The Effect of Isometric Exercise Training on Resting Blood Pressure with Specific Reference to Peripheral Vascular Function and Structure

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ABSTRACT

Isometric exercise training has been shown to bring about beneficial reductions in resting blood pressure, however the physiological mechanism responsible remain equivocal. It is currently unknown whether isometric exercise training-induced reductions in resting blood pressure are associated with improvements in vascular endothelial function and structure. The aim of this thesis was to investigate the effect of an 8-week isometric exercise training intervention on resting blood pressure, conduit artery vascular endothelial function, artery diameter and blood flow patterns at rest. Fourteen healthy young males (mean age: 23 ± 4 years; body mass: 80.7 ± 11.0kg; height: 178.8 ± 6.2cm) completed 8-weeks of isometric bilateral leg extension exercise training at high intensity (105.4% 2-min $torque_{peak}$). Three exercise sessions were performed each week and each session consisted of four 2-minute isometric muscular contractions each separated by a 3-minute recovery period. Resting blood pressure was measured using brachial artery oscillometric methods at pre, mid and post intervention. Brachial artery flow-mediated vasodilatation and resting artery diameter and blood flow were assessed at pre, week 2, mid, week 6 and post intervention using high-resolution duplex Doppler ultrasound. Total peripheral resistance and cardiac output were determined at pre and post intervention using high-resolution echocardiography. A generalised estimating equation analysis was used to estimate the effect of exercise versus non-exercise intervention over time on resting blood pressure, brachial artery flow-mediated vasodilatation, artery diameter and blood flow, total peripheral resistance and cardiac output. Isometric bilateral leg extension exercise training reduced resting systolic blood pressure (training: 117 vs. control: 121 mmHg) (P=0.002) and resting mean arterial pressure (training: 88 vs. control: 91 mmHg) (P=0.001) following 4 weeks of exercise training compared to the control condition. Brachial artery flow-mediated vasodilatation was increased (mid training: 8.65 ± 1.02% vs. mid control: 6.38 ± 1.14%) (P=0.011). Superficial femoral artery diameter, antegrade blood flow and antegrade shear rate were increased (all $P \leq 0.05$), whilst superficial femoral retrograde shear rate was decreased (P=0.013) following 2 weeks of exercise training compared to the control condition. There were no significant condition by time interactions observed in resting blood pressure, vascular endothelial function, artery diameter or blood flow patterns following 8 weeks of exercise training (all $P \geq 0.05$). Cardiac output was not significantly different following 8 weeks of exercise training (P=0.148). Total peripheral resistance was increased following 8 weeks of exercise training compared to the non-exercising control condition (P=0.054). Isometric bilateral leg extension exercise training can effectively lower resting blood pressure and increase conduit artery endothelium-dependent vasodilatation in healthy young men after 4 weeks with a concomitant reduction in resting blood pressure. These beneficial adaptations were no longer evident from mid to post exercise training in healthy normotensive young men.

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Abbreviations and symbols

DBP- Diastolic blood pressure

ECG- Electrocardiography

EMG- Electromyography

EMG_{peak}- Peak electromyography

FMD- Flow-mediated vasodilatation

HF- high frequency

HR- Heart rate

HRV- Heart rate variability

LF- low frequency

LF/HF ratio- low frequency/high frequency ratio

MAP- Mean arterial blood pressure

MSNA- Muscle sympathetic nerve activity

MVC- Maximal voluntary contraction

$\dot{Q}$- Cardiac output

RBP- Resting blood pressure

SBP- Systolic blood pressure

SV- Stoke volume

TPR- Total peripheral resistance

Δ Delta (Change)

CHAPTER 1: INTRODUCTION

High blood pressure is the leading preventable cause of cardiovascular disease in men and all-cause mortality in women (Garber et al., 2011). The cardiovascular protective effects of exercise training and the efficacy of exercise training to lower resting blood pressure have been well documented (Vanhees et al., 2012; Blair and Morris, 2009; Grau et al., 2009; Joyner and Green, 2009; Green et al., 2008). Therefore, the lowering of resting blood pressure has important implications for maintaining health. Isometric exercise training has been shown to lower resting blood pressure (RBP) in both normotensive and hypertensive populations despite differences in exercise training duration, exercise intensity and duration of muscular contraction (Millar et al., 2013; 2008; 2007; McGowan et al., 2007a; 2007b; 2006, Badrov et al., 2013; Baross et al., 2013; 2012; Devereux et al., 2010; Wiles et al., 2010; Howden et al., 2002; Ray and Carrasco, 2000; Wiley et al., 1992). More specifically, bilateral isometric leg extension exercise training has been shown to lower RBP in both healthy young and prehypertensive middle aged men (Baross et al., 2013; 2012; Devereux et al., 2010; Wiles et al., 2010).

Isometric exercise training studies investigating the physiological mechanisms responsible for the blood pressure lowering effect of exercise training have thus so far produced equivocal results (Devereux et al., 2010; Wiles et al., 2010; Ray and Carrasco, 2000). Exercise training-induced reductions in RBP are understood to occur through alterations in the determinants of arterial blood pressure, namely cardiac output ($\dot{Q}$) and/or total peripheral resistance (TPR) (Pescatello et al., 2004; Delp and O'Leary, 2004; Wiley et al., 1992). The evidence to date indicates that a reduction in TPR is likely to explain exercise training-induced reductions in RBP (Smith and Fernhall, 2011; Fagard and Cornelissen, 2007), with evidence of a reduction in TPR and RBP in unhealthy individuals (Hambrecht et al., 2000) and evidence of a reduction in $\dot{Q}$ and RBP following exercise training in healthy individuals (Wiles et al., 2017). A

reduction in TPR following exercise training may be mediated as a consequence of the widening of the vasculature within the peripheral circulation, this would permit less resistance to blood flow and therefore a lower pressure would be required to drive a given blood flow through the systemic circulation. In particular, alterations in peripheral vasculature function and structure are considered to significantly influence the resistance to blood flow (Wiedeman, 1962). An increase in vascular function and structure may be the physiological mechanism responsible for mediating exercise training-induced reductions in RBP. Recent evidence has demonstrated that improvements in indices of vascular function (Badrov et al., 2013; Tinken et al., 2010) and structure (Spence et al., 2013; Baross et al., 2012) can occur following exercise training. Despite this proposed concept, the physiological mechanism(s) responsible for mediating alterations in $\dot{Q}$ and/or TPR following isometric exercise training remain unknown (Carlson et al., 2014; Millar et al., 2014; Cornelissen and Smart, 2013; Devereux et al., 2010; Wiles et al., 2010). Therefore, investigation into the effect of isometric exercise training on peripheral vascular function and structure and the associations with reductions in RBP will form the focus of this thesis.

CHAPTER 2: LITERATURE REVIEW

2.1 Understanding isometric exercise

2.1.1 An overview of isometric exercise and cardiovascular adjustments in response to an acute bout of isometric exercise

Static (isometric) exercise involves the development of a relatively large muscular force with little change or no change in muscle length or joint movement (Mitchell et al., 2005). Isometric exercise causes an increase in intramuscular pressure at a localised level within active muscle fibres. Subsequently, isometric muscular contraction will change the normal blood flow patterns within the blood vessels, and ultimately lead to impedance of blood flow within the active muscles referred to as blood flow occlusion (Rowell, 1993). It has been demonstrated that once an isometric muscular contraction reaches 10 to 15% of an individual's maximal voluntary contraction (MVC) the muscle fails to secure adequate muscle blood flow (Rowell, 1993); and this appears to be dependent on the muscle group examined (Lind, 1983; Shepherd et al., 1983; Edwards et al., 1972). The occlusive effect of an isometric muscular contraction results in an exaggerated pressor response (Rowell, 1993). It is possible that occlusion to blood flow during isometric exercise provides a unique stimulus that may be related to improved cardiovascular health following exercise training (Horiuchi and Okita, 2012; Evans et al., 2010; Takarada et al., 2000). Under fatiguing isometric muscular contraction, the substantial energy demands must be derived through the processes of anaerobic metabolism and the reduction of local blood flow to skeletal muscle may elicit ischemic conditions (Hietanen, 1984).

Isometric muscular contractions that result in fatigue have been shown to elicit progressive increases in arterial blood pressure, heart rate, left ventricular contractility and cardiac output ($\dot{Q}$) (Lind, 1983; Schibye et al., 1981; Helfant et al., 1971). The increase in $\dot{Q}$ is disproportionally high for the volume of oxygen consumed (Lind et al., 1964) due to changes

in regional blood flow in the vascular beds (Gandevia and Hobbs, 1990). An increase in $\dot{Q}$ contributes to the observed increase in blood pressure during isometric exercise in healthy individuals (Lind et al., 1964). Alterations in autonomic function, more specifically an increase in heart rate (HR) due to vagal withdrawal and sympathetic cardiac stimulation have been held accountable (McAllister, 1979; Lind et al., 1964). Early research has established that at the immediate onset of exercise an increase in mean arterial pressure (MAP) and HR were brought about via excitatory drive from the cerebral cortex in the brain termed 'cortical irradiation' (Krogh and Lindhard, 1913). This central control mechanism is referred to as 'central command' (Goodwin et al., 1972) and it is considered responsible for eliciting immediate cardiovascular adjustments to static exercise (Gandevia and Hobbs, 1990; Davies and Starkie, 1985; Hultman and Sjoholm, 1982).

Peripheral reflexes initiated from within the active skeletal muscle, in conjunction with central command, play an important role in initiating the cardiovascular response during dynamic and isometric exercise (Rowell and O'Leary, 1990; Mitchell, 1990). The existence of a blood pressure-raising reflex originating from the contracting skeletal muscle is likely to be related to the muscle metaboreflex (Alam and Smirk, 1937). This reflex control mechanism is termed the 'exercise pressor reflex' (Mitchell et al., 1983; McCloskey and Mitchell, 1972; Coote et al., 1971) and cardiovascular responses during static exercise have also been associated with this reflex response (Kaufman and Forster, 1996; Friedman et al., 1992). Muscle metaboreceptors (unmyelinated group IV axons) located within the active skeletal muscle are sensitive to chemical changes (Rowell and O'Leary, 1990; Mitchell, 1990) and these changes may contribute to the exercise pressor response (Rusch et al., 1981). In addition, rapid increases in HR by the withdrawal of the vagal inhibition have been documented during external muscle compression and muscle contraction through the stimulation of muscle mechanoreceptors (myelinated group III axons) (Carrington et al., 2003; Gladwell and Coote, 2002).

More recently, the arterial baroreflex has been shown to play an important role in the neural regulation of cardiovascular adjustments to isometric exercise (Mitchell, 2013). The ability of the arterial baroreflex to be reset during exercise is considered to regulate the increase in blood pressure and perfusion pressure to active muscle at the onset of exercise (Coote and Dodd, 1976). Arterial baroreceptors are pressure sensitive (mechanosensitive) nerve fibre endings situated in the adventitia of systemic arteries and the heart including the aortic arch and carotid sinuses (Kougias et al., 2010). Evidence suggests the concomitant increase in blood pressure and HR is brought about during the initial onset of exercise via the resetting the central operating point (Joyner, 2006; Raven et al., 2006; Melcher and Donald, 1981). Indeed, an appropriately functioning arterial baroreflex has been recognised as essential for modulating the normal neural cardiovascular responses known to occur during exercise (Fadel, 2008). A recent review by Inchinose et al. (2012) has reiterated that an interplay between central command, muscle metaboreflex and arterial baroreflex are pivotal in modulating cardiovascular responses to isometric exercise. Although the mechanisms controlling the modulation of the arterial baroreflex are not fully understood (Raven, 2008), an interaction exists during isometric exercise between the arterial baroreflex and the muscle metaboreflex which helps to appropriately modulate sympathetic nervous activity (Inchinose et al., 2012; 2006; Kamiya et al., 2001).

2.2 Isometric exercise training and resting blood pressure

2.2.1 Contextualising the importance of exercise in the management of resting blood pressure

Hypertension affects more than one in four adults in England and is the second largest risk factor for premature death and disability (Health and Social Care Information Centre, 2013; Murray et al., 2013). Furthermore, hypertension is a leading risk factor for global disease burden (Lim et al., 2012). The economic burden to the National Health Service in England from conditions attributable to hypertension, including stroke, coronary heart disease and chronic kidney disease, exceeds two billion pounds (Optimity Matrix, 2014). The findings from the Framingham Heart Study state that a population-wide reduction in resting diastolic blood pressure (DBP) of 2 mmHg can have a clinically relevant impact, with an estimated 17% decrease in the prevalence of hypertension and a 6% reduction in the risk of coronary heart disease (Cook et al., 1995).

There is overwhelming evidence with regard to the detrimental impact hypertension has on public health and the economy, it is therefore surprising that significant discrepancies remain in the strategies used for the detection, management and prevention of high blood pressure. The most recent scientific statement from The American Heart Association suggested that isometric exercise training may be a suitable lifestyle intervention strategy to act as a non-pharmacological alternative to lower RBP (Brook et al., 2013). Further still, Public Health England (2014) have stated that there is a necessity for research into innovative lifestyle modifications strategies to lower RBP in those individuals classified with prehypertension (120/80 mmHg to 139/89 mmHg).

2.2.2 The overall effectiveness of resistance, aerobic and combined exercise training to induce reductions in resting blood pressure

A meta-analysis of 93 trials has summarised the overall effectiveness of exercise training to lower RBP in populations of varying blood pressure classifications (Cornelissen and Smart, 2013). The results from the meta-analysis revealed no significant differences in exercise training-induced reductions in resting systolic blood pressure (SBP) and DBP between endurance, dynamic resistance and combined exercise training modalities (Cornelissen and Smart, 2013). Whereas, isometric exercise training induced significantly larger reductions in resting SBP (10.9 mmHg) and DBP (6.2 mmHg) compared to endurance, dynamic resistance and combined endurance and dynamic resistance exercise training (See Table 1). A systemic review and meta-analysis of 9 studies was conducted to assess the overall effectiveness of isometric exercise training to lower RBP (Carlson et al., 2014). The results from this research further demonstrated the effectiveness of isometric exercise training to lower resting SBP, DBP and MAP in the normotensive population by 7.8 mmHg, 3.1 mmHg and 3.6 mmHg, respectively. Furthermore, a recent publication by Inder et al. (2016) reported similar findings in a review of 11 randomised controlled trials. It may be construed from the evidence of these meta-analyses that isometric exercise training is effective to lower RBP. Isometric exercise training-induced reductions in RBP appear to be similar in magnitude compared to those induced by endurance, dynamic resistance and combined modes of exercise training. What is more, consistent reductions in RBP of at least 2 mmHg have been reported following isometric exercise training, a reduction that is considered to be clinically significant for cardiovascular disease prognosis (Chobanian et al., 2003).

Table 1. The mean difference in resting blood pressure from pre to post exercise training for various training modalities (adapted from Cornelissen and Smart, 2013).

Training modality	SBP (mmHg)	DBP (mmHg)
Endurance	- 3.5 (-4.6 to -2.3)	- 2.5 (-3.2 to -1.7)
Dynamic resistance	- 1.8 (-3.7 to -0.011)	- 3.2 (-4.5 to -2)
Isometric resistance	- 10.9 (-14.5 to -7.4)	- 6.2 (-10.3 to -2.0)
Combined endurance and resistance	- 1.4 (4.2 to 1.5)	- 2.2 (-3.9 to -0.48)

Note: Data presented as mean difference (upper and lower 95% confidence limits). SBP: systolic blood pressure; DBP: diastolic blood pressure.

2.2.3 The effectiveness of isometric exercise training in reducing resting blood pressure in normotensive, prehypertensive and hypertensive individuals

Isometric handgrip exercise training

Isometric handgrip exercise training has been shown to induce significant reductions in RBP in normotensive, prehypertensive and hypertensive individuals. A summary of the programme variables and findings from exercise training studies involving isometric handgrip exercise has been compiled (See Table 2). Early research by Wiley et al. (1992) demonstrated significant RBP reductions following exercise training. Resting SBP and DBP were significantly reduced (13 mmHg and 15 mmHg, respectively) in prehypertensive individuals after just 4 weeks of isometric exercise performed 3 days per week at an exercise intensity of 30% MVC (Wiley et al., 1992). Similarly, large reductions in resting SBP and DBP (10 mmHg and 9 mmHg, respectively) in normotensive individuals were evident in a shorter period (after 3 weeks) when participants completed four 45-second contractions performed at 50% MVC (Wiley et al., 1992). Furthermore, Ray and Carrasco (2000) also confirmed significant reductions in resting DBP and MAP (5 mmHg and 4 mmHg, respectively) in healthy young participants following

5 weeks of exercise training. Taylor et al. (2003) also reported the effectiveness of a 10-week training intervention in bringing about reductions in resting SBP and MAP (19 mmHg and 11 mmHg, respectively). Reductions in resting SBP and MAP were evident from week 5 onwards, an indication that an isometric exercise training intervention of short duration can be effective in lowering RBP. In a subsequent study by Badrov et al. (2013) resting SBP was significantly reduced by 6 mmHg in normotensive participants following 8 weeks of training either 3 or 5 days per week; the analysis revealed that training more frequently (5 days per week) resulted in these RBP reductions occurring at a faster rate. Garg et al. (2014) reported significant reductions in resting SBP and DBP (10 mmHg and 6 mmHg, respectively) for 10 weeks of training. McGowan et al. (2007a) and Millar et al. (2008) reported similar reductions in resting SBP by 5 mmHg vs. 6 mmHg after 8 weeks of exercise training in normotensive individuals. The combined findings from Wiley et al. (1992), Ray and Carrasco (2000), Badrov et al. (2013) and Taylor et al. (2003) clearly demonstrate the ability of RBP to be lowered within 3 to 5 weeks of isometric handgrip exercise training within both normotensive and prehypertensive populations. For isometric exercise training studies with a total duration of more than 8 weeks, consistent reductions in RBP have been reported following exercise training within the normotensive population.

Inherent methodological differences between programme variables are likely to explain the magnitude and time course of exercise training-induced adaptations in RBP. The majority of exercise training research within normotensive, prehypertensive and hypertensive populations has utilised unilateral or alternating unilateral isometric handgrip exercise, predominantly consisting of four 2-minute contractions at 30% MVC performed 3 times per week for a total duration of 8 to 10 weeks (See Table 2). Isometric exercise training involving handgrip exercise has induced significant reductions in blood pressure ranging from 5 mmHg to 19 mmHg for resting SBP, and 3 mmHg to 11 mmHg for resting DBP (Millar et al., 2013; Badrov et al.,

2013; McGowan et al., 2006, Peters et al., 2006; Taylor et al., 2003; Wiley et al., 1992). A notable finding from the existing literature, isometric exercise training-induced reductions in RBP tended to be lower in magnitude in normotensive populations compared to prehypertensive populations. The most effective isometric exercise training programme variables required to elicit optimal RBP reductions within specific populations remain to be established.

Isometric bilateral leg extension exercise training

Previous research has investigated the effectiveness of isometric bilateral leg extension exercise training to lower RBP in normotensive (Wiles et al., 2010; Devereux et al., 2010; Howden et al., 2002) and prehypertensive individuals (Baross et al., 2013; 2012). However, the number of studies investigating isometric lower limb exercise training is limited in comparison to isometric handgrip exercise training. Isometric bilateral leg exercise training involves the recruitment of a larger muscle mass compared to handgrip exercise and there is evidence to show that the magnitude of cardiovascular responses to an acute bout of isometric exercise are positively correlated with the amount of muscle mass involved (Gálvez et al., 2000; Seals, 1989; Mitchell et al., 1980). It is currently unclear to what extent muscle mass recruitment influences the magnitude of subsequent RBP reductions following isometric exercise training. It is possible that isometric exercise training involving the larger muscle groups, may result in RBP reductions comparable to upper body isometric exercise training which may be achieved at a lower relative exercise intensity or in a shorter total training duration. The majority of research involving isometric bilateral leg extension exercise training has focused on investigating the effect of exercise intensity on the magnitude of RBP reductions. A number of studies have shown that exercise training-induced RBP reductions are dependent on exercise intensity (Baross et al., 2012; Devereux et al., 2010; Wiles et al., 2010).

A summary of the programme variables and findings from exercise training studies involving isometric lower limb exercise has been compiled (See Table 3).

Wiles et al. (2010) performed isometric bilateral leg extension exercise training at a constant level of electromyography equivalent to 75% and 95% of peak HR whilst all other programme variables remained the same. Resting SBP, DBP and MAP were significantly reduced following 8 weeks of isometric bilateral leg extension training in the lower intensity group compared to the control group, indicating that exercise intensity ranging between 75% and 95% peak HR (equivalent to 10 and 21% MVC, respectively) can elicit comparable reductions in RBP. In alignment with the findings presented by Wiles et al. (2010), Devereux et al. (2010) reported that 4 weeks of isometric bilateral leg extension exercise training performed at 95% of peak HR significantly lowered resting SBP, DBP and MAP (5 mmHg, 3 mmHg and 3 mmHg, respectively). There is limited data on the effectiveness of isometric bilateral leg extension exercise training to lower RBP within the prehypertensive population, however previous research has reported significant reductions in resting SBP and MAP (11 mmHg and 5 mmHg, respectively) following 8 weeks of training performed 3 days per week at 85% of peak HR (Baross et al., 2012). Interestingly, within a middle-aged (age 55 ± 6 years) prehypertensive population Baross et al. (2012) reported no significant changes in RBP following 8 weeks of low intensity exercise training at 70% of peak HR whilst all other programme variables remained the same. This would suggest that exercise training-induced RBP reductions are dependent on training intensity in a middle-aged prehypertensive population.

The application of alternative methods of prescribing isometric exercise intensity, other than the traditional percentage of maximum voluntary force (% MVC), have been explored in previous studies (Devereux et al., 2011; Wiles et al., 2007). Devereux et al. (2011) investigated

the relationship between several markers of isometric exercise intensity and reductions in RBP following 4 weeks of exercise training. Interestingly, the prescription of exercise intensity using a torque-based method (2-minute $torque_{peak}$) was most strongly associated with reductions in resting SBP and MAP. These results would suggest that the optimal method to prescribe intensity for isometric bilateral leg extension exercise may be to relate exercise training torque to the highest average torque that can be sustained for the final two minutes of a discontinuous incremental exercise test (2-minute $torque_{peak}$) (Devereux et al., 2011). These findings led the author to state that it may be advantageous to prescribe exercise intensity based upon a marker which mimics the actual training bout duration; and training at 105% 2-minute $torque_{peak}$ appears most likely to induce significant reductions in resting SBP after 4 weeks of isometric bilateral leg extension exercise training (Devereux et al., 2011).

Both isometric upper body and lower body exercise training protocol are effective in lowering RBP within normotensive and prehypertensive populations. Studies involving isometric bilateral leg extension exercise training have focused on investigating the effect of exercise intensity on the magnitude of blood pressure reductions within normotensive populations. These studies have consistently demonstrated modest reductions around 5 mmHg for resting SBP and 3 mmHg for resting MAP at exercise intensities equivalent to 20 to 24 % MVC performed 3 times per week for a total of 4 to 12 weeks (Howden et al., 2002; Wiles et al., 2010; Devereux et al., 2010). Based upon the programme variables utilised in previous isometric lower limb exercise training research, the protocol utilised within the present thesis will include four 2-minute bilateral isometric leg extension contractions performed 3 days per week for a total of 8 weeks. Furthermore, a discontinuous incremental isometric exercise test (Section 3.2.11) will be used to prescribe exercise intensity using a torque-based method (2-minute $torque_{peak}$) (Devereux et al., 2011) to investigate the effectiveness of this exercise intensity prescription method to elicit favourable exercise training-induced reductions in RBP.

2.3 An overview of proposed physiological mechanisms responsible for exercise training-induced reductions in resting blood pressure

The exact physiological mechanism(s) responsible for mediating isometric exercise training-induced reductions in RBP remains unclear. Thus so far, potential physiological mechanisms have been broadly categorised into the following areas: cardiac autonomic regulation, cardiac functional and structural adaptations, neural regulation of vascular tone, vascular functional and structural adaptations and oxidative stress (Millar et al., 2014).

2.3.1 Cardiac autonomic regulation

Isometric exercise training-induced reductions in RBP may be brought about via reduced stimulation of the sympathetic cardioaccelerator nerves and/or increased stimulation of the parasympathetic cardioinhibitory nerves. An adaptation in cardiac autonomic function could lead to a lower HR which would permit increased diastolic filling, end-diastolic volume and thus greater systolic emptying resulting in a more efficient $\dot{Q}$. A dynamic balance between parasympathetic and sympathetic nervous outflow to the heart in order to generate a heartbeat is an essential biological process for optimal cardiovascular health. The cardiovascular centre in the medulla of the brainstem responds to afferent impulses from sensory neurons and higher brain centres by adjusting HR via changes in the relative balance between sympathetic and parasympathetic outflow (Shaffer and Venner, 2013). The measurement of heart rate variability (HRV) is considered to provide an insight into the potential cardiac autonomic regulation of RBP; it should be noted that the precise physiological interpretation of HRV is subject to debate (McCraty and Shaffer, 2015; Shaffer et al., 2014). Reduced HR variability and high resting HR are considered independent predictors of cardiovascular disease and mortality (Tsuji et al., 1996). It is widely assumed that a lower low frequency to high frequency ratio reflects greater parasympathetic activity relative to sympathetic activity, whereas a greater

Table 2. A summary of the programme variables and findings from exercise training studies involving isometric upper body exercise.

Isometric handgrip exercise							
	Journal	**Sex and age**	**Exercise Intensity**	**Exercise protocol**	**Total duration**	**RBP**	**Potential mechanism for RBP reduction**
Normotensive	Wiley *et al.* (1992)	Males	50% MVC	4 x 45-seconds 5 days per week Alternating unilateral	8 weeks	SBP ↓10mmHg DBP ↓9mmHg	None investigated
	Ray and Carrasco (2000)	Males	30% MVC	4 x 3-mins 4 days per week Unilateral	5 weeks	SBP ↓5mmHg MAP ↓4mmHg	No change resting MSNA
	Howden et al. (2002)	Males and Females	30% MVC	4 x 2-mins 3 days per week Bilateral	5 weeks	SBP ↓12mmHg	
	McGowan *et al.* (2007)	Males and Females	30% MVC	4 x 2-mins 3 days per week Unilateral	8 weeks	SBP ↓5mmHg	No change peripheral vascular endothelial function
	Badrov *et al.* (2013)	Females	30% MVC	4 x 2-mins 5 days per week Alternating unilateral	8 weeks	SBP ↓6mmHg	Improved resistance vessel function
	Badrov *et al.* (2013)	Females	30% MVC	4 x 2-mins 3 days per week Alternating unilateral	8 weeks	SBP ↓6mmHg	Improved resistance vessel function
	Garg *et al.* (2014)	Males and Females	30% MVC	5 x 3-mins 3 days per week	10 weeks	SBP ↓10mmHg DBP ↓6mmHg	None investigated
Prehypertensive	Wiley *et al.* (1992)	Males	30% MVC	4 x 2-mins 3 days per week Unilateral	8 weeks	SBP ↓13mmHg DBP ↓15mmHg	None investigated
Hypertensive	Taylor *et al.* (2003)	Males and Females	30% MVC	4 x 2-mins 3 days per week Alternating unilateral	10 weeks	SBP ↓19mmHg MAP ↓11mmHg	Reduced LF:HF ratio
	Peters *et al.* (2006)	Males and Females	50% MVC	4 x 45-secs 3 days per week Alternating unilateral	6 weeks	SBP ↓13mmHg DBP ↓2mmHg	No change HRV
	McGowan *et al.* (2006)	-	30% MVC	4 x 2-mins 3 days per week Unilateral	8 weeks	↔	Improved peripheral vascular endothelial function
	Stiller-Moldovan *et al.* (2012)	Females	30% MVC	4 x 2-mins 3 days per week Alternating unilateral	8 weeks	↔	

Table 2 continued.

Millar *et al.* (2013)	Males and Females	30% MVC	4 x 2-mins 3 days per week Unilateral	8 weeks	SBP ↓5mmHg MAP ↓3mmHg
Badrov *et al.* (2013a)	Males and Females	30% MVC	4 x 2-mins 3 days per week Alternating unilateral		SBP ↓8mmHg DBP ↓5mmHg MAP ↓6mmHg

Table 3. A summary of the programme variables and findings from exercise training studies involving isometric lower body exercise.

Isometric bilateral leg exercise							
	Journal	**Sex and age**	**Exercise Intensity**	**Exercise protocol**	**Total duration**	**RBP**	**Potential mechanism for RBP reduction**
Normotensive	Howden *et al.* (2002)	Males	20% MVC	4 x 2-mins 3 days per week	5 weeks	SBP↓10mmHg DBP ↔ MAP ↔	None investigated
	Wiles *et al.* (2010)	Males	75% HRpeak (~10%MVC)	4 x 2-mins 3 days per week	8 weeks	SBP↓4mmHg DBP↓3mmHg MAP↓3mmHg	No change $\dot{Q}$, TPR or HRV
	Wiles *et al.* (2010)	Males	95% HRpeak (~21% MVC)	4 x 2-mins 3 days per week	8 weeks	SBP↓5mmHg DBP↓3mmHg MAP↓3mmHg	
	Devereux *et al.* (2010)	Males	95% HRpeak (~24% MVC)	4 x 2-mins 3 days per week	4 weeks	SBP↓5mmHg DBP↓3mmHg MAP↓3mmHg	No change $\dot{Q}$, TPR or HRV

Table 3 continued.

	Gill *et al.* (2015)	Males	20% EMGpeak (~23% MVC)	4 x 2-mins 3 days per week	3 weeks	SBP↓4mmHg DBP↓4mmHg MAP↓4mmHg	None investigated
	Gill *et al.* (2015)	Males	30%EMGpeak (~34% MVC)	4 x 2-mins 3 days per week	3 weeks	SBP↓2mmHg DBP ↔ MAP ↔	
Prehypertensive	Baross *et al.* (2012)	Males	70% HRpeak ~7% MVC)	4 x 2-mins 3 days per week	8 weeks	SBP ↔ DBP ↔ MAP ↔	
	Baross *et al.* (2012)	Males	85% HRpeak (~14% MVC)	4 x 2-mins 3 days per week	8 weeks	SBP↓11mmHg DBP ↔ MAP↓5mmHg	Peripheral vascular adaptation

low frequency to high frequency ratio reflects higher sympathetic activity relative to parasympathetic activity (Shaffer et al., 2014).

The majority of the studies which have incorporated measurement of HRV have reported no significant change in power spectral or time-domain measures of HRV following isometric exercise training in either normotensive or hypertensive individuals (Millar et al., 2013; Stiller-Moldovan et al., 2012). In addition, the majority of research suggests that resting HRV is not altered following dynamic resistance training in young healthy or prehypertensive individuals (Collier et al., 2009; Heffernan et al., 2007; Cooke and Carter, 2005). Researchers who have reported isometric exercise training-induced reductions in RBP in healthy and hypertensive individuals have also reported no concomitant change in linear (frequency or time domain) or non-linear HRV (Badrov et al., 2013; Wiles et al., 2010; Ray and Carrasco, 2000). In contrast, Taylor et al. (2003) reported a decrease in the low frequency component and a significant increase in the high frequency component resulting in a non-statistically significant trend toward a reduction in the low frequency to high frequency ratio following isometric exercise training. These results may suggest improved vagal modulation of HR is responsible for the observed reductions in RBP within the hypertensive population.

There are conflicting results with regard to changes in resting HR following isometric exercise training, some studies have reported no significant change (Devereux et al., 2010; Ray and Carrasco, 2000; Wiley et al., 1992) whilst another study has reported significant reductions in resting HR (Baross et al., 2012). Furthermore, although aerobic training has been shown to lower resting HR and efferent sympathetic nerve activity to the sinoatrial node in the heart (Carter et al., 2003); there is limited evidence to suggest that dynamic resistance training (Kingsley and Figueroa, 2014) or isometric exercise training (Millar et al., 2013; Badrov et al.,

2013) has any such effect on HRV or resting HR in individuals with normal cardiac autonomic modulation (Millar et al., 2014).

2.3.2 Cardiac structure and function

Adaptations in cardiac function and structure have been proposed as a potential mechanism responsible for exercise training-induced reductions in RBP (Saltin et al., 2000). There is extremely limited research available which has investigated the effect of isometric exercise training on adaptations in cardiac structure and function, therefore existing literature will be reviewed which has utilised dynamic resistance and aerobic exercise training in order to explore this potential mechanism for exercise training-induced reductions in RBP. Isometric exercise training has been shown to result in no significant difference in resting stroke volume (SV) and $\dot{Q}$ from pre to post training (Devereux et al., 2010; Wiles et al., 2010). In contrast, a recent study by Wiles et al. (2017) has shown decreased $\dot{Q}$ at rest following 8-weeks of home-based wall squat exercise training, however this study did not investigate any further measures of cardiac function or structure. Previous research has shown that resistance exercise trained individuals have higher SV at rest as well as enhanced left ventricular diastolic function compared to their sedentary counterparts (Adler et al., 2008). Some studies have reported improved left ventricular diastolic function, systolic function and ejection fraction in animals and humans following dynamic resistance exercise training thereby indicative of improved cardiac function (Alves et al., 2014; Gielen et al., 2010; Levinger et al., 2005). Whereas, combined aerobic and resistance exercise training in unhealthy populations has been shown to elicit no change in cardiac function (Fontes-Carvalho et al., 2015; Haykowsky et al., 2007).

There are a limited number of experimental studies available that have investigated cardiac structural adaptations following dynamic resistance exercise training. The findings are conflicting, with reports of increased cavity size (eccentric hypertrophy) or increased wall

thickness (concentric hypertrophy) (Naylor et al., 2008; Haykowsky et al., 2000). Aerobic exercise training has been shown to alter cardiac dimensions, namely increased left ventricular hypertrophy, left ventricular diameter and left ventricular wall thickness (Schmidt-Trucksäss et al., 2003; Pluim et al., 2000), which can lead to increased SV and reduced HR under rested conditions. There appears to be limited evidence to support alterations in cardiac structure and function as a physiological mechanism responsible for RBP reductions after isometric exercise training. Similarly, exercise training-induced reductions in RBP tend not to be associated with decreases in cardiac sympathetic modulation in healthy individuals (Section 2.3.1). In light the current review of existing literature, physiological mechanisms other than cardiac function and structure will take precedence for investigation in their role as a mediator of exercise training-induced reductions in RBP.

2.3.3 Neural regulation of vascular tone

Exercise training-induced reductions in RBP may be mediated through attenuated sympathetic neural regulation of vascular tone within the peripheral vasculature (Millar et al., 2014). Vascular tone is defined as the degree of tension exerted by the vascular smooth muscle relative to its maximal dilated state (Korthius, 2011). Vascular tone is the term used to describe changes in contractile tension of blood vessels that cause vasoconstriction or vasodilatation where necessary to ensure adequate tissue perfusion pressure and blood flow (Levick, 2010). There are two main approaches for the assessment of sympathetic innervation of vascular tone: muscle sympathetic nerve activity (MSNA) which uses microneurography to record sympathetic outflow from postganglionic sympathetic neurons controlling vascular tone in humans (Bruno et al., 2012), and to a lesser extent whole body and regional norepinephrine spill over (Joyner et al., 2010). It is widely accepted that human MSNA is modulated by the arterial baroreflex mechanism and this is integral to the short-term regulation of vascular tone and blood pressure (Bruno et al., 2012; Charkoudian and Rabbits, 2009).

Studies involving predominantly lower body aerobic, whole body resistance, and isometric or isotonic hand grip exercise have produced equivocal results, either demonstrating unchanged (Ray and Carter et al., 2010; Saito et al., 2009; Carter et al., 2003; Cooke et al., 2005; Ray and Carrasco, 2000; Ray, 1999; Sheldahl et al., 1994; Svedenhag et al., 1984), decreased (Grassi et al., 1994) or increased MSNA (Sinoway et al., 1996) at rest in healthy individuals following exercise training. Ray and Carrasco (2000) demonstrated no significant change in MSNA following 5 weeks of isometric handgrip exercise training despite significant reductions in resting MAP and DBP (4 mmHg and 5 mmHg, respectively) in healthy young individuals. It was inferred that reductions in RBP in healthy normotensive individuals were not dependent on attenuated efferent sympathetic innervation of peripheral vasculature. In addition, several researchers have reported no significant change in resting MSNA following exercise training in healthy young men (Ray and Carter, 2010; Saito et al., 2009; Cooke et al., 2005; Svedenhag et al., 1984). Six weeks of one-legged cycling performed 4 days per week had no effect on resting MSNA (Ray, 1999) and 8 weeks of whole body resistance training performed 3 days per week has been shown to have no effect on resting MSNA (Carter et al., 2003). Saito et al. (2009) reported that 4 weeks of isometric handgrip exercise training performed 4 days per week had no effect on resting MSNA. Furthermore, a recent study has reported that concurrent aerobic and resistance exercise training had no effect on indices of resting cardiac and vascular sympathetic modulation (Alex et al., 2013), perhaps indicative that the cardioprotective effects of exercise training are unlikely to be mediated by changes in resting sympathetic nerve activity in healthy young individuals. Overall, there appears to be limited evidence to support the notion that attenuated neural regulation of vascular tone is a potential mechanism for isometric exercise training-induced reductions in RBP.

Muscle sympathetic nerve activity reactivity is a technique used to assess sympathoexcitation to an applied stressor and it has been suggested that attenuation in MSNA reactivity may be

advantageous to cardiovascular health (Carter and Ray, 2015). A reduction in MSNA reactivity would imply that when a stressor is applied less sympathoexcitation occurs. Research has shown that an attenuation in MSNA reactivity can occur even when resting MSNA is either unchanged or decreased in unhealthy populations (Martinez et al., 2011; Stranznicky et al., 2010; Laterza et al., 2007; Ray et al., 1999; Grassi et al., 1994). One study has demonstrated that 4 months of aerobic cycling exercise training can reduce RBP and a concomitant attenuation in resting MSNA and MSNA reactivity (Laterza et al., 2007). Sinoway et al. (1996) reported a reduction in MSNA reactivity to stressors following 4 weeks of rhythmic handgrip training in trained individuals possibly due to the desensitisation of the metabolically sensitive muscle afferents due to chronic exposure to products of anaerobic metabolism. Carrington et al. (1999) have suggested that desensitisation of both the mechanoreceptors and mechanoreceptors muscle afferent inputs are responsible for exercise training-induced reductions in RBP potentially mediated via an attenuation in MSNA. Further evidence demonstrates that reductions in MSNA at rest following rhythmic handgrip exercise training occur with concomitant reductions in lactate production (Mostoufi-Moab et al., 1998). This supports the proposal that altered afferent muscle metaboreceptor responses are involved in the attenuation of efferent MSNA reactivity response following static forearm conditioning (Somers et al., 1992).

Blood pressure variability has also been proposed as a marker of baroreflex-mediated peripheral sympathetic modulation of vascular tone (Pagani et al., 1988). Indeed, the low-frequency spectra of systolic blood pressure variability has been shown to significantly decrease with concomitant reductions in RBP following 10 weeks of isometric exercise training (Taylor et al., 2003). These findings suggest that attenuated peripheral sympathetic modulation may play a role in the isometric exercise training-induced reductions in RBP in hypertensive participants, yet there is limited evidence to suggest this is the primary mechanism responsible

for RBP reductions in healthy individuals. It has been postulated that enhanced vascular endothelial function and reduction in the protein expression of angiotensin type II receptors in the paraventricular nucleus of the hypothalamus and rostral ventrolateral medulla of the brainstem could be the underlying mechanisms for any observed reductions in MSNA (Carter and Ray, 2015). The interaction between the sympathetic nervous system and vascular endothelial function may help to explain the high within individual variation in resting MSNA in normotensive individuals with similar RBP (Skarphedinsson et al., 1997). It is likely that the invasive nature of MSNA measurement techniques has contributed to the lack of investigation in this area. Although there is some evidence of improved SBP variability in unhealthy individuals following isometric exercise training, there is limited evidence to support attenuated neural regulation of vascular tone as a physiological mechanism responsible for isometric exercise training-induced reductions in RBP.

2.3.4 Peripheral vascular function and structure

Vascular resistance is a key regulator of skeletal muscle blood flow and therefore alterations in local control mechanisms relating to the function and structure of the vascular endothelium (Hamann et al., 2004) and vascular smooth muscle cells (Clifford and Hellsten, 2004) may provide the greatest insight into chronic adaptations to exercise training (Whyte and Laughlin, 2010). There is growing evidence to suggest that reductions in RBP occur predominantly through locally mediated changes in vascular physiology (Prior et al., 2004; 2003; Green et al., 2004a; 2004b; Brown, 2003). Vascular resistance is primarily brought about by the magnitude of contraction of the vascular smooth muscle surrounding the resistance arteries, which is itself, initiated via the vascular endothelium (Thomas and Segal, 2004; Delp and Laughlin, 1998). Improvements in the functioning of the peripheral vasculature may lead to enhanced relaxation of the vascular smooth muscle surrounding the resistance arteries, widening of the vessel and therefore lowered vascular resistance. Intrinsic mechanisms which regulate vascular tone

include: the secretion of substances from the endothelium (nitric oxide, prostaglandins and endothelium derived hyperpolarising factor), the release of vasoactive metabolites generated by the active tissues (adenosine), myogenic responses and the release of vasoactive paracrine secretions (autacoids) (Levick, 2010).

The vascular endothelium is considered the intrinsic mechanism most likely to modulate the control of muscle blood flow and thus vascular resistance at the local level through the release of vasoactive substances (Sarelius and Pohl, 2010). The vascular endothelium is a monolayer of cells between the vessel lumen and the vascular smooth muscle cells (Tousoulis et al., 2012), which is pivotal for normal bodily functions including the exchange of respiratory gases and nutrients between blood and tissue, regulation of vascular tone and prevention of inflammation. Homocellular gap junctions transmit ions and membrane potentials between neighbouring endothelial cells and heterocellular gap junctions transmit signals between the endothelial cells and the vascular smooth muscle (Félétou, 2011). The vascular endothelium detects changes in blood flow and shear stress through the lumen ultimately supporting the movement of bolus flow at the capillary level (Weinbaum et al., 2007). Vasodilator substances released locally such as nitric oxide, prostacyclin and endothelium derived hyperpolarising factor, hydrogen peroxide and potassium ions as well as vasoconstrictor substances such as angiotensin-II and endothelin all play a role in the modulation of vascular tone (Bellien et al., 2008; Vanhoutte et al., 2005). Vascular endothelial nitric oxide located within the skeletal muscle has been shown to contribute to the control of vascular tone (Grange et al., 2001; Lau et al., 2000). In addition, vascular tone is also affected by changes in vascular smooth muscle via extrinsic regulation including autonomic regulation of the heart (HRV) and peripheral vasculature (MSNA) as well as circulating hormones (Levick, 2010); it should be acknowledged that there is high variability in the measurement of HRV and MSNA (Section 2.3.1 and 2.3.3).

Brachial artery flow-mediated dilatation (FMD) is a commonly used technique in clinical and research settings to evaluate conduit artery endothelial function and is an accepted index of nitric oxide-mediated endothelium dependent vasodilatation (Joannides et al., 1995; Kooijman et al., 2008). Artery diameter and arterial wall thickness are commonly used measures to assess vascular structure also referred to as arterial remodelling (Dinenno et al., 2001). Furthermore, conduit artery vasodilator capacity is widely accepted as a surrogate marker of arterial remodelling (Naylor et al., 2005). Substantial evidence from animal studies shows enhanced endothelium-dependent dilator responses, increased nitric oxide production and endothelial nitric oxide synthase gene expression within 7 to 10 days of exercise training in conduit arteries and arterioles (Koller et al., 1995; Wang et al., 1993; Sessa et al., 1994). In addition, improved vascular endothelial function and increased endothelial nitric oxide synthase protein levels have been shown following 2 to 4 weeks of exercise training in skeletal muscle arterioles in an animal model (Sun et al., 1994). Moreover, exercise training studies using animal models have shown significant enlargement of arterial diameter which have been reported to be endothelium- and nitric oxide-dependent (Prior et al., 2003; Rudic et al., 1998; Lash and Bohlen, 1992). This historical research has led to the modern day consensus that efficient functioning of the vascular endothelium is essential for maintaining healthy vessel walls and vasomotor tone in conduit and resistance blood vessels (Padilla et al., 2014; Whyte and Laughlin, 2010).

Epidemiological studies have suggested that changes in cardiovascular disease risk factors such as blood pressure, cholesterol, body weight, triglycerides and insulin sensitivity explain a large proportion (approximately 60%) of the observed beneficial effect of exercise training (Mora et al., 2007). This has led researchers to suspect that the remaining risk reduction may be attributed to changes in vascular parameters such as endothelial function, arterial remodelling and vessel compliance (Thijssen et al., 2010; Green et al., 2009; 2008; Mora et al., 2007). The

results from the meta-analysis conducted by Inaba et al. (2010) demonstrated that for every 1% improvement in brachial artery FMD there is a potential to reduce the risk of a cardiovascular event by 13% and therefore maintaining optimal vascular endothelial health would inevitably be beneficial. Current evidence from the Framingham Offspring Cohort suggests that hypertension is positively associated with the degree of endothelial dysfunction (Benjamin et al., 2004); and endothelial dysfunction has been shown to precede hypertension (Rossi et al., 2004; Sander et al., 1999). Endothelial dysfunction is defined as the progressive development of irregular vascular function mainly due to a reduction in nitric oxide bioavailability and abnormal regulation of vascular tone (Yao et al., 2010).

Only a few studies have attempted to explain exercise training-induced reductions in RBP through improvements in systemic vascular endothelial function. Improvements in RBP have been associated with enhanced vascular endothelial function in healthy individuals (Badrov et al., 2013), young prehypertensive individuals (Beck et al., 2013), formerly preeclamptic women and aged matched controls (Scholten et al., 2014), postmenopausal women (Swift et al., 2012) and in rats (Mota et al., 2014). In contrast, research has demonstrated no association between improvements in vascular endothelial function and reductions in RBP in obese (Cotie et al., 2014) or healthy individuals (McGowan et al., 2007b). Increases in artery diameter have been reported to occur in the exercised-trained limb following training (Spence et al., 2013; Hunt et al., 2013; Miyachi et al., 2001), with some reports of increased artery diameter in the systemic peripheral vasculature (Stebbings et al., 2013). Isometric lower limb exercise training-induced reductions in RBP have been associated with increased conduit artery diameter in the leg (Baross et al., 2012). Structural adaptations in the form of artery diameter are likely to contribute to benefit of exercise training on RBP; however, there is limited research confirming the existence of a relationship between increases in resting conduit artery diameter and reductions in RBP. It could be inferred that improvements in vascular endothelial function via

a nitric oxide-mediated mechanism might at least in part explain isometric exercise training-induced reductions in RBP.

2.3.5 The role of nitric oxide and shear stress in mediating improvements in vascular function and structure

The signalling molecule nitric oxide is produced by the endothelium and is considered primarily responsible for the relaxation of the vascular smooth muscle (Dudzinski and Michel, 2007; Sessa, 2005). Arteriole and resistance vessel function is upregulated in response to increased wall shear stress and is most likely mediated via endothelium-derived nitric oxide and prostaglandins (Koller *et* al., 1995) and upregulate endothelial nitric oxide synthase expression (Sun et al., 1998; Delp and Laughlin, 1997). Together in sufficient quantities this may counteract neurogenic and myogenic constrictor influences over the vasculature (Hecker et al., 1993). Several types of nitric oxide-dependent vasodilator mechanisms have been identified which may bring about vasodilatation: flow-mediated, mechanically-induced (Clifford et al., 2006), muscle-activation related mechanisms (Van Teeffelen and Segal, 2006), muscle-metabolite related mechanisms (Clifford and Hellsten, 2004) and red blood cell oxyhaemoglobin desaturation mechanisms for vasodilatation (Ellsworth, 2004). In particular, flow-mediated vasodilatation is a vasodilatory mechanism that permits the rapid normalisation of heightened shear stress (Dimmeler and Zeiher, 2003; Niebauer and Cook, 1996; Koller and Kaley, 1991). In response to heightened blood flow, the release of endothelial-derived nitric oxide can contribute to the propagation of vasodilatation upstream from sites of metabolic demand to increase blood flow in upstream vessels (Bagher and Segal, 2011; Cohen and Sarelius, 2002).

It is likely that multiple mechanistic pathways involving both mechanical (shear stress and circumferential stress) and chemical stimuli (oxidative stress) exert an influence over the

subsequent production and release of nitric oxide from the vascular endothelium (McLean et al., 2015). Early animal studies were the first to highlight that structural adaptations in vessel diameter allow heightened shear stress forces to be returned to near baseline levels (Zarins et al., 1987; Kamiya and Togawa, 1980) which negated the need for continued improvement in nitric oxide-mediated vascular endothelial function. The endothelium is an essential transducer of haemodynamic shear stress signals since the removal of the endothelium abolished any previously observed changes in vascular structure in the carotid artery in rats (Langille and O'Donnell, 1986) and in the canine femoral artery (Rubanyi et al., 1986). The endothelium is a key sensor of shear stress and previous research has demonstrated that vasodilatation is largely dependent on the integrity of the endothelium (Guzman et al., 1997; Sessa et al., 1994; Holtz et al., 1984). Improvements in endothelial function and structural enlargement of blood vessels (outward arterial remodelling) are considered to be mediated via a shear stress mechanism (Green et al., 2011c; 2009; Jasperse and Laughlin, 2006).

2.4 Exploration of vascular adaptations as a mechanism for exercise training-induced reductions in resting blood pressure

2.4.1 The effect of acute exercise on conduit artery vascular endothelial function

The beneficial effects of chronic exercise training on resting vascular endothelial function have been reasonably well studied (Green et al., 2011c), whereas the effects of a single bout of isometric exercise on vascular endothelial function are largely unknown. It has been proposed that the vascular endothelial function response within an acute exercise model (Padilla et al., 2007) may permit an enhanced understanding of the effects of exercise training particularly in relation to RBP (Thompson et al., 2001). It has been theorised that changes in blood flow and shear rate magnitude and/or pattern during exercise have significant implications for exercise training-induced adaptations in vascular endothelial function and structure (Green et al.,

2011c). Therefore, it is surprising that few studies have examined both acute changes in brachial artery FMD and exercise-induced changes in haemodynamic parameters such as blood flow and shear rate patterns (Birk et al., 2013; Padilla et al., 2007). In addition to this, the relationship between acute changes in brachial artery FMD immediately following exercise and chronic changes in resting brachial artery FMD following training are unknown.

The effect of an acute bout of resistance exercise on brachial artery FMD has received relatively little attention in comparison to aerobic exercise (Atkinson et al., 2015; Choi et al., 2014; Gonzales et al., 2011; Varady et al., 2010; Tinken et al., 2009; Gori et al., 2010; Phillips et al., 2011; Jurva et al., 2006; McGowan et al., 2006). High intensity aerobic exercise has been shown to immediately reduce brachial artery FMD, whereas low to moderate intensity aerobic exercise has been shown to significantly increase brachial artery FMD after exercise (Dawson et al., 2013). Previous resistance exercise training studies have investigated the effect of acute isometric handgrip exercise (Gori et al., 2010; Tinken et al., 2009; McGowan et al., 2006), acute isotonic handgrip exercise (Atkinson et al., 2015; Gonzales et al., 2011), acute eccentric elbow flexor exercise (Choi et al., 2014) and acute lower body dynamic resistance exercise (Phillips et al., 2011; Varady et al., 2010; Jurva et al., 2006).

Brachial artery FMD has been shown to significantly decrease in sedentary individuals at 1 hour following acute maximal effort leg press exercise (7.1% to 2.5%) whereas this impairment was not evident in trained weightlifters indicating the likely importance of resistance training status (5.6% to 7.1%) (Jurva et al., 2006). Similarly, brachial artery FMD has been shown to be significantly impaired in sedentary individuals 30-minutes following acute leg press exercise above 80% of an individual's one repetition maximum (8.0% to 5.7%), whereas brachial artery FMD improved in trained weightlifters performing the same exercise protocol (6.7% to 8.8%) (Phillips et al., 2011). Furthermore, research involving lower limb exercise has

reported an exercise-intensity dependent brachial artery FMD response, with an immediate decrease in brachial artery FMD following high (6.6% to 3.6%) and moderate (6.1% to 4.7%) but not low intensity (6.3% to 5.9%) lower body cycling exercise (Birk et al., 2013).

Upper body resistance exercise can elicit a transient post exercise decrease in brachial artery FMD (Gonzales et al., 2011). Gonzales et al. (2011) reported that dynamic handgrip exercise performed at a slow velocity reduced brachial artery FMD at 30-minutes post exercise in healthy young individuals (6.9% to 3.6%). In addition, McGowan et al. (2006) has reported that an acute bout of isometric handgrip exercise significantly attenuated brachial artery FMD immediately post exercise in medicated hypertensive individuals (acute FMD response before training: pre 3.1% to post 2.1%). Most studies involving an acute bout of resistance exercise have demonstrated a transient reduction in brachial artery FMD, this is in agreement with the proposal that a biphasic response is likely to predominate in healthy individuals immediately following an acute bout of exercise (Dawson et al., 2013). A biphasic response is characterised as an immediate decrease in vascular endothelial function followed by normalisation or supra-normalisation at later time points, and this may have important implications for the long-term upregulation of vascular function and structure. The effect of an acute bout of exercise on vascular endothelial function has been investigated over recent years, however the intensity and duration of the exercise protocol is markedly varied. To date, no research exists which has investigated the effect of an acute bout of isometric lower limb exercise on nitric oxide-mediated endothelium-dependent vasodilatation assessed via brachial artery FMD.

2.4.2 The influence of exercise intensity on conduit artery vascular endothelial function

Previous research has shown that high intensity lower body aerobic exercise (60 to ≤ 80% $\dot{V}O_{2max}$) results in a larger reduction in brachial artery FMD immediately post exercise (Birk et al., 2013; Johnson et al., 2012b; Llewellyn et al., 2012). Birk et al. (2013) compared the effect

of 30 minutes cycling exercise at different intensities (50%, 70% and 85% $\dot{V}O_{2max}$) on post exercise brachial artery FMD in healthy young men and reported an exercise intensity-dependent decrease in brachial artery FMD following both moderate and high intensity aerobic exercise; this was not evident at the lowest intensity. Llewellyn et al. (2012) examined the effect of moderate intensity aerobic treadmill running exercise for 30 minutes at 60% $\dot{V}O_{2max}$ and reported a significant decrease in brachial artery FMD (adjusted for baseline artery diameter) at 30-minutes post moderate intensity exercise (pre to post: 8.89% to 5.83%). Furthermore, Johnson et al. (2012b) reported an increase in brachial artery FMD immediately post moderate intensity exercise at 50% $\dot{V}O_2$ peak (pre: 5.0% to post: 7.2%) and decrease in brachial artery FMD immediately post high intensity exercise at 80% $\dot{V}O_{2peak}$ (post: 4.7%). Several other studies have reported an increase in FMD at lower exercise intensities (Zhu et al., 2010; Tinken et al., 2009; Harris et al., 2008; Cosio-Lima et al., 2006; Harvey et al., 2005). Together these results suggest that intensity-dependent changes in vascular endothelial function can occur. Furthermore, it may be inferred from the existing literature that moderate to high intensity exercise is most likely to result in a transient decrease in brachial artery FMD as opposed to performing exercise at a low intensity.

Interestingly, the reduction in brachial artery FMD reported by Birk et al. (2013) returned to baseline levels at 1-hour post exercise exemplifying the existence of a biphasic response in an acute exercise model. In addition, Llewellyn et al. (2012) reported that brachial artery FMD was non-statistically elevated above pre exercise levels at 1-hour post high intensity exercise. Moreover, brachial artery FMD was higher at 1-hour post high intensity exercise in comparison to exercise performed at moderate intensity (Llewellyn et al. 2012). These findings further support the notion that an acute bout of high exercise intensity can elicit a biphasic response in vascular endothelial function. Collectively, the findings from Birk et al. (2013), Johnson et al. (2012b) and Llewellyn et al. (2012) support the notion that *supercompensation* in vascular

endothelial function occurs within an acute exercise model. *Supercompensation* is a basic theory of athletic training whereby periods of progressive increases in perturbations in cellular and systemic environments are followed by sufficient periods of recovery. This process allows beneficial adaptations in physiological systems that permit bodily systems to surpass initial baseline levels of functioning (Bompa and Haff, 2009). This integrative biological response will ultimately return the body to a higher level of homeostasis via acute changes that temporarily allow increased exercise capacity to deviate from resting homeostasis (Stanley et al., 2013). It would appear beneficial to assess the acute effects of isometric bilateral leg exercise on brachial artery FMD at two different exercise intensities (high and low) to provide an insight into the possible existence of an intensity-dependent biphasic response.

2.4.3 The timing of flow-mediated vasodilatation assessment post exercise

Flow-mediated vasodilatation has been assessed in the brachial artery following an acute bout of exercise ranging from immediately post exercise bout up to 48 hours post exercise, with assessments most typically occurring within 30-minutes to 1-hour post exercise. Brachial artery FMD has been shown to decrease (Franklin et al., 2014; Birk et al., 2013; Hwang et al., 2012; Bailey et al., 2012; Johnson et al., 2012b; Gori et al., 2010; Llewellyn et al., 2012; Gonzalez et al., 2011; Phillips et al., 2011; Dawson et al., 2008; McGowan et al., 2006; Jurva et al., 2006; Silvestro et al., 2002), increase (Birk et al., 2013; Johnson et al., 2012b; Phillips et al., 2011; Gonzalez et al., 2011; Zhu et al., 2010; Tinken et al., 2009; Cosio-Lima et al., 2006) or remain unchanged (Atkinson et al., 2015; Hwang et al., 2012; Dawson et al., 2008; Cosio-Lima et al., 2006; Silvestro et al., 2002) when measured within 30-minutes of performing a bout of aerobic or resistance exercise. Those studies that reported an immediate increase or no change in brachial artery FMD within 30-minutes post exercise predominantly consist of low to moderate

intensity exercise, the majority of which predominantly utilise the aerobic energy system or a small muscle mass.

The majority of the studies involving resistance exercise or predominantly lower limb exercise that have reported a decrease in brachial artery FMD immediately following an exercise bout also report an increase at later time points (≥60 minutes) (Birk et al., 2013; Katayama et al., 2013; Johnson et al., 2012b; Gonzales et al., 2011; Goel et al., 2007). This finding reinforces the likely existence of a biphasic brachial artery FMD pattern, which may be a normal response within the vasculature to large muscle group exercise modalities performed at moderate to high exercise intensities. A transient decrease in brachial artery FMD following an acute bout of exercise is considered to act as a catalyst for vascular adaptations when an exercise bout is performed repeatedly (Padilla et al., 2011a; Suvorova and Kojda, 2007). The classic physiological concept of *hormesis* (Schulz, 1888) is defined as a process whereby mild stress activates endogenous mechanisms of repair and maintenance to protect cells against subsequent stresses (Durand and Gutterman, 2014; Dawson et al., 2013; Gems and Partridge, 2008; Zhang et al., 2008). The *hormesis* response within the vasculature is understood to be activated by mild stressors including oxidative, metabolic and thermal stressors (Calabrese et al., 2012). Resistance trained individuals have been shown to better off-set an immediate attenuation in brachial artery FMD following acute exercise compared to their untrained counterparts (Phillips et al., 2011; Jurva et al., 2006). It could be that *vascular hormesis* is a viable concept to explain improvements in vascular responses over time to the same exercise stimulus, possibly due to the protective mechanisms accrued over time via upregulation to exercise-induced haemodynamic and metabolic stressors (Thorin-Trescases and Thorin, 2010).

2.4.4 Blood flow and shear rate patterns during exercise

Exercise-induced haemodynamic parameters including blood flow, shear rate patterns and exercising blood pressure have been suggested as potential physiological stimuli responsible for mediating immediate post exercise changes in vascular endothelial function (Gonzales et al., 2011, 2008; Bilfinger and Stefano, 2000). The physical forces exerted upon the luminal vessel wall and endothelial cell surface are pivotal in the regulation of blood vessel structure and function (Gimbrone et al., 2000). The endothelium is constantly exposed to a frictional force per unit area known as shear stress which ranges between 10 and 70 dyne/cm^2 (Malek et al., 1999; Davies, 1995). Shear stress is typically pulsatile and unidirectional in relatively straight and healthy human conduit arteries at rest and yields a predominantly positive value over the cardiac cycle (Davies, 1995). Pulsatile shear stress is considered to promote the release of favourable substances from the endothelium that inhibit smooth muscle cell proliferation, adhesion of leukocytes and blood coagulation (Adams et al., 2005; Woodman et al., 2005; Osanai et al., 2000; Niebauer and Cooke, 1996). Low endothelial shear stress is by definition unidirectional with a periodically varying magnitude over the cardiac cycle yielding a low time-average (below 10 to 12 dyne/cm^2), and oscillatory shear stress is bidirectional with a periodically varying magnitude over the cardiac cycle yielding a very low time-average close to zero (Chatzizisis et al., 2007). Oscillatory shear stress and low levels of shear stress have been shown to promote oxidative stress, attenuate nitric oxide-dependent vasodilatation, increase permeability to low-density lipoproteins as well as promote inflammation and vascular smooth muscle cell proliferation (Chatzizisis et al., 2007).

The profile of the blood flow waveform can be further classified into antegrade and retrograde components to order to provide an insight into blood flow and shear rate patterns and allow for the determination of oscillatory shear stress (Padilla et al., 2014). In studies involving isolated blood vessels and cultured cells, an elevated mean shear rate has been shown to elicit an anti-

atherogenic phenotype whereas augmented retrograde shear rate and low mean shear rate have been shown to elicit a pro-atherogenic phenotype (Hastings et al., 2007; Dai et al., 2004; Stone et al., 2003; Ziegler et al., 1998a; 1998b; De Keulenaer et al., 1998). Augmented antegrade shear rate has been shown to improve vascular endothelial function (Tinken et al., 2009), whereas augmented retrograde shear rate has been shown to evoke endothelial dysfunction in humans (Thijssen et al., 2009b). Although there is evidence to support the notion that chronic reductions in mean shear rate and augmented oscillatory shear index are potent pro-atherogenic signals to the conduit arteries, few studies have confirmed the association between disturbed blood flow and impaired vascular endothelial function *in vivo* (Padilla et al., 2014).

Research has established that muscular contractions can generate high oscillatory blood flow patterns, characterised by high forward direction blood flow (antegrade blood flow) and high backward direction blood flow (retrograde blood flow) (Rådegran, 1997; Robergs et al., 1997; Walløe and Wesche, 1988; Anrep, 1934). Earlier research has shown that low levels of shear stress are produced during dynamic forearm exercise with negligible contributions from retrograde blood flow (Green et al., 2005; 2002). In comparison, a bout of cycling exercise produced high levels of mean shear stress with significant contributions from elevations in both antegrade and retrograde blood flow (Green et al., 2005; 2002). Green et al. (2005) concluded that increased retrograde blood flow during lower limb cycling exercise led to enhanced blood flow pulsatility, which forms an integral part of the shear stress stimulus acting on the endothelium to stimulate the release of endothelial-derived nitric oxide bringing about vasodilatation and thus increased total blood flow capacity.

The majority of studies which have investigated blood flow and shear rate patterns during lower limb aerobic exercise have reported increased shear rate in upstream conduit arteries feeding non-exercising skeletal muscles (Birk et al., 2013; Johnson and Wallace, 2012; Simmons et al.,

2011; Thijssen et al., 2009a; Tanaka et al., 2006; Green et al., 2005; 2002). Nonetheless, these findings suggest that haemodynamic changes induced by lower body exercise modalities impact upon the shear rate in the systemic vasculature. Therefore, the haemodynamic stimuli afforded by isometric bilateral isometric leg exercise could provide a plausible mechanism for mediating systemic vascular adaptations. Studies that have assessed exercise-induced blood flow and/or shear rate patterns within the active skeletal muscle during lower (Gonzales et al., 2008) and upper limb exercise (Atkinson et al., 2015; Green et al., 2005; 2002) have demonstrated changes in localised blood flow during muscular contractions. In addition, the change in blood flow immediately upon cessation of muscular contraction known as post exercise hyperaemia (Osada et al., 2003) may be a potent exercise-induced haemodynamic stimulus in its own right.

Previous studies have measured the total magnitude of blood flow to the lower limbs during dynamic knee extensor and cycling exercise (Parker et al., 2007; Lutjemeier et al., 2005; Rådegran and Saltin, 1998; Rådegran, 1997), during isometric lower limb exercise (Osada et al., 2015; McNeil et al., 2015; Wigmore et al., 2006; Wesche, 1986; Sadamoto et al., 1983), and the total magnitude of blood flow to the upper limbs during isometric upper limb exercise (Thompson et al., 2007; Hunter et al., 2006; Jensen et al., 1993; Kagaya and Homma, 1997). Previous research involving single leg isometric exercise protocol have reported that localised total blood flow is elevated compared to baseline levels, however it is proportionately reduced in relation to graded intensity isometric leg exercise (ranging from 5% to 50% MVC) (Gaffney et al., 1990; Sjøgaard et al., 1988). Similarly, the magnitude of total blood flow upon cessation of sustained forearm isometric muscular contraction has been shown to increase linearly with contraction intensity (Wigmore et al., 2006). Furthermore, significant increases in total blood flow have been reported to occur in the exercising limb following repeated brief 10-second

isometric muscular contraction of the quadriceps muscles in proportion to the workload (Osada et al., 2015).

Few published research studies have investigated blood flow and shear rate patterns (antegrade, retrograde and oscillatory components) during muscular contraction or immediately post cessation of muscular contraction (Atkinson et al., 2015; Johnson and Wallace, 2012; Gonzales et al., 2011; Tinken et al., 2009; Gonzales et al., 2008; Green et al., 2005; 2002). Gonzales et al. (2008) investigated the impact of single leg dynamic knee extensor exercise at fast and slow contraction velocities on blood flow and shear rate patterns in the common femoral artery. Retrograde blood flow was significantly increased compared to baseline during both fast contraction velocity exercise and slow contraction velocity exercise (121.7 $ml.min^{-1}$ vs.11.2 $ml.min^{-1}$, respectively). Furthermore, the fast contraction velocity exercise produced significantly greater antegrade blood flow compared to slow contraction exercise condition (2012 $ml.min^{-1}$ vs. 1745.6 $ml.min^{-1}$, respectively). The findings from Gonzales et al. (2008) are in alignment with those of Green et al. (2005; 2002) who suggested that blood flow became more pulsatile in nature. It may be that this increased blood flow pulsatility is a potent modulator of improved nitric oxide-mediated endothelium-dependent vasodilatation, however it is currently unknown to what extent lower limb isometric exercise alters antegrade and retrograde components of blood flow and shear rate.

Previous research has alluded to specific blood flow and shear rate patterns during exercise bouts (Gonzales et al., 2008; Green et al., 2005; 2002) and immediately upon cessation of exercise (Johnson and Wallace, 2012). Johnson and Wallace (2012) explored shear rate patterns in the brachial artery immediately upon cessation of running at different intensities. The findings revealed that oscillatory shear index and retrograde shear rate were significantly decreased immediately post high intensity moderate duration treadmill running exercise (80%

$\dot{V}O_{2peak}$ for 30 minutes), moderate intensity moderate duration (50% $\dot{V}O_{2peak}$ for 30 minutes) and moderate intensity long duration treadmill running exercise (50% $\dot{V}O_{2peak}$ for 60 minutes). In addition, a significant intensity-dependent increase in antegrade and mean shear rate immediately post exercise was observed with the greatest increases following high exercise intensity 80% $\dot{V}O_{2peak}$ (Johnson and Wallace, 2012). Shear rate patterns during high intensity isometric muscular contractions and during the recovery period in between contractions have been investigated (Smith, 2014) as part of a graded intensity isometric bilateral leg exercise test. These data demonstrated an intensity-dependent increase in both antegrade and retrograde shear rate. Whilst this standardised graded exercise testing protocol has been used to prescribe isometric exercise intensity (Baross et al., 2012; Devereux et al., 2011; Wiles et al., 2010; 2007), it does not reflect a typical bout of isometric exercise commonly used as part of a chronic exercise training intervention. Therefore, the acute exercise study within this thesis aimed to more clearly quantify the blood flow and shear rate patterns during a bout of isometric lower limb exercise in the same way it would be utilised as part of a training intervention. To date there is no data investigating how blood flow and shear rate patterns manifest during lower limb isometric muscular contractions or upon cessation of muscular contractions.

2.4.5 The relationship between exercise-induced blood flow and shear rate patterns and peripheral conduit artery vascular endothelial function

The impact of exercise-induced blood flow and shear rate patterns at various exercise intensities on vascular endothelial function remains largely under investigated. Atkinson et al. (2015) recently summarised the exercise-induced blood flow and shear rate patterns across 30-second epochs at 5-minute intervals throughout a 30-minute unilateral isotonic handgrip exercise bout. Mean and antegrade blood flow and shear rate were increased above baseline, the largest increases were evident during performance of the highest exercise intensity (15%

MVC) and there were no reported changes in retrograde blood flow and shear rate. Atkinson et al. (2015) revealed that brachial artery FMD was increased at 60-minutes post exercise at the highest exercise intensity only, and this may be due to intensity-dependent increases in antegrade blood flow and shear rate. It is likely that this improvement in vascular function post exercise is related to the lessor haemodynamic, metabolic and neurohumoral responses to small muscle group exercise (Atkinson et al., 2015). Isometric bilateral lower limb exercise is likely to cause a markedly different stimulus due to the larger amount of muscle mass recruited during exercise, it is currently unknown how these exercise-induced haemodynamic patterns will affect acute vascular endothelial function.

Tinken et al. (2009) has also examined blood flow and shear rate patterns induced by different exercise modalities and their subsequent ability to mediate acute changes in vascular endothelial function. Shear rate was manipulated during an acute 30-minute bout of bilateral forearm heating, recumbent leg cycling and bilateral handgrip exercise using one arm as a control limb (non-cuffed arm) and the other as the experimental limb (cuffed limb) in healthy young men. Antegrade shear rate was increased to a similar extent during all three exercise intensity conditions which resulted in a similar increase in brachial artery FMD. However, the modulated reduction in antegrade shear rate, which was induced by sub-diastolic cuff inflation, prevented this improvement in brachial artery FMD from occurring. These findings from Tinken et al. (2009) further demonstrate that the magnitude of antegrade shear rate could provide an important stimulus to enhance vascular endothelial function in humans.

Recent studies have reported that increases in retrograde shear rate, modulated via cuff inflation without impacting upon antegrade shear rate, induced a dose-dependent attenuation in brachial artery FMD in healthy young men (Thijssen et al., 2009b). These findings were the first to report that when retrograde shear rate is increased in isolation it could be a potential stimulus

for endothelial dysfunction in humans. Further evidence had emerged to support the concept that a reduction in mean blood flow and/or an increase in retrograde blood flow and oscillatory shear rate evokes endothelial injury *in vivo* as evidenced by local increases in the concentration of endothelial micro-particles (Jenkins et al., 2013) although this has not been confirmed in humans. Interestingly, confounding results were later reported in older men with pre-existing endothelial dysfunction whereby FMD in both the brachial artery and superficial femoral artery were unaffected by acute elevations in retrograde shear rate (Schreuder et al., 2015a). These conflicting results demonstrate that this area remains largely unexplored, and although the same intervention was applied, there was a drastically different vascular function response to the same isolated retrograde shear rate stimulus dependent on the population examined.

Gonzales et al. (2011) investigated the effect of local exercise-induced blood flow and shear rate patterns on vascular function. Brachial artery FMD was significantly reduced 30-minutes following slow contraction velocity exercise (6.90% to 3.61%) and unchanged 30-minutes following fast contraction velocity exercise (5.46% to 6.14%). The findings from Gonzales et al. (2011) show that estimated contractile work was higher during slow versus fast contraction velocity, and those with higher estimated contractile work tended to have higher exercising blood pressure and higher retrograde shear rate during exercise. Further subgroup analysis indicated that those with higher exercising MAP during the slow contraction velocity exercise tended to experience the largest decrease in brachial artery FMD measured 30-minutes post exercise. Higher estimated contractile work was proposed to be due to the greater amount of time spent in contraction (Gonzales et al., 2011) which may go some way to explain the observed transient reduction in brachial artery FMD post exercise.

Acute changes in vascular endothelial function may require a critical threshold of exercise intensity, which is likely related to increases in exercising blood pressure as well as shear stress

(Hallmark et al., 2014). An exercising blood pressure threshold has been proposed to exist above which vascular endothelial function may be impaired following acute exercise (Gonzales et al., 2011). Although alterations in vascular endothelial function are predominantly considered to be a shear stress-mediated phenomenon, other factors such as a transmural pressure related mechanism may also mediate changes in vascular endothelial function particularly at a systemic level via exercising blood pressure. It is extremely difficult at this time to disassociate between transmural pressure and shear stress related mechanisms *in vivo* (Green et al., 2011a).

2.5 Exploring peripheral vascular adaptations as a mechanism for exercise training-induced reductions in resting blood pressure

2.5.1 The effects of exercise training on vascular endothelial function and the association with resting blood pressure reductions

Research into the effect of exercise training on resting vascular endothelial function in conduit and resistance arteries has reported improvements in healthy (Birk et al., 2012; Rakobowchuk et al., 2008; Thijssen et al., 2007; Goto et al., 2003; Gocke et al., 2002; DeSouza et al., 2000; Green et al., 1994) and unhealthy populations (Grace et al., 2015; McGowan et al., 2007b; Hambrecht et al., 2003; Walsh et al., 2003; Linke et al., 2001; Maiorana et al., 2001; 2000). Whole body dynamic resistance (Beck et al., 2014; Spence et al., 2013), upper body dynamic resistance (Zoeller et al., 2009) and upper body isometric and isotonic handgrip exercise training (Badrov et al., 2013; Tinken et al., 2010; 2008) can improve in conduit artery or resistance vascular endothelial function in healthy individuals.

A meta-analysis by Ashor et al. (2015) revealed similar effectiveness of aerobic, resistance and combined exercise training to significantly improve conduit artery vascular endothelial function in both healthy and unhealthy populations by 2.79%, 2.52% and 2.07%, respectively.

A small number of studies have reported no change in conduit artery or resistance vascular endothelial function in healthy individuals following whole body resistance training (Rakobowchuk et al., 2005), combined aerobic and resistance exercise training (Maiorana et al., 2001) and isometric handgrip exercise training (McGowan et al., 2007a). Early research by Kingwell et al. (1997) reported improved nitric oxide-mediated forearm resistance vessel function, assessed via venous occlusion plethysmography, following 4 weeks of cycling exercise training in healthy young men. Clarkson et al. (1999) reported improved brachial artery FMD following 10 weeks of combined aerobic and anaerobic training as part of basic military training, whereby brachial artery FMD increased significantly from 2.2% to 3.9%. There were no concomitant reductions in RBP and enhanced endothelial-dependent function was independent of RBP and only related to improvements in exercise performance (Clarkson et al., 1999).

The research investigating the effect of isometric exercise training on RBP and vascular endothelial function is relatively sparse. Badrov et al. (2013) has reported improved vascular endothelial function at the resistance vessel level with concomitant reductions in RBP following isometric exercise training in normotensive individuals. The research by Badrov et al. (2013) consisted of randomised controlled trials involving three conditions: alternating unilateral isometric handgrip exercise either 3 or 5 days per week for a total of 8 weeks, and a non-exercising control condition. Resting SBP was significantly reduced by 6 mmHg in both exercise conditions, with resistance vessel endothelial function was improved by 57% in the 5 days per week condition compared to 42% in the 3 days per week condition.

McGowan et al. (2007a) also reported that unilateral isometric handgrip exercise contractions at 30 % MVC performed three days per week for a total of 8 weeks, the same intensity as Badrov et al. (2013), induced significant reductions in resting SBP of 5 mmHg. There was no

significant interaction observed for brachial artery FMD between the exercised and control arm. McGowan et al. (2007a) proposed that the assessment of the conduit artery may not be the most relevant measurement site in healthy individuals in order to detect changes in vascular endothelial function but rather at the level of resistance vessels since this level of the arterial tree is considered primarily responsible for determining blood pressure (Haddy et al., 1968). McGowan et al. (2007b) also reported increased endothelial nitric oxide-dependent vasodilatation localised to the exercised limb and a reduction in RBP in a medicated hypertensive population. This result may infer that resting conduit artery vascular endothelial function can be improved at a localised rather than systemic level in a hypertensive population.

Numerous studies demonstrate that exercise training leads to systemic improvement in vascular endothelial function (Birk et al., 2012; Padilla et al., 2011a; 2011c; Maiorana et al., 2003). Localised improvements in vascular function and structure have been shown to be mediated through repeated increases in shear stress (Birk et al., 2012; Tinken et al., 2009; 2008). Furthermore, evidence has confirmed that brachial artery vasodilatation in the inactive limb during lower limb exercise is also shear rate-dependent (Padilla et al., 2011a). It is clear from the research available that exercise training, regardless of modality, can induce favourable benefits upon vascular endothelial function at both a local and systemic level within both conduit and resistance vessels. Previous research has predominantly investigated the effectiveness of aerobic exercise training on vascular endothelial function and further investigation into the effectiveness of dynamic resistance and isometric exercise training on vascular endothelial function to explain exercise training-induced reductions in RBP may prove worthwhile.

2.5.2 The effects of exercise training on vascular structure and its association with resting blood pressure reductions

Adaptations in artery diameter following exercise training appear to be highly dependent on the mode of training and the location of vascular bed examined in relation to the exercising limb (Black et al., 2016). There is some evidence in the form of cross-sectional studies examining vascular structure demonstrating that racket sports players possess a significantly larger artery diameter and lower arterial wall thickness in the dominant versus non-dominant arm (Rowley et al., 2011a). Brachial artery diameters were significantly larger in athletes involved in sports requiring predominantly upper body orientated movements and superficial femoral artery diameters were significantly larger in runners and cyclists in comparison to control subjects (Rowley et al., 2011b). This has led to speculation that increases in artery diameter are a localised phenomenon (Green et al., 2011c; Huonker et al., 2003; Schmidt-Trucksäss et al., 2000).

Localised increases in artery diameter to the exercising limb can occur following exercise training in healthy individuals following upper body resistance exercise training (dynamic free weight and fixed weight exercises and isotonic handgrip) (Stebbings et al., 2013; Zoeller et al., 2009; Tinken et al., 2010; 2008) and lower body rhythmic aerobic exercise training (Thijssen et al., 2007). Furthermore, 8 weeks of lower limb isometric exercise training increased common femoral artery diameter within the exercised limbs in middle-aged prehypertensive men (Baross et al., 2012). In addition to this, a significant correlation was reported between common femoral artery diameter and exercise training-induced reductions in resting SBP (Baross et al., 2012). Those who experienced the largest reductions in RBP had the largest resting common femoral artery diameter. Interestingly these observed improvements in peripheral vascular structure, which occurred during the latter stages of exercise training, were intensity-dependent and localised to the trained limbs (Baross et al., 2012). Miyachi et al. (2001) has reported that

6 weeks of lower body endurance exercise at 80% of one-legged peak oxygen uptake ($\dot{V}O_{2peak}$) significantly increased vessel cross sectional area of both the femoral artery by 16% and vein by 46%, unchanged RBP and a significant reduction in resting HR. In contrast, Schreuder et al. (2015b) and Thijssen et al. (2013; 2011a) have both reported unchanged localised and systemic artery diameter following lower limb aerobic exercise training in healthy young individuals.

There is conflicting evidence to date about the nature of localised versus systemic adaptations in artery diameter, and recent research supports systemic improvement in artery diameter following dynamic resistance exercise training although RBP was unchanged pre to post training (Stebbings et al., 2013). Adaptations in artery diameter following training appear to be dependent on the location of the vascular bed examined in relation to the exercising limb (Black et al., 2016) and the nature of the programme variables forming the exercise protocol. Based upon the current evidence, adaptations in both localised and systemic resting artery diameter may be a potential mechanism responsible for RBP reductions following isometric exercise training in young healthy individuals.

2.5.3 The time course of exercise training-induced changes in vascular endothelial function and structure

There has been a long standing interest in the measurement of vessel wall structural adaptations (Thijssen et al., 2013; DeVan and Seals, 2012; van Duijnhoven et al., 2010; Billinger et al., 2009; Dinenno et al., 2001) and endothelium-dependent vasodilatation following exercise training (Allen et al., 2003; Delp and Laughlin, 1997). Acetylcholine-induced relaxation was significantly higher at week 4 and week 10 in rats who completed 10 weeks of aerobic exercise training in comparison to sedentary controls. Furthermore, Allen et al. (2003) assessed the time course of changes in conduit artery vascular endothelial function twice per week in healthy

young men throughout a 4-week isotonic handgrip exercise training. Brachial artery FMD improved by 62% relative to baseline at the end of week 4 and changes were localised to the trained arm only (baseline: 4.82% vs. end of week 4: 5.20%). Brachial artery FMD was significantly improved at the end of the first week of training and this improvement was maintained up to week 4 (Allen et al. 2003). This finding from Allen et al. (2003) is in agreement with earlier studies showing rapid improvements in vascular endothelial function in animal studies within 1 to 10 days of exercise training (Delp and Laughlin, 1997; Sessa et al., 1994; Wang et al., 1993). This is also in alignment with the original hypothesis that functional responses are superseded by structural adaptations (Laughlin, 1995).

Relatively few studies have measured vascular functional and structural adaptations simultaneously throughout an exercise training intervention (Tinken et al., 2010; 2008; Vita et al., 2008; Allen et al., 2003). This is particularly pertinent given that a sequential time course has been proposed to exist between artery function and structure (Laughlin, 1995). Based upon Seyle's Theory of *Stress-Response-Adaptation* (1978), Laughlin (1995) indicated that structural adaptations supersede functional responses in order to normalise the shear stress stimulus. There is limited data on the time course of functional and structural vascular adaptations when measured simultaneously in healthy young men following lower limb dynamic resistance training or isometric exercise training. The time course of functional and structural vascular adaptations have previously been reported in isolation in dynamic resistance and isometric exercise training studies (Baross et al., 2012; Rakobowchuk et al., 2005), thereby allowing some preliminary insight into vascular adaptations at regular time intervals.

Rakobowchuk et al. (2005) reported that brachial artery FMD was unchanged at mid (week 6) and post (week 13) compared to baseline following a whole body dynamic resistance exercise training. Resting brachial artery diameter as well as peak and 10-second post cuff occlusion

reactive hyperaemia were significantly increased at mid and post training (Rakobowchuk et al., 2005), possibly indicating the presence of increased systemic artery diameter and improved vascular endothelial function at the level of the resistance vessels. It was not possible to determine from the aforementioned study whether the observed increases in resting brachial artery diameter were attributable to the release of vasoactive substances that affect vascular tone or structural changes to the vessel itself. Furthermore, the aforementioned study did not assess vascular function and structure at regular time points. This may have prevented an adequate period with which to observe the potential transition of functional into structural vessel adaptations. Baross et al. (2012) investigated whether localised common femoral artery or systemic brachial artery peripheral vascular adaptations occurred at pre, mid and post an 8-week isometric bilateral leg extension exercise training intervention. Common femoral artery blood flow velocity, artery diameter and vascular conductance were significantly increased following 8 weeks of isometric exercise training only.

A research study by Tinken et al. (2008) examined the time course of changes in vascular function and structure throughout an 8-week lower limb cycling exercise training intervention. Healthy young men performed aerobic exercise 3 times per week for 8 weeks at 80% of their heart rate reserve. Flow-mediated vasodilatation and vasodilator capacity of the brachial artery and popliteal artery were examined fortnightly. Brachial artery FMD was significantly higher at week 2 and 4 of training compared to baseline and vasodilator capacity was significantly higher at week 2, 4, 6 and 8 of training compared to baseline. Popliteal artery FMD was significantly higher at week 2, 4, and 6 of training compared to baseline and popliteal artery vasodilator capacity was significantly higher at week 4, 6 and 8 of training compared to baseline. The results revealed brachial and popliteal artery FMD were highest at week 2 and these functional changes were followed by structural adaptations, thus permitting both local and systemic endothelial function to return to near baseline values. The results from Tinken et

al. (2008) exposed a rapid time course of both localised and systemic endothelial function in humans at regular intervals throughout an exercise training intervention that were followed by both local and systemic indices of structural adaptations.

Furthermore, Tinken et al. (2010) conducted a novel study whereby healthy young men performed 8 weeks of bilateral isotonic handgrip training at 30 contractions per minute for 30-minutes performed three times per week on localised brachial artery FMD. A pneumatic cuff (60mmHg) was positioned around one forearm to arrest blood flow and shear rate during each exercise bout and the other was non-cuffed to establish shear stress as a modulator for changes in vascular function and structure. Tinken et al. (2010) also examined brachial artery FMD in response to ischemic exercise, an index of resistance artery size or remodelling (Naylor et al., 2005). Flow-mediated vasodilatation in the experimental (non-cuffed) limb was significantly increased at week 2 (6.5%), week 4 (6.9%) and week 6 (5.8%) compared to baseline (4.4%). Ischemic exercise dilatation of the brachial artery was significantly increased in the non-cuffed limb at week 2 (10.0%), week 4 (11.5%), week 6 (14.0%) and week 8 (15.6%) compared to baseline (9.8%) indicating progressive increases in conduit vessel adaptations during the latter phase of the training intervention. Both studies by Tinken et al. (2010; 2008) have reinforced the effectiveness of lower body and upper body dominant training to induce rapid functional changes in the vascular endothelium. This functional response was normalised after 4 weeks due to the presence of structural vascular adaptations.

In contrast to the findings form Tinken et al (2010; 2008), recent evidence has undermined the original hypothesis that functional changes occur rapidly and precede structural adaptations (Spence et al., 2013). Conduit artery diameter, wall thickness and endothelial function were assessed in healthy individuals during a 24-week of dynamic resistance or aerobic training intervention (Spence et al., 2013). This research was the first to demonstrate that continual

improvements in both functional and structural parameters can occur for much longer periods of time than initially hypothesised when appropriate progressive overload of the exercise stimulus is applied. Resistance exercise training resulted in a significant improvement post training resting brachial artery diameter (pre: 3.8 mm vs. post: 4.1 mm) and peak FMD diameter (0.2 mm) (Spence et al., 2013). Furthermore, aerobic training induced a significant improvement in post training resting femoral artery diameter, peak FMD diameter and percentage FMD. These results are in agreement with others who have reported the localised effects of training on artery function and structure (Green et al., 2011c; Rowley et al., 2011b). To date, the magnitude and time course of change in vascular function and structure throughout an 8-week isometric bilateral leg extension exercise training intervention are unknown. Moreover, it is currently unclear whether isometric bilateral leg extension exercise training is likely to induce a change in systemic vascular function and structure and whether this adheres to the original hypothesis that functional responses precede structural adaptations (Laughlin, 1995).

2.6 Contextualisation of existing research for purpose of this thesis

The magnitude and pattern of exercise-induced blood flow and shear rate within the peripheral vasculature has been recognised as a likely mechanism responsible for mediating adaptations in vascular function and structure. There is no research to date which has quantified the blood flow and shear rate patterns induced during an acute bout of isometric lower limb exercise. The maintenance of a sufficient exercise stimulus over several weeks may result in enhanced vascular endothelial function superseded by adaptations in vascular structure. Furthermore, there is considerable evidence to suggest that enhanced functional and structural adaptations within the peripheral vasculature may be a mechanism responsible for the well-documented RBP lowering effects of isometric exercise training. To date, there has been no attempt to

establish the role of peripheral vascular adaptations as a physiological mechanism responsible for isometric bilateral leg exercise training-induced reductions in RBP and therefore it is essential to investigate this proposed mechanism.

The aim of the first study within this thesis was to assess the reliability of resting artery diameter, blood flow velocity, and blood flow within the brachial artery, common femoral artery and superficial femoral artery as well as cardiac output, stroke volume and resting blood pressure. These measurements were performed to ascertain measurement error and to inform sample size estimation for subsequent studies conducted as part of this thesis. The reliability coefficients would also enable the researcher to contextualise any observed changes, and to provide information on biological, equipment and operator reliability.

The aim of the second study within this thesis was to investigate the effect of acute isometric bilateral leg extension exercise performed at different intensities on the primary outcome variable brachial artery FMD. In addition, the aim of the second study was to investigate the effect of an acute bout of isometric bilateral leg extension exercise at different intensities on conduit artery diameter, blood flow and shear rate patterns within the exercising limb during muscular contractions and the recovery period in between muscular contractions. The aim of the third study within this research thesis was to investigate the effects of 8-weeks of isometric bilateral leg extension exercise training on the primary outcome variables including RBP, $\dot{Q}$, TPR and brachial artery FMD. In addition, the aim of the third study was to establish whether alterations occurred in resting heart rate variability, and artery diameter and blood flow patterns within the brachial artery, common femoral artery and superficial femoral artery.

CHAPTER 3: GENERAL METHODS

3.1 Participant information and testing criteria

Healthy young men were recruited to take part in the studies within this thesis. Prospective volunteers were provided with an information sheet for the relevant research study they wished to participate in advance of their visit to the Sport and Exercise Science Laboratories and were required to adhere to the strict testing criteria as outlined in the participant information sheets (Appendices 2 to 4). Volunteers were required to complete a health and fitness questionnaire (Appendix 1) and provide their written voluntary informed consent form during their initial visit. It was clearly communicated to all participants that they were free to withdraw from the study at any time without adverse consequences. Prospective participants were required to undergo a preliminary RBP measurement during their first visit to the laboratory to warrant normotensive blood pressure status in accordance with British Hypertension Society Guidelines (<120/<80 mmHg) (Williams et al., 2004). Healthy young men were recruited in order to minimise the risk of confounding variables since health status, sex and age have been shown to affect an individual's response to an isometric exercise stimulus (Kent-Braun et al., 2002; Smolander et al., 1998; Ettinger et al., 1998; Petrofsky and Lind, 1975). Participants were considered healthy if they were free from any clinically diagnosed cardiovascular, respiratory and/or metabolic condition.

Participants who were smokers, previous smokers, or who had donated blood less than 6 weeks prior since this has been shown to affect heart rate, blood pressure and blood volume (Bouchard et al., 1995) were not permitted to take part in the studies. Participants fasted for at least 6 hours prior to testing procedures (Thijssen et al., 2011d). Participants abstained from caffeinated products and alcohol for 12 hours prior to testing and were required to refrain from strenuous exercise for 24 hours prior to testing (Wiles et al., 2010). Participants were instructed to maintain their regular physical and dietary activity behaviours throughout the study. All repeat

resting measures were conducted at approximately the same of time of day (± 1 hour) since cardiovascular variables are known to be influenced by circadian rhythms (Jones et al., 2008; Atkinson and Reilly, 1996). Participation was voluntary and there were no monetary benefits. Volunteers were members of staff, undergraduate students and alumni student at Canterbury Christ Church University. All recruitment and data handling procedures were conducted in alignment with the University's research governance framework following the publication by the Department of Health Research Governance Framework for Health and Social Care (Department of Health, 2005). The Faculty Research Ethics Committee at Canterbury Christ Church University approved all of the studies within this thesis. All testing was completed at the Canterbury Christ Church University Sport and Exercise Science Laboratories.

3.2 Testing Procedures

3.2.1 Blood pressure

Resting blood pressure was measured using an automated oscillometric blood pressure monitoring device (Dinamap Carescape V100, GE Medical Systems, Berkshire, United Kingdom). There are several documented advantages to using the oscillometric technique of determining non-invasive blood pressure (NIBP) in comparison to using the auscultation technique such as elimination of potential investigator bias and errors of interpretation (O'Brien et al., 2010; 2003; Coe and Houghton, 2002). A pneumatic cuff was placed around the participant's upper left arm approximately 1.5 cm above the antecubital fossa and it was supported at the mid-sternal level throughout the blood pressure measurements (Figure 1). The participants arm circumference was measured whilst in a relaxed state using an anthropometric measuring tape (SECA, Germany). The largest arm circumference was used to ensure the appropriate pneumatic cuff size (Critikon Duracuf, GE Medical Systems, Berkshire, United

Kingdom). The same pneumatic cuff size was used for each participant during subsequent measurements.

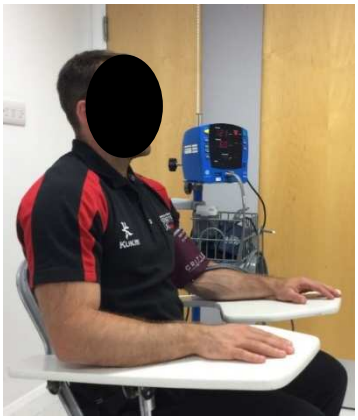


Figure 1. Demonstrates the appropriate equipment set up to measure resting blood pressure using the Dinamap Carescape V100.

A microprocessor within the device controlled the inflation and deflation of the pneumatic cuff to a predetermined pressure known as the Dinamap NIBP determination sequence. During each stage of the determination sequence the transducer within the pneumatic cuff detected changes in pressure oscillation. During the deflation sequence, the oscillations were measured in conjunction with the cuff pressure. The microprocessor deflated the pneumatic cuff one step down the determination sequence every time two pulses of relatively equal amplitude were detected. The microprocessor stores the information regarding cuff pressure, matched pulse amplitude and the time between successive pulses. Mean arterial pressure was determined at the highest amplitude that oscillations occurred during the determination sequence and subsequently systolic and diastolic pressures were derived using a reference algorithm (Dinamap Carescape V100 Service Manual (2011), GE Medical Systems, United Kingdom).

The Dinamap Pro Care (100 to 400), Pro Care 120 and Carescape V100 automated oscillometric blood pressure monitoring devices have fulfilled the accuracy criteria of the International Protocol for the European Society of Hypertension (2010) (O'Brien et al., 2010),

American Association of Medical Instrumentation (ANSI/AAMI SP-10) (1992) and the British Hypertension Society revised protocol grade A/A (1993) (de Greeff et al., 2007; Reinders et al., 2006). The Dinamap Pro 200 device has been shown to be a reliable automated oscillometric blood pressure monitoring device with no significant differences between trials or between days for repeated assessment of resting SBP, DBP and MAP (Wiles et al., 2010). The coefficients of variation were 3.54%, 4.73% and 2.83% for resting SBP, DBP and MAP respectively. Given that reliability can differ between versions of equipment and this can directly affect sample size calculation, a reliability study will be conducted as part of this thesis (see Section 3.2.2).

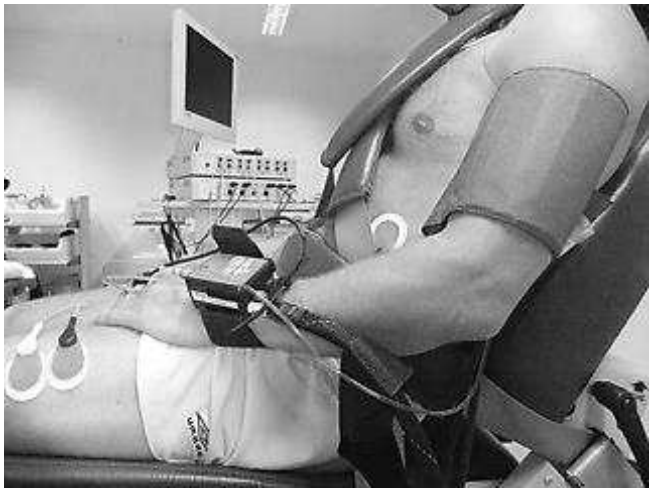


Figure 2. The standardised assessment of blood pressure using the Finometer® Pro during isometric exercise on the Isokinetic Dynamometer.

A non-invasive continuous finger blood pressure measurement device (Finometer® Pro, FMS, Finapres Medical Systems BV, Amsterdam, The Netherlands) was also used to measure blood pressure in the studies within this research thesis. Participants were required to maintain a seated position with the arm supported at the mid-sternal level during blood pressure measurements. An appropriately fitted pneumatic cuff was positioned around the participant's upper left arm approximately 1.5cm above the antecubital fossa. An appropriately fitting finger

cuff was positioned around the intermediate phalanx of the middle finger on the left hand (Figure 2). The finger cuff was comprised of a thin inflatable air bladder, which was inflated by an air hose connected to a front-end microprocessor strapped to the participant's left wrist. The fast pneumatic proportional valve in the front-end microprocessor unit modulates the air pressure, which changes the finger cuff pressure to allow for adjustments in continuous blood pressure measurement.

The Finometer® Pro device utilises infrared photoplethysmography to measure blood pressure. An infrared light source and infrared sensing photodiode photocell is built into the finger cuff and measured fluid blood tissue inside the artery. The photoplethysmograph inside the finger cuff was positioned in front of the artery and the light source (light emitting diode, LED) and light detector (photodiode) were placed symmetrically on each side of the finger. The Finometer® Pro utilises the volume-clamp method of Peñás to continuously evaluate transmural pressure in the finger which is calculated as the difference between intra-arterial and finger cuff pressure (Bogart and Lieshout, 2005). When transmural pressure equals zero the pressure applied by the adjustable finger cuff must equal intra-arterial pressure, this adjustment is considered representative of prevailing blood pressure levels. The inflatable finger cuff with in-built infrared photoplethysmography responds to the pulsatile unloading of the arterial wall of the finger. The unloading of the finger arterial wall is achieved through the volume-clamp method, whereby blood volume is detected by the photodiode and kept constant through the application of corresponding pressure by the inflatable finger cuff. This continuous counter pressure is required to keep finger arterial blood volume constant and directly corresponds to arterial blood pressure.

The PhysioCal criteria of Wessling technology is incorporated into the Finometer to calibrate the pressure detected from the finger cuff with that of the brachial artery obtained from an upper arm pneumatic cuff (Wessling et al., 1995). This process is performed at regular intervals

during continuous measurements to adjust for changes in vascular tone of the arterial walls. Finger arterial pressure measurement was calibrated using brachial artery pressure waveform reconstruction and height level correction (Gizdulich et al., 1997; Bos et al., 1996). Brachial artery pressure waveform reconstruction and height level correction are considered to compensate for the distortion of the pressure waveform along the brachial artery. The Riva-Rocci upper arm pneumatic cuff was positioned near the level of the heart and the finger cuff was consistently positioned at mid-sternal level. It was essential that the two ends of height correction sensor were placed at both the level of heart and finger. A pressure transducer measured the difference in height of the liquid column between the sensors at two levels and this was subtracted from the finger pressure. The brachial artery pressure waveform reconstruction method has been shown to fulfil the accuracy criteria outlined by the American Association of Medical Instrumentation (ANSI/AAMI SP-10) (1992) (Guelen et al., 2003; Bos et al., 1996). The weighted accuracy across 43 studies measuring continuous finger blood pressure using a Finometer has been shown to fall within the 5 mmHg limits outlined by the ANSI/AAMI SP-10 (1992) criteria (Imholz et al., 1998). The Finometer has been shown to satisfy the ANSI/AAMI SP-10 (1992) criteria for accuracy and achieved an overall A/B grading according to the British Hypertension Society criteria (Schutte et al., 2004). Previous studies have published reliability data using previous versions of the Finometer that may have a direct impact upon sample size calculation. A reliability study will be conducted as part of this thesis, it is necessary to ascertain that it is a reliable measure since this will be used to monitor BP during exercise (see Section 3.2.2).

3.2.2 The reliability of blood pressure measurement using a Dinamap Carescape V100 and Finometer® Pro device

Aims

The aim of this preliminary study was to assess the intra-tester reproducibility of the Dinamap Carescape V100 and Finometer® Pro device to measure blood pressure across an 8-week period.

Methods

Participants

Ten healthy males volunteered to participate in this preliminary study (age: 26 ± 9 years; height: 178.1 ± 5.7 cm; weight: 79.9 ± 14.2 kg) (mean ± SD). All participants were required to adhere with the testing criteria as outline din the participant information sheet (Appendix 2). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed a Health and Fitness Questionnaire (Appendix 1) and provided written informed consent (Appendix 2), thereby adhering to the guidelines set by the Declaration of Helsinki (1964).

Testing procedures

Prior to the study commencing all participants confirmed their adherence to the testing criteria. Following an initial visit to the laboratory for familiarisation, two trials were performed at week 1 and a third trial at week 8 to mimic the proposed duration of the subsequent exercise training study. Participants visited the laboratory at approximately the same time of day on each visit. Following completion of this initial process, participants were asked to rest in a seated position for 20-minutes. Measurements were first performed using the Dinamap Carescape V100 device. Following the fitting of an appropriately sized blood pressure cuff at the start of the rest period, 3 automated blood pressure measurements were performed each separated by a 1-

minute rest period and the lowest value was used for analysis of RBP (Wiles et al., 2010). Following the removal of the Dinamap Carescape V100 device blood pressure cuff, the participant was asked to remain in a seated position whilst the Finometer® Pro blood pressure cuff was fitted. Upon completion of the Finometer® Pro calibration, a continuous 5-minute measurement was recorded interfaced Labchart 7 software (AD Instruments Ltd, Australia). Systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and heart rate values were recorded and used for further analysis.

Statistical Analysis

Data was analysed using the statistical software programme SPSS 21.0 for Windows. Data was assessed for conformity with parametric assumptions (Field, 2000). Parametric data was analysed using a repeated measures analyses of variance (ANOVA) to assess for differences between manual and automated software techniques and trials (2-1 and 3-2). Where data was found to have violated parametric assumptions a non-parametric Freidman test was used. The significance level was set at $P \leq 0.05$. A single coefficient of variation (CV%) was derived by logarithmically transformed two-way ANOVA, intra-class correlation coefficient (ICC) and 95% confidence limits for the CV% were calculated using root mean square error (RMSE) (Atkinson and Neville, 2001). Data are presented as mean ± SD. The sample size (n) was estimated for a crossover design exercise training study using the within-subject variation expressed as coefficient of variation (s) and the change score (d) (Figure 3).

$$n = 16 * s^2 / d^2$$

Figure 3. Sample size estimation equation **(**Hopkins et al., 1999).

Results

The systolic, diastolic and mean arterial blood pressure data was normally distributed for the Dinamap Carescape V100 and Finometer® Pro automated blood pressure monitoring devices (P >0.05). There were no significant differences between trials for resting SBP (P =0.510) when measured using either the Finometer® Pro device or the Dinamap Carescape V100 device. There was a significant difference between technique for resting SBP (P =0.050), resting SBP was significantly higher (5 ± 2 mmHg) when measured using the Finometer® Pro device compared to the Dinamap Carescape V100 device. There were no significant differences between trials for resting DBP (P =0.720) when measured using either the Finometer® Pro device or the Dinamap Carescape V100 device. There was a significant difference between technique for resting DBP (P =0.002), resting DBP was significantly higher (7 ± 2 mmHg) when measured using the Finometer® Pro device compared to the Dinamap Carescape V100 device. There was no significant difference between trials for resting MAP (P =0.355) when measured using either the Finometer® Pro device or the Dinamap Carescape V100 device. There was a significant difference between technique for resting MAP (P =0.007), which was significantly higher by (5 ± 1 mmHg) when measured using the Finometer® Pro device compared to the Dinamap Carescape V100 device. Based on the reliability study data, estimated sample size using the Dinamap device is between n=9 and 16 participants. Table 4 displays the group mean coefficient of variation and intra class correlation coefficient for the Dinamap Carescape V100 and Finometer® Pro device.

Table 4. Resting blood pressure measures obtained from repeated measures (*n*=10).

Variable	Visit 1	Visit 2	Visit 3	CV%	ICC
Dinamap Carescape V100					
SBP	120 ± 9	124 ± 10	127 ± 10	3.05 (2.09 - 5.56)	0.87
DBP	73 ± 7	73 ± 6	74 ± 7	6.05 (4.16 - 11.05)	0.58
MAP	90 ± 5	92 ± 5	93 ± 6	2.80 (1.93 - 5.11)	0.79
Finometer® Pro					
SBP	127 ± 6	130 ± 10	130 ± 10	4.76 (3.28 - 8.70)	0.67
DBP	76 ± 5	80 ± 7	81 ± 6	3.26 (2.24 - 5.94)	0.90
MAP	93 ± 5	97 ± 8	98 ± 7	3.78 (2.60 - 6.90)	0.82

Note: Data presented as group mean CV% (lower and upper 95% confidence intervals). ICC= intra class correlation coefficient. SBP= systolic blood pressure, DBP= diastolic blood pressure, MAP= mean arterial blood pressure. Data presented as mean ± SD.

Discussion

The aim of this preliminary study was used to estimate the sample size requirement for a crossover study conducted over an 8-week period. Resting blood pressure parameters were independently incorporated into the sample size equation (Hopkins et al., 1999) (Figure 3). Change scores were incorporated using the data from research whereby similar blood pressure measurement protocol using a Dinamap Pro 200 were utilised (Wiles et al., 2010). The results from this study demonstrate that both the Dinamap Carescape V100 and the Finometer® Pro device report reliable resting blood pressure values over a prolonged period of time with coefficients of variation ranging between 3.05% and 6.05%. A coefficient of variation less than 10% should be considered good and less than 20% should be considered acceptable (Scott, Randolph and Leier, 1989).

The operation manual for the Dinamap Pro Series (100 to 400) specifies that the blood pressure values obtained using the device correspond to comparisons with intra-aortic values, which either meet or exceed the American National Standards Institute (ANSI)/AAMI SP-10 standards for accuracy (a mean difference of ≤ 5 mmHg, and a standard deviation of ≤ 8 mmHg) (GE Medical Systems, 2002). There appears to be very few publications that report the reliability of the Dinamap Carescape V100. The reliability of the Dinamap Pro 200 for the measurement of blood pressure was explored in the PhD thesis of Wiles (2008) using ten healthy male participants within a 1-week testing period. When the lowest blood pressure value of each of the three trials were compared, a coefficient of variation of 3.54% (CI: 2.47-4.95%), 4.73% (CI: 3.29-6.62%) and 2.83% (CI: 1.97-3.96%) for resting SBP, DBP and MAP respectively (Wiles, 2008). The coefficients of variation in the present study are comparable to those reported by (Wiles, 2008). Whilst the validity of the Finometer device has been established, there is less information existing related to the reproducibility of non-invasive finger blood pressure (Parati et al., 2001). Good short-term reproducibility of the Finapres with

coefficients of variation below 10% has been reported (Højgaard et al., 2005; Parati et al., 2001), however there appears to be few studies that have measured the reliability of RBP using the Finometer® Pro. The results of this preliminary study should contribute to the existing body of research surrounding the reproducibility of the Finometer® Pro device. The Dinamap Carescape V100 provided lower resting blood pressure values compared to the Finometer® Pro (See Table 4). These findings are in agreement with the findings reported by O'Brien *et al.* (1993) that DBP measured using the Dinamap 8100 tends to be underestimated by 7.6 mmHg, which does not meet the AAMI criteria and only a grade D on the BHS criteria. At this point, it is essential to be aware that in the aforementioned study the Dinamap blood pressure was compared to those measured using the auscultatory method. This is also an indirect measure of blood pressure and not a gold standard method for reference, which has potential sources for measurement error (Friedman, 1997).

Summary

The results from this study demonstrate that the Dinamap Carescape V100 and the Finometer® Pro device report reliable resting blood pressure values over time in healthy populations. The reliability reported by this research appears to be adequate for the detection of meaningful changes in the primary outcome variable resting blood pressure when a relatively moderate sample size of between $n=9$ and 16 are utilised. The blood pressure reproducibility results from this study are likely to contribute to the limited existing research and this has the potential to inform future studies of sample size estimation using the same measurement tools. The reliability was similar between devices, and therefore for the purpose of continuity between studies the Dinamap Carescape V100 will be used to measure change in RBP in the exercise training study conducted as part of this thesis.

3.2.3 Heart rate and heart rate variability

Heart rate and heart rate variability components were measured and recorded via electrocardiography using a 16-channel chart recorder, PowerLab/SP16 with Chart 7 software (AD Instruments Ltd, Australia). A standard three-lead bipolar electrocardiogram arrangement was used as recommended by AD Instruments, Australia. Participants were fitted with three blue sensor R electrode pads (Ambu Inc., Maryland, USA). A three-lead shielded Bio Amp cable (MLA2340, AD Instruments Ltd, Australia) was attached to the electrode pads and the cable was connected to the PowerLab/SP16 via a Bio Amp CF Amplifier (ML132, AD Instruments Ltd, Australia). Electrodes were placed inferior to the right (earth) and left (negative) clavicle both midway between the conoid tubercle and the costal tuberosity and an electrode was also placed over the tenth rib (positive) on the left side (Klabunde, 2011). Prior to electrode application the skin was prepared during each laboratory visit. The area was cleaned using a steret isopropyl alcoholic skin wipe (Medlock Medical Ltd, United Kingdom) and allowed time to dry before the electrodes were positioned. Resting HR and HRV were measured and recorded following a resting period of 20-minutes in a supine position in a quiet room. In order to avoid uncertain spectral components participants were instructed to breathe at a pace of 12 breaths per minute using a metronome during resting HR and HRV measurements (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Participants were asked to relax and minimise movement during the resting 5-minute HRV measurement and recording period.

Heart rate variability represents the difference in duration between successive R-R intervals measured during the electrocardiography recording and is considered to represent the state of control of the cardiovascular system through the sympathetic and parasympathetic branches of the autonomic nervous system (Malliani, 1999). A signal for each QRS complex was detected during the electrocardiography recording using Chart 7 software to identify heartbeats before

undertaking power spectral density analysis. An R-wave detection threshold was established for each individual participant immediately prior to the testing protocol (Figure 4). A sample rate of 1000 Hz was used for both HR and HRV measurements as recommended to reduce the risk of signal noise being included within the HRV spectrum (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). A visual check of the electrocardiography recording was performed off-line in conjunction with the automatic Chart 7 software interpolation check before further analysis (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). If the percentage of ectopic beats during a 5-minute electrocardiography recording exceeded 10% of the total measurement it was deemed unacceptable for HRV analysis (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Ectopic heartbeats are defined as abnormal heartbeats originating from a self-excitable site in the atria or ventricles as opposed to originating from the sinoatrial node. Ectopic heart beats can produce erroneous HRV data and subsequently result in an abnormal QRS complex which is usually evident with extended R-R interval in comparison to an sinoatrial node derived normal heartbeat.

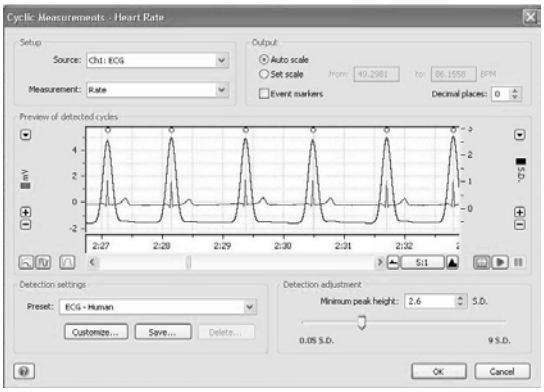


Figure 4. R-wave detection thresholds as indicated by circular events marker above the signal using Chart 7 Labchart software.

Heart rate variability data was divided into consecutive sections comprising 1024 data points (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996) and a Fast Fourier Transform algorithm was applied to each of the sections. The Fast Fourier Transform algorithm transforms the signal from time domain to frequency domain data expressed in cycles per beat and converted to hertz by dividing by beats per second. Power spectral density analysis of HRV demonstrates how power distributes as a function of frequency and allows the identification of three main spectral frequency components. The spectral frequency bands for HRV analysis were standardised for all participants and consisted of high frequency (0.15 – 0.4 Hz) (HF), low frequency (0.04 – 0.15 Hz) (LF) and very low frequency (< 0.04 Hz) (VLF) components. The spectral frequency bands for HRV were reported in absolute values of power (ms^2) which included the components total power (TP), high frequency, low frequency, very low frequency components and low frequency to high frequency ratio (LF:HF). Total power represents the variance of all R-R intervals across a 5-minute recording. The spectral frequency bands for HRV were reported in relative values of power expressed as normalised units (nu) (Malliani et al., 1991) (Figure 5).

$$LFnu = LF / (TP – VLF) \times 100$$

$$HFnu = HF / (TP – VLF) \times 100$$

Figure 5. Spectral frequency bands for heart rate variability reported as normalised units (Malliani et al., 1991).

In accordance with recommendations outlined by the Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology (1996) the absolute and relative HRV parameters were recorded. Relative HRV parameters are considered to represent the state of control of the cardiovascular system through the sympathetic and parasympathetic branches of the autonomic nervous system (Malliani, 1999), therefore these

parameters took precedence over absolute parameters within this thesis. The use of calculated normalised units reduces signal noise and minimise the effect of changes in total power on the low frequency and high frequency components. Relative HRV parameters have been reported to be more appropriate when evaluating the effect of an intervention on participants particularly when large differences in total power are evident (Malliani, 1999). Considerable day-to-day variation has been demonstrated in HRV measures (Pinna et al., 2007; Sandercock et al., 2004; Jáuregui-Renaud et al., 2001). Mean coefficients of variation for frequency domain components of HRV have been reported to vary between 32% and 93% using three instruments (Sandercock et al., 2004).

3.2.4 Ultrasonography- Artery diameter and blood flow velocity

Doppler ultrasound is a non-invasive technique, underpinned by the Doppler ultrasound principle, to provide quantitative data regarding the structure and function of blood vessel (www.bmus.org). Duplex ultrasound involves the simultaneous acquisition of artery diameter and blood flow velocity through B-mode and pulse wave Doppler imaging. Artery diameter and blood flow velocity were measured using Two-Dimensional Duplex Doppler ultrasound (LOGIQ e book, GE Healthcare, United Kingdom) and an 8L-RS probe (GE Healthcare, United Kingdom) operating at a variable Doppler frequency of 4 to 10MHz. Blood vessels were imaged in the longitudinal plane in order to identify the clear vascular wall boundaries (Pignoli et al., 1986) (Figure 6. A). Doppler beam vessel angle of isonation of 60 degrees in relation to the vessel orientation was used to permit the most accurate estimation of Doppler velocities whilst maintaining optimal B-mode artery diameter images (Gerhard-Herman et al., 2006). The sample volume cursor was positioned in the centre of the blood vessel and the gate width was as wide as possible without encompassing on the vessel walls (Harris et al., 2010) and mean blood flow velocity was measured as determined by the sample size volume (Thijssen et al., 2011d). Resting vascular ultrasound parameters were measured following 20-

minutes rest in the supine position on a portable bed in a quiet room. The ultrasound settings and transducer position on the limb were noted and remained constant for each participant throughout each study. Participants were instructed to remain in supine position and minimise movement during testing.

To examine brachial artery diameter and blood flow velocity, the arm was extended at approximately 80 degrees to the participant's torso and supported at heart level. The brachial artery was imaged by positioning the probe approximately 9 cm proximal to the medial epicondyle (Figure 7. A). To examine common femoral artery diameter and blood flow velocity, the transducer was positioned distal to the inguinal ligament and approximately 2 to 3 cm above the bifurcation of the common, superficial and profunda femoral artery (Figure 6. B to D). To examine superficial femoral artery diameter, the transducer was positioned distal to the bifurcation of the common, superficial and profunda arteries. An electrocardiogram trace was recorded simultaneously using an in-built system within the ultrasound device (GE Healthcare, United Kingdom) to identify distinct phases within each cardiac cycle.

Artery diameter and blood flow analysis

Brachial, common femoral and superficial femoral artery diameter is defined as the perpendicular measure from the inter-lumen to inter-lumen space (Peiffer et al., 2007). Brachial, common femoral and superficial femoral artery diameter was determined off-line using automated edge-detection wall tracking software (Woodman et al., 2001). Artery diameter was determined across the complete cardiac cycle and calculated as the average from the highest quality 30-second video recording. To perform analysis using the edge detection and wall tracking software, B-mode ultrasound video recordings of the artery were stored as audio video interleaves onto the computer that contained the software. A region of interest was selected which encompassed the desired section of the artery to be analysed. The artery

diameter was then calibrated against the image size on the computer by drawing a region between two points on the ultrasound image of a known distance apart. A parallel-prong rake algorithm of 200 to 400 parallel lines within the region of interest with subsequent quadratic spline interpretation at 20 to 30 Hz was used to calculate the diameter (Green et al., 2002). Brachial, common femoral and superficial femoral artery diameter arterial blood flow velocity was determined off-line by the operator using in-built ultrasound trace software (LOGIQ e, GE Healthcare, United Kingdom). Due to incapability of the software with the ultrasound model, following the analysis of artery diameter using edge detection and wall tracking software, in order to determine blood flow the cross-sectional area of the artery was calculated as πr^2, where *r* represents the half of the artery diameter (Naylor et al., 2005).

Brachial, common femoral and superficial femoral artery blood flow velocity were calculated as the average of all velocity time integrals from the highest quality 30-second video recording, which was the same video recording used to calculate artery diameter. Using an average over multiple cardiac cycles appears advantageous to minimise the normal physiological variation depending on the relationship between the cardiac cycle, intramuscular pressure and pulse pressure (Harris et al., 2010; Rådegran, 1997). The velocity-time integral curve was determined off-line by the operator using in-built velocity trace measurement software (GE Healthcare, United Kingdom) Antegrade blood flow velocity ($cm.s^{-1}$) was calculated as the average of the maximum velocity time integrals measured as the highest velocity in a Doppler spectrum within each cardiac cycle. Retrograde blood flow velocity ($cm.s^{-1}$) was calculated using the average of the minimum velocity time integrals measured as the lowest velocity in a Doppler spectrum within each cardiac cycle. Antegrade blood flow ($ml.min^{-1}$) was calculated as the product of the cross sectional area of the artery and antegrade blood flow velocity.

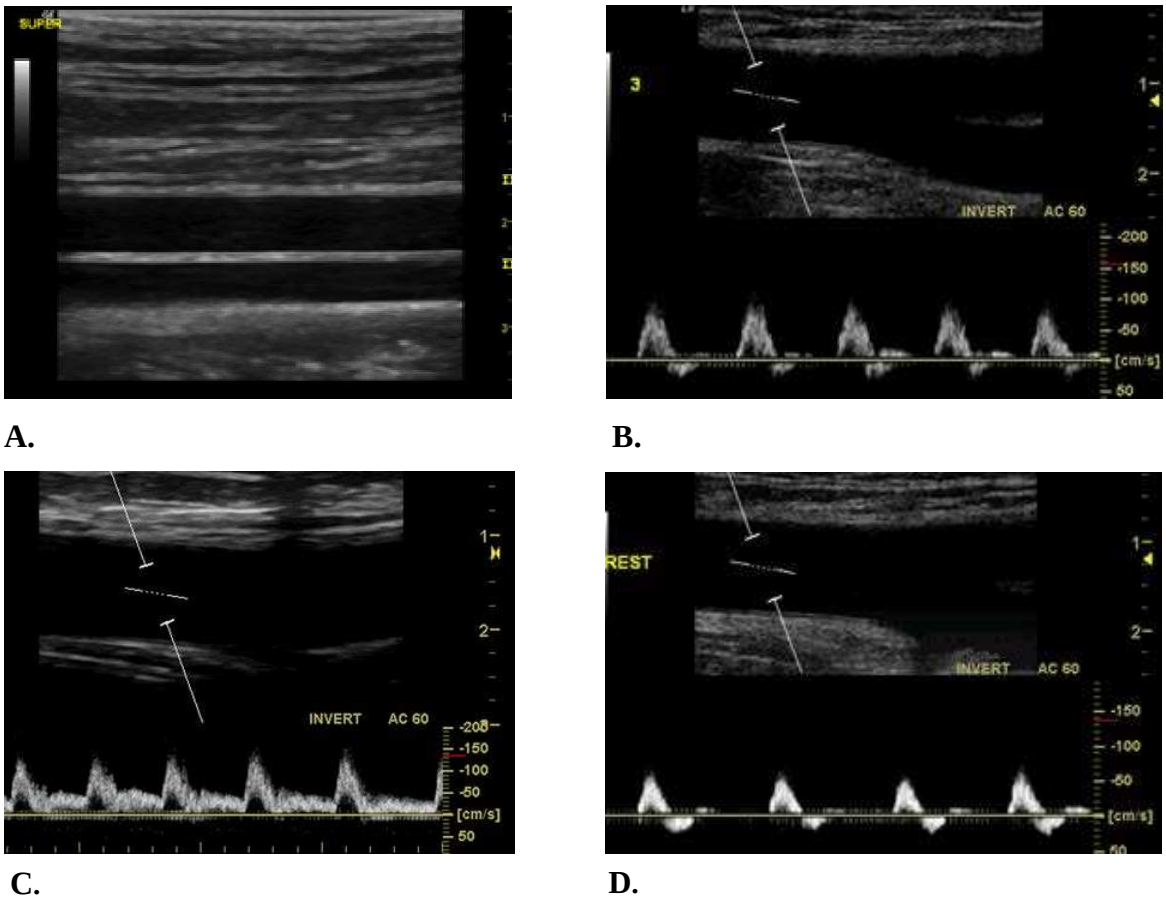


Figure 6. A. Two-dimensional B-mode ultrasound image of the superficial femoral artery and femoral vein in longitudinal axis view. **B.** Two-dimensional B-mode ultrasound image of the common femoral artery during muscular contraction, **C.** Upon cessation of muscular contraction and **D.** Under rested conditions.

Retrograde blood flow ($ml.min^{-1}$) was calculated as the product of the cross sectional area of the artery and retrograde blood flow velocity). Antegrade shear rate (s^{-1}) was calculated as four times and antegrade blood flow velocity divided by the cross sectional area of the artery. Retrograde shear rate (s^{-1}) was calculated as four times and retrograde blood flow velocity divided by the cross sectional area of the artery. The antegrade and retrograde components of blood flow velocity, blood flow and shear rate were calculated accordance with world leading research within this field (Green et al., 2005; 2002). Shear rate, an estimate of shear stress without accounting for blood viscosity, was used to avoid the implementation of invasive

testing procedures (Pyke and Tschakovsky, 2005). Oscillatory shear index was calculated using the following equation: [retrograde shear rate / (antegrade shear rate + retrograde shear rate)] (Newcomer et al., 2008; Wu et al., 2004). Oscillatory shear index values near zero indicate unidirectional shear rate throughout the cardiac cycle and oscillatory shear index values near 0.5 indicates oscillatory flow (Padilla et al., 2011a; 2011b).

Day-to-day reliability coefficients of variations have been reported for resting common femoral artery diameter (2.42%), brachial artery diameter (3.06%), common femoral artery blood flow velocity (3.19%), brachial artery blood flow velocity (3.45%), common femoral artery blood flow (2.76%) and brachial artery blood flow (3.73%) (Baross et al., 2012). Walther et al. (2006) has reported lower mean coefficients of variation for resting common femoral artery diameter (1.8%) and larger mean coefficients of variation for common femoral artery blood flow velocity (13.8%). Shoemaker et al. (1996) has reported coefficients of variation of 4.1% for resting brachial artery diameter and 13.1% for brachial artery antegrade blood flow. As part of this thesis, the tester reliability needs to be ascertained, as this is the main component of variability seen in this measurement. Furthermore, this may have a direct impact upon sample size estimation (see Section 3.2.5).

3.2.5 The reliability of ultrasonography measurements using the LOGIQ e ultrasound (GE Healthcare) device

Aims

The aim of this preliminary study was to assess the reliability of the observer to measure brachial artery and common femoral artery diameter, blood flow velocity and blood flow using the LOGIQ e ultrasound. An additional aim of this study was to compare the reliability of the observer performing repeated peripheral conduit artery diameter and blood flow measures determined using manual and automated software techniques.

Methods

Participants

Eighteen healthy males volunteered to participate in this preliminary study (age: 25 ± 5 years; height: 177.5 ± 5.7 cm; weight: 80.9 ± 10.6 kg) (mean ± SD). All participants were required to adhere with the strict testing criteria as outlined in the participant information sheet (Appendix 2). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed a Health and Fitness Questionnaire (Appendix 1) and provided written informed consent (Appendix 2), thereby adhering to the guidelines set by the Declaration of Helsinki (1964).

Testing procedures

Prior to the study commencing all participants confirmed their adherence to the testing criteria. Participants visited the laboratory for an initial visit and two subsequent trials each separated by 7 days. Participants visited the laboratory at approximately the same time of day on each visit. Following completion of this initial process, participants were asked to rest in a supine position for 20-minutes. Before the commencement of ultrasound imaging the participant was fitted with an in-built ultrasound 3-lead electrocardiogram arrangement to measure the QRS complex. Following a 20-minute rest period in the supine position, brachial artery and common femoral artery diameter and blood flow velocity were measured using two-dimensional duplex ultrasound (LOGIQ e book, GE Healthcare).

The brachial and common femoral artery were imaged in accordance with the protocol outlined in the general method (Section 3.2.4). The highest quality 30-second video recordings of the brachial and common femoral artery were determined by the researcher and used for further analysis. Two different methods of artery diameter analysis were investigated as part of this study. Manual analysis was performed using the in-built ultrasound software where the

diameter was measured as the perpendicular distance from inter-lumen to inter-lumen space and averaged across 3 cardiac cycles at peak systole (LOGIQ e, GE Healthcare, United Kingdom). Automated analysis was performed using edge-detection and wall tracking software (Woodman et al., 2001) where the diameter was calculated as the average across the cardiac cycle in a continuous 30-second recording as outlined in the general method (Section 3.2.4). Subsequently, blood flow was calculated for the brachial and common femoral artery as the product of blood flow velocity and artery diameter obtained for both the manual analysis or automated analysis method (Section 3.2.4). All measurements of brachial artery, common femoral artery diameter and blood flow velocity were repeated in the same order of testing during subsequent visits to the laboratory.

Statistical analysis

Data was analysed in an identical manner to the previous reliability study (see Section 3.2.2).

Results

Based on initial data checks the data obtained for brachial artery and common femoral artery diameter, blood flow velocity and blood flow were normally distributed. There were no significant differences between trials for the assessment of brachial artery diameter (P =0.248). Brachial artery diameter was significantly higher using the manual analysis technique compared to using edge-detection and wall tracking software analysis technique (P =0.003). There was no significant difference between analysis technique (P =0.349) or trials (P =0.351) for the assessment of common femoral artery diameter. There was no significant difference between trials for the assessment of brachial artery blood flow velocity (P =0.229) or brachial artery blood flow (P =0.295). Brachial artery blood flow was significantly higher when calculated using artery diameter obtained from the manual analysis technique (P <0.01)

Table 5. Resting ultrasonography brachial artery and common femoral artery measures (diameter, *n*=18, blood flow velocity, *n*=12, blood flow, *n*=12) measures obtained from repeated measures.

Variable	Visit 1	Visit 2	Visit 3	CV%	ICC
Brachial artery					
Manual (in-built ultrasound software)					
Diameter (mm)	0.42 ± 0.04	0.42 ± 0.04	0.43 ± 0.03	3.15 (2.55 - 4.13)	0.87
Blood flow velocity ($cm.s^{-1}$)	70 ± 15	73 ± 18	68 ± 15	10.09 (7.80 - 14.28)	0.86
Blood flow ($ml.min^{-1}$)	60 ± 16	62 ± 15	59 ± 12	10.98 (8.49 - 15.54)	0.89
Automated (edge-detection and wall tracking software)					
Diameter (mm)	0.39 ± 0.04	0.40 ± 0.04	0.40 ± 0.04	5.42 (4.39 - 7.11)	0.78
Blood flow ($ml.min^{-1}$)	54 ± 17	57 ± 14	53 ± 13	12.93 (10.0 - 18.30)	0.88
Common femoral artery					
Manual (in-built ultrasound software)					
Diameter (mm)	0.92 ± 0.10	0.92 ± 0.09	0.92 ± 0.10	1.95 (1.57 - 2.55)	0.97
Blood flow velocity ($cm.s^{-1}$)	56 ± 9	57 ± 12	60 ± 12	5.96 (4.61 - 8.43)	0.92
Blood flow ($ml.min^{-1}$)	226 ± 51	231 ± 48	242 ± 57	8.39 (6.49 -11.87)	0.88
Automated (edge-detection and wall tracking software)					
Diameter (mm)	0.89 ± 0.11	0.88 ± 0.10	0.90 ± 0.11	3.69 (2.99 - 4.84)	0.92
Blood flow ($ml.min^{-1}$)	214 ± 56	218 ± 48	237 ± 57	11.07 (8.56 -15.67)	0.82

Note: Data presented as group mean CV% (lower and upper 95% confidence intervals). ICC= intra class correlation coefficient. Data presented as mean ± SD.

presumably because of the higher brachial artery diameter. There was a significant difference between trials 1 and 3 for the assessment of common femoral artery blood flow velocity (P =0.037). There was no significant difference between trials for common femoral artery blood flow (P =0.055) or analysis technique (P =0.097). Table 5 displays the group mean coefficient of variation and intra class correlation coefficient for the brachial artery and common femoral artery measures.

Discussion

The results from this study were used to assess the potential impact of the researcher in collecting this data and demonstrate that the researcher had the ability to reliably measure peripheral vascular conduit artery diameter and blood flow velocity over an 8-week time period. The measurement of brachial artery diameter was higher using the manual analysis technique given that measurements were are performed at peak systole, whereas the automated analysis technique which utilises edge-detection and wall tracking software measures the diameter across the entire cardiac cycle. Common femoral artery blood flow velocity was slightly higher at trial 3 compared to trial 1, however this did not affect the calculation of blood flow**.** The manual method of analysing artery diameter is potentially more time intensive in comparison to using edge-detection and wall tracking software and subject to intra-observer bias.

Summary

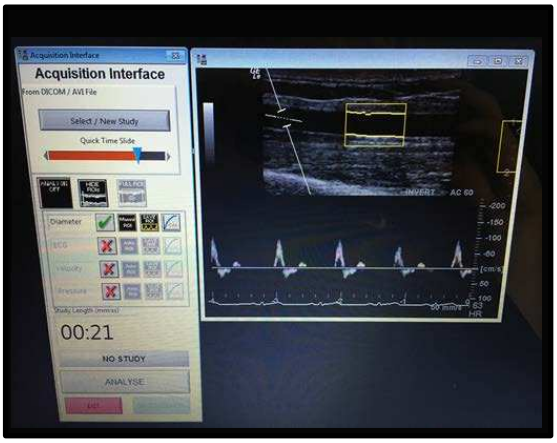
The findings from this reliability study show that the researcher can reliably measure peripheral vascular conduit artery parameters over an 8-week period. Furthermore, due to the similar coefficients of variation but less time intensive requirements, it may be advantageous to continue using the edge-detection and wall tracking software to analyse artery diameter and determine blood flow compared to the manual analysis method. Furthermore, the results from

this reliability study appear to demonstrate favourable estimations of sample size. Change scores from previous research were incorporated into the sample size equations (Spence et al., 2013; Stebbings et al., 2013; Baross et al., 2012; Hambrecht et al., 2000). The sample size for a cross over designed study was estimated to be *n=1* participant for brachial artery blood flow and *n=8* for brachial artery diameter using the automated edge-detection and wall tracking software analysis method. The sample size for a cross over designed study was estimated to be *n=2* participants for both common femoral artery blood flow and diameter using the automated edge-detection and wall tracking software analysis method.

3.2.6 Ultrasonography- Brachial artery flow-mediated vasodilatation

Flow-mediated vasodilatation (FMD) is the dilator response of the blood vessel to an increase in blood flow or more precisely shear stress (Corretti et al., 2002). The brachial artery FMD technique is a valid non-invasive technique used as an indicator of nitric oxide-mediated endothelium-dependent vasodilatation (Celermajer et al., 1992). Participants rested for 20-minutes in the supine position on a portable bed in a quiet room. Duplex ultrasound was used to measure brachial artery FMD thus allowing the simultaneous quantification of shear stress (Padilla et al., 2008; Pyke and Tschakovsky, 2007; 2005). The right arm was extended approximately 80 degrees to the participant's torso and supported at heart level by an adjustable table in order to measure the brachial artery (Figure 7. A). The transducer was positioned in the distal one third of the upper arm. The ultrasound settings and transducer position on the limb were recorded and maintained for each participant throughout each study.

A.



B.

Figure 7. A. The standardised arrangement for all brachial artery flow-mediated vasodilatation (FMD) assessments **B.** Brachial artery flow-mediated vasodilatation (FMD) analysis using edge-detection and wall tracking software (Woodman et al., 2001).

A rapid inflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned around the forearm, immediately distal to the olecranon process to provide a stimulus for forearm ischemia (Corretti et al., 2002). The placement of the cuff distal to the site of measurement has been shown to produce predominantly endothelium-dependent vasodilatation (Betik et al., 2004; Berry et al., 2000). Following collection of baseline brachial artery diameter and blood flow velocity measurements, the rapid inflation pneumatic cuff was inflated to supra systolic blood pressure (200 mmHg) for 5-minutes to provide an ischemic stimulus (Parker et al., 2006). Brachial artery diameter and blood flow velocity recordings were resumed prior to deflation of the pneumatic cuff. Following deflation of the pneumatic cuff, artery diameter and blood flow velocity continued for 3-minutes thereafter in accordance with technical specifications (Thijssen et al., 2011d). Blood pressure was recorded prior to and following the assessment (Pyke and Tschakovsky, 2008).

Brachial artery flow-mediated vasodilatation analysis

Baseline and peak brachial artery diameter was determined off-line using automated edge-detection wall tracking software (Woodman et al., 2001). For further detail regarding the software processing algorithms, see Section 3.2.4. Baseline artery diameter was calculated as the mean across the entire cardiac cycle from the highest quality 60-second video recording. Peak artery diameter was calculated as the highest diameter from the 3-minute video recording following pneumatic cuff deflation. Peak artery diameter was detected according to an algorithm that identified the maximum bracket of data in the post cuff deflation period (Woodman et al., 2001) (Figure 7. B). The automated software uses designated time bins to locate the maximum diameter throughout the entire post cuff deflation period, as opposed to using a predetermined time window that is more likely to lead to error in calculation (Luini et al., 2010). The post cuff deflation shear rate area under the curve was determined off-line by the operator using in-built ultrasound trace software (LOGIQ e, GE Healthcare, United Kingdom) from the maximum velocity time integrals up to the time of peak diameter and calculated using the trapezoid rule (Harris et al., 2010; Tinken et al., 2008).

An allometric model involving brachial artery peak diameter as the outcome and baseline diameter as the covariate was used and back-transformed to represent percentage mean values of FMD 'adjusted' for baseline diameter (Atkinson and Batterham, 2013a). Shear rate area under the curve is considered to represent the total stimulus involved in the FMD response and was calculated as the total shear rate response from immediate cuff deflation up to the time of peak diameter, (Pyke and Tschakovsky, 2007). The normalisation of brachial artery FMD to shear rate area under the curve is considered to eliminate the potential influence of the variation in shear rate profile created by cuff ischemia (Padilla et al., 2008). Studies have used different mathematical equations and timings to determine the total shear rate response, McGowan et al. (2006) used the peak shear rate obtained during cuff release whilst others have similarly used

blood flow averaged across the cardiac cycle up to the point of peak diameter (Llewellyn et al., 2012; Pyke and Tschakovsky, 2007; 2005; Pyke et al., 2004).

The normalisation of FMD to the shear stress stimulus should not be adopted if the relationship between shear stress and FMD is weak (≥1) (Atkinson and Batterham, 2013a; 2013b; Atkinson et al., 2009). Therefore, adherence to the relevant assumptions underlying the reliable use of FMD ratios were tested to determine whether normalisation of brachial artery FMD to shear stress should be applied. It has been demonstrated that the mean intra-observer coefficient of variation of repeated measures of FMD is significantly lower using computerised software (6.7%) compared to manual analysis (24.8%) techniques using the intima-lumen interfaces (Woodman et al., 2001). The coefficient of variation for between visit reproducibility of brachial artery FMD was 14.7% using the software (Woodman et al., 2001).

3.2.7 Echocardiography- Aortic diameter and blood flow velocity

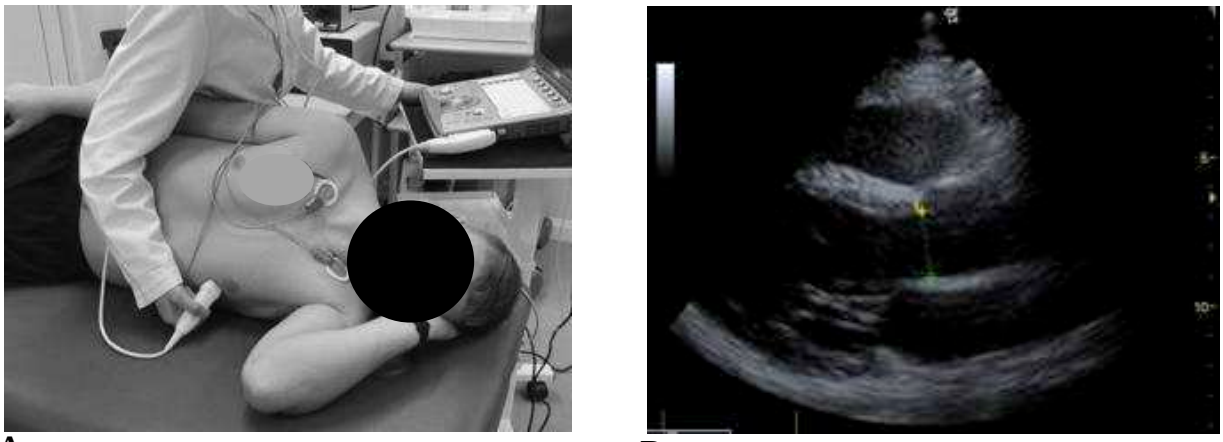
Transthoracic Doppler ultrasound is a non-invasive technique used to provide quantitative data regarding the direction and velocity of blood flow through the heart during the complete cardiac cycle, also known as echocardiography (Oxborough, 2008). Doppler ultrasound is considered a safe technique when undertaken in accordance with the British Medical Ultrasound Society Guidelines (www.bmus.org). This technique involves the emission of ultrasound waves from a transducer at a given frequency, which are reflected from the moving blood flow within the heart and vasculature at a different frequency to the transducer. This shift in frequency is proportional to the reflecting object's velocity vector in the direction of the incident wave as represented by the following Doppler equation (Figure 8).

$$v = \frac{c\,(f_r - f_t)}{2f_t\,(\cos\theta)}$$

Figure 8. Doppler Shift equation.

Whereby v is velocity of the blood, c is speed of sound in the medium, f_r is the frequency received by the transducer, f_t is the frequency transmitted by the transducer and θ is the angle between the transmitted ultrasound waves and the direction of blood flow (Celermajer et al., 1992; Coats, 1990). The transducer angulation was optimised throughout measurements to ensure that the angle of incidence between blood flow velocity and the transmitted ultrasound waves was approximately at zero or below 20 degrees (Feigenbaum, 1994; Lewis et al, 1984). All echocardiographic parameters were measured following 20-minutes rest in the supine position in a quiet room.

Aortic diameter and aortic blood flow velocity were measured using two-dimensional Doppler ultrasound (LOGIQ e book, GE Healthcare, United Kingdom) and 3S-RS probe (GE Healthcare, United Kingdom) operating at a pulse wave Doppler frequency of 2.2MHz. Pulse wave and colour Doppler ultrasound was used to depict the direction of blood flow, depth discrimination and locate specific blood flow sites. Participants were instructed to remain in the left lateral decubitus position and raise the left arm above shoulder height in order to maximise the echocardiographic acoustic window (Lang et al., 2005). An electrocardiogram trace was recorded simultaneously using a system in-built in the ultrasound device (GE Healthcare, United Kingdom) and displayed in real time on the ultrasound monitor to identify distinct phases of the cardiac cycle.



A. **B.**

Figure 9. A. Echocardiography assessment in the parasternal long axis view. **B.** Parasternal view of the aortic valve and diameter measurement of left ventricular outflow tract during peak systole.

The parasternal long axis view was used to measure the cross-sectional area of the aorta (Figure 9. A). The transducer was positioned between the third and fourth intercostal space against the left sternal border. The index point of the transducer was angled by the operator towards the participant's right shoulder. The aortic diameter was measured at the aortic valve hinge points as the perpendicular distance from inter-lumen to inter-lumen space (Figure 9. B) and determined off-line by the operator using in-built ultrasound measurement calipers (GE Healthcare, United Kingdom). Aortic diameter was calculated as the average diameter across three cardiac cycles at peak systole. The apical five chamber view was used to measure aortic blood flow velocity. The participant was instructed to move into a slightly more supine position in order to optimise image acquisition in the apical view. The transducer was positioned between the sixth and seventh intercostal space in the mid axilla. The index point of the transducer was angled by the operator towards the participant's left shoulder. Upon acquisition of the desired view, the sample pulse wave volume was measured immediately proximal to the aortic valve. Aortic blood flow velocity was determined off-line by the operator using in-built ultrasound velocity trace software (GE Healthcare, United Kingdom). The Doppler signal received by the transducer for each heart beat was displayed as a blood flow velocity envelope

over time also known as a velocity-time integral. The area beneath the velocity-time integral curve was determined off-line by the operator using in-built velocity trace measurement software (GE Healthcare, United Kingdom) (Figure 10). Aortic blood flow velocity was calculated as the average across 10 consecutive velocity-time integrals from the highest quality 30-second video.

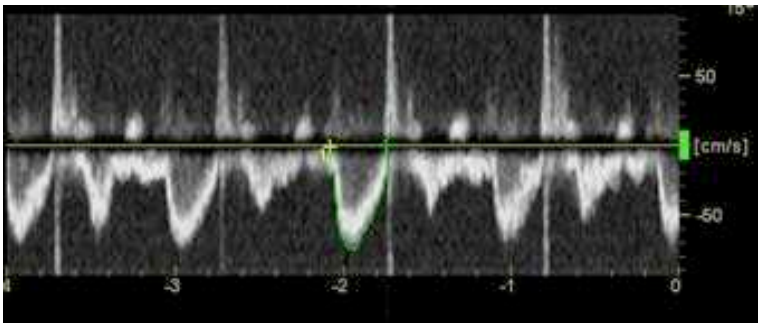


Figure 10. Velocity time integral spectrum trace (highlighted in green) of aortic blood velocity with in-built ultrasound velocity trace measurement software (GE Healthcare, United Kingdom).

Determination of stroke volume, cardiac output and total peripheral resistance

The aortic artery cross sectional area was calculated as πr^2, where *r* represents the half of the artery diameter (Lewis et al., 1984). Stroke volume was calculated as the product of aortic blood flow velocity and aortic cross-sectional area (Ihlen et al., 1987). Cardiac output was calculated as the product of SV and resting HR (Opie, 2004). Total peripheral resistance was calculated as resting MAP divided by $\dot{Q}$ (Wiles et al., 2010; Levick et al., 2010). Correlation coefficients have been reported between pulse wave Doppler ultrasound using the left ventricular outflow tract method and thermodilution techniques to measure SV (r=0.95, SEE=0.6 mL/min) and $\dot{Q}$ (r= 0.91, SEE=0.63 mL/min) (Lewis et al., 1984). The within-day reproducibility for $\dot{Q}$ using aortic methods, when expressed as coefficients of variation, range between 4% and 10%. The between-day reproducibility for $\dot{Q}$ using aortic methods when expressed as coefficients of variation range between 9% and 14% (Coats, 1990). As part of

this thesis, a reliability study will be conducted in order to able to interpret statistical significance.

3.2.8 The reliability of echocardiography measurements using the LOGIQ e ultrasound (GE Healthcare) device

Aims

The aim of this preliminary study was to assess the reliability of the observer to measure aortic artery diameter, SV and $\dot{Q}$ using the LOGIQ e ultrasound.

Methods

Participants

Eighteen healthy males volunteered to participate in this preliminary study (age: 25 ± 5 years; height: 177.5 ± 5.7 cm; weight: 80.9 ± 10.6 kg) (mean ± SD). All participants were required to adhere with the strict testing criteria as outlined in the participant information sheet (Appendix 2). Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed a Health and Fitness Questionnaire (Appendix 1) and provided written informed consent (Appendix 2), thereby adhering to the guidelines set by the Declaration of Helsinki (1964).

Testing procedures

Prior to the study commencing all participants confirmed their adherence to the testing criteria. Participants visited the laboratory for an initial visit and two subsequent trials each separated by 7 days. Participants visited the laboratory at approximately the same time of day on each visit. Following completion of this initial process, participants were asked to rest in a supine position for 20-minutes. Before the commencement of ultrasound imaging the participant was fitted with an in-built ultrasound 3-lead electrocardiogram arrangement to measure the QRS

complex. Following a 20-minute rest period in the supine position, aortic diameter and aortic blood flow velocity were measured using two-dimensional duplex ultrasound (LOGIQ e book, GE Healthcare, United Kingdom).

Upon acquisition of the desired aortic diameter and aortic blood flow velocity ultrasound images the recordings were saved under each participant's unique patient identification number on the ultrasound device. The highest quality 30-second video recording of the aortic diameter and aortic blood flow velocity were used for further analysis. Aortic artery diameter was measured using the left ventricular outflow tract method and aortic blood flow velocity was measured in the apical five chamber view (Section 3.2.7). Stroke volume and $\dot{Q}$ were calculated as outlined in the general method (Section 3.2.7). Aortic diameter was calculated as the average across three cardiac cycles at peak systole using the in-built ultrasound software. Aortic blood flow velocity was calculated as the average of 10 consecutive velocity time integrals using the in-built ultrasound software from the same highest quality 30-second video recording as the aortic artery diameter. All echocardiography measurements were repeated in the same order of testing during subsequent visits to the laboratory.

Statistical analysis

Data was analysed in an identical manner to the previous reliability study (see section 3.2.2).

Results

Based on initial data checks the data obtained for brachial artery and common femoral artery diameter, blood flow velocity and blood flow were normally distributed. The data for SV was normally distributed. The data for $\dot{Q}$ was not normally distributed. There was no significant difference between trials for the assessment of the SV (P =0.645) or $\dot{Q}$ (P =0.395). Table 6

displays the group mean coefficient of variation and intra class correlation coefficient for the echocardiographic parameters.

Table 6. Resting stroke volume and cardiac output (*n*=14) measures obtained from repeated measures.

Variable	Visit 1	Visit 2	Visit 3	CV%	ICC
Stroke volume ($ml.min^{-1}$)	89 ± 12	90 ± 12	91 ± 15	4.36 (3.43 - 5.97)	0.89
Cardiac output ($L.min^{-1}$)	5.1 ± 0.9	5.1 ± 1.0	5.3 ± 1.2	7.59 (5.98 - 10.40)	0.89

Note: Data presented as group mean CV% (lower and upper 95% confidence intervals). ICC= intra class correlation coefficient. Data presented as mean ± SD.

Discussion

The results from this study were used to assess the potential impact of the researcher in collecting this data and to estimate the sample size requirement for a crossover study conducted over an 8-week period. The results of this study demonstrate that the researcher conducting the ultrasound imaging had the ability to reliably measure $\dot{Q}$ and SV over an 8-week time period. In order to estimate sample size, change scores were incorporated using the data from research whereby cardiac parameters were utilised (Spence et al., 2013; Stebbings et al., 2013; Baross et al., 2012; Hambrecht et al., 2000). The sample size for a crossover designed study was estimated to be *n=1* participant for SV and *n=25* for $\dot{Q}$. A number of measures investigated as part of this preliminary study would require very large sample sizes, unless the changes detected by the current study are extremely large (e.g. $\dot{Q}$), however this data was still collected to potentially inform future study design.

Summary

The results of this study demonstrate that the researcher conducting the ultrasound imaging had the ability to reliably measure echocardiographic parameters over an 8-week time period. The reliability reported by this research appears to be adequate for the detection of meaningful changes in selected cardiac variables when a relatively moderate sample size of between *n=1* and *25* are utilised. The reproducibility results from this study are likely to contribute to the limited existing research and this has the potential to inform future studies of sample size estimation using the same measurement tools.

3.2.9 Isometric torque

Isometric toque was measured using a calibrated Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems Inc., Shirley, New York) (Figure 11. A). The Biodex System 3 Pro isokinetic dynamometer allowed for isometric muscular force to be applied to a movement arm using a hydraulic servo-controlled mechanism to create resistance against applied force. The isokinetic dynamometer was connected to a computer using Biodex Advantage software for Windows XP (Biodex Medical Systems Inc., Shirley, New York). This allowed the operator to program the commands relating to the desired protocol. A data link from the Biodex remote access to a 16-channel chart recorder (PowerLab, AD Instruments Ltd, Australia) enabled the synchronisation of the time component of surface electromyography and muscular force recordings during incremental isometric exercise tests and subsequent isometric exercise sessions. The Biodex System 3 Pro isokinetic dynamometer has been shown to have good intra-day and inter-day reliability for the measurement of torque (ICC=0.99) and position (ICC=0.99) throughout the complete range of motion (Drouin et al., 2004).

Participants were instructed to sit in an upright position on the dynamometer seat with 90 degrees of flexion at the hip and knee (Alkner et al., 2000) which was measured using a

calibrated goniometer (MIE Medical Research, Leeds, United Kingdom) to maintain and standardise this position. The lateral femoral condyle of the participant's right leg was aligned with the centre of rotation of the dynamometer head (Croisier et al., 2005). The backrest was adjusted to ensure the back of the knees fitted comfortably over the front edge of the seat and adequate support of the lumbar spine region. The seat position was adjusted for each individual according to their anthropometric measurements. Participants were secured into the dynamometer seat using a waist strap and thoracic straps crossed over the front of the body at mid-sternum level to prevent any extraneous movement. The dynamometer was fitted with a modified hip attachment that inserted into the standard knee attachment. The modified hip attachment allowed both legs to be secured to a single attachment. The modified hip attachment was secured 1 cm superior to the medial malleoli of the ankles. The 30 cm wide modified hip attachment strap was padded with 3 cm thick high-density foam facing the anterior portion of the shin and a 15 cm high-density foam pad facing the posterior lower leg on the dynamometer arm (Devereux et al., 2010; Wiles et al., 2010). Participants were instructed to avoid using their upper body musculature during isometric bilateral leg extension exercise in order to satisfy standardised levels of stabilisation and to avoid confounding effects on force (Magnusson et al., 1993). Isometric torque was measured during the discontinuous incremental isometric exercise test and isometric exercise sessions.

3.2.10 Electromyography

Surface electromyography was used to measure myoelectric signals formed by physiological variations in the state of the muscle fiber membranes (Basmajian and DeLuca, 1985) during the discontinuous incremental isometric exercise test and exercise sessions. Surface electromyography was recorded using 16-channel Powerlab chart recorder with Chart 7 software for Windows XP (AD Instruments Ltd, Australia). A five-lead shielded Bio Amp cable (MLA2540, AD Instruments Ltd, Australia) was used in conjunction with a dual bio-

amplifier to measure the electrical activity of the left and right vastus lateralis simultaneously. The vastus lateralis of the quadriceps muscle group was examined since it has been shown to exhibit a linear relationship between electromyography and force during isometric leg extension exercise ($r = 0.996$, $P < 0.01$) (Alkner et al., 2000). Surface electromyography of the vastus lateralis measured during maximal isometric knee extension and repeated isometric knee extension contractions at 50% MVC have demonstrated high day to day reliability (ICC>0.70) (Mathur et al., 2005; Rainoldi et al., 2001).

Two electrodes (Blue Sensor T Electrode pads, Ambu A/S, Denmark) were positioned 20mm apart (from centre to centre) on each vastus lateralis muscle approximately two-thirds on the line from the anterior spina iliaca superior to the lateral side of the patella in the direction of the muscle fibres (Figure 11. B) (Cram and Criswell, 1998). A reference electrode was also positioned on the olecranon at the proximal end of the ulna (Cram and Criswell, 1998). Electrode placements were consistent among participants using the vastus lateralis location as outlined by Surface Electromyography for the Non-Invasive Assessment of Muscles guidelines (SENIAM) (www.seniam.org). Prior to electrode application, the skin was prepared and a skin impedance check was performed during each laboratory visit which required the measurement of electromyography. Each participant was assigned their own disposable razor and the electrode placement area was dry shaven and prepared using 2 to 3 gentle strokes with ultra-fine abrasive paper. The area was cleaned using a steret isopropyl alcoholic skin wipe (Medlock Medical Ltd., United Kingdom) and allowed time to dry before the electrodes were positioned. Skin impedance was measured using an impendence testing unit (UFI Checktrode Model 1089 mk III, Morro Bay, California) following each electrode application with impedance values below 10 kΩ were classified as acceptable (Hermens et al., 2000).

The root mean square of the raw electromyography signal was computed using the Chart 7 and smoothed using a window of 1.5ms (Chart 7 for Windows XP, AD Instruments Ltd, Australia). The electromyography signal was sampled at 2000 Hz with the bioamp set to apply a 10Hz high pass filter, 500Hz low pass filter and a 50Hz notch filter (Wiles et al., 2010). The root mean square electromyography from each leg was summed and the total was averaged over 1 second to provide a single root mean square electromyography value (Wiles et al., 2010). This value was deemed to represent the total effort required to maintain a given force per unit time. This single electromyography value was displayed in real time as millivolts x 10^6 on a computer monitor to participants during the discontinuous incremental isometric exercise test and isometric exercise sessions to provide a participant specific 'target' exercise intensity that equated to a predetermined percentage of peak electromyography.

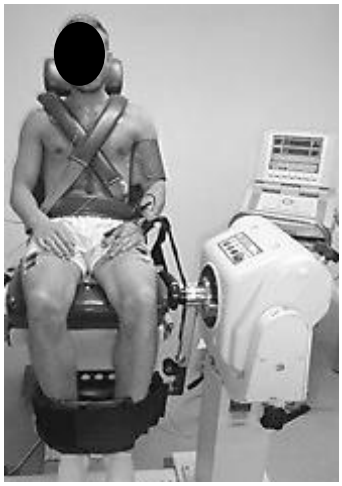
A.  **B.** 

Figure 11. A. The standardised dynamometry arrangement for all exercise tests and exercise training sessions. **B.** The standardised electromyography electrode placement on the vastus lateralis muscle.

3.2.11 Maximal voluntary contraction and discontinuous incremental isometric exercise test protocol

An individual's maximum voluntary contraction was determined before the discontinuous incremental isometric exercise test using a calibrated strain gauge (Advanced Forced Gauge 2500N, Mecmesin, West Sussex, United Kingdom). Participants were appropriately fitted into the seat of the isokinetic dynamometer in an upright position, with 90 degrees of flexion at the hip and with the thighs supported. Bilateral isometric leg extension was performed at a knee angle of 90 degrees (Alkner et al., 2000) which was measured and set at the start of each test using a calibrated goniometer (MIE Medical Research, Leeds, United Kingdom). A standardised warm up consisting of steady state cycling for 5-minutes and a series of predominantly lower body stretching exercises were completed prior to the start of exercise testing (Appendix 5). The primary aim of the warm up was to prepare the leg musculature and knee joints for maximum exertion during the maximum voluntary contraction test. Three maximum voluntary contractions were performed each terminated after 2 to 3 seconds, and each was separated by a 2-minute rest period (Wiles et al., 2007). The strain gauge device consisted of a digital unit connected to a modified leg attachment, which was padded with 3cm thick high-density foam. The strain gauge device was attached to the base of the isokinetic dynamometer with the modified leg attachment positioned on the anterior portion of the lower leg and 1cm superior to the medial malleoli of the ankles. Peak electromyography (EMG_{peak}) was determined from the maximum voluntary contraction attempt which produced the highest torque. Peak electromyography was established as the mean of the electromyography activity recorded 0.25 seconds immediately prior to the highest electromyography value recorded, as used by Wiles et al. (2010).

The peak electromyography value was used to calculate percentage 'targets' of peak electromyography for increments during the discontinuous incremental isometric exercise test.

This incremental test was performed using the isokinetic dynamometer (Baross et al., 2012; Devereux et al., 2010; Wiles et al., 2010). The discontinuous incremental isometric exercise test has demonstrated a linear relationship between heart rate and electromyography ($r = 0.93$ to 1.00, $P < 0.05$ in all cases) (Devereux et al., 2010). Isometric exercise intensity has since been prescribed using an individual target electromyography value that equates to a given percentage of each participant's peak HR (Baross et al., 2012; Wiles et al., 2010; Devereux et al., 2010). Participants began bilateral-leg isometric exercise during the incremental test at 10% EMG_{peak} for a period of 2 minutes. Thereafter, the intensity increased in 5% increments interspersed by 5-minute rest periods, up to volitional fatigue or failure to maintain electromyography signal within +/- 5% of the electromyography 'target' value. The final 60-seconds of each increment were used for further analysis. The reliability of the incremental isometric exercise test has been reported with a coefficient of variation of 17.5%, 5.2% and 5.3% for EMG_{peak}, HR and SBP respectively (Wiles et al., 2007). Electromyography, torque and heart rate were continuously recorded throughout the maximal voluntary contraction test and discontinuous incremental isometric exercise test.

CHAPTER 4: ACUTE ISOMETRIC EXERCISE STUDY

4. The effect of acute isometric bilateral leg extension exercise at high and low intensities on flow-mediated vasodilatation with specific reference to isometric exercise-induced haemodynamic parameters

This work was presented at the European College of Sports Science Annual Conference, 26th-29th June 2013, Barcelona.

4.1 Introduction

Adaptations within the peripheral vasculature following exercise training may account, at least in part, for the incompletely explained reduction in traditional cardiovascular risk factors following exercise training (Green et al., 2011c; Mora et al., 2007). The maintenance of the overall health of the vasculature is imperative for maintaining optimal blood pressure. Maintaining optimal vascular health appears increasingly important given that endothelial dysfunction has been associated with increased risk of developing hypertension (Dharmashankar and Widlansky, 2010; Corrado et al., 2005; Panza et al., 1990).

There is evidence to suggest that the blood pressure lowering effects of exercise training are attributable to changes in the function and structure of the peripheral vasculature (Green et al., 2009; Joyner and Green, 2009; Laughlin and McAllister, 1992). Furthermore, previous research has highlighted that exercise training intensity influences the magnitude of endothelium-dependant dilatation due to the increased production of endothelial nitric oxide and decreased levels of oxidative stress (Goto et al., 2003). In order to further understanding of the peripheral vascular mechanisms that may elicit improvements RBP following isometric exercise training, it is important to explore the acute impact of acute impact of isometric exercise on endothelium-dependent flow-mediated vasodilatation as this may inform the adaptations that occur following repeated bouts.

It has been theorised that exercise-induced changes in blood flow and shear rate patterns within the exercising limb are likely exert a significant influence over subsequent exercise training-induced vascular adaptations (Green et al., 2011a; 2011c). There is conflicting evidence surrounding the impact different blood flow and shear rate patterns exert immediately upon the vascular endothelium. What is more, the long-term influence of antegrade and retrograde blood flow and shear rate patterns upon vascular adaptations in humans are unknown. Recent evidence has proposed that a 'critical threshold' of antegrade shear rate exists during an acute exercise bout which is necessary to enhance vascular endothelial function (Atkinson et al., 2015). In contrast, elevations in retrograde shear rate during an acute exercise bout have been reported to have a potentially negative impact on vascular endothelial function (Thijssen et al., 2009b). Isometric muscular contractions elicit substantial increases in intramuscular pressure, blood flow occlusion, metabolite accumulation and impaired delivery of oxygen to the exercising muscle (Sejersted et al., 1984; Barnes, 1980) which are unique and challenging physiological stimuli. Therefore, the changes in blood flow and shear rate caused by isometric muscular contractions may prove a potent modulator for adaptations in vascular endothelial function. To date, the patterns of blood flow and shear rate within the exercising limbs during acute isometric exercise remains largely unknown.

The main aims of this study were to investigate the effect of an acute bout of isometric bilateral leg extension exercise at high and low intensities on brachial artery FMD immediately and at 30-minutes post exercise, and to establish the blood flow and shear rate patterns within the exercising limb during an acute bout of isometric bilateral leg extension exercise. It was hypothesised that (i) peripheral vascular endothelial function will be reduced immediately post high intensity isometric exercise and will remain unchanged immediately following low intensity isometric exercise, and (ii) high intensity isometric exercise results

in a more pronounced post exercise hyperaemic response upon cessation of muscular contraction compared to low intensity isometric exercise**.**

4.2 Methods

Participants

Eleven healthy normotensive males (mean age: 22 ± 3 years; body mass: 78.2 ± 9.2 kg; height: 178.0 ± 6.5 cm; hours spent exercising per week: 6.0 ± 1.5 hours) volunteered to participate in a repeated measures design study. Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed a Health and Fitness Questionnaire (Appendices 1 and 3), and provided written informed consent, thereby adhering to the guidelines set by the Declaration of Helsinki (1964).

Study design

All participants adhered to the testing criteria as outlined in the general methods (see Section 3.1). This study required the participant to visit the laboratory on eight separate occasions. The first visit to the laboratory acted as a familiarisation for the maximal voluntary contraction test and discontinuous incremental isometric exercise test (see Section 3.2.11). Participants returned to the laboratory between 2 and 7 days later to repeat the maximal voluntary contraction test and discontinuous incremental isometric exercise test. Participants returned for a further 6 visits to complete the exercise sessions. The exercises session consisted of isometric bilateral leg extension exercise whereby four contractions were held for 2-minutes each separated by 3-minute rest periods (Figure 12). The isometric exercise sessions were performed in a random order each separated by at least 48 hours to avoid potential confounding effects of serial measurements (Padilla et al., 2007). Two isometric exercise sessions were performed at high intensity, two isometric exercise sessions

performed at low intensity and two non-exercising control sessions. Brachial artery FMD was assessed prior to, immediately post and 30-minutes post cessation of the exercise session. Artery diameter and blood flow velocity were measured continuously in the common femoral artery of the participant's right leg throughout the exercise session using Doppler Ultrasound. Blood pressure, heart rate, torque and electromyography were measured continuously throughout the exercise session.

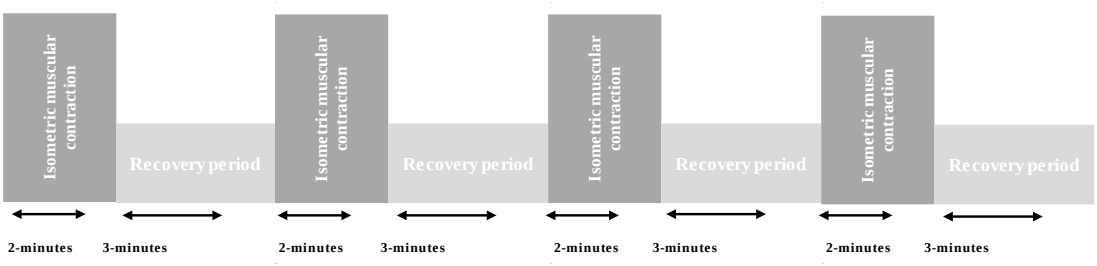


Figure 12. A schematic representation of the isometric exercise session protocol

4.3 Experimental procedures

4.3.1 Maximal voluntary contraction test

Three maximum voluntary contractions were performed each terminated after 2 to 3 seconds, and each separated by a 2-minute rest period. Peak electromyography (EMG_{peak}) was determined during the maximal voluntary contraction test attempt which produced the highest overall mean torque. Peak electromyography (EMG_{peak}) was established as the mean electromyography activity recorded in the 0.25 seconds immediately prior to the highest identified electromyography value (Wiles et al., 2010). The EMG_{peak} was used to calculate percentage electromyography 'targets' for the subsequent discontinuous incremental isometric exercise test which is described in detail in the general methods (Section 3.2.11).

4.3.2 Discontinuous incremental isometric exercise test

Following completion of the maximal voluntary contraction test, participants rested for 5-minutes in a seated position and then performed a discontinuous incremental isometric exercise test. Two-minute isometric bilateral leg extension muscular contractions were performed at a constant level of electromyography. The discontinuous incremental isometric exercise test started at a level of 10% EMG_{peak} and the intensity increased in 5% increments thereafter. During the discontinuous incremental isometric exercise test each 2-minute muscular contraction increment was interspersed by 5-minute rest period; performed up to volitional fatigue or failure to maintain electromyography signal within +/- 5% of the electromyography 'target' value.

4.3.3 Isometric exercise

All isometric exercise sessions and exercise tests were conducted using a calibrated Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems Inc., Shirley, New York). Participants sat in the dynamometer in an upright position, with 90 degrees of flexion at the hip and knee with the thighs supported (Section 3.2.9). The Biodex System 3 Pro isokinetic dynamometer was used to synchronize muscular force recordings the time component of surface electromyography, which is described in detail in the general methods (Section 3.2.9 and 3.2.10).

4.3.4 Exercise intensity

Participants performed high intensity exercise at a participant-specific torque value derived by using the methods of Devereux et al. (2010). This equated to 105.4% of the average torque achieved during the last 60-seconds of the final increment of the discontinuous incremental isometric exercise test (105.4% 2-min $torque_{peak}$). Participants performed low intensity isometric exercise at a participant-specific torque value which equated to 75% of

the average heart rate interpolated from the regression line of heart rate versus torque ascertained during the last 60-seconds of the final increment of the discontinuous incremental isometric exercise test (75% HR_{peak}). Further details of the discontinuous incremental exercise test have been reported in previous research (Devereux et al., 2012; Wiles et al., 2010). Two high intensity sessions required the participant to exercise at 105.4% 2-min $torque_{peak}$ and two low intensity sessions required the participant to exercise at a torque value that equated to 75% HR_{peak}. During the exercise session, participants performed four isometric bilateral leg extension muscular contractions each lasting for 2-minutes in duration and each separated by 3-minute rest periods (see Figure 12). Exercise sessions were separated by a minimum of 48 hours but no longer than 7 days.

4.3.5 Exercising electromyography

Surface electromyography was recorded throughout the discontinuous incremental isometric exercise test and exercise sessions using a five-lead shielded Bio Amp cable (MLA2540, AD Instruments Ltd, Australia), in conjunction with a dual bio-amplifier to measure the electrical activity from both vastus lateralis' simultaneously and interfaced with the 16-channel Powerlab chart recorder with Chart 7 software for Windows XP (AD Instruments Ltd, Australia) which is described in detail in the general methods (Section 3.2.10).

4.3.6 Exercising blood pressure and heart rate

Blood pressure was measured continuously throughout the exercise sessions using a non-invasive continuous finger blood pressure measurement device (Finometer® Pro, FMS, Finapres Medical Systems BV, Amsterdam, The Netherlands), interfaced with the 16-channel chart recorder (Powerlab/SP16 with Chart 7 Software, AD Instruments Ltd, Australia) which is described in detail in the general methods (Section 3.2.1). Heart rate was continuously recorded using a standard three lead bipolar electrocardiogram arrangement,

as recommended by AD Instruments (NSW, Australia) interfaced with the 16-channel chart recorder (PowerLab/SP16 with Chart 7 Software, AD Instruments Ltd, Australia) which is described in detail in the general methods (Section 3.2.3).

4.3.7 Exercising blood flow and shear rate

Common femoral artery diameter and blood flow velocity were measured continuously throughout each exercise session. Measurements were performed during each isometric muscular contraction (the average of four 2-minute contractions) during the recovery period in between each contraction (the average of three 3-minute recovery periods in between contractions) and at baseline (the average of a 30-second recording under rested conditions) prior to the start of the exercise session. Common femoral artery diameter and blood flow velocity were measured continuously using two-dimensional Duplex Doppler ultrasound (LOGIQ e book, GE Healthcare, United Kingdom). Common femoral artery diameter was analysed using automated edge-detection and wall tracking software (Woodman *et al.*, 2001) and blood flow velocity was analysed using in-built ultrasound trace software (LOGIQ e, GE Healthcare, United Kingdom) (Section 3.2.4). Common femoral artery antegrade and retrograde blood flow, antegrade and retrograde shear rate and oscillatory shear index were calculated in accordance with the general methods outlined (Section 3.2.4).

4.3.8 Brachial artery flow-mediated vasodilatation

Brachial artery FMD was assessed at baseline following a 20-minute rest period using in accordance with recently published guidelines (Thijssen et al., 2011d). A rapid inflation/deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned around the forearm distal to the olecranon process and inflated to suprasystolic pressure (200 mmHg) for 5-minutes. Baseline and peak artery diameter were measured during the FMD assessment and analysed using automated edge-detection and wall tracking software

(Woodman et al., 2001). Blood flow velocity was analysed using in-built ultrasound trace software (GE Healthcare, United Kingdom). Brachial artery FMD was adjusted for baseline diameter and back transformed to represent an adjusted percentage FMD as outlined in the general methods (Section 3.2.6).

4.4 Statistical analysis

Data was assessed for conformity with parametric assumptions (Field, 2000). Where data was not normally distributed, data transformation was performed where appropriate. An alpha level of $P \leq 0.05$ was set for statistical significance. The change in brachial artery FMD pre to post exercise was assessed using General Estimating Equations (GEE) using the methods outlined by Atkinson and Batterham (2013a). Condition (high exercise intensity, low exercise intensity and non-exercising control) was entered as the independent variable and logarithmically transformed baseline artery diameter was entered as a covariate. Data was back transformed in order to calculate covariate adjusted percentage brachial artery FMD. General estimating equation analysis was used to assess for change in exercise-induced haemodynamic variables: common femoral artery diameter, antegrade and retrograde blood flow velocity, antegrade and retrograde blood flow, antegrade and retrograde shear rate and oscillatory shear index. Condition (high and low exercise intensity) and time (contraction, recovery period and baseline) were entered as independent variables. General estimating equation analysis was used to assess for change in exercising cardiovascular and neuromuscular parameters including blood pressure, heart rate, torque and electromyography. A paired samples t-test was used to assess for differences in mean arterial blood pressure and heart rate which was measured prior to and immediately upon completion of the FMD assessment; where data was deemed non-parametric an equivalent Wilcoxon test was used. Repeated measures correlation analysis was performed to assess for linear dependence between changes in brachial artery FMD and exercise-induced

haemodynamic and exercising cardiovascular parameters. Data is presented as mean ± SE for brachial artery FMD, common femoral artery diameter, antegrade and retrograde blood flow velocity, blood flow, shear rate and oscillatory shear index. Data is presented as mean ± SD for all other parameters unless otherwise stated.

4.5 Results

4.5.1 Brachial artery FMD assessment post exercise

There was a significant condition effect for brachial artery FMD from pre to immediately post exercise (P =0.002). Change in brachial artery FMD from pre to immediately post exercise was significantly lower in the high exercise intensity condition compared to the non-exercising control condition (mean difference: -5.78 ± 2.70 %, P <0.001) (Figure 13). Change in brachial artery FMD from pre to immediately post exercise was significantly lower in the high exercise intensity condition compared to the low exercise intensity condition (mean difference: -5.08 ± 1.70 %, P =0.050) (Figure 13).

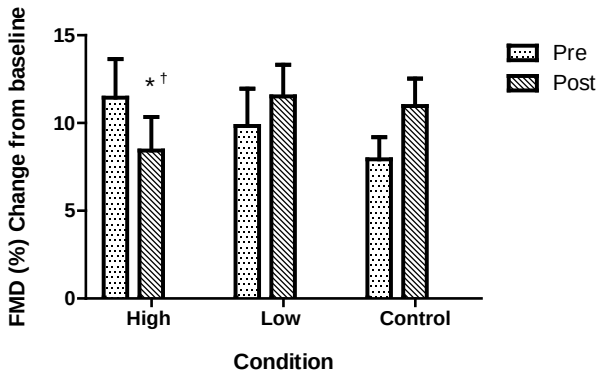


Figure 13. Brachial artery flow-mediated vasodilatation (FMD %) measured at pre and immediately post isometric bilateral leg extension exercise and non-exercising control conditions. *Note:* * indicates statistically significant compared to low exercise intensity. † indicates statistically significant compared to control condition.

Change in brachial artery FMD from pre to immediately post exercise was not different between low exercise intensity condition and non-exercising control condition (mean

difference: -0.74 ± 2.45 %, *P* =0.761). There was no significant condition effect for brachial artery FMD from pre to 30-minutes post exercise (*P* =0.966) (Figure 14). Tables 7 and 8 show brachial artery characteristics at pre and post high exercise intensity, low exercise intensity and non-exercising control condition.

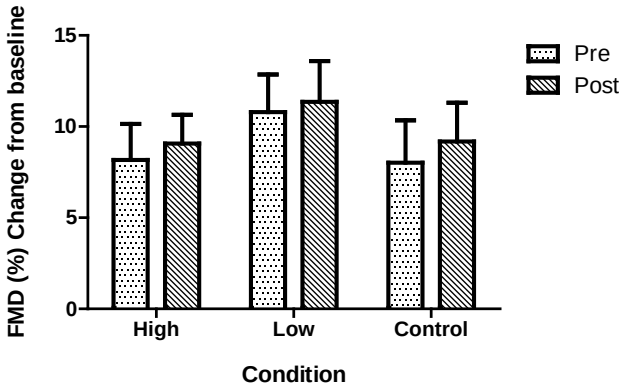


Figure 14. Brachial artery flow-mediated vasodilatation (FMD %) measured at pre and 30-minutes post isometric bilateral leg extension exercise and non-exercising control conditions. *Note:* * indicates statistically significant compared to low exercise intensity. † indicates statistically significant compared to control condition.

Table 7. Brachial artery characteristics measured at pre and immediately post high intensity exercise, low intensity isometric bilateral leg extension exercise and non-exercising control conditions.

	Condition						
	High exercise intensity		**Low exercise intensity**		**Control**		**P value**
Variable	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	
Adjusted FMD (%)	11.44 ± 2.21	8.43 ± 1.91*†	9.83 ± 2.13	11.52 ± 1.80	7.93 ± 1.26	10.97 ± 1.56	0.002
Unadjusted FMD (%)	11.55 ± 6.52	7.82 ± 3.90	10.11 ± 6.44	12.17 ± 6.12	8.28 ± 6.19	11.16 ± 6.36	
Baseline diameter (mm)	4.00 ± 0.28	4.09 ± 0.26	3.99 ± 0.33	3.94 ± 0.39	3.98 ± 0.40	3.99 ± 0.27	
Peak diameter (mm)	4.46 ± 0.38	4.41 ± 0.39	4.38 ± 0.38	4.41 ± 0.40	4.29 ± 0.33	4.42 ± 0.27	
SR AUC (S^{-1} x 10^3)	3.91 ± 1.94	5.13 ± 2.01	3.55 ± 1.52	3.24 ± 1.61	4.03 ± 2.01	3.90 ± 1.96	

Note: FMD = Flow-mediated vasodilatation * indicates statistically significant compared to low exercise intensity. † indicates statistically significant compared to control condition Adjusted FMD (%) presented as mean ± SE, all other data presented as mean ± SD.

Table 8. Brachial artery characteristics measured at pre and 30-minutes post high intensity exercise, low intensity isometric bilateral leg extension exercise and non-exercising control conditions.

	Condition						
	High exercise intensity		**Low exercise intensity**		**Control**		**P value**
Variable	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	
Adjusted FMD (%)	8.17 ± 1.97	9.07 ± 1.57	10.79 ± 2.07	11.36 ± 2.23	8.02 ± 2.32	9.18 ± 2.13	0.966
Unadjusted FMD (%)	8.14 ± 4.63	8.80 ± 4.31	11.68 ± 5.61	11.71 ± 10.82	8.33 ± 6.22	9.11 ± 6.28	
Baseline diameter (mm)	4.04 ± 0.28	4.08 ± 0.28	3.92 ± 0.40	4.04 ± 0.30	4.01 ± 0.32	4.08 ± 0.45	
Peak diameter (mm)	4.38 ± 0.40	4.43 ± 0.30	4.36 ± 0.41	4.50 ± 0.35	4.34 ± 0.42	4.45 ± 0.52	
SR AUC (S^{-1} x 10^3)	3.96 ± 2.08	4.62 ± 2.13	3.17 ± 1.20	3.16 ± 1.86	2.80 ± 1.02	3.63 ± 2.03	

Note: FMD = Flow-mediated vasodilatation * indicates statistically significant compared to low exercise intensity. † indicates statistically significant compared to control condition. Adjusted FMD (%) presented as mean ± SE, all other data presented as mean ± SD.

4.5.2 Exercise-induced haemodynamic variables specific to the exercising limb

Table 9 displays the summary data for the exercise-induced haemodynamic variables.

Common femoral artery diameter

There was no significant condition by time interaction observed for artery diameter (P =0.081). There was no significant difference in artery diameter at baseline or during isometric muscular contractions between exercise intensities (P =0.458 and P =0.649). There was a trend for lower artery diameter during muscular contractions in the high exercise intensity condition (P =0.087). This was also the case for artery diameter which was elevated during the recovery period in the high exercise intensity condition compared to low exercise intensity condition (P =0.055).

Common femoral artery antegrade and retrograde blood flow velocity

There was a significant condition by time interaction observed for antegrade blood flow velocity (P <0.001). Antegrade blood flow velocity during contractions and the recovery period was elevated compared to baseline for both high and low exercise intensities (all P <0.001). There was no significant difference in antegrade blood flow velocity at baseline or during isometric muscular contractions between intensities (P =0.143). Antegrade blood flow velocity was greater during the recovery period in the high compared to low exercise intensity condition (P <0.001).

There was a significant condition by time interaction observed for retrograde blood flow velocity (P <0.001). Retrograde blood flow velocity was significantly elevated during high intensity contractions and lower during the recovery period compared to preceding baseline (P =0.001 and P <0.001). Retrograde blood flow velocity was not significantly altered during low intensity contractions or the recovery period compared to baseline (P =0.171 and

P =0.282 respectively). Retrograde blood flow velocity was greater during high compared to the low intensity contractions (*P* <0.001). Retrograde blood flow velocity was lower during the recovery period in the high compared to low exercise intensity condition (*P* =0.002).

Common femoral artery antegrade and retrograde blood flow

There was a significant condition by time interaction observed for antegrade blood flow (*P* <0.001). Antegrade blood flow during contractions and recovery period was elevated above baseline for both high and low exercise intensities (*P* <0.001 for all cases). There was no significant difference in antegrade blood flow during contractions between intensities (*P* =0.345). Antegrade blood flow was greater during the recovery period in the high compared to the low exercise intensity (*P* <0.001).

There was a significant condition by time interaction observed for retrograde blood flow (*P* <0.001). Retrograde blood flow was elevated during high intensity contractions and lower during the recovery period compared to baseline (*P* =0.001 and *P* <0.001 respectively). Retrograde blood flow was significantly elevated during low intensity contractions and unaltered during the recovery period compared to baseline (*P* =0.031 and *P* =0.896 respectively). Retrograde blood flow was greater during contractions (*P* =0.002) and lower during the recovery period for high compared to the low exercise intensity condition (*P* =0.002).

Common femoral artery antegrade and retrograde shear rate

There was a significant condition by time interaction observed for antegrade shear rate (*P* <0.001). Antegrade shear rate was elevated during contractions and the recovery period compared to baseline for both conditions (*P* <0.001 for all cases). There was no significant

difference in antegrade shear rate during contractions between exercise intensities (P=0.122). Antegrade shear rate was greater during the recovery period in the high compared to low exercise intensity condition (P <0.001).

There was a significant condition by time interaction observed for retrograde shear rate (P <0.001). Retrograde shear rate was elevated during high intensity contractions and lower during the recovery period compared to baseline (P =0.001 and P <0.001 respectively). Retrograde shear rate during low intensity contractions and the recovery period was not significantly different compared to baseline (P =0.295 and P =0.143 respectively). Retrograde shear rate was greater during high compared to the low intensity contractions (P =0.001). Retrograde shear rate was lower during the recovery period in the high compared to the low exercise intensity condition (P =0.002).

Common femoral artery oscillatory shear index

There was a significant condition by time interaction observed for the oscillatory shear index (P <0.001). The oscillatory shear index was lower during contractions and the recovery period compared to baseline values for both high and low intensities (P <0.001 for all cases). The oscillatory shear index was greater during contractions in the high compared to low exercise intensity condition (P=0.009). The oscillatory shear index was lower during the recovery period in the high compared to the low exercises intensity condition (P <0.001).

Table 9. Mean common femoral artery diameter, blood flow velocity, blood flow and shear rate patterns at baseline, during bilateral isometric leg extension exercise muscular contractions and recovery periods in between contractions at high and low exercise intensities.

	Condition					
	High exercise intensity			**Low exercise intensity**		
Variable	*Baseline*	*Contraction*	*Recovery*	*Baseline*	*Contraction*	*Recovery*
Diameter (cm)	0.96 ± 0.02	0.97 ± 0.02	0.95 ± 0.02	0.97 ± 0.03	0.98 ± 0.03	0.98 ± 0.02
Antegrade BFV ($cm.s^{-1}$)	6.6 ± 0.4	9.5 ± 0.6*	12.9 ± 0.5*†	6.5 ± 0.4	8.9 ± 0.5*	9.0 ± 0.7*
Retrograde BFV ($cm.s^{-1}$)	-3.7 ± 0.2	-4.4 ± 0.2*	-2.1 ± 0.4*†	-3.4 ± 0.1	-3.7 ± 0.3	-3.2 ± 0.2
Antegrade BF ($ml.min^{-1}$)	284 ± 19	427 ± 33*	549 ± 21*†	286 ± 18	404 ± 28*	404 ± 30*
Retrograde BF ($ml.min^{-1}$)	-162 ± 11	-197 ± 14	-96 ± 19*†	-148 ± 7	-170 ± 16*	-146 ± 13
Antegrade SR (s^{-1})	28 ± 2	39 ± 2*	55 ± 3*†	28 ± 2	37 ± 2*	37 ± 3*
Retrograde SR (s^{-1})	-16 ± 1	-18 ± 1*†	- 9 ± 2*†	-14 ± 1	-15 ± 1	-13 ± 1
OSI (au)	0.36 ± 0.01	0.32 ± 0.02*	0.14 ± 0.03*†	0.34 ± 0.01	0.30 ± 0.02*	0.27 ± 0.02*

Note: * indicates statistically significant compared to baseline. † indicates statistically significant compared to same time point between exercise intensities. BFV= blood flow velocity, BF= blood flow, SR= shear rate OSI=oscillatory shear index. Data presented as mean ± SE.

4.5.3 Exercising blood pressure, torque, electromyography and heart rate

The group mean 'target' torque for the high and low exercise intensity conditions was 135 ± 39 Nm and 63 ± 17 Nm respectively. The group mean exercising torque achieved from the vastus lateralis of both legs during the high and low intensity isometric exercise sessions was 129 ± 35 Nm and 62 ± 15 Nm respectively. The group mean exercising electromyography from the vastus lateralis of both legs during the high and low intensity isometric exercise sessions was 122 ± 48 mV and 60 ± 24 mV respectively. The group mean exercising blood pressure and heart rate during the high and low intensity exercise sessions are presented in Table 10. There was no significant difference between MAP measured following the pre exercise FMD assessment (85 ± 5 mmHg) and prior to the post exercise FMD (87 ± 5 mmHg) (P <0.001). There was no significant difference between heart rate measured following the pre exercise FMD assessment (64 ± 9 bpm) and prior to the post exercise FMD assessment (63 ± 11 bpm) (P =0.544).

Table 10. Blood pressure and heart rate during high and low intensity isometric exercise compared to baseline conditions.

	High exercise intensity		**Low exercise intensity**	
Variable	Baseline	Exercising	Baseline	Exercising
SBP (mmHg)	119 ± 6	163 ± 11	120 ± 7	144 ± 15
DBP (mmHg)	64 ± 5	101 ± 12	63 ± 5	90 ± 7
MAP (mmHg)	85 ± 4	107 ± 14	85 ± 5	108 ± 9
HR (bpm)	63 ± 9	121 ± 14	64 ± 10	83 ± 11

Note: Data presented as mean ± SE.

4.5.4 Associations between vascular endothelial function and exercising haemodynamic variables

There was a significant negative correlation of moderate to high strength between change in brachial artery FMD immediately post exercise and exercising DBP (P =0.018, r= -0.67), (Figure 15.A). A significant negative correlation of moderate strength was observed between change in brachial artery FMD immediately post exercise and exercising SBP (P =0.045, r= -0.59). The results also show a significant negative correlation of moderate strength between change in brachial artery FMD immediately post exercise and exercising MAP (P =0.031, r= -0.62) (Figure 15.B).

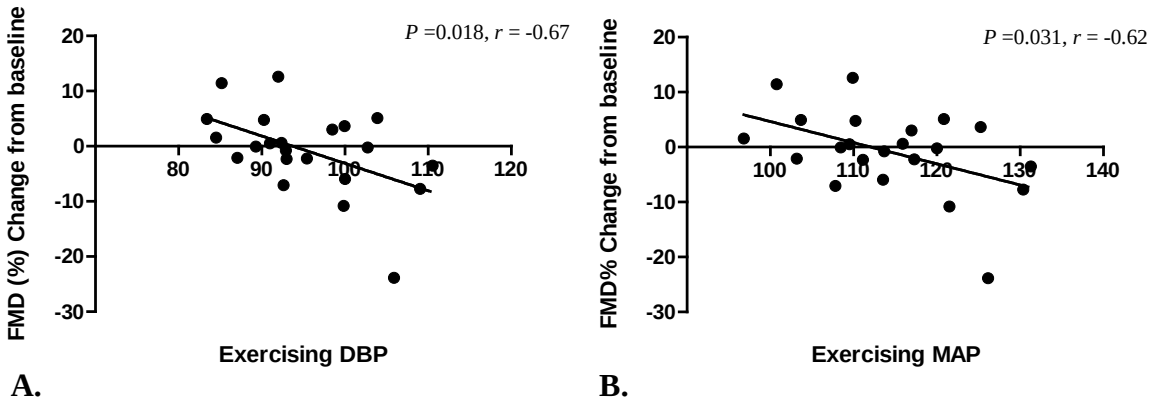


Figure 15. A. The relationship between change (pre to post exercise) in brachial artery vascular endothelial function and exercising diastolic blood pressure and **B.** brachial artery vascular endothelial function and exercising mean arterial blood pressure.

4.6 Discussion

4.6.1 The effect of acute isometric exercise on vascular endothelial function

The results from the present study confirm the existence of an intensity-dependent decrease in brachial artery FMD when assessed immediately post high intensity isometric bilateral leg extension exercise (FMD pre to post: 11.44 to 8.43%) (See Figure 13). The immediate reduction in brachial artery FMD observed following high intensity isometric exercise was

comparable in magnitude to the reduction in brachial artery FMD reported following high intensity cycling exercise (FMD pre to post: 6.6% to 3.6%) (Birk et al., 2013). Indeed, numerous studies have reported an immediate reduction in brachial artery FMD following an acute bout of high intensity or maximal effort resistance (Franklin et al., 2014; Choi et al., 2014; Gonzales et al., 2010; McGowan et al., 2006; Jurva et al., 2006) and aerobic exercise (McClean et al., 2015; Birk et al., 2013; Llewellyn et al., 2012; Gori et al., 2010; Rognmo et al., 2008). High intensity isometric bilateral leg extension exercise was the only condition to elicit a significant transient reduction in brachial artery FMD, which returned near to baseline levels when measured again at the 30-minute time point. Brachial artery FMD was unchanged immediately following low intensity isometric bilateral leg extension exercise within the present study (FMD pre to post: 9.83 to 11.52%) (See Figure 13). This finding of unchanged brachial artery FMD immediately post low intensity isometric exercise is in agreement with those of Birk et al. (2013) who reported unchanged brachial artery FMD immediately following low intensity cycling exercise (FMD pre to post: 6.3% to 5.9%). It is evident from the results of the present study that isometric exercise intensity performed at 105.4% 2-min $torque_{peak}$ was of a sufficient threshold to elicit a significant change in FMD, whereas this was not the case for the low intensity condition performed at 75%HR_{peak}. The findings from the present study indicate that exercise intensity is likely to play an important role in modulating vascular adaptations to exercise, with higher intensity isometric exercise associated with a transiently larger decrease in brachial artery FMD.

The results from the present study demonstrate brachial artery FMD was unchanged compared to pre-exercising values at 30-minutes post high (FMD pre to post: 8.17 to 9.07%), low intensity bilateral isometric leg extension exercise (FMD pre to post: 10.79 to 11.36%) and following non-exercising control condition (FMD pre to post: 8.02 to 9.18%) (See Figure 14). These findings are in alignment with the results presented by Birk et al. (2013) who reported

that brachial artery FMD had returned to near pre exercise levels at 60-minutes post cycling exercise under low (FMD pre to post; 50%HR_{Max}: 6.4% to 6.9%), moderate (70%HR_{Max}: 6.7% to 5.9%) and high exercise intensity conditions (85%HR_{Max}: 6.1% to 6.8%). These findings tend to align with those of Johnson et al. (2012b) who reported an exercise intensity-dependent decrease in brachial artery FMD immediately post high intensity exercise only, which returned to baseline levels when measured again at 60-minutes post exercise. Interestingly, the results from this study showed that brachial artery FMD returned towards pre-exercise levels at a faster rate than reported in previous studies where typically repeat measurements of FMD have been performed at 1-hour post exercise.

Atkinson et al. (2015) reported a significant increase in brachial artery FMD at 60-minutes following high intensity isotonic handgrip exercise only (pre to 60-minutes post: 2.5% to 4.4%) and unaltered brachial artery FMD immediately post isotonic handgrip exercise. The findings from the study by Atkinson et al. (2015) contradict the traditional biphasic response hypothesis (Section 2.4.1 and 2.4.2) and are in contrast to the pattern of change in brachial artery FMD reported immediately and 30-minutes post isometric exercise in the present study. Atkinson et al. (2015) have stated that small changes in exercise-induced retrograde shear rate may explain the observed immediate improvement in brachial artery FMD at the higher exercise intensity. The results from the present study tend to support this proposal, since the amount of retrograde shear rate was greater during lower limb isometric muscular contractions versus isotonic handgrip contractions (Atkinson et al. 2015). Therefore, the discrepancies within existing research regarding vascular endothelial function may well be attributed to distinct differences in the shear rate stimulus afforded by the exercise protocol. It is not possible to definitively conclude whether a biphasic response in vascular endothelial function exists following an acute bout of isometric bilateral leg extension exercise, since vascular function was not assessed at numerous consecutive time points beyond 30-minutes. Although speculative, the biphasic

response may exist since an initial decrease in brachial artery FMD was observed immediately following high intensity isometric exercise, which returned near to baseline levels within 30-minutes post exercise.

Previous research has attributed the immediate attenuation in brachial artery FMD in young healthy individuals to heightened oxidative stress (Llewellyn et al., 2012; Silvestro et al., 2002). Normal regulatory vasodilator pathways dependent on the synthesis of nitric oxide via the availability of L-arginine at the level of the vascular endothelium are limited (Jin and Loscalzo, 2010), and therefore increased levels of oxidative stress are likely to further reduce nitric oxide bioavailability and production. Whilst the present study did not assess indices of oxidative stress, previous studies have demonstrated that high intensity exercise is associated with increased production and circulation of reactive oxidant species (Powers and Jackson, 2008; Peters et al., 2006; Cooper et al., 2002). Goto et al. (2003) reported that in trained individuals high intensity exercise increases indices of oxidative stress, although endothelial function remained unchanged. Goto et al. (2003) also reported that in trained individuals moderate intensity exercise significantly improved endothelial function and a trend for increased oxidative stress was present. This clearly demonstrates the role of oxidative stress in modulating the acute vascular function response to exercise which is dependent on exercise intensity. In the present study only high intensity exercise caused a significant change in vascular endothelial function post exercise, and therefore this intensity warrants further investigation in future training studies examining vascular adaptations.

The influence of shear rate and baseline artery diameter during the FMD assessment itself and following an acute bout of exercise have been implicated as a potential mechanism that may explain the FMD response to an acute bout of exercise. Baseline artery diameter was elevated immediately post exercise in the present study, with the largest increase in baseline diameter

occurring post high intensity isometric exercise (See Table 7). The concomitant elevation in baseline diameter and decrease in peak diameter occurred only in the high intensity exercise condition, which consequently resulted the largest decrease in brachial artery FMD. In the current study, the slope of the relationship between logarithmically transformed baseline and peak diameters were different to 1 and thus the need to control for baseline diameter using an appropriate allometric model was required. A statistical analysis approach was utilised in the present study which controlled for potential changes in baseline artery diameter (Atkinson et al., 2015). Following covariate control for baseline diameter an exercise intensity dependant decrease in brachial artery FMD was still present immediately post high intensity isometric bilateral leg exercise. The results from the present study are in alignment with those reported by (Katayama et al., 2013) whereby increased post exercise baseline diameter can influence the magnitude of post exercise changes in FMD. As part of the present study, prior data checks were also conducted to determine the relationship between FMD and shear rate area under the curve and it was established that this normalisation technique was not appropriate due to the weak relationship between shear rate and FMD post exercise (Atkinson et al., 2009).

4.6.2 Exercise-induced blood flow and shear rate patterns

Antegrade blood flow was elevated above baseline by 50% and 41%, during both high and low intensity isometric muscular contractions respectively when compared to baseline values. Moreover, high intensity isometric exercise elicited greater antegrade blood flow during the recovery period versus low intensity isometric exercise with a 93% increase in antegrade blood flow during the recovery period above baseline (see Table 9). The change in retrograde blood flow brought about during an acute bout of isometric bilateral leg extension exercise showed a distinctly different pattern at high versus low exercise intensity. Retrograde blood flow was elevated by 22% and 15% above baseline during both high and low intensity isometric

muscular contractions respectively. Moreover, high intensity isometric exercise elicited significantly greater retrograde blood flow during isometric muscular contractions versus low intensity isometric exercise. Of particular interest, high intensity isometric exercise lowered retrograde blood flow by 42% below initial baseline values during the recovery period (see Table 9). Whereas, low intensity exercise resulted in unchanged retrograde blood flow during the recovery period compared to initial baseline values. This result demonstrates that lower intensity isometric bilateral leg extension exercise had minimal effect on retrograde blood flow.

Antegrade shear rate was elevated by 41% and 33% above baseline during high and low intensity isometric muscular contractions respectively. Perhaps most interestingly, there was a greater increase in antegrade shear rate during the recovery period in the high versus low intensity isometric exercise condition. There was an increase in antegrade shear rate during the recovery period of 98% above baseline following high intensity isometric exercise, and an increase of 35% above baseline following low intensity isometric exercise. Retrograde shear rate increased during isometric muscular contractions by 15% from baseline in the high intensity isometric exercise condition and unchanged in the low intensity isometric exercise condition. More remarkably, retrograde shear rate was significantly lowered during the recovery period by 46% in the high intensity isometric exercise condition and unchanged in the low intensity isometric exercise condition. Oscillatory shear index was lowered during isometric muscular contractions by 11% and 12% below baseline values for high and low exercise intensities respectively. During the recovery period oscillatory shear index was lowered by 61% in the high intensity isometric exercise condition and by 21% in the low intensity isometric exercise condition. This reduction in oscillatory shear index during the recovery period in between muscular contractions indicates that blood flow became less oscillatory in nature; most likely due to a concomitant increase in antegrade shear rate and decrease in retrograde shear rate upon cessation of muscular contraction.

High intensity isometric bilateral leg extension exercise appears to provide a unique stimulus whereby upon the release of isometric muscular contractions there is a substantial increase in blood flow through the common femoral artery, commonly referred to as post exercise hyperaemia. Only high intensity exercise elicited a heightened antegrade and reduced retrograde pattern of blood flow upon cessation of the isometric muscular contraction. It is plausible that an intensity-dependent increase in post exercise hyperaemia exists upon cessation of isometric bilateral leg extension muscular contractions. This notion is also supported by the absent heightened post exercise hyperaemia response following low intensity lower limb isometric. McLoughlin et al. (2012) have reported complete reversion of retrograde blood flow upon cessation of muscular contractions during handgrip exercise, a similar pattern was observed in the present study. McLoughlin et al. (2012) have indicated this is suggestive of an adaptive response in downstream microvasculature. Although speculative, increased antegrade blood flow and reduced retrograde blood flow during the recovery period in between high intensity isometric muscular contractions may be due to an increase in vasodilatation downstream from the exercising muscle beds. It is feasible to suggest that the increase in antegrade blood flow and shear rate observed within the common femoral artery during the present study may also be due to conducted vasodilatation from the level of resistance vessels and microvasculature to upstream conduit feed arteries likely due to a nitric oxide-mediated vasodilator mechanism (Markos et al., 2013).

The observed transient reduction in FMD occurred in response to an exercise stimulus that elicited both a concomitant increase in antegrade shear rate and retrograde shear rate during contractions and a concomitant increase in antegrade shear rate and decrease in retrograde shear rate upon cessation of muscular contraction. It has been hypothesised that an attenuation in post exercise brachial artery FMD occurs in response to increased levels of retrograde shear rate during exercise (Johnson et al., 2012a; 2012b). What is more, increases in retrograde blood

flow and retrograde shear rate have been associated with a reduction in nitric oxide availability mediated by an increase in superoxides due to activation of the nicotinamide adenine dinucleotide phosphate-oxidase system (Godbole et al., 2009; Lu and Kassab, 2004). However, it is also interesting to consider that isometric lower limb exercise resulted in heightened antegrade blood flow and shear rate and diminished retrograde blood flow and shear rate during the recovery period. These are seemingly favourable blood flow patterns within the peripheral vasculature for promoting an anti-atherogenic environment (Adams et al., 2005; Woodman et al., 2005). Although, investigation into the exercise-induced haemodynamic parameters responsible for mediating acute changes in brachial artery FMD was beyond the scope of this thesis; it may be that the magnitude of reduction in brachial artery FMD immediately post exercise is associated with lower retrograde blood flow and retrograde shear rate and higher antegrade shear rate in the lower limbs during the recovery period. It is currently unknown whether a transient decrease in conduit artery vascular endothelial function post exercise proves a potentially beneficial or harmful stimulus when utilised as part of a long-term exercise training intervention.

4.7 Summary

Isometric bilateral leg extension exercise performed at a high intensity elicits a transient reduction in systemic endothelium-dependent vasodilatation. Isometric bilateral leg exercise performed at a high intensity was the only condition to result in a transient reduction in brachial artery FMD and temporarily diminish retrograde blood flow and retrograde shear rate upon cessation of isometric muscular contractions. The exercise-induced haemodynamic stimulus and systemic vascular endothelial function are distinctly different at the high and low exercise intensities investigated as part of this acute exercise study. In light of the findings from the present study, the haemodynamic challenge afforded during a bout of high intensity isometric bilateral leg extension exercise may be an important physiological stimulus for reductions in

RBP following isometric exercise training given the transient nature of the FMD reduction combined with the associated pro-atherogenic effects of lower retrograde and oscillatory shear stress.

CHAPTER 5: ISOMETRIC EXERCISE TRAINING STUDY

5. The effect of an eight-week isometric exercise training intervention on resting blood pressure with a specific focus upon functional and structural peripheral vascular adaptations

This work was presented at the American College of Sports Medicine Annual Meeting 27th *May-31st May, Orlando, USA.*

5.1 Introduction

Isometric exercise training has been shown to be an effective training method by which to lower blood pressure, however the causal physiological mechanisms responsible remain equivocal. Alterations in TPR and/or $\dot{Q}$ have been inferred as the mechanism(s) for exercise training-induced reductions in RBP, with reductions in TPR modulated by improvements in peripheral vascular function and structure (Pescatello et al., 2004; Huonker et al., 1996). There is considerable evidence which has shown improvement in conduit artery vascular endothelial function following exercise training (Section 2.5), and it has been proposed that these adaptations in functional responses are superseded by structural adaptations within peripheral conduit arteries (Laughlin, 1995). Existing research supports the notion that vascular endothelial function is improved within the first 2 to 4 weeks of isometric handgrip exercise training followed by progressive increases in indices of resistance artery remodelling (Tinken et al., 2010). Improvements in vascular function and structure have been inferred as the most likely mechanism responsible for mediating exercise training-induced reductions in RBP; this

theory remains to be elucidated for isometric bilateral leg extension exercise training-induced reductions in RBP in healthy individuals.

The findings from previous isometric bilateral leg extension exercise training studies clearly demonstrate reductions in RBP are possible following as little as 4 weeks of isometric exercise training (Devereux et al., 2010; Howden et al., 2002). Research has investigated the optimal exercise intensity for lower body isometric exercise and it has been reported by Devereux et al. (2011) exercise training at the average torque sustained during the final 2-minute increment of a graded exercise test correlated most strongly reductions in RBP. The exercise intensity indicated by Devereux et al. (2011) equated to the high intensity workload participants completed in Chapter 4; this intensity demonstrated a heightened haemodynamic response during exercise compared to a lower intensity exercise session and was the only exercise intensity to impact upon vascular endothelial function. Following the findings from the acute exercise study (Chapter 4) which demonstrated a transient reduction in brachial artery FMD immediately post high intensity isometric exercise, it is important to explore the effect of repeated bouts of high intensity isometric exercise as a training intervention on functional and structural vascular adaptations and associated reductions in RBP.

The main aims of this study were to investigate the effect of an 8-week high intensity isometric exercise training intervention on RBP, resting brachial artery FMD and resting artery diameter and blood flow patterns. It was hypothesised that (i) isometric exercise training-induced reductions in resting blood pressure will be associated with improvements in peripheral vascular endothelial function and structure and thus reductions in total peripheral resistance; and (ii) isometric exercise training will significantly reduce resting blood pressure by week 4, and this reduction will be maintained following 8-weeks of isometric exercise training.

Structural changes in artery diameter will supersede vascular functional changes as the programme progresses.

5.2 Method

Participants

Fourteen healthy normotensive males (mean age: 23 ± 4 years; body mass: 80.7 ± 11.0kg; height: 178.8 ± 6.2cm) (mean ± SD) volunteered to participate in a repeated measures experimental crossover design study. Prior to testing, and after receiving institutional ethical approval, each participant received a written explanation of the procedures including any potential risks, completed a Health and Fitness Questionnaire (Appendices 1 and 4), and provided written informed consent, thereby adhering to the guidelines set by the Declaration of Helsinki (1964).

Study design

All participants adhered to the testing criteria as outlined in the general methods (see Section 3.1). This study required the participants to visit the laboratory as part of an experimental repeated measures crossover training study design. Sixteen participants volunteered, one participant withdrew from the study during the intervention and one participant's data was withdrawn from analysis (see Figure 16). Participants were required to complete an 8-week exercise training condition and an 8-week non-exercising control condition. Participants were randomly assigned to an order of experimental condition with a four-week transition period used to separate the completion of the exercise training and non-exercising control condition. All participants were required to visit the laboratory at the same time of day (± 1 hour) for baseline measurements of RBP, $\dot{Q}$, TPR, brachial artery FMD, heart rate variability, conduit artery diameter, blood flow and shear rate patterns and oscillatory shear index. Participants returned to the laboratory between 2 and 7 days later to repeat the baseline measurements.

Those participants who were randomly assigned to the exercise training condition were required to visit the laboratory to perform a maximal voluntary contraction test followed by a discontinuous incremental isometric exercise test (Section 3.2.11) in order to determine their subsequent exercise training intensity. Those participants randomly assigned to the non-exercising control condition were required to visit the laboratory to perform the maximal voluntary contraction test only. Following the collection of all baseline measurements, participants assigned to the exercise training condition visited the laboratory to perform an isometric exercise session 3 days per week and each session was separated by 24 to 48 hours. The maximal voluntary contraction test and discontinuous incremental isometric exercise test were repeated following 4 weeks of exercise training. Exercising blood pressure of the upper arm was measured continuously throughout each exercise session. Exercising torque and electromyography of the lower limbs was measured continuously throughout each exercise session.

All participants were required to visit the laboratory every 2 weeks for repeat measurement of vascular endothelial function using the brachial artery FMD technique (Section 3.2.6) and measurement of resting conduit artery diameter and blood flow velocity (Section 3.2.4). Resting conduit artery blood flow, shear rate patterns and oscillatory shear index were subsequently calculated (Section 3.2.4). All participants were required to visit the laboratory at the end of week 4 (mid training) and end of week 8 (post training) for repeat measurement of resting blood pressure and heart rate (Section 3.2.1). All participants were required to visit the laboratory at the end of week 8 for repeat measurement of $\dot{Q}$, TPR, SV (Section 3.2.7) and HRV (Section 3.2.3).

5.3 Experimental procedures

5.3.1 Maximal voluntary contraction test

Three maximum voluntary contractions were performed each terminated after 2 to 3 seconds, and each separated by a 2-minute rest period. Peak electromyography (EMG_{peak}) was determined during the maximal voluntary contraction test attempt that produced the highest overall mean torque. Peak electromyography was established as the mean electromyography activity recorded in the 0.25 seconds immediately prior to the highest identified electromyography value (Wiles et al., 2010). The EMG_{peak} was used to calculate percentage electromyography 'targets' for the subsequent discontinuous incremental isometric exercise test which is described in detail in the general methods (Section 3.2.11).

5.3.2 Discontinuous incremental isometric exercise test

Following completion of the maximal voluntary contraction test, participants rested for 5-minutes in a seated position and then performed a discontinuous incremental isometric exercise test. Two-minute isometric bilateral leg extension muscular contractions were performed at a constant level of electromyography. The discontinuous incremental isometric exercise test started at a level of 10% EMG_{peak} and the intensity increased in 5% increments thereafter. During the test each 2-minute muscular contraction was interspersed by 5-minute rest period. The discontinuous incremental isometric exercise test was performed up to volitional fatigue or failure to maintain electromyography signal within +/- 5% of the electromyography 'target' value. The final 2-minute increment completed during the discontinuous incremental isometric exercise test was used to derive exercise intensity for subsequent isometric exercise sessions (Section 3.2.11). Electromyography, torque and heart rate were continuously monitored and recorded throughout the test.

5.3.3 Isometric exercise

All isometric exercise sessions and exercise tests were conducted using a calibrated Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems Inc., Shirley, New York). Participants sat in the dynamometer in an upright position, with 90 degrees of flexion at the hip and knee with the thighs supported (Section 3.2.9). The Biodex System 3 Pro isokinetic dynamometer was used to synchronize the time component of surface electromyography and muscular force recordings, which is described in detail in the general methods (Section 3.2.10). Each exercise session consisted of four 2-minute bouts of isometric bilateral leg extension exercise performed at a participant-specific intensity determined from the discontinuous incremental isometric exercise test. Each 2-minute isometric muscular contraction was separated by a 3-minute recovery period (see Figure 12).

5.3.4 Exercise intensity

Participants performed high intensity exercise at a participant-specific torque value derived by using the methods of Devereux et al. (2010). This equated to 105.4% of the average torque achieved during the last 60-seconds of the final 2-minute increment of the discontinuous incremental isometric exercise test (105.4% 2-min $torque_{peak}$). Participants were required to complete at least 95% of the 24 isometric exercise sessions to demonstrate adherence to the training intervention. If this requirement was not fulfilled the participant was required to withdraw from the exercise training study.

5.3.5 Brachial artery flow-mediated vasodilatation

Brachial artery flow-mediated vasodilatation was assessed at baseline following a 20-minute rest period using the brachial artery FMD technique in accordance with recently published guidelines (Thijssen et al., 2011d). A rapid inflation/deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned around the forearm distal to the olecranon process and inflated

to suprasystiolic pressure (200 mmHg) for 5 minutes. Baseline and peak artery diameter during FMD assessment was analysed using edge-detection and wall tracking software (Woodman et al., 2001) and blood flow velocity was analysed using in-built ultrasound trace software (GE Healthcare, United Kingdom). Brachial artery FMD was adjusted for baseline diameter and back transformed to represent an adjusted percentage brachial artery FMD as outlined in the general methods (Section 3.2.6).

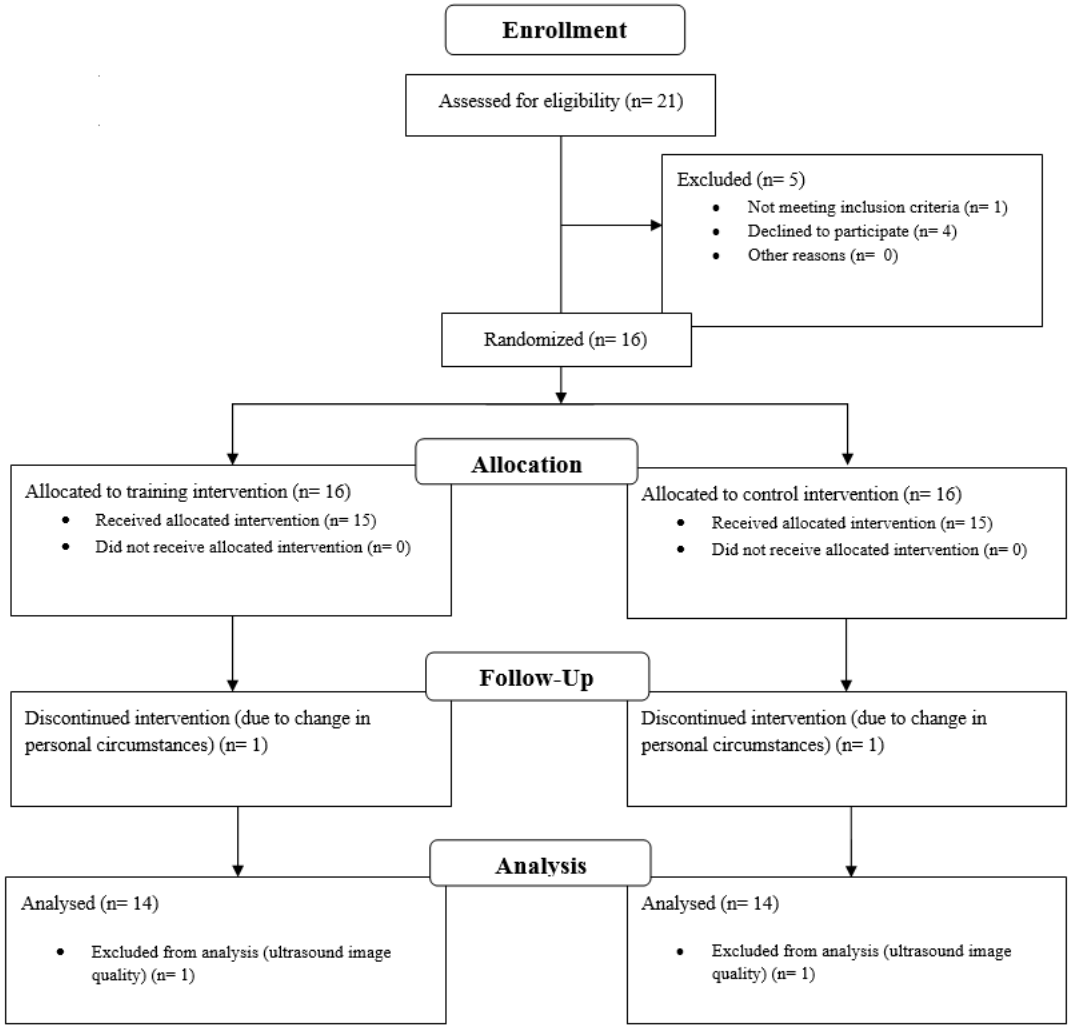


Figure 16. A CONSORT diagram for the isometric exercise training study

5.3.6 Resting artery diameter, blood flow and shear rate

Artery diameter and blood flow velocity were assessed in the brachial, common femoral and superficial femoral artery following a 20-minute rest period. Artery diameter and blood flow velocity were measured continuously using duplex Doppler ultrasound (LOGIQ e book, GE Healthcare, United Kingdom). Artery diameter was analysed using automated edge-detection and wall tracking software and blood flow velocity was analysed using in-built ultrasound trace measurement software (LOGIQ e, GE Healthcare, United Kingdom). Antegrade and retrograde blood flow, shear rate and oscillatory shear index were calculated as outlined in the general methods (Section 3.2.4).

5.3.7 Resting Cardiac Output and Total Peripheral Resistance

Aortic blood flow velocity and cross-sectional area of the aorta were measured using two dimensional Doppler ultrasound (LOGIQ e book, GE Healthcare, United Kingdom) and an 3S-RS probe (GE Healthcare, United Kingdom). Aortic diameter was calculated as the average across 3 cardiac cycles at peak systole and the cross-sectional area of the aortic artery was calculated, which is described in detail in the general methods (Section 3.2.7). Aortic blood flow velocity was calculated as the average across 10 consecutive velocity time integrals from the highest quality 30-second video recording. Stroke volume was calculated as the product of aortic blood flow velocity and aortic cross-sectional area, and $\dot{Q}$ was calculated as the product of SV and resting HR. Total peripheral resistance was calculated as resting MAP divided by $\dot{Q}$, which is described in detail in the general methods (Section 3.2.7).

5.3.8 Exercising electromyography

Surface electromyography was recorded throughout the discontinuous incremental isometric exercise test and exercise sessions using a five-lead shielded Bio Amp cable (MLA2540, AD Instruments Ltd, Australia), in conjunction with a dual bio-amplifier to measure the electrical

activity from both vastus lateralis' simultaneously and interfaced with the 16-channel Powerlab chart recorder with Chart 7 software for Windows XP (AD Instruments Ltd, Australia) which is described in detail in the general methods (Section 3.2.10).

5.3.9 Exercising blood pressure and heart rate

Blood pressure was measured continuously throughout the exercise sessions using a non-invasive continuous finger blood pressure measurement device (Finometer® Pro, FMS, Finapres Medical Systems BV, Amsterdam, The Netherlands), interfaced with the 16-channel chart recorder (Powerlab/SP16 with Chart 7 Software, AD Instruments Ltd, Australia) which is described in detail in the general methods (Section 3.2.1). Heart rate was continuously recorded using a standard three lead bipolar electrocardiogram arrangement, as recommended by AD Instruments (NSW, Australia) interfaced with the 16-channel chart recorder (Powerlab/SP16 with Chart 7 Software, AD Instruments Ltd, Australia) which is described in detail in the general methods (Section 3.2.3).

5.4 Statistical analysis

Data was assessed for conformity with parametric assumptions (Field, 2000). Where data was not normally distributed, data transformation was performed where appropriate. An alpha level of $P \leq 0.05$ was set for statistical significance. Changes in resting blood pressure and heart rate were assessed using General Estimating Equations (GEE) using the methods outlined by Atkinson and Batterham (2013a). Condition (exercise training and non-exercising control) and time (pre, mid and post intervention) were entered as independent variables and baseline resting blood pressure was entered as a covariate (Millar et al., 2007). General estimating equation analysis was used to assess changes in resting heart rate variability, velocity time integrals, SV, $\dot{Q}$ and TPR. Condition (exercise training and non-exercising control) and time (pre and post intervention) were entered as independent variables. General estimating equation analysis was

used to assess changes in brachial artery FMD as outlined by Atkinson and Batterham (2013a). Condition (exercise training and non-exercising control) and time (pre, week 2, mid, week 6 and post intervention) were entered as independent variables. Logarithmically transformed baseline resting artery diameter was entered as a covariate and data was back transformed in order to calculate covariate adjusted percentage brachial artery FMD. General estimating equation analysis was used to assess changes in resting artery diameter, antegrade and retrograde blood flow, blood flow, shear rate and oscillatory shear index in the common femoral artery, superficial femoral artery and brachial artery at pre, week 2, mid, week 6 and post intervention. Repeated measures correlation analysis was performed to assess for linear dependence between changes in resting blood pressure and heart rate with artery diameter, brachial artery FMD, heart rate variability, $\dot{Q}$ and TPR. Data is presented as mean ± SE for all parameters unless otherwise stated.

5.5 Results

5.5.1 Resting systolic blood pressure

There was a significant condition by time interaction for resting SBP (P =0.007). The results demonstrate a reduction in resting SBP (-4 mmHg) following 4 weeks of exercise training compared to the control condition (P =0.002) (see Figure 18.A). There was a trend towards a reduction in resting SBP (-3 mmHg) following 8 weeks of exercise training compared to the control condition (training: 117 ± 1 mmHg vs. control: 119 ± 1 mmHg) (P =0.082).

5.5.2 Resting diastolic blood pressure

There was a significant condition by time interaction for resting DBP (P =0.048). There was a significant main effect observed for condition (training: 72 ± 1 mmHg vs. control: 73 ± 1 mmHg, P =0.022). There was a reduction in resting DBP (-3 mmHg) from pre to week 4 within the exercise training condition (pre training: 74 ± 1 mmHg vs. mid training: 71 ± 1 mmHg) (P

=0.001) (see Figure 18. B). There was a trend towards a reduction in resting DBP (-2 mmHg) following 4 weeks of exercise training compared to the control condition (training: 71 ± 1 mmHg vs. control: 73 ± 2 mmHg) (*P* =0.072). There was no change in resting DBP (-1 mmHg) following 8 weeks of exercise training compared to the control condition (training: 72 ± 1 mmHg vs. control: 73 ± 1 mmHg) (*P* =0.403).

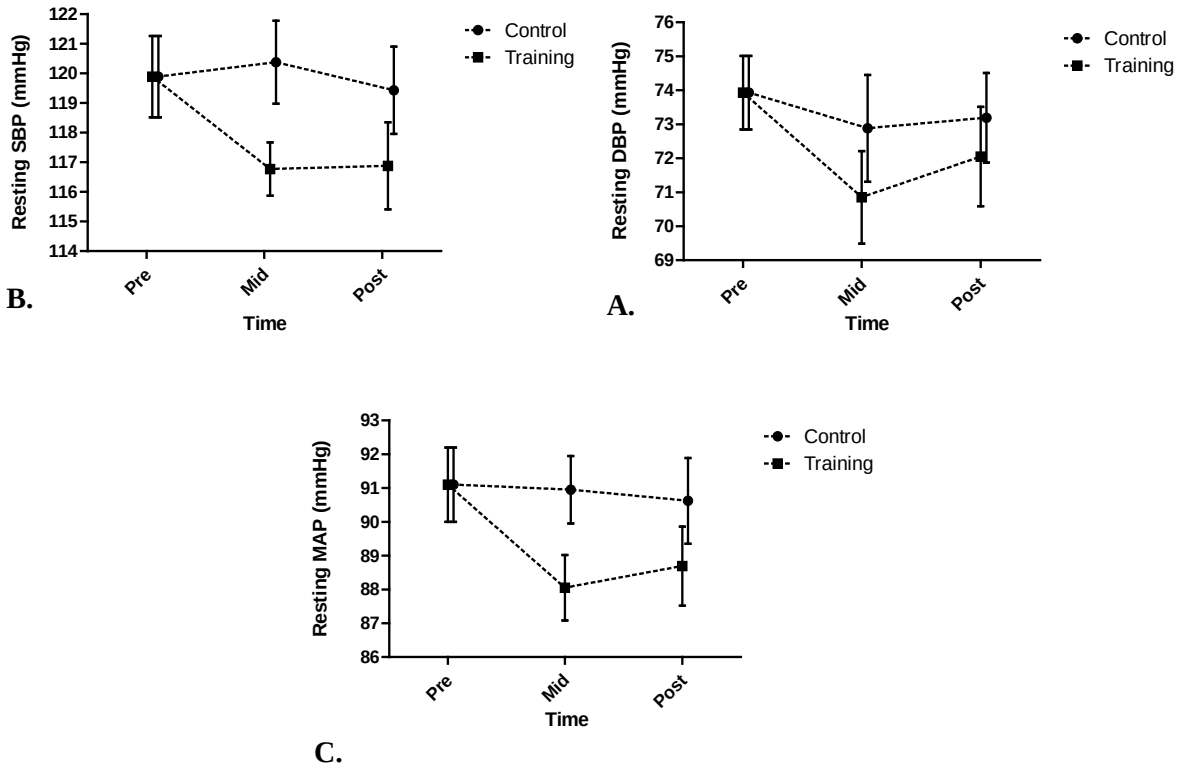


Figure 18. A. Change in resting systolic blood pressure, **B.** Change in resting diastolic blood and **C.** Change in resting mean arterial blood pressure measured throughout an 8-week exercise and non-exercising control condition.

5.5.3 Resting mean arterial pressure

There was a significant condition by time interaction for resting MAP (*P* <0.001). The results demonstrate a reduction in resting MAP (-3 mmHg) following 4 weeks of exercise training compared to the control condition (training: 88 ± 1 mmHg vs. control: 91 ± 1 mmHg) (*P* =0.001) (see Figure 18.C). There was a trend towards a reduction in resting MAP (-2 mmHg)

following 8 weeks of exercise training compared to the control condition (training: 89 ± 1 mmHg vs. control: 91 ± 1 mmHg) (P =0.059).

5.5.4 Resting heart rate

There was no significant main effects or interaction observed for resting HR (P =0.712). The results demonstrate no change in resting HR (-2 bpm) following 4 weeks of exercise training compared to the control condition (training: 69 ± 2 bpm vs. control: 71 ± 3 bpm) (P =0.588) or following 8 weeks (P= 0.454) (see Figure 19).

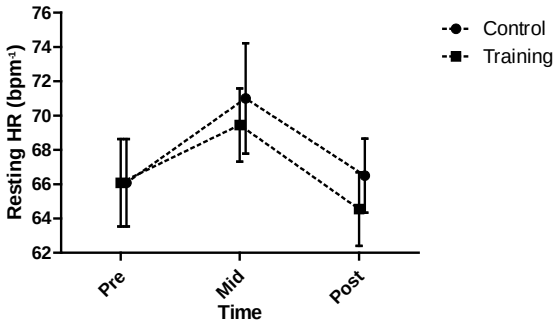


Figure 19. Change in resting heart rate measured throughout an 8-week exercise training period compared to non-exercising control condition.

5.5.5 Resting heart rate variability

There was no significant condition by time interaction for low frequency normalised units following 8 weeks of exercise training (P =0.756). There was also no significant interaction observed following 8 weeks of exercise training for high frequency normalised units (P =0.605); for low frequency high frequency ratio (LF/HF) (P =0.445); or for very low frequency normalised units following 8 weeks of exercise training (P =0.894). There was a significant

condition by time interaction or for total power (P =0.029), with lower total power following 8 weeks of exercise training compared to the control condition (see Table 15).

5.5.6 Brachial Artery Flow-Mediated Vasodilatation

There was significant condition by time interaction for brachial artery FMD (P =0.011). There was a significant increase brachial artery FMD at mid exercise training compared to mid control condition (P =0.013) (see Table 11). Brachial artery FMD increased by 2.27% following 4 weeks of exercise training compared to the control condition (Figure 20.C). A significant difference was observed for the covariate baseline diameter (P =0.001)

Table 11. Brachial artery flow-mediated vasodilatation measured throughout an 8-week isometric bilateral leg extension exercise training and non-exercising training control condition.

		Time				
Variable	**Condition**	*Pre*	*Week 2*	*Mid*	*Week 6*	*Post*
Adjusted FMD (%)	Training	8.36 ± 1.13	7.68 ± 1.15	8.65 ± 1.02*	6.78 ± 1.03	8.21 ± 1.65
	Control	7.57 ± 1.25	6.81 ± 1.42	6.38 ± 1.14	8.81 ± 1.29	8.35 ± 1.35
Unadjusted FMD (%)	Training	8.25 ± 1.12	7.41 ± 1.60	8.63 ± 1.18	6.72 ± 1.18	8.75 ± 1.74
	Control	7.63 ± 0.98	6.64 ± 1.34	6.75 ± 1.43	9.16 ± 1.71	8.54 ± 1.19
Baseline diameter (mm)	Training	3.99 ± 0.13	4.03 ± 0.13	3.96 ± 0.11	3.98 ± 0.13	3.91 ± 0.15
	Control	3.96 ± 0.14	4.02 ± 0.15	3.92 ± 0.14	3.94 ± 0.16	3.95 ± 0.15
Peak diameter (mm)	Training	4.31 ± 0.12	4.32 ± 0.11	4.30 ± 0.11	4.24 ± 0.13	4.22 ± 0.12
	Control	4.26 ± 0.15	4.28 ± 0.15	4.17 ± 0.13	4.29 ± 0.16	4.28 ± 0.16
Mean SR AUC (au)	Training	1949 ± 176	2949 ± 420	2237 ± 299	2885 ± 411	2297 ± 282
	Control	2334 ± 226	2769 ± 369	3426 ± 358	2891 ± 211	2985 ± 276

Note: FMD: flow-mediated vasodilatation, SR AUC: shear rate area under the curve. * indicates statistically significant compared to non-exercising control condition.

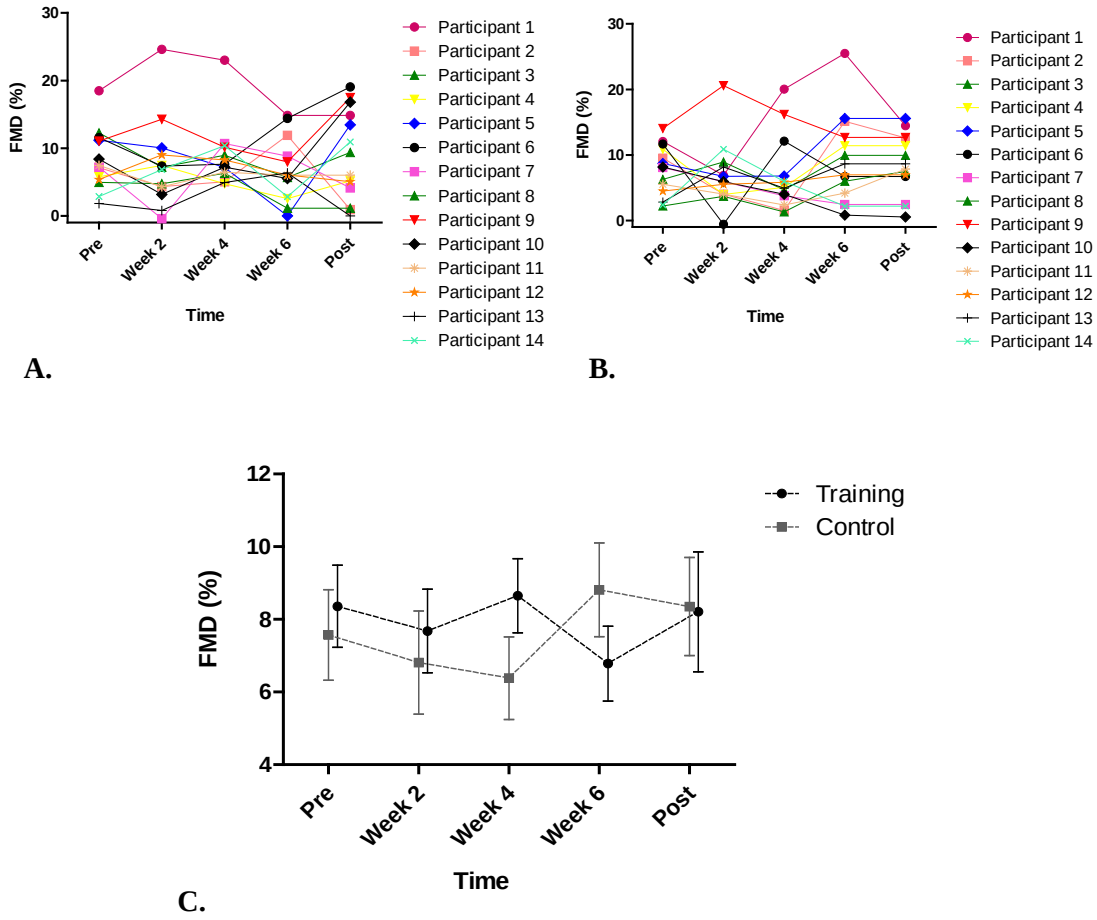


Figure 20. A. Individual change in brachial artery FMD (%) in the exercise training group. **B.** Individual change in brachial artery FMD (%) in the non-exercising control group. **C.** Mean exercise training and control group change in brachial artery FMD (%) throughout an 8-week intervention.

5.5.7 Resting Vascular Artery Diameter and Blood Flow Patterns

There was a significant condition by time interaction for brachial artery antegrade blood flow (P =0.001). There was a significant main effect observed for time (P >0.001), whereby brachial artery antegrade blood flow was higher in the exercise training condition compared to control condition (all P ≥0.005). There was no significant condition by time interaction for brachial artery diameter (P =0.895), retrograde blood flow (P =0.582), antegrade shear rate (P =0.134), retrograde shear rate (P =0.639) or oscillatory shear index (P =0.781) (see Table 12). There was no significant condition by time interactions for common femoral artery diameter (P =0.867), antegrade blood flow (P =0.566), antegrade shear rate (P =0.673), retrograde blood flow (P =0.364), retrograde shear rate (P =0.673) or oscillatory shear index (P =0.908) (see Table 13).

There was a significant condition by time interaction for superficial femoral artery diameter (P =0.028). Superficial femoral artery diameter was higher following 2 weeks of exercise training compared to the control condition (P=0.006). There was a significant condition by time interaction for superficial femoral artery antegrade blood flow (P =0.015), antegrade shear rate (P =0.027) and retrograde shear rate (P =0.002) (see Table 14). Superficial femoral artery antegrade blood flow was higher following 2 weeks of exercise training compared to the control condition (P=0.015). Superficial femoral artery antegrade shear rate demonstrated a trend for higher following 2 weeks of exercise training compared to the control condition (P =0.055). Superficial femoral artery retrograde shear rate was lower following 2 weeks of exercise training compared to the control condition (P =0.013). There was no significant condition by time interaction for superficial femoral artery retrograde blood flow (P =0.445) or oscillatory shear index (P =0.506) (see Table 14).

Table 12. Characteristics of the brachial artery measured throughout an 8-week isometric bilateral leg extension exercise training and non-exercising training control condition.

		Time				
Variable	**Condition**	*Pre*	*Week 2*	*Mid*	*Week 6*	*Post*
Diameter (mm)	Training	3.99 ± 0.15	4.03 ± 0.13	3.96 ± 0.11	3.98 ± 0.13	3.91 ± 0.15
	Control	3.96 ± 0.14	4.00 ± 0.15	3.92 ± 0.14	3.94 ± 0.16	3.95 ± 0.15
Antegrade BF ($ml.min^{-1}$)	Training	51.4 ± 3.7	60.3 ± 4.8	52.6 ± 4.0	51.6 ± 4.2	47.2 ± 4.4
	Control	54.7 ± 5.0	55.1 ± 4.3	42.7 ± 4.3	49.6 ± 4.8	50.7 ± 4.5
Retrograde BF ($ml.min^{-1}$)	Training	15.7 ± 1.8	15.5 ± 2.2	13.4 ± 1.5	13.9 ± 2.2	13.7 ± 1.9
	Control	16.2 ± 1.9	15.2 ± 1.8	13.9 ± 2.3	12.5 ± 1.7	14.6 ± 2.3
Antegrade SR (s^{-1})	Training	69.5 ± 4.2	76.7 ± 3.6	71.3 ± 3.7	68.8 ± 3.1	66.5 ± 3.7
	Control	73.6 ± 3.4	75.0 ± 6.0	67.4 ± 4.3	69.4 ± 4.5	69.6 ± 3.2
Retrograde SR (s^{-1})	Training	19.9 ± 1.4	18.4 ± 1.5	17.8 ± 1.4	17.1 ± 1.9	17.9 ± 1.2
	Control	21.2 ± 1.3	19.5 ± 1.3	17.9 ± 1.4	16.5 ± 1.7	18.4 ± 1.3
OSI (au)	Training	0.23 ± 0.01	0.20 ± 0.02	0.20 ± 0.01	0.20 ± 0.02	0.22 ± 0.01
	Control	0.22 ± 0.01	0.21 ± 0.02	0.21 ± 0.01	0.20 ± 0.02	0.21 ± 0.01

Note: BF: blood flow, SR: shear rate, OSI: oscillatory shear index. * indicates statistically significant compared to non-exercising control condition.

Table 13. Characteristics of the common femoral artery measured throughout an 8-week isometric bilateral leg extension exercise training and non-exercising training control condition.

		Time				
Variable	**Condition**	*Pre*	*Week 2*	*Mid*	*Week 6*	*Post*
Diameter (mm)	Training	9.38 ± 0.22	9.28 ± 0.26	9.12 ± 0.22	9.25 ± 0.30	9.13 ± 0.29
	Control	9.30 ± 0.30	9.33 ± 0.37	9.20 ± 0.31	9.44 ± 0.29	9.42 ± 0.29
Antegrade BF ($ml.min^{-1}$)	Training	285.6 ± 16.3	280.5 ± 18.9	255.8 ± 14.4	266.2 ± 16.5	256.1 ± 18.1
	Control	271.8 ± 13.2	265.2 ± 17.9	245.4 ± 17.5	271.6 ± 14.6	303.7 ± 27.8
Retrograde BF ($ml.min^{-1}$)	Training	165.0 ± 11.4	154.0 ± 12.2	139.6 ± 9.7	152.3 ± 11.8	148.8 ± 10.1
	Control	147.5 ± 10.7	152.9 ± 10.9	132.4 ± 11.2	152.2 ± 11.5	163.1 ± 17.3
Antegrade SR (s^{-1})	Training	30.1 ± 2.2	30.1 ± 1.9	29.3 ± 2.3	29.3 ± 2.1	29.9 ± 1.8
	Control	30.5 ± 2.9	29.1 ± 2.3	27.6 ± 2.2	29.0 ± 2.7	31.2 ± 2.2
Retrograde SR (s^{-1})	Training	17.0 ± 0.9	16.3 ± 0.8	15.7 ± 1.1	16.4 ± 1.0	16.9 ± 1.2
	Control	15.7 ± 0.8	15.9 ± 0.9	14.4 ± 0.7	15.2 ± 0.6	16.3 ± 0.9
OSI (au)	Training	0.37 ± 0.01	0.36 ± 0.02	0.35 ± 0.01	0.36 ± 0.01	0.37 ± 0.01
	Control	0.35 ± 0.01	0.33 ± 0.03	0.35 ± 0.01	0.36 ± 0.02	0.35 ± 0.01

Note: BF: blood flow, SR: shear rate, OSI: oscillatory shear index. * indicates statistically significant compared to non-exercising control condition.

Table 14. Characteristics of the superficial femoral artery measured throughout an 8-week isometric bilateral leg extension exercise training and non-exercising training control condition.

		Time				
Variable	**Condition**	*Pre*	*Week 2*	*Mid*	*Week 6*	*Post*
Diameter (mm)	Training	6.99 ± 0.20	7.15 ± 0.20	6.97 ± 0.20	6.91 ± 0.21	6.88 ± 0.21
	Control	6.82 ± 0.24	6.67 ± 0.23	6.86 ± 0.22	6.97 ± 0.27	6.93 ± 0.27
Antegrade BF ($ml.min^{-1}$)	Training	128.4 ± 8.7	156.6 ± 8.6	145.5 ± 7.1	134.1 ± 9.5	138.3 ± 10.2
	Control	145.2 ± 7.3	131.3 ± 7.4	141.2 ± 8.8	146.3 ± 9.4	137.0 ± 8.8
Retrograde BF ($ml.min^{-1}$)	Training	64.8 ± 4.8	72.0 ± 3.1	70.7 ± 5.0	69.5 ± 5.7	69.6 ± 6.6
	Control	71.1 ± 4.5	66.7 ± 66.7	70.8 ± 7.3	75.2 ± 5.5	68.2 ± 4.3
Antegrade SR (s^{-1})	Training	32.4 ± 2.1	36.9 ± 2.0	37.5 ± 2.4	34.4 ± 1.6	36.3 ± 2.1
	Control	42.2 ± 4.3	39.5 ± 2.9	39.1 ± 3.7	38.6 ± 3.4	37.1 ± 3.3
Retrograde SR (s^{-1})	Training	16.3 ± 1.1	17.2 ± 1.1	18.1 ± 1.3	17.8 ± 1.1	17.9 ± 1.1
	Control	20.1 ± 1.7	19.9 ± 1.5	18.8 ± 1.8	19.5 ± 1.3	18.4 ± 1.5
OSI (au)	Training	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
	Control	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.33 ± 0.01

Note: BF: blood flow, SR: shear rate, OSI: oscillatory shear index. * indicates statistically significant compared to non-exercising control condition.

5.5.8 Cardiac Output, Stroke Volume and Total Peripheral Resistance

Stroke volume was significantly lower following 8 weeks of exercise training compared to the control condition (P =0.039). There was no significant difference observed for $\dot{Q}$ following 8 weeks of exercise training compared to the control condition (P =0.148). There was a trend for higher total peripheral resistance following 8 weeks of exercise training compared to the control condition (P =0.054) (see Table 15).

Table 15. Cardiac parameters measured pre and post an 8-week isometric bilateral leg extension exercise training and non-exercising training control condition.

		Time		
Variable	**Condition**	***Pre***	***Post***	**P value**
Cardiac output ($L.min^{-1}$)	Training	4.52 ± 0.26	4.50 ± 0.25	0.148
	Control	4.42 ± 0.24	4.84 ± 0.27	
Stroke volume ($ml.min^{-1}$)	Training	68.7 ± 2.5	69.6 ± 2.7*	0.039
	Control	66.6 ± 2.3	72.7 ± 2.8	
Velocity time integrals ($cm.s^{-1}$)	Training	19.2 ± 0.8	19.4 ± 0.8*	0.035
	Control	18.6 ± 0.7	20.31 ± 0.8	
Total peripheral resistance ($mmHg \cdot mL^{-1} \cdot min^{-1}$)	Training	21.0 ± 1.4	20.9 ± 1.3	0.054
	Control	21.6 ± 1.1	19.4 ± 0.9	
Heart rate variability				
LFnu	Training	65.2 ± 4.8	67.2 ± 2.8	0.713
	Control	69.1 ± 3.6	69.0 ± 4.0	
HFnu	Training	26.2 ± 3.6	26.9 ± 2.2	0.605
	Control	27.1 ± 3.6	25.5 ± 3.4	
LF/HF	Training	3.02 ± 1.24	2.62 ± 1.15	0.445
	Control	2.89 ± 1.21	3.04 ± 1.24	
TP (ms^2)	Training	4966 ± 534	3933 ± 547*	0.029
	Control	3564 ± 566	4142 ± 503	

Note: LFnu: low frequency normalised units, HFnu: high frequency normalised units, LF/HF: low/high frequency ratio, TP: total power. * indicates statistically significant compared to non-exercising control condition.

5.5.9 Maximal voluntary contraction torque

There was no significant condition by time interaction for maximal voluntary contraction torque (P =0.430). Maximal voluntary contraction torque was 417 ± 25 Nm and 432 ± 21 Nm for pre and post exercise training condition respectively. Maximal voluntary contraction torque was 432 ± 21 Nm and 433 ± 24 Nm for pre and post control condition respectively.

5.5.10 Exercising torque, electromyography and blood pressure

The group mean exercising torque values remained within the target training zone (± 5%) during weeks 1 to 4 (-4.53%) and weeks 5 to 8 (-3.50%). The group mean exercising torque was higher at week 5 compared to week 1 and week 4 (P =0.003 and P <0.001 respectively). The group mean exercising torque was higher at week 8 compared to week 4 and week 1 (P <0.001 and P =0.005 respectively). There was a significant main effect over time observed in mean exercising electromyography (P <0.001). The group mean exercising electromyography was higher during week 1 compared to week 4 of training (P <0.001). The group mean exercising electromyography during week 5 was higher compared to week 1 and week 4 (P =0.010 and P <0.001, respectively). There was no significant difference between mean exercising electromyography during week 5 and week 8 (P =0.055). The group mean exercising electromyography at week 8 was not significantly different from week 1 (P =0.237). There was no significant main effect observed for time in exercising SBP (P =0.088), exercising DBP (P =0.699) or exercising MAP (P =0.375). All exercising torque, electromyography and blood pressure data are presented in Table 16.

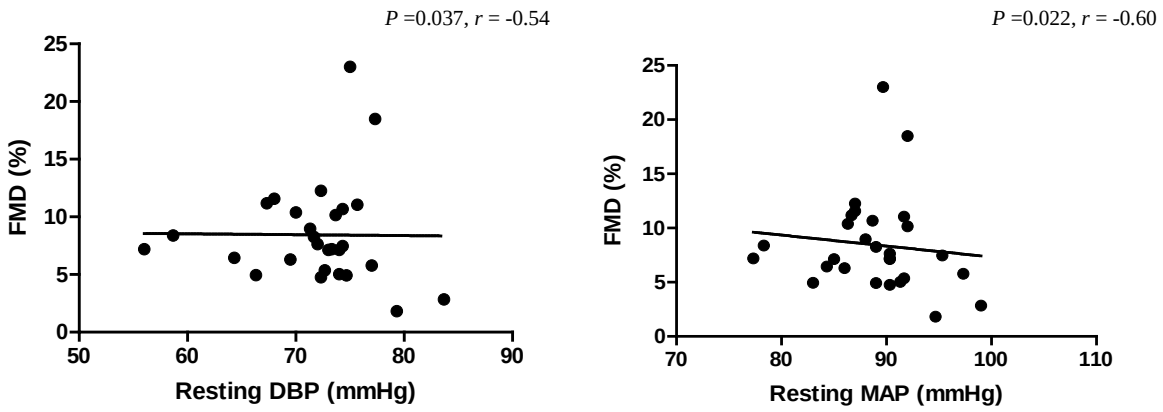
Table 16. Group exercise training data for exercising blood pressure, electromyography and torque during an 8-week isometric bilateral leg extension exercise training.

	Time			
Variable	*Week 1*	*Week 4*	*Week 5*	*Week 8*
Torque (Nm)	118 ± 1	115 ± 1	137 ± 1*†	136 ± 1*†
EMG (mV)	114 ± 6†	104 ± 6	131 ± 7*†	124 ± 9
SBP (mmHg)	167 ± 4	164 ± 4	171 ± 3†	-
DBP (mmHg)	108 ± 1	107 ± 1	110 ± 1	-
MAP (mmHg)	127 ± 1	126 ± 1	130 ± 1	-

Note: EMG: electromyography, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure. * indicates statistically significant compared to week 1, † indicates statisticall significant compared to week 4.

5.5.11 Relationship between exercise training-induced resting blood pressure reductions and selected cardiovascular and neuromuscular variables

The results show a significant negative correlation of moderate strength between resting brachial artery FMD and resting DBP measured at pre to mid intervention (P =0.037, r= -0.54) (Figure 21. A). The results also show a statistically significant moderate to strong negative correlation between resting brachial artery FMD and resting MAP measured at pre to mid intervention (P =0.022, r= -0.60) (Figure 21. B). The results show a positive correlation of weak strength close to statistical significance between resting brachial artery FMD and resting brachial artery retrograde shear rate (P = 0.058, r= 0.25) (Figure 22). The results demonstrated a negative correlation of low strength between resting SBP and brachial artery antegrade blood flow (P =0.087, r= -0.32); and a positive correlation of low strength between resting MAP and superficial femoral artery retrograde blood flow (P =0.096, r= 0.33). The results show a positive correlation of low strength between resting DBP and superficial femoral artery retrograde blood flow (P =0.066, r= 0.36) and a negative correlation of low strength between resting DBP and superficial femoral artery antegrade blood flow (P =0.079, r= -0.34).



A. **B.**

Figure 21. A. The relationship between change in brachial artery vascular endothelial function (BA FMD %) and exercise training-induced reductions in resting diastolic blood pressure (DBP). **B.** The relationship between change in brachial artery vascular endothelial function (BA FMD %) and exercise training-induced reductions in resting mean arterial blood pressure (MAP).

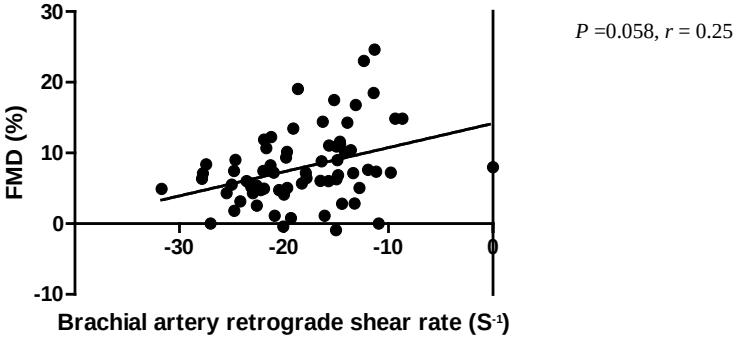


Figure 22. The relationship between change in brachial artery vascular endothelial function (FMD %) and resting brachial artery retrograde shear rate.

5.6 Discussion

5.6.1 Exercise training-induced reductions in resting blood pressure

Four weeks of isometric bilateral leg extension exercise resulted in a significant reduction in both resting SBP (4 mmHg) and MAP (3 mmHg) compared to the non-exercising control

condition. There was a trend for a reduction in resting DBP following 4 weeks of isometric bilateral leg extension exercise training (2 mmHg) compared to the non-exercising control condition. Furthermore, there was a trend for reductions in resting SBP and MAP following 8 weeks of isometric exercise training (3mmHg and 2mmHg, respectively) of which the magnitude is still considered a clinically relevant reduction (Chobanian et al., 2003). The reductions in RBP reported in the present training study are comparable to those reported by Wiles et al. (2010) following high and low intensity isometric exercise training (SBP: 4 mmHg and 5 mmHg; MAP: 3 mmHg and 3 mmHg for high and low intensity, respectively). The reductions in RBP reported in the present training study are also similar in magnitude to those RBP reductions reported by Devereux et al. (2010) following 4 weeks of high intensity bilateral leg isometric exercise training in young normotensive males (SBP: 5 mmHg; MAP: 3 mmHg). This discrepancy between isometric exercise training-induced reductions in RBP from week 1 to 4 and week 5 to 8 may to relate to the existing optimal levels of RBP at baseline (Millar et al., 2007). This unexpected finding may be due to the negative feedback response of the cardiovascular system to prevent any further decrease in RBP. Further to this, another potential explanation may relate to the change in absolute exercise intensity following the repeat of the discontinuous incremental isometric exercise test after week 4 of the training intervention; the implications and future directions related to this finding will be discussed further in Chapter 6.

Research by Devereux et al. (2011) has inferred that reductions in resting SBP could be achieved following 4 weeks of training at a specific marker of intensity relating to a constant force may provide a markedly different exercise stimulus compared to exercising at a constant electromyography value. Devereux et al. (2011) reported strong associations between the average 2-minute torque sustained during the final stage of an incremental isometric exercise test (105.4% of 2-min $torque_{peak}$) and isometric exercise training-induced reductions in RBP. Thereby suggesting that those individuals who trained at this specific marker of intensity may

be guaranteed to experience a reduction in resting SBP of at least 5 mmHg. The RBP reductions observed in the current study does not support the previous suggestion by Devereux et al. (2011); and this discrepancy may be partly explained through the fact that 'responders and non-responders' are documented following various types of exercise training intervention in both RBP and FMD (Green et al., 2014b; Millar et al., 2007). Indeed, the fact that only 7 participants demonstrated reductions in resting SBP, 9 participants demonstrated reductions in resting DBP and 8 participants demonstrated reductions in resting MAP (out of 15 participants) across the first 4 weeks of training tends to support the existence of this phenomena following isometric exercise training. Since the statistical analysis used by Devereux et al. (2011) did not account for responder/non-responder concept, the prediction of a training-induced reduction in resting SBP of 5 mmHg for all participants exercising at 105.4% of 2-min torque$_{peak}$ may not be an accurate representation of how all individuals respond.

5.6.2 Exercise training-induced changes in resting vascular endothelial function, diameter and blood flow patterns

A significant improvement in brachial artery FMD occurred following 4 weeks of isometric bilateral leg extension exercise training. Those individuals who experienced the largest isometric exercise training-induced reductions in resting MAP and DBP tended to demonstrate the largest increase in resting brachial artery FMD (Section 5.5.11). This is a particularly important finding considering that for every 1% improvement in brachial artery FMD, the likelihood of the occurrence of cardiovascular events is reduced by 13% (Inaba et al., 2010). According to the results of a meta-analysis by Inaba et al. (2010), isometric bilateral leg extension exercise training has the potential to be effective lifestyle intervention to alter future cardiovascular outcomes and reduce the risk of cardiovascular disease since this study demonstrated an improvement in brachial artery FMD of 2.27%. The results from the present study are in agreement with those from a recent meta-analysis by Ashor et al. (2015) who

reported that aerobic, resistance and combined exercise training modalities increased brachial artery FMD from 2.0 to 2.8%.

The present training study has demonstrated a systemic improvement in brachial artery FMD, which is in agreement with a large body of research having reported improved systemic vascular endothelial function in healthy populations (Spence et al., 2013; Birk et al., 2012; Simmons et al., 2011; Tinken et al., 2008; Clarkson et al., 1999; Kingwell et al., 1997). The magnitude of improvement in brachial artery FMD within the present study of 2.27% is greater than that reported by an earlier study by McGowan et al. (2006). McGowan et al. (2006) reported an improvement of 1.9% in medicated hypertensive individuals following 8 weeks of isometric handgrip exercise training. The results from the present study are also comparable to those demonstrating that 24-weeks of whole body resistance training improved brachial artery FMD by 1.9% in healthy young individuals (Spence et al., 2013). Brachial artery FMD in the present training study was also similar to recent research whereby lower limb cycling exercise elicited significant improvements in resting brachial artery FMD at week 2 in healthy young individuals (Birk et al., 2012). This would lead to the conclusion that the overall magnitude of improvement in brachial artery FMD induced by lower limb isometric exercise training is comparable to other modalities of lower body aerobic and dynamic resistance exercise training.

The results from the present study demonstrate no change in resting artery diameter in the brachial or common femoral throughout an 8-week bilateral leg isometric exercise training intervention. This is in agreement with a number of studies that have reported no change in localised and systemic resting artery diameter following lower limb aerobic exercise training (Schreuder et al., 2015b; Thijssen et al., 2013; 2011a). This would suggest that structural adaptations, when assessed via resting artery diameter in the brachial and common femoral artery, are not a mechanism responsible for reductions in RBP following exercise training.

There was a significant improvement in superficial femoral artery diameter at week 2 of isometric exercise training. It could be suggested from this finding that arterial remodelling may have occurred downstream from the exercising muscle, however it is unlikely that structural adaptations could occur within such short period of time. It is unlikely that arterial remodelling had occurred at this early stage in the exercise training intervention given that changes in femoral artery structure are typically reported at later time points (Stebbings et al., 2013). Therefore, the changes in diameter are most probably a functional vasodilatory response as opposed to structural adaptations. This response may have been necessary to cope with the significant hemodynamic stimuli produced at the onset of exercise with the rapid elevations in blood flow within the exercising lower limbs.

There was a significant increase in superficial femoral artery antegrade blood flow and shear rate following 2 weeks of exercise training, this may be an indicator of improved blood flow capacity downstream from the exercising limb. This transient increase in resting diameter and antegrade blood flow suggests that functional vasodilation had occurred. The results from the present study demonstrate that isometric lower limb exercise training performed at a high intensity in healthy young men can elicit more favourable blood flow patterns that are associated with promoting an anti-atherogenic environment. Recent research has alluded to the potentially negative implications of excessive transient elevations in retrograde shear rate on acute vascular endothelial function (Jenkins et al., 2013; Thijssen et al., 2009b), and interestingly there was a significant decrease in superficial femoral artery retrograde shear rate following 2 weeks of exercise training. The results of the present study further emphasise the beneficial impact isometric exercise training may have upon the resting haemodynamic parameters within the peripheral vasculature in the lower limbs. Although brachial artery retrograde shear rate was not significantly altered as a result of isometric exercise training, improved brachial artery FMD was associated with lower brachial artery retrograde shear rate

(P =0.058) (Section 5.5.11). Collectively, these findings highlight the potential influence resting blood flow and shear rate patterns may have upon vascular endothelial function.

The improvement in brachial artery FMD at week 4 would infer that an improvement in peripheral vascular tone had occurred; and supports the theory that exercise training-induced reductions in RBP occur via reductions in TPR (Fagard and Cornelissen, 2007). Heart rate variability remained unchanged at week 8 of the training intervention in young healthy normotensive males; however the interpretation of no statistical significance for this parameter is limited due to the high variability previously reported (Section 2.3.1). It is unlikely that an attenuation in cardiac autonomic nerve activity was responsible for isometric exercise training-induced RBP reductions considering there were no significant changes observed in resting heart rate at week 4 or week 8. Previous research investigating the effect of dynamic resistance and isometric exercise training on neural-mediated vasoconstrictor tone has predominantly shown no change in this measure in healthy individuals at rest (Section 2.3.3). Nonetheless, a change in this parameter cannot be completely discounted as a potential mechanism for isometric exercise training-induced reductions in RBP particularly as it was not measured at week 4 of the intervention. The unexpected increase in TPR, reduction in SV, and unchanged $\dot{Q}$ following 8 weeks of isometric exercise training may help to explain why the reduction in RBP in healthy young men was not statistically significant at week 8. Those individuals who experienced RBP reductions below their optimal blood pressure classification may have experienced an increase in TPR in order to prevent RBP falling into hypotensive ranges (c.f. responses to hypovolaemia [Länne and Ludvall, 1992]). Albeit speculative, this may provide an explanation for the heightened TPR following 8 weeks of high intensity isometric exercise training.

Few studies have explored the relationship between exercise training-induced improvements in RBP and enhanced vascular endothelial function (Badrov et al., 2013; Beck et al., 2013; Swift et al., 2012). Therefore, the existence of a strong relationship between exercise training-induced reductions in RBP and improvements in vascular endothelial function in the present study ought to be recognised. It is not possible to unequivocally conclude whether the observed improvements brachial artery FMD were mediated via the systemic effects of shear stress. This can only be inferred from existing evidence within the literature that has manipulated shear stress using a pneumatic cuff to determine its role in mediating improvements in FMD (Carter et al., 2013; Tinken et al., 2010). It is plausible that the improvement in brachial artery FMD in the present study is, at least in part, mediated through a shear stress mechanism as this has been demonstrated in inactive upper limbs in response to lower limb exercise (Birk et al., 2012; Simmons et al., 2011). The brachial artery FMD technique is considered an index of nitric-oxide mediated endothelium dependent vasodilatation, and therefore the present study assumes that the improvement in FMD is nitric-oxide mediated and endothelium-dependent. An improvement in vasodilator function as a result of improved vascular smooth muscle function cannot be eliminated entirely since endothelium-independent vasodilatation was not measured in the present study (Corretti et al., 2002). The majority of the evidence suggests that improvement in conduit artery vasodilator function is predominantly due to improvement in endothelium-dependent function (Conraads et al., 2015; Luk et al., 2012). It should be acknowledged that an attenuation in sympathetic nervous system mediated vasoconstrictor tone may have contributed to the observed improvement in brachial artery FMD (Thijssen et al., 2014; Green et al., 2011c); however the role of the sympathetic nervous system in meditating changes in vascular tone within the peripheral vasculature cannot be elucidated in the present study.

5.7 Summary

High intensity isometric bilateral leg extension exercise training is an effective strategy for lowering RBP and enhancing brachial artery FMD. Adaptations were evident following 4 weeks of training, and with a trend for a maintenance in the reduction in RBP following 8 weeks of training. There was no change in resting artery diameter in the brachial and common femoral artery throughout the 8-week training intervention; however measures of superficial femoral artery diameter and blood flow were enhanced following 2 weeks of exercise training. Furthermore, lower retrograde shear rate within the peripheral vasculature may be inferred as having an important mechanistic role in mediating exercise training-induced reductions in RBP and improvement in brachial artery FMD. The improvements in vascular endothelial function were systemic in nature however, it is possible given the early localised improvements in superficial femoral artery diameter and blood flow, that adaptations within downstream peripheral conduit arteries or within the lower limb vasculature may be important mechanisms in bringing about training-induced reductions in RBP. Notwithstanding the limitations with heart rate variability data, the current findings suggest that cardiac autonomic regulation is not the mechanism responsible for mediating beneficial reductions in RBP. Isometric bilateral leg exercise training offers an opportunity to improve systemic vascular endothelial function, and most notably those participants who experienced the largest exercise training-induced reductions in RBP demonstrated the greatest improvement in systemic vascular endothelial function.

CHAPTER 6: GENERAL DISCUSSION

6.1 Executive summary of the findings

The primary aim of this thesis was to elucidate the role of peripheral vascular adaptations as the physiological mechanism responsible for the RBP lowering effects of isometric exercise training. The secondary aim was to define the time course of vascular adaptations in an attempt to explain training-induced reductions in RBP. The main findings from this research are presented below:

- Isometric bilateral leg exercise training performed at a high intensity is effective in lowering resting SBP and MAP following 4 weeks of training (Section 5.5.1 and 5.5.3);
- There was a trend for reductions in resting SBP, DBP and MAP following 8 weeks of isometric bilateral leg exercise training although the results did not reach statistical significance (Sections 5.5.1 to 5.5.3);
- Isometric bilateral leg exercise training performed at a high intensity enhanced resting brachial artery FMD following 4 weeks of training (Section 5.5.6);
- Isometric exercise training-induced reductions in resting MAP were associated with improvements in resting brachial artery FMD (Section 5.5.11);
- There was a trend for resting brachial artery FMD to be associated with reductions in resting brachial artery retrograde shear rate (Section 5.5.11);
- Isometric bilateral leg exercise training performed at a high intensity increased resting superficial femoral artery diameter, antegrade blood flow and antegrade shear rate and lowered retrograde shear rate following 2 weeks of training (Section 5.5.7);
- Total peripheral resistance was elevated and stroke volume was reduced following 8 weeks of isometric bilateral leg exercise training (Section 5.5.8);

- An acute bout of high intensity (but not low) isometric bilateral leg extension exercise transiently reduced brachial artery FMD immediately post exercise, which returns to near baseline levels by 30 minutes post exercise (Section 4.5.1);
- Isometric bilateral leg extension exercise performed at high intensity demonstrates a trend for decreased common femoral artery diameter during muscular contractions and an elevated common femoral artery diameter during the recovery period (Section 4.5.2);
- High intensity isometric bilateral leg extension exercise produces a distinctly different blood flow and shear rate pattern with greater retrograde blood flow and shear rate during contraction and greater increases antegrade blood flow and shear rate and lower retrograde blood flow upon cessation of contraction compared to low intensity (Sections 4.5.2);
- High intensity isometric bilateral leg extension exercise produces lower oscillatory shear index during the recovery period compared to low intensity isometric bilateral leg extension exercise (Section 4.5.2).

Overall, it would appear that isometric bilateral leg exercise training performed at a high intensity is an effective strategy in reducing RBP in healthy young men. The results from the present exercise training intervention demonstrate that reductions in RBP in the initial 4 weeks were mediated via adaptations in peripheral vascular endothelium-dependent vasodilatation, which are likely to contribute to a decrease in TPR. The results from the present exercise training intervention study demonstrate that alterations in peripheral conduit artery resting diameter and cardiac autonomic regulation were not the primary physiological mechanisms responsible for the observed exercise training-induced reductions in RBP.

6.2 The effects of isometric exercise training on resting blood pressure in healthy young men

Isometric bilateral leg exercise training consisting of four 2-minute muscular contractions performed 3 days per week significantly reduced resting SBP (4 mmHg) and MAP (3 mmHg) after 4-weeks of exercise training in young normotensive males. Although not statistically significant, these reductions in resting SBP (3mmHg) and MAP (2mmHg) were maintained until week 8 of exercise training. The observed magnitude of reduction in resting SBP was comparable to the effectiveness of previously reported aerobic and dynamic resistance exercise training modalities (Cornelissen and Smart, 2013). The observed magnitude of reduