to induce AD remodeling, however this was in vivo, and therefore not comparable to an exercise model SR stimulus such as that utilised in this current research thesis. Research examining the acute response of SR immediately post-exercise have documented peak increases in SR of ~70 s^{-1} in the brachial artery after high intensity running exercise (Johnson & Wallace, 2012). This is in comparison to the smaller peak post-exercise SR of 40.8 ± 5.9 s^{-1} documented in the common femoral artery in this current research thesis (Chapter 4) after high intensity bilateral isometric leg extension exercise. Therefore it could be suggested that the intensity of bilateral ILEET utilised in this current research study may not have induced a peak SR stimulus of a great enough magnitude at higher exercise intensities to induce conduit artery adaptation, and subsequent statistically significant RBP change in all participants of the HI group. This would imply that there may be a minimum threshold of SS needed to induce vascular adaptation, that not all participants in the HI group met, to lead to a RBP adaptation statistically greater than that observed in the LO group. Furthermore, it must also be taken into consideration that some participants in the LO group also experienced a reduction in RBP following IET, despite performing IET with a low post-exercise haemodynamic challenge. This would suggest that for some participants a low level of haemodynamic stimulus may have been sufficient enough to lead to a RBP adaptation. This highlights that the minimum threshold for SS to induce vascular adaptation may differ between individuals. Indeed, whilst the participants within this current study were all classified as normotensive, it is likely that the robustness of BP homeostasis and adherence to an individual 'norm' may be stronger in some participants then others. As a result some

individuals may have a greater minimum SS threshold for vascular adaptation than other individuals, and consequently these participants would need to be exposed to higher values of SS during IET to induce vascular adaptation for significant RBP adaptations to be observed. The results of study 4 therefore suggest that the specific magnitude of a haemodynamic challenge needed to consistently induce conduit AD adaptation, and in turn a RBP adaptation is largely varied depending upon the specific individual and an individualised minimum SS threshold level for vascular adaptation. As such, unless each individual's specific SS threshold level for vascular adaptation is identified, prescribing IET based upon the magnitude of the haemodynamic challenge is not a successful method to induce consistently significantly greater RBP adaptation.

It is proposed that increases in SS stimulate endothelial cells to release endothelial factors that cause endothelial dependent vasodilation (Pohl et al, 1986). As a result, the endothelium dilates and SS is normalised (Dimmeler & Zeiher, 2003; Koller & Kaley, 1991; Niebauer & Cooke, 1996; Zarins et al, 1987). Green et al (2004) suggests that exercise training induced structural enlargement of conduit vessels may be an adaptive response which acts to mitigate the increases in wall stress brought about by repeated exercise bouts, and thus remove the need for ongoing functional dilation. It may be possible that the post-exercise haemodynamic challenge that the HI group was repeatedly exposed to may not have been of a consistently great enough magnitude to repeatedly overload the vascular endothelium for continued normalisation of SS. This may have consequently reduced the ability of this haemodynamic stimulus to maximise eNOS expression and NO production for vascular adaptation that may have also led to a more pronounced reduction in RBP following IET. Tinken et al (2010) also utilised a haemodynamic challenge during 8 weeks of dynamic handgrip exercise training to establish the effects of repeated exposure to this physiological stimulus on endothelial adaptations. Results demonstrated a significant artery remodeling process had taken place. However it was apparent that at every 2 weeks of the exercise training, Tinken had increased the required target %MVC for dynamic handgrip exercise by 10 %. It is likely that this would have repeatedly overloaded the endothelium for continued normalisation of SS via a dilation process that over time may have up regulated the bioavailability of NO for artery remodeling. This suggests that whilst this current study focused on ensuring that the HI group always trained to a greater haemodynamic challenge than that of the LO group, it may also be important that the haemodynamic challenge be continuously increased throughout the training period to ensure the

effectiveness of a haemodynamic stimulus as the physiological stimulus for RBP reductions following IET.

The common femoral artery typically demonstrates an unresponsive nature to dilate in response to an increased SS stimulus (Gonzales et al, 2009; Radegran et al, 1997; Radegran et al, 2000; Wray et al, 2005). Thijssen et al (2008) attributes this lack of response to the vessel wall to lumen ratio, in that the larger the lumen of the artery, the less smooth muscle relative to elastic laminae in the vessel wall, which reduces the vessels capabilities to respond to a vasodilatory substances stimulated by SS. It may be speculated that a significantly large SS would be needed to induce a vasodilatory response in the common femoral artery, and that the post-exercise SR the HI group of this study performed IET at was not of a sufficient enough magnitude to stimulate the possible mechanism for RBP reductions after IET in all HI group participants. This may also demonstrate the need for continuously increasing the haemodynamic stimulus over the training period. However, the vasodilatory responses of the common femoral artery to different magnitudes of shear stimuli has not been widely investigated, and therefore this reasoning must be considered as largely speculative.

One important factor that may have reduced the effectiveness of the haemodynamic challenge in the HI group to initiate an adaptation for greater RBP reduction after IET is the influence of dilatory metabolites. It is well established that isometric exercise contraction at high levels may reduce muscle BF which may result in the accumulation of vasodilatory metabolites such as K^+, H^+, adenosine, prostaglandins, lactate & bradykinin (Bangsbo & Hellten, 1998; Pippoli et al 1994; Rowell & O'Leary, 1990). Upon release of isometric contraction these vasodilatory metabolites are released into the circulation and have been shown to induce vasodilation post-exercise (Osada et al, 2003). Whilst study 3 of this current research thesis suggested that the increased haemodynamic response seen immediately post-isometric leg extension exercise may be attributed to a downstream vasodilation in the profunda artery in response to increased SS, it is also possible that this downstream vasodilation occurred in response to the increased release of vasodilatory metabolites upon immediate cessation of exercise contraction, and not as a direct response to an increase in SS. As Laughlin et al (2008) suggested, increases in BF do not necessarily produce increases in SS if the radius of the artery also increases via other vasodilatory influences. In this instance, eNOS expression is unlikely to increase (Laughlin et al, 2008), and therefore a vascular adaptation for possible reduction in RBP is unlikely to occur. As isometric exercise

intensity increases, the production of vasodilatory metabolites is likely to be increased in response to the mismatch between demand for O_2 versus BF that is somewhat restricted due to mechanical compression of the blood vessels during static contraction. Whilst this current study did not measure the presence of vasodilatory metabolites, and therefore their influence on the increased haemodynamic challenge in this study is undetermined, this may go some way as to explaining the lack of a significant RBP change in the HI group of this current study versus the LO and CON groups.

These findings also highlights a limitation of this current research thesis, in that it was not possible to record real time blood haemodynamic data from the profunda femoral artery at the level of intramuscular pressure created from the isometric contraction of the quadriceps. Whilst the common femoral artery was imaged as a viable alternative due to the fact that it feeds the downstream profunda artery, Pyke & Tschakovsky (2005) suggested vessels with different diameters may have the same flow, but experience different levels of SS and therefore a different degree of stimulus. As the post-exercise haemodynamic challenge established in this current research thesis was determined at the level of the common femoral artery, it is not known as to whether a similar stimulus in terms of shear magnitude and characteristics would be seen at the downstream profunda level. It is also apparent that the common femoral artery and the profunda femoral artery respond differently to a haemodynamic stimulus. Wray et al (2005) identified that an increased shear stimulus created during dynamic exercise increased vascular reactivity in the profunda femoral artery, whilst no improvements in vascular reactivity were noted in the common femoral artery. Despite their close proximity, this demonstrates the differences between the ability of these arteries to respond to an increased haemodynamic challenge. Therefore it must be considered that the haemodynamic challenge and associated physiological response in the common femoral artery may not necessary reflect the same challenge and response experienced at the profunda artery level. As the profunda artery was not directly assessed, it could be speculated that this possible unestablished downstream haemodynamic stimulus at high intensities of isometric exercise may not have been optimal for increased eNOS expression and increased NO production for vascular adaptation mediated RBP reductions. This may explain why the findings of this current study suggest that performing IET to a high haemodynamic challenge (as measured from the common femoral artery) does not influence the degree of RBP change from those who perform IET with a low or no haemodynamic challenge.

The effectiveness of a haemodynamic challenge as the physiological stimulus for RBP reductions after IET is largely dependent upon the ability of this haemodynamic challenge to induce a vascular functional or structural adaptation via increased expression of eNOS and NO production. However, it must be taken into consideration that eNOS and NO values were not assessed in this current research thesis, and therefore it remains to be established as to whether the post-exercise haemodynamic challenge utilised in this current research thesis was able to increase expression of eNOS and production of NO. Whilst NO is a well-established contributor to an increased BF response to arm exercise in the brachial artery (Duffy et al, 1999a, 1999b; Dyke et al, 1995; Gilligan et al, 1994), it is less established whether NO has the same influence during leg exercise. For example, Radegran & Saltin (1999) found that L-NMMA (a known antagonist of NO) infusion had no effect on BF during steady state or peak knee extension exercise. Bradley et al (1999) also noted that L-NMMA did not reduce BF during cycling exercise. In contrast, Hickner et al (1997) did observe a reduction in BF when L-NMMA was administered during dynamic leg exercise. Whilst Green et al (2004) suggested that these marked differences between the contribution of NO during arm exercise versus leg exercise may be due to a greater dilution of NO antagonists during leg exercise (due to a greater muscle mass and blood supply), the possibility still remains that a haemodynamic challenge during leg exercise may not up regulate eNOS expression and NO production. In addition to this, whilst very few studies have established the role of NO to the post-exercise response after a bout of isometric exercise, Endo et al (1994) demonstrated that BF post-isometric handgrip contraction for 3 minutes was not affected by L-NMMA infusion, suggesting that the increased post-exercise BF and vasodilatory response observed was not mediated by NO. It could therefore be speculated that the increased haemodynamic response observed after high intensities of isometric leg extension exercise in this current research thesis may not be NO mediated. In this instance, it is therefore unlikely that a greater magnitude shear stimulus would provide the necessary physiological stimulus for greater vascular adaptation and subsequent greater RBP reductions following IET.

In summary, whilst studies 2, 3 and 4 of this research thesis found evidence of a post-exercise haemodynamic challenge at higher isometric exercise intensities, it is apparent from the findings of study 5 that this haemodynamic challenge does not appear to be a primary physiological stimulus necessary for RBP reductions after IET. A reduced

magnitude of the haemodynamic challenge and ineffective shear pattern characteristics from performing IET to an individualised %EMG_{peak} may have contributed to the reduced success of this haemodynamic stimulus for RBP adaptation. When interpreting these findings, it must be considered that it was not possible to determine the characteristics of this post-exercise haemodynamic challenge and the associated vascular endothelium response at the level of the exercising musculature in the profunda artery. There is also supporting evidence to suggest that post-exercise haemodynamic SS during leg exercise may not be a stimulus for increased eNOS expression and NO production. The effects of a isometric exercise induced haemodynamic stimulus on eNOS expression and NO production must first be examined before a final conclusion can be drawn as to whether a haemodynamic stimulus is the physiological stimulus for RBP reductions following IET.

7.4 According to the findings of study 5 (Chapter 6) is local conduit artery diameter remodeling the mechanism for blood pressure reductions after isometric exercise training?

In study 5 it was initially proposed that local conduit artery remodeling in the common femoral artery may be the mechanism for BP reductions after IET. This was based on the observation that acute bouts of isometric exercise can induce a significantly elevated post-exercise BF and SR (including ASR and RSR) response (please refer to Chapters 3, 4 & 5 for the haemodynamic response to acute isometric exercise). Regular exposure to increased SS that results from increases in BF during bouts of exercise is currently considered to be a primary training stimulus for the release of dilatory substances that may also be the signal for artery remodeling (Laughlin et al, 2008). Rudic et al (1998) suggested that the vascular endothelium senses the mechanical forces elicited by the flow of blood (and therefore SS), and coordinates these signals into biochemical events that regulate vascular tone and ultimately structure. In response to this haemodynamic challenge, eNOS may act as a mechanosensor for NO release to long term haemodynamic change that regulates aspects of extracellular matrix turnover, endothelial and smooth muscle cell proliferation, migration, organisation and responsiveness to growth factors; all of which are events associated with vascular remodeling (Rudic et al, 1998). Consequently it is widely acknowledged that the exercise training induced enlargement of conduit artery lumen diameter is a chronic adaptation that is mediated by the expression of eNOS and NO release from the

endothelium in response to SS (Green et al, 2004; Rudic et al, 1998). It was hypothesised that repeated exposure to a post-exercise haemodynamic challenge during high intensity IET may lead to up regulation of eNOS and subsequent NO production at rest, and induce a conduit AD remodeling process, that in turn may reduce TPR, and consequently lower RBP.

The findings of study 5 demonstrate that local conduit AD changes in the common femoral artery do not correlate with the significant changes in RBP that were observed within both the HI and LO groups of this study. This therefore suggests that local conduit AD remodeling is not a primary mechanism for determining BP reductions after IET in young normotensive male participants, and that other physiological mechanism(s) must be responsible for these RBP adaptations.

Only one other study has examined the role of local conduit artery remodeling in BP reductions following IET. Baross et al (2012) observed significant reductions in resting SBP, DBP and MAP in middle aged men that performed 8 weeks of a bilateral ILEET program at 85% HR_{peak}. Changes in common femoral AD at rest also correlated with these BP reductions, leading Baross et al (2012) to suggest that a possible SS mediated local AD change may be responsible for the BP adaptations observed. It is apparent that the results of study 5 are not in agreement with the findings of Baross et al (2012), in that significant BP changes within the HI and LO groups of this current study were not correlated to changes in common femoral AD.

One possible explanation that may provide greater understanding regarding the differences between the findings of Baross et al (2012) and the findings of study 5 (Chapter 6) of this research thesis, could be related to the SS stimulus utilised in both of these studies to directly induce local conduit AD remodeling. As this discussion has previously identified, it could be suggested that training to a individualised $\%EMG_{peak}$ to elicit a specific post-exercise haemodynamic challenge (as utilised in study 4), may not have elicited the correct shear pattern characteristics, and/or have been of a great enough magnitude to induce an adaptation in conduit AD. In contrast, the training methods utilised by Baross et al (2012) of performing isometric exercise to $85\%HR_{peak}$ may have provided an effective haemodynamic stimulus in terms of shear pattern characteristics and magnitude that is capable of increasing eNOS expression and NO production at rest to induce a significant diameter change that may be responsible for a large component of the BP adaptations observed following IET. However, since Baross

et al (2012) did not measure the haemodynamic response in the common femoral artery to isometric bilateral leg exercise, it is impossible to make a direct comparison between the SS stimulus utilised in the current study and that of Baross et al (2012). Therefore it remains unknown as to what are the best shear pattern characteristics and magnitude of a SS stimulus required to induce common femoral AD changes that may lead to reductions in RBP after IET.

A further explanation that may also help to explain the difference between the findings of Baross et al (2012) and the results of this current study is the type of participants used. Baross et al (2012) used older pre-hypertensive participants (resting SBP ~138 mmHg), whilst this current study used young normotensive participants (resting SBP ~ 116 mmHg). Whilst exercise induced vascular remodeling in healthy participants has not been widely investigated, McGowan et al (2007b) and Maiorana et al (2001a) have demonstrated "unhealthy" populations seem to respond to an exercise training stimulus and subsequently experience improvements in vascular function. The pre-hypertensive participants utilised by Baross et al (2012) may fall into this "unhealthy" category as hypertension is largely associated with endothelial dysfunction (Iiyama et al, 1996; Modena et al, 2002; Perticone et al, 2001; Vanhoutte, 1996; Widlansky et al, 2003). This may go some way as to explaining why Baross et al (2012) observed AD adaptations to exercise training that may have been responsible for RBP reductions after IET. In comparison, the participants of this current study were normotensive and classified as healthy, and therefore may have been less responsive to an exercise training stimulus. Previous evidence has demonstrated that normotensive populations may be less responsive to an exercise induced adaptation in the vasculature. For example, McGowan et al (2007a & 2007b) demonstrated that endothelial function improved after 8 weeks of isometric handgrip exercise in hypertensive participants, whilst no significant changes in endothelial function was seen in the normotensive participants. Similar results have also been observed by Maiorana et al (2001a & 2001b), as improvements in endothelial function were seen only in type 2 diabetic participants, and not healthy participants after 8 weeks of whole body exercise training. In line with this existing evidence, the group of normotensive participants in this current study may have been less likely to experience improvements in vascular function and subsequent conduit AD remodeling, despite sub-group RBP reductions. In addition, it must also be considered that the RBP adaptations commonly observed in normotensive participants following IET may be attributable to small undetectable changes in a

number of mechanisms in sequence, as opposed to just one mechanism such as that seen in hypertensive participants. As normotensives are classified as "healthy", it seems unlikely that the human body would allow a large significant change in just one physiological mechanism as it may alter the homeostatic balance of an already 'optimally' functioning cardiovascular system. In light of this theory, it is possible that the normotensive participants of this current study may have experienced a small adaptation in conduit AD that was undetected by the methods used in this thesis to measure resting AD. This small adaptation may have formed part of a sequence of small adaptations in other physiological mechanisms within this healthy normotensive population that could partly explain the reductions in RBP observed within each training group of this current study. Ultimately, this highlights that the RBP reductions that normotensive and pre-hypertensive / hypertensive populations experience following IET is likely to be governed by differing mechanisms.

It must be acknowledged that it is still possible that local conduit artery remodeling may be the mechanism for RBP reductions after IET, and that this adaptation may have taken place in another artery rather than the common femoral artery. For example, several studies have demonstrated repeated exposure to increased SS as a result of exercise training increases the peak vasodilator response in the smaller resistance arteries, thus suggesting luminal expansion adaptation has occurred (Green et al, 1996; Naylor et al, 2006; Rakobowchuck et al, 2005a; Sinoway et al, 1986). It is possible that structural adaptation to a larger lumen diameter may have occurred in the resistance vessels in response to repeated exposure to a SS stimulus via IET in study 5. Given that a reduced resistance vessel lumen diameter is closely associated with the occurrence of hypertension (Folkow, 1971; Mulvany, 1993; Zervoudaki & Toutouzas, 2003), this may go some way as to explaining the reductions in RBP observed. In addition, as the common femoral artery is the main conduit artery that feeds the distal leg arterial supply, it is likely that these distal arteries may also have experienced an increased haemodynamic challenge in the immediate periods post-exercise. In particular, it could be speculated that the profunda femoral artery may have experienced conduit artery remodeling adaptation in response to repeated exposure to a high haemodynamic challenge post-exercise. This suggestion is based upon earlier discussion (Chapter 4) where it was proposed that the downstream profunda artery (which directly supplies the quadriceps with blood) could be directly affected by mechanical compression from isometric leg extension contraction, resulting in a possible reduced BF and metabolite

accumulation at higher exercise intensities. When contraction is released, it is plausible to suggest that the increased perfusion pressure flow in response to mechanical compression combined with the release of vasodilatory metabolites may lead to a large FMD response in the profunda artery as a result of increased SR from the increased post-exercise BF. Repeated exposure to a SS stimulus has been shown to improve vascular function and induce artery remodeling via up regulation of NO synthase and production of NO (Green et al, 2004; Rudic et al, 1998; Zoeller et al, 2009). It might be suggested that AD remodeling may have occurred in the downstream profunda artery that may also have been responsible for the sub-group BP changes observed in this current study. However this is largely speculation, as it was not possible to accurately measure the profunda artery at rest or during exercise due to its deep location with the leg musculature, and therefore the possibility of profunda vasculature adaptations to IET requires further investigation.

The presence of a specific timeline for vascular adaptation may also provide insight as to why in this current study, conduit AD remodeling does not appear to be the mechanism for RBP reductions after IET. Tinken et al (2010) established that there is a time course to the occurrence of vascular adaptations in the brachial artery after 8 weeks of rhythmic handgrip exercise training. Exercise training was initially associated with functional improvements in the vasculature, which gradually returned to baseline by week 8 and were consequently superseded by artery remodeling. It is well established that the brachial artery is more reactive to a SS stimulus than the common femoral artery in terms of vasodilation (Gonzales et al, 2009; Radegran, 1997; Radegran & Saltin, 2000; Shoemaker et al, 1997; Wray et al, 2005). It is currently unknown as to whether a similar timeline of vascular adaptation is observed in the common femoral artery. It might be argued that a vascular adaptation process may occur at a quicker rate in the brachial artery as opposed to the less reactive common femoral artery. If this is the case, then it is possible that a period of 8 weeks exercise training may not be long enough to significantly induce a change in common femoral AD, and that a longer training intervention and subsequent exposure to a SS stimulus is required. When resting common femoral AD measurements were taken after the 8 week exercise intervention in this current study, it is possible that the artery may have still been in undergoing improvements in vascular function, which would make it unlikely for changes in common AD to be observed. However, as local improvements in vascular

function were not assessed in this current study, it is impossible to verify this theory without further investigation.

A possible methodological limitation that may help to explain why changes in conduit AD did not correlate with changes in RBP may be due to the method used to measure common femoral AD at rest. In this current study, edge detection and wall tracking software was used to measure AD in a resting state (please see page 53 in Chapter 2 for more details). Naylor et al (2005) suggests that conduit AD at rest is dependent upon sympathetic tone, circulating hormone modulation and local paracrine effects, and therefore measuring the artery at rest may be a poor index of vascular structure. As an alternative, Naylor et al (2005) suggests that conduit AD should be assessed in response to a maximal stimulus so as to diminish the influence of these functional factors at rest. Whilst this may be seen as a viable alternative for conduit AD remodeling, it could be suggested that measuring common artery femoral AD in response to a maximal stimulus may not best reflect any conduit AD changes that are responsible for BP changes at rest. As RBP is determined by TPR at rest, it would thus seem reasonable to measure AD whilst the artery is in a resting state and not in response to a maximal stimulus.

The results of this investigation suggest that changes in common AD are not the primary mechanism for BP reductions after IET intervention in young, healthy normotensive males. It could be suggested that an ineffective haemodynamic stimulus may be responsible for this finding. In addition to this, it is recommended that the vascular health of the participants used and the time course for vascular adaptations should be considered before local conduit AD changes are assessed as a mechanism for RBP adaptations to isometric exercise intervention.

7.5 According to the findings of study 5 (Chapter 6), do other non-invasive cardiovascular variables play a role in the resting blood pressure reductions following isometric exercise training?

The findings of study 5 presented in Chapter 6 of this research thesis established that other non-invasive measures of cardiovascular variables (which include systemic AD remodeling, autonomic balance, TPR, and cardiac adaptations) did not correlate with the BP changes observed within each exercise training group. This finding ostensibly suggests that none of these proposed variables are related to RBP reductions after 8

weeks of IET. This was a surprising finding as RBP is determined by $\dot{Q}$ and/or TPR (Wiley et al, 1992), and therefore a change in one of these variables must have been responsible for the sub-group RBP changes observed. Other studies utilising bilateral isometric leg extension exercise training in normotensive males also found similar findings, in that they too observed changes in RBP that could not be attributed to changes in $\dot{Q}$ or TPR (Wiles et al, 2010; Devereux et al, 2010b). Care must be taken in the interpretation of these findings from this current study as several of these measures did not have adequate statistical power to draw firm conclusions based upon non statistically significant differences. The consideration of each of these variables in the role that they may play in the RBP reductions associated with IET is discussed below.

-*Systemic conduit artery remodeling*

Previous work by Tinken et al (2009) and Thijssen et al (2009a) has demonstrated that lower limb exercise (cycling and walking) can induce an oscillatory pattern in BF in the brachial artery that leads to increased NO release. It could be suggested that isometric contraction may also induce a OSI pattern that leads to increased NO release in the non-exercising limbs. This is largely speculative, as no previous study has examined the haemodynamic response and characteristics in the non-exercising limb in response to isometric exercise contraction. Repeated exposure to an OSI in the non-exercising limbs may induce a conduit AD remodeling process that in turn may mediate a BP reduction after IET. However the results of this current study demonstrate that changes in brachial AD pre-post isometric intervention did not correlate with the changes observed in the sub-group BP reductions observed. This suggests that systemic conduit AD remodeling may not be the mechanism for BP reductions after IET.

This finding is in agreement with those of Baross et al (2012) who found that there was no significant difference in brachial AD pre-post an 8 week isometric exercise intervention, and therefore was not the mechanism for the significant reductions in RBP that they observed. McGowan et al (2007b) also explored the possibility of a systemic vascular adaptation as the mechanism for RBP reductions after 8 weeks of isometric handgrip training in participants medicated for hypertension. Despite significant reductions in RBP, the 8 week isometric intervention only improved local brachial artery vascular function, whilst no changes were seen in the systemic vasculature. McGowan et al (2007b) concluded that local vascular functional changes could not be the mechanism for RBP reductions after IET as RBP is controlled by a systemic

operating system and therefore a systemic change in vascular function would be necessary to influence a RBP adaptation. However, it should be considered that a local AD change induced by a SS mediated up regulation of NO may influence systemic RBP regulation. Whilst NO may play a role in lowering RBP via local vascular mechanisms (such as conduit artery remodeling and improved endothelial function), several studies have demonstrated that NO may also act in the central nervous system to reduce vascular sympathetic tone (Guyenet, 2006; Pechanova, 2010; Togashi et al, 1992). This may help to explain how a local vascular adaptation might induce a systemic RBP adaptation following IET. Furthermore, AD changes that may occur within the leg vasculature (such as that seen by Baross et al, 2012) may have a greater impact on RBP control as opposed to the smaller brachial artery, possibly due to a greater surface area of the leg vasculature to determine resistance to BF, and hence RBP. Theoretically this may support the notion of a local leg vascular leg adaptation as the mechanism for RBP reductions after IET (such as that observed by Baross et al, 2012), whilst a local adaptation in the smaller brachial artery may not have a large enough impact on RBP for a local vasculature adaptation to be considered as a mechanism in studies such as that of McGowan et al (2007b) that utilises isometric handgrip exercise training.

When reviewed as a whole, the work of Baross et al (2012) and McGowan et al (2007b) in addition to the findings of this current research thesis suggest that systemic AD remodeling is unlikely to be the mechanism for RBP reductions following 8 weeks of IET. It is therefore suggested that future studies should also focus upon the haemodynamic response in the non-exercising limbs during and immediately after acute bouts of isometric exercise contraction to fully establish the likelihood of systemic AD remodeling as a mechanism for RBP adaptation in response to IET.

- *Autonomic balance*

Autonomic balance is the balance between parasympathetic nervous system withdrawal and sympathetic nervous system activation (Millar et al, 2013). As hypertension is associated with a negative change in sympathovagal balance through an increased sympathetic nervous system activity, it has been proposed that reductions in RBP may come about via a positive change in sympathovagal balance, where the sympathetic nervous system has less input (Taylor et al, 2003; Miller et al, 2013). Heart rate variability is a measure of the magnitude of HR fluctuations that are related to beat to beat regulation of HR by sympathovagal balance (Miller et al, 2013). Therefore HRV

changes may provide an insight into the changes in sympathovagal balance that may occur with IET, and consequently may be the mechanism for RBP reduction. The results of this study suggest that HRV and therefore changes in sympathovagal balance may not be the mechanism for BP reductions after IET in normotensives, as within group RBP changes were not associated with changes in HRV.

Other studies utilising bilateral ILEET intervention in normotensive healthy individuals also found no changes in HRV pre-post exercise training, despite marked reductions in RBP (Baross et al, 2012; Devereux et al, 2010b; Wiles et al, 2010). In contrast, Taylor et al (2003) found significant reductions in RBP in hypertensive participants that coincided with a significant changes in BPV, suggesting that a change in sympathovagal balance was responsible for the observed reductions in RBP. Taken together, these studies suggest that changes in sympathovagal balance may be the mechanism for reductions in RBP after IET in hypertensive participants, but that a different mechanism may be responsible for the BP reductions observed in normotensive populations.

Miller et al (2013) suggested that the traditional methods of measuring HRV (as used in this current study) may not be sensitive enough to detect small changes in sympathovagal balance that may be significant for RBP reductions. It is suggested instead that non-linear measures of HRV may provide the sensitivity needed to detect any small change in sympathovagal balance (Miller et al, 2013). With this in mind, the methods used to assess HRV in this current study may not have been sensitive enough to detect any changes in sympathovagal balance which may have been responsible for the sub-group RBP changes observed. It is apparent that the use of non - linear measures of HRV should be examined further in normotensive populations that do not typically demonstrate changes in sympathovagal balance using traditional measures of HRV to fully determine the role of autonomic function in the RBP reductions associated with IET.

-*Total peripheral resistance*

As Wiley et al (1992) identified, the mechanisms responsible for reductions in RBP after IET must involve adjustment of one or more of the components that determine RBP - $\dot{Q}$ or TPR. However the results of this study demonstrate that changes in TPR were not related to the changes in RBP observed within each exercise training group. This therefore suggests that changes in TPR may not have been the mechanism for RBP

reductions in this current study. This in line with the findings that changes in local and systemic AD (common femoral and brachial arteries) were also not related to the changes in RBP observed within the HI and LO groups of this current study.

This finding is in agreement with other studies utilising bilateral ILEET, which also did not find a significant change in TPR pre-post isometric exercise intervention, despite significant reductions in RBP (Baross et al, 2012; Devereux et al, 2010b; Wiles et al, 2010). This is particularly surprising in regards to the work of Baross et al (2012), as RBP reductions were associated with a enlargement of the common femoral AD, implying that resistance to flow must have decreased in order for RBP to reduce. Yet no changes in TPR were observed.

Since $\dot{Q}$ does not typically change after exercise training, it is suggested that alterations to TPR must be responsible for any changes to resting arterial pressure (Pescatello et al, 2004). This becomes apparent as prior research demonstrates that once $\dot{Q}$ values are normalised, those with a greater TPR typically have a higher RBP, (Julius & Conway, 1968). It is possible that alterations in TPR in line with BP changes may not have been observed in this current study and in the studies of Wiles et al (2010), Devereux et al (2010b) and Baross et al (2012) because the methods used to measure TPR were not sensitive enough to detect small but significant changes likely in normotensive participants. Total peripheral resistance in this current study was calculated from MAP/$\dot{Q}$, with MAP measurements made using an automated BP monitor, whilst $\dot{Q}$ was measured using echocardiography (Please refer to Chapter 2 page 50 for methodology). Chapter 2 (page 72) reports that the reproducibility of the TPR calculation is not very precise, meaning that a small change in TPR may be seen as insignificant without a substantially large sample size. Whilst it is likely that a TPR related mechanism remains the mechanism for RBP reductions after IET, a more sensitive method of detecting changes in TPR within normotensive participants may be needed to fully establish the role of TPR in RBP adaptation.

- *Cardiac adaptations*

As previously stated, changes in $\dot{Q}$ do not typically occur with IET (Pescatello et al, 2004). In light of this, few IET studies have directly measured $\dot{Q}$ changes after isometric exercise intervention. However in those studies that have, no changes in $\dot{Q}$ have been observed despite significant reductions in RBP (Baross et al, 2012; Devereux et al,

2010b; Wiles et al, 2010). The results of this current study are also in agreement with these findings, as changes in $\dot{Q}$ were not related to the changes in sub-group RBP. Therefore it is unlikely that reductions in RBP are mediated by $\dot{Q}$ adaptation. However it should be noted that the methods of measuring $\dot{Q}$ are not very precise (as established in Chapter 2 page 70), and therefore may not be sensitive enough to detect a small change in $\dot{Q}$ that may occur.

This study also explored the role of HR, IVRT and LVET in terms of a adaptation to $\dot{Q}$ that may lead to a reduction in RBP. Isovolumic relaxation time represents the time between the closing of the aortic valve to the onset of filling by the opening of the mitral valve (Thomas & Weyman, 1991), and therefore changes in IVRT represent an adaptation to diastolic cardiac function. Left ventricular ejection time is the time interval from the opening to the closure of the aortic valve and is used as a measure of systolic function (Hirschfield et al, 1975). Therefore changes to LVET may represent an adaptation to systolic cardiac function. The results of this current study demonstrated that changes in HR, IVRT and LVET did not relate to the sub-group BP changes observed in this current study, and therefore strengthen the notion that an adaptation in $\dot{Q}$ is not the mechanism for RBP reductions after isometric exercise intervention.

7.6 Conclusion

The primary aim of this research thesis was to examine whether BF hemodynamics play a significant role in the BP reductions commonly observed after IET. Rationale for this research question was largely based upon the work of Wiles et al (2010) and Devereux et al (2010b) which identified that the magnitude of RBP reductions after IET were exercise intensity dependent, and that the stimulus for these reductions was more prominent when fatigue was at its greatest. It was hypothesised that a haemodynamic response may be greatest at higher levels of fatiguing isometric exercise, and therefore may be the physiological stimulus for RBP adaptation following IET.

The results presented in studies 2, 3 and 4 provided conceptual support for this hypothesis in that the acute haemodynamic response to bilateral isometric leg extension exercise was greatest at higher intensities of isometric exercise that induced higher levels of fatigue. However, repeated exposure to this increased haemodynamic stimulus at higher intensities of isometric exercise over an 8 week intervention period (study 5)

did not induce a change in RBP that was statistically significant from those who performed isometric exercise intervention with a low or no haemodynamic stimulus. This may be attributed to the fact that this haemodynamic stimulus was induced by performing IET to a individualised $\%EMG_{peak}$ exercise intensity, that may have reduced the effectiveness of the haemodynamic challenge in terms of magnitude and/or shear characteristics. In addition, it is also possible that the post-exercise haemodynamic SS stimulus was not able to increase eNOS expression and NO production, and consequently did not induce a vascular adaptation for RBP reduction. It must also be considered that this post-exercise haemodynamic stimulus was not determined directly in the vascular endothelium at the level of the exercising musculature, and therefore it is currently unknown as to whether a haemodynamic stimulus at this level may be more effective as the stimulus for BP reductions after IET. However based upon the evidence presented in this research thesis, it is unlikely that BF haemodynamics play a role in the BP reductions commonly observed after IET performed to a individualised $\%EMG_{peak}$.

This research thesis also set out to explore whether local conduit AD remodeling may be a significant mechanism responsible for RBP reductions after IET. Results suggest that it is unlikely that local conduit AD remodeling is a significant mechanism responsible for BP adaptation in normotensive participants. Further investigations into other cardiovascular variables that could be considered as the mechanism for BP reductions after IET did also not explain the BP changes observed within the sub-groups of study 5. However, as the participants in this study were normotensive and healthy, it is unlikely that a significant change in just one physiological mechanism would explain the RBP adaptations observed, and instead small changes in a number of mechanisms may altogether contribute to induce a RBP reduction. These small changes may be too small to be considered significant by the techniques that this current study has used to measure these cardiovascular variables, and consequently significant changes in these variables may have gone undetected as potential mechanisms for RBP reductions following IET.

7.7 Implications of thesis findings.

An important finding of this thesis is that IET caused a reduction in RBP of ~2 mmHg in healthy male normotensive participants. This demonstrates that IET is indeed an effective form of exercise training for RBP in normotensives. The implications of this

significant finding is that this research adds to the increasing amount of evidence that demonstrates the success of IET as an effective and viable form of exercise training for RBP reduction, and potentially health gains for otherwise healthy normotensive male populations. These findings may also give some justification to future research exploring the use of IET prescription to unhealthy populations that would greatly benefit in terms of their health, and risk of morbidity and mortality by reducing their RBP.

A second significant finding from the work completed in this thesis is that training to a high post-exercise haemodynamic stimulus does not induce statistically significant greater gains in RBP adaptation then when training to a low post-exercise haemodynamic stimulus. This finding implies that it is unlikely for the haemodynamic challenge present when performing isometric exercise to be the primary physiological stimulus for RBP reductions following IET in normotensive participants. The implication of this finding is that whilst the physiological stimulus for RBP reductions in normotensives remains unidentified, these results will allow future research to focus upon other physiological variables that are associated with fatiguing isometric exercise, and therefore could be considered as stimuli for IET induced RBP reductions. A similar implication can be said of the third significant finding of this thesis, that conduit AD remodeling is not the definitive mechanism that adapts in response to IET to directly cause a reduction in RBP in normotensives. This may allow other researchers to investigate new theories surrounding the mechanisms for RBP adaptation following IET. For example, this thesis proposed that it may be possible that small adaptations occur in a number of physiological mechanisms in response to IET in normotensive participants (as opposed to a large adaptation in just one primary mechanism), and together these small changes combine to induce a RBP reduction. This may provide a new focus for future research exploring the mechanisms for IET induced RBP reductions.

It is evident that the findings of this thesis have contributed to the current state of knowledge surrounding isometric exercise and its ability to induce a RBP reduction. This thesis introduced a novel line of investigation that has not previously been explored in the existing literature, in that this work focused upon identifying the physiological stimulus for RBP reductions associated with exercise training variables rather then the common research aim of previous isometric research to identify the

physiological mechanism for RBP adaptation. This thesis may therefore provide a new line of investigation for future isometric research with the aim of adding to the existing knowledge in this field.

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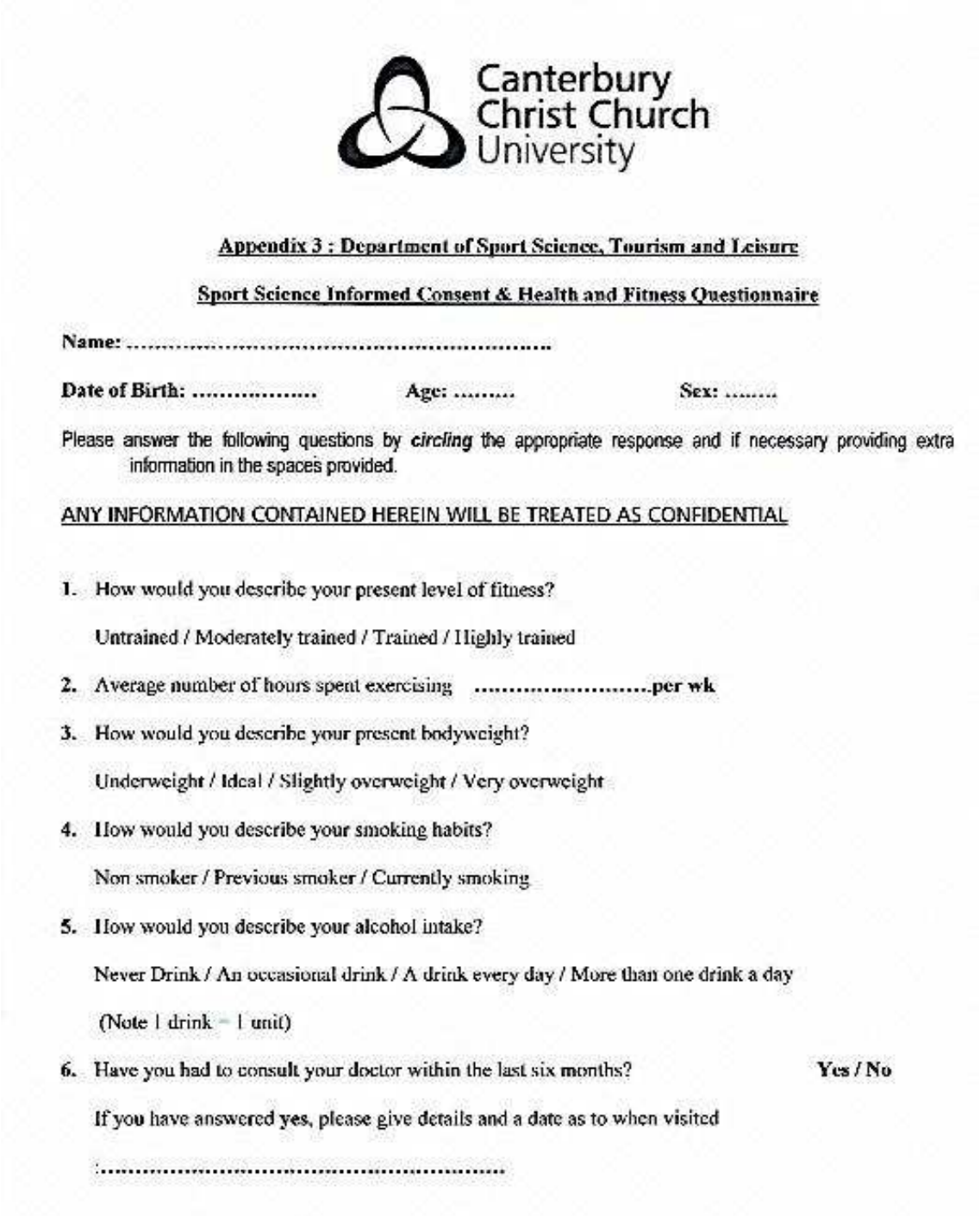
Appendices

Appendix 1: Participant Health Questionnaire



Canterbury Christ Church University

Appendix 3 : Department of Sport Science, Tourism and Leisure

Sport Science Informed Consent & Health and Fitness Questionnaire

Name: .....

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 wk**

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 and a date as to when visited

   :.....

7. Are you presently taking any form of medication or have you taken any medication in the last 4 weeks? **Yes / No**

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

8. Are you presently taking any substances or have you taken any substances in the last 4 weeks that may affect your blood pressure (e.g. Creatine)? Yes/No

   If you have answered yes please give details.....

9. Have you given blood in the last 6 weeks? Yes/No

10. 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.

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

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

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

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

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

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

14. 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: .....

Signature of Subject: .....

Signature of Sport Scientist: .....

Date: .....

Appendix 2: Participant information sheet for study 1.

Canterbury Christ Church University

The reliability and validity of a non invasive Doppler Ultrasound to measure femoral and brachial blood flow velocity, femoral and brachial artery diameter, and cardiac output.

***Lay Title**: "The reliability and validity of non invasive sound waves (Doppler ultrasound) to measure how fast blood flows in the main leg artery (femoral artery) and the main arm artery (brachial artery); how wide the arteries in the leg and arm are (artery diameter); and lastly how much blood is pumped from the heart in one minute (cardiac output)."*

PARTICIPANT INFORMATION

A research study is being conducted at Canterbury Christ Church University (CCCU) as part of a PhD thesis by Jenna Smith.

Background

Doppler ultrasound is simply the projection of sound waves into the human body using a probe that is placed against human skin, producing visual images of organs, blood vessels and body tissue. As a result it is possible to see inside the human body without using complicated and invasive procedures.

Because of its non invasive nature, doppler ultrasound is commonly used in Sport and Exercise Science as it allows the measurement of variables that would otherwise be very difficult to measure accurately. However before doppler ultrasound can be utilised in the Sport and Exercise department at CCCU, it must be checked as to whether it can actually measure what it is supposed to measure (i.e. Its validity) and whether or not it can measure these things accurately over a number of occasions (i.e. its reliability).

The study has been approved within the University by the Faculty of Social and Applied Sciences research ethics committee.

What will you be required to do?

Participants in this study will be required to:

- Visit the laboratory on 5 different days, preferably with only one or two days apart between visits.
- To visit the laboratory at the same time of day on each of these days.

To participate in this research you must:

- Be male, with normal blood pressure.
- Be a non-smoker.
- Have no known medical conditions.
- Not be taking any medication.

Procedures

You will be asked to complete a health and fitness questionnaire about your current state of health and fitness. You will then be required to lie down for 15 minutes, resting. After the 15 minutes rest period, a probe will be placed against your thigh, then against the inside of the upper arm, and lastly on your chest (against the chest bone, and then the ribs).

Testing will be completed at the same time of day for every visit. You will be asked to follow the following criteria:

- No food within 3 hours of testing.
- Drink ONLY WATER with 3 hours before testing.
- No alcohol within 24 hours.
- Not to be taking any medication for the preceding 4 weeks.
- Not to have given blood during the preceding 6 weeks.
- Free from illness/ infection during the preceding 2 weeks.
- Not to have exercised strenuously in the 36 hours leading up to testing.

Please note, you are required to wear **shorts** to provide access to the thigh, and will be asked to **remove you t-shirt** to provide access to the chest.

Please read the continuing sheet that details the ingredients of the ultrasound gel in order to make sure that you are not allergic to one or some of the ingredients.

Feedback

You will receive feedback of all your results. Should anything abnormal be discovered you will be informed, and all information will be passed onto your GP for diagnosis and further investigation.

Confidentiality

All data and personal information will be stored securely within CCCU premises or in accordance with the Data Protection Act 1998 and the University's own data protection requirements. Data can only be accessed by Jenna Smith, Dr Jim Wiles, Dr Damian Coleman & Dr Ian Swaine. After completion of the study, all data will be made anonymous (i.e. all personal information associated with the data will be removed).

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 **Jenna Smith** on 01227 767700 (ext 3145) or 07929278579 or email jes36@canterbury.ac.uk
Department of Sport Science, Tourism & Leisure, North Holmes Road, Canterbury CT1 1QU

Aquasonic Ultrasound Transmission Gel Ingredients

Reserve osmosis (RO) water

Humectants

Polymer

Preservatives

Water soluble fragrance

FD&C color

Propyl paraben and methyl paraben in bacteriostatic concentration

ph range 6.50-6.95.

Appendix 3: Participant information sheet for studies 2,3 and 4.

Canterbury Christ Church University

Participant information sheet - Study 1:

"The relationship between acute cardiovascular responses, exercise intensity, torque patterns and metaboreceptor stimulation during an incremental isometric test."

Lay Title: "The relationship between the immediate heart and blood vessel responses (cardiovascular responses), how hard the subject is exercising (exercise intensity), the amount of force they are producing (torque patterns), and how much the exercise is causing them to become tired (fatigue in relation to metaboreceptor stimulation (metaboreceptors are stimulated when an individual becomes fatigued, please see lay summery for details)), during exercise where the muscle stays the same length during contraction, thus producing no movement (isometric exercise) where the exercise gradually gets harder (incrementally) as time goes on."

PARTICIPANT INFORMATION

A research study is being conducted at Canterbury Christ Church University (CCCU) as part of a PhD thesis by Jenna Smith.

Background

Muscle contraction in which the leg or arm does not move (isometric exercise) when performed over a number of weeks, has been shown to cause changes to the heart and blood vessels (cardiovascular adaptation). Previous work completed in this department suggests that the harder the isometric exercise, the greater the cardiovascular adaptation. This suggests that the mechanisms that are stimulated when an individual is doing harder isometric exercise play an important part in any cardiovascular adaptation that might take place. This study aims to examine this theory further. Here we will investigate what happens to these mechanisms during and after a single bout of isometric exercise.

This study has been approved within the University by the faculty of Social and Applied Sciences research ethics committee.

What will you be required to do?

Participants in this study will be required to:

- Visit the laboratory on 5 different days, with a minimum of 48 hours between visits.
- To visit the laboratory at the same time of day on each of these visits.

To participate in this research you must:

- Be a healthy, non smoking male.

- Not have a musculo skeletal injury.
- have no known medical problems that may impair your ability to participate in the study in any way and/or 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.
- not have undergone blood donation during the preceding 6 weeks or during the course of the study. (Bouchard, 1995).

Procedures

You will be asked to complete a health and fitness questionnaire about your current state of fitness. You will then be required to sit in the isokinetic dynamometer (see image 1) whilst both your ankles are strapped into the immovable brace.

Image 1: isokinetic dynamometer

Electrodes (pads that stick to your body to allow electrical signals to be read) will be placed on your collar bones, ribs and thighs to record your heart rate and any electrical activity that may occur in your muscles. In order to attach the electrodes to your legs, a small area of hair will need to be shaved first. You will also be required to wear a finger cuff and an upper arm cuff to measure your blood pressure.

One the first session in the laboratory you will be asked to extend your legs against the immovable brace as hard as possible 3 times for 2 seconds each time. This will record the maximum force you can produce. After this, you will rest for 15 minutes in a seated position.

After the resting period, the incremental isometric test protocol will start. This involves you extending your legs against the immovable leg brace for 2 minute, 5 times. You will have 5 minutes rest between each 2 minutes of extending your legs. Each 2 minute bout will be harder than the last.

It should be noted that you will be performing exercise that may feel result in you feeling discomfort or shortness of breath. You are free to terminate the test at any point if you feel you can no longer meet the exercise demands placed upon you.

Testing will be completed the same time of day each time you visit. Testing sessions should take no more time than 1 hr 15 minutes. You will be asked to follow the following criteria:

- No food within 3 hours of testing.
- Drink only water within 3 hours before testing.
- No alcohol within 24 hours.

- Not to have been taking any medication for the preceding 4 weeks.
- Not to have given blood during the preceding 6 weeks.
- Free from illness/ infection during the preceding 2 weeks.
- Not to have exercised strenuously (i.e. to exhaustion) in the 36 hours leading up to testing.

Please note, you are required to wear shorts to provide access to the thigh, and will be asked to remove your t-shirt to provide access to the chest.

Feedback

You will receive feedback of all your results. Should anything abnormal be discovered you will be informed, and all information will be passed on to your GP for diagnosis and further investigation.

Confidentiality

All data and personal information will be stored securely within CCCU premises or in accordance with the Data Protection Act 1998 and the University's own data protection requirements. Data can only be accessed by Jenna Smith, Dr Jim Wiles, Dr Damian Coleman & Dr Ian Swaine. After completion of the study, all data will be made anonymous (i.e. all personal information associated with the data will be removed).

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 Jenna Smith on 01227 767700 (ext 3145) or 07929278579 or email jes36@canterbury.ac.uk
Department of Sport science, Tourism & Leisure, North Holmes road, Canterbury, CT1 1QU.

Canterbury Christ Church University

Participant information sheet - Study 2:
"The relationship between post exercise hyperemia and an incremental isometric test."

Lay Title: "The relationship between how much and how fast blood flows (hyperemia) after exercise (post exercise) where the muscle stays the same length during contraction, thus producing no movement (isometric exercise), and the exercise gradually gets harder (incrementally) as time goes on."

PARTICIPANT INFORMATION

A research study is being conducted at Canterbury Christ Church University (CCCU) as part of a PhD thesis by Jenna Smith.

Background

Muscle contraction in which the leg or arm does not move (isometric exercise) when performed over a number of weeks, has been shown to cause changes to the heart and blood vessels (cardiovascular adaptation). Previous work completed in this department suggests that the harder the isometric exercise, the greater the cardiovascular adaptation. This suggests that the mechanisms that are stimulated when an individual is doing harder isometric exercise play an important part in any cardiovascular adaptation that might take place. This study aims to examine this theory further. Here we will investigate what happens to these mechanisms during and after a single bout of isometric exercise.

This study has been approved within the University by the faculty of Social and Applied Sciences research ethics committee.

What will you be required to do?

Participants in this study will be required to:

- Visit the laboratory on 5 different days, with a minimum of 48 hours between visits.
- To visit the laboratory at the same time of day on each of these visits.

To participate in this research you must:

- Be a healthy, non smoking male.
- Not have a musculo skeletal injury.
- have no known medical problems that may impair your ability to participate in the study in any way and/or 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.
- not have undergone blood donation during the preceding 6 weeks or during the course of the study. (Bouchard, 1995).

Procedures

You will be asked to complete a health and fitness questionnaire about your current state of fitness. You will then be required to sit in the isokinetic dynamometer (see image 1) whilst both your ankles are strapped into the immovable brace.

Image 1: Isokinetic dynamometer

Electrodes (pads that stick to your body to allow electrical signals to be read) will be placed on your collar bones, ribs and thighs to record your heart rate and any electrical activity that may occur in your muscles. In order to attach the electrodes to your legs, a small area of hair will need to be shaved first. You will also be required to wear a finger cuff and an upper arm cuff to measure your blood pressure.

One the first session in the laboratory you will be asked to extend your legs against the immovable brace as hard as possible 3 times for 2 seconds each time. This will record the maximum force you can produce. After this, you will rest for 15 minutes in a seated position.

After the resting period, the incremental isometric test protocol will start. This involves you extending your legs against the immovable leg brace for 2 minutes, 5 times. You will have 5 minutes rest between each 2 minutes of extending your legs. Each 2 minutes bout will be harder than the last. During this test two ultrasound probes will be placed against your thigh in order to view the arteries in your leg.

It should be noted that you will be performing exercise that may feel result in you feeling discomfort or shortness of breath. You are free to terminate the test at any point if you feel you can no longer meet the exercise demands placed upon you.

Testing will be completed the same time of day each time you visit. Testing sessions should take no more time than 1 hr 15 minutes. You will be asked to follow the following criteria:

- No food within 3 hours of testing.
- Drink only water within 3 hours before testing.
- No alcohol within 24 hours.
- Not to have been taking any medication for the preceding 4 weeks.
- Not to have given blood during the preceding 6 weeks.
- Free from illness/ infection during the preceding 2 weeks.

Appendix 4: Participant information sheet for study 5

Canterbury Christ Church University

"The effects of isometric training at two different levels of metaboreceptor stimulation on cardiovascular measures."

Lay Title: "The effects of holding a static muscle position against an immovable force (isometric exercise), on a number of occasions over a number of weeks (training), in relation to two different levels of muscular fatigue (metaboreceptor stimulation) on resting adaptations in blood pressure, the structure of human arteries and the efficiency of the heart to pump blood (cardiovascular measures)."

PARTICIPANT INFORMATION

A research study is being conducted at Canterbury Christ Church University (CCCU) as part of a PhD thesis by Jenna Smith.

Background

Muscle contraction that results in no limb movement (i.e. squeezing a tennis ball) is known as isometric exercise. When performed over a number of weeks, it has been shown to lower resting blood pressure. Recently some researchers have shown that this type of exercise might also change the size of the arteries in the muscles being exercised. It has been suggested that both resting blood pressure and artery changes after this type of exercise training might depend on how hard the training is. So, we want to look at blood pressure and artery changes in three groups: those who exercise quite hard, those who exercise a bit easier and those who don't do the exercise at all. In theory, the harder exercise group should experience larger effects on blood pressure and artery size, the gentler exercise group should experience smaller changes and the group who don't exercise should experience no changes at all.

What will you be required to do?

You will be asked to commit to a 10-week study. If you are allocated to one of the two exercise groups, the exercise will involve 'tensing' of both legs (at the same time), either at a moderate-to-vigorous level or a gentle level of effort, depending on which exercise group you are allocated to. The visits of the 10-week study will be broken down into:

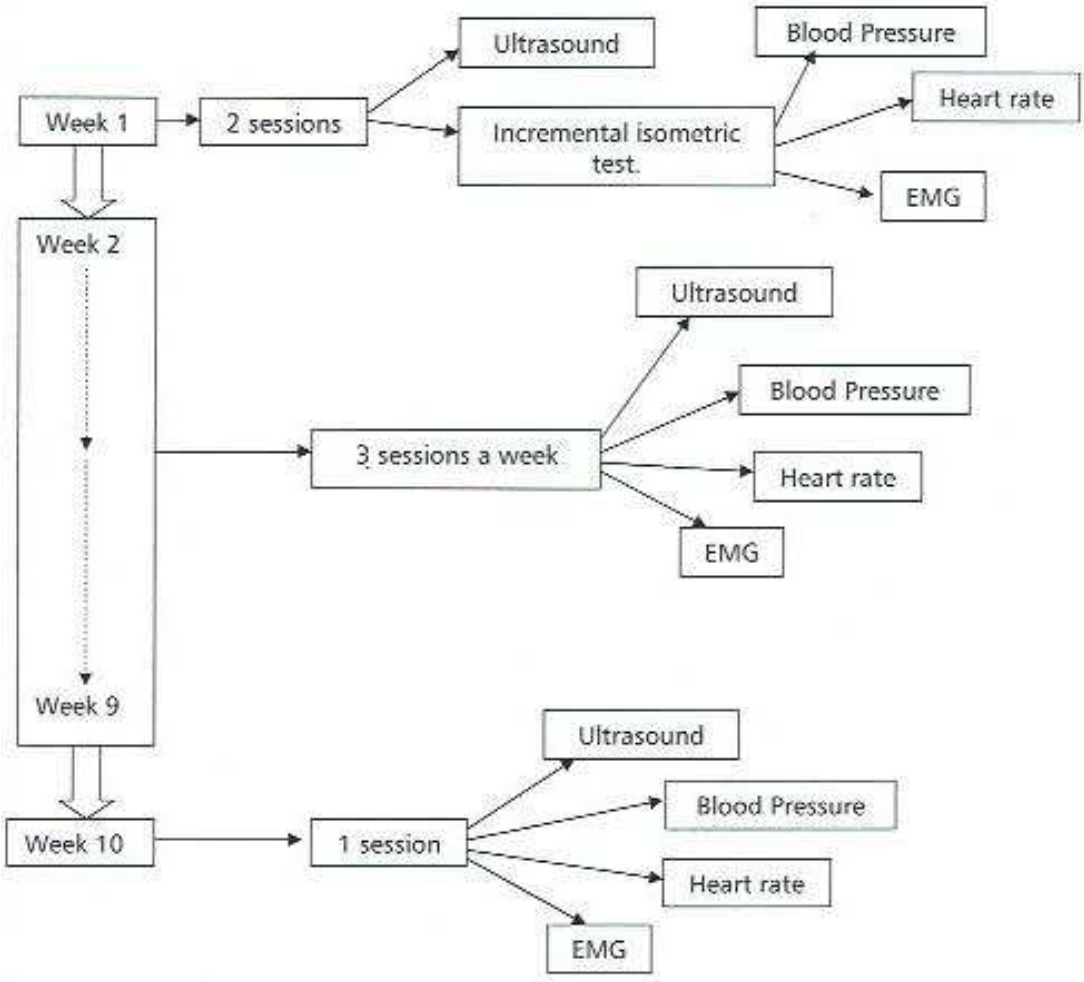
- **Week 1** = 2 visits
- **Week 2-9** = 3 visits a week (on Mondays, Wednesdays, and Fridays), every week.
- **Week 10** = 1 visit

If you want to take part we need you to commit to all of these sessions, and you'll be asked to visit the laboratory at the same time of day on each of these visits.

To participate in this research you must:

- Be free from ill-health, and a non-smoking male.
- Not have a muscle, joint or bone injury.
- Have no known medical problems that may affect your ability to participate in the study in any way and/or have been free from illness/infection for the preceding 2 weeks to testing.
- Not to be receiving treatment for any medical condition.
- Not to be on any drugs or medication that might interfere with the physiological measures of the study and/not to have been on medication for the preceding 4 weeks prior to testing.
- Not to have undergone blood donation during the preceding 6 weeks or during the course of the study.

Procedures



Before testing commences, you will be asked to complete a health and fitness questionnaire which will ask you questions about your current state of health and your current level of fitness.

Ultrasound

Ultrasonography will be used to scan your heart, leg arteries and arm arteries. This involves a small probe being placed against the skin, aided by ultrasonic gel, to produce an image of the required component. Please refer to the end of this participant sheet to check for allergies against the ultrasonic gel. You will be asked to remove any clothing around the chest area so that your heart can be

scanned. Baggy shorts and a t-shirt would be ideal to allow access to the thigh and upper arm. Insert a diagram here showing where the ultrasound probe will be placed.

Incremental Isometric test

During week 1, you will be asked to complete an incremental isometric exercise test in order to determine your appropriate exercise intensity for the future training sessions. Firstly, you will be required to tense your legs as hard as you can so we can establish your maximal force. Once this has been completed you will then be asked to repeat the same leg tensing exercise, this time holding the tensing for 2 minutes at a time, with 5 minutes rest in between. Each bout of tensing will get harder in effort as time goes on. The test will continue until you feel like you can't do any more.

Blood Pressure

In weeks 1 and 10 blood pressure will be recorded using a machine called a 'finometer'. It's called this because it involves wearing a finger blood pressure cuff, which pulsates slightly when worn. An upper arm blood pressure cuff also needs to be worn, however it is only inflated at the start of the procedure and remains deflated for the rest of the test.

During weeks 2-9 your resting blood pressure will be monitored using a common hospital-type blood pressure device (a dinamap). This involves the customary upper arm blood pressure cuff, which is worn and inflated upon a few times to get an average resting reading.

Heart rate

In weeks 1 and 10, your resting heart rate will be measured. This involves placing 3 small circular sticky pads (electrodes) on your chest that measure the basic electrical activity of your heart.

EMG

The electrical activity of your leg muscles will be recorded whilst you perform the double leg tensing exercise. In a similar way to measuring your heart rate, small sticky pads (electrodes) will be placed on your thighs. To ensure that these pads make a good contact with your skin, it might be necessary to shave a small area of your skin on your thigh.

You should note that during the leg-tensing exercise, you might feel some discomfort or shortness of breath, however this is similar to what you would experience during any normal exercise activity. Of course, you are free to stop the exercise at any point, without reason, if you want to.

The assessment session in week 1 should last no more than a couple of hours. From week 2 onwards the sessions will last no more than about an hour. You will be asked to follow the following criteria before each visit:

- No food within 3 hours of testing
- Drink only water within 3 hours before testing
- No alcohol within 24 hours
- Not to have been taking any drugs or medication for the preceding 4 weeks.
- Not to have given blood during the preceding 6 weeks

- To have kept your salt intake the same throughout the 10 week testing period.
- To be free from illness/infection during the preceding 2 weeks.
- Not to have exercised strenuously (i.e. to exhaustion) in the 36 hours leading up to testing.

Feedback

If you would like feedback of all your results please ask for them. In the unlikely event of anything out of the ordinary being discovered when making measurements, you will be informed, and with your permission, we can provide information for your GP, so that you can get it checked out.

Confidentiality

All data and personal information will be stored securely (locked) within CCCU premises in accordance with the Data Protection Act 1998 and the University's own data protection requirements. Data can only be accessed by Jenna Smith, Dr Jim Wiles, Dr Damian Coleman & Dr Ian Swaine. During the study, all data will be made anonymous (i.e. all personal information associated with the data will be removed) so that you cannot be identified from the data.

Dissemination of results

The results of the study may be published, however all participants will be made anonymous so that you cannot be identified from the data.

Deciding Whether to Participate

If you have any questions or concerns about the nature of the study, its procedures or requirements, 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 Jenna Smith on 01227 767700 (ext 3145) or 07929278579 or email
i.e.smith36@canterbury.ac.uk
Department of Sport Science, Tourism and Leisure, North Holmes Road, Canterbury CT1 1QU

Ultrasound ingredients
Reserve osmosis (RO) water
Humectants
Polymer
Preservatives
Water soluble fragrance
FD & C color
Propyl paraben and methyl paraben in bacteriostatic concentration
Ph range 6.50-6.95

Appendix 5: Example of participant consent form used in studies 1,2,3,4 and 5.

Canterbury Christ Church University

CONSENT FORM

Title of Project: "The reliability and validity of a non invasive Doppler Ultrasound to measure femoral and brachial blood flow velocity, femoral and brachial artery diameter, and cardiac output."

Name of Researcher: Jenna Smith

Contact details:

Address: 1 Crossways
Sittingbourne
Kent
ME10 4RH

Tel: 01227 767700 (ext 3145) or 07929278579

Email: jes36@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 : r value for each significant correlation between each haemodynamic variable and relative exercise intensity.

Variable	r value
MBF	0.669
PBF	0.61
PE-MBF	0.742
PE-PBF	0.66
ΔBF	0.574
MSR	0.726
PSR	0.668
PE-MSR	0.839
PE-PSR	0.868
ΔSR	0.511
MASR	0.859
PASR	0.893
PE-MASR	0.895
PE-PASR	0.899
ΔASR	0.64
MRSR	-0.546
PRSR	-0.527
PE-MRSR	-0.427
PE-PRSR	-0.647
PE-MOSI	-0.404

Appendix 7. *CON Group mean values for resting cardiovascular variables at pre- training, mid-training and post-training time points.*

Resting cardiovascular variable	Pre-training point (0 weeks)	Mid-training point (4 weeks)	Post-training point (8 weeks)
Common femoral AD (cm)	0.93 ± 0.07	0.95 ± 0.09	0.87 ± 0.08
Brachial AD (cm)	0.38 ± 0.04	0.37 ± 0.05	0.38 ± 0.04
TPR	15.75 ± 4.18	16.97 ± 5.18	16.06 ±4.70
HR (beats ·min^{-1})	62 ± 11	60 ± 8	65 ± 9
TP (ms^2)	14142 ± 13003	11585 ± 7198	11435 ± 7320
HF (ms^2)	4732 ± 5417	3300 ± 3757	1902.11 ± 1527.85
LF (ms^2)	3939 ± 4031	2724 ± 1891	5468 ± 4941
HFnu	44.15 ± 16.05	39.51 ± 18.47	28.26 ± 7.97
LFnu	48.10 ± 20.68	49.15 ± 19.04	65.95 ± 11.04
LF/HF	1.47 ± 1.36	1.49 ± 1.33	2.64 ± 1.36
$\dot{Q}$ (L·min^{-1})	5.69 ± 2.17	5.56 ± 2.16	5.64 ± 1.93
IVRT (msec)	64.5 ± 11.1	72.4 ± 16.9	61.5 ± 8.9
LVET (msec)	297.2 ± 13.7	310.5 ± 21.6	302.4 ± 15.1

Common femoral artery diameter (AD), brachial artery diameter (AD), total peripheral resistance (TPR), heart rate (HR), total power (TP), high frequency (HF), low frequency (LF), high frequency normalized units (HFnu), low frequency normalized units (LFnu). Low frequency / high frequency ratio (LF/HF), Cardiac output (Q), isovolumic relaxation time (IVRT), and left ventricular ejection time (LVET).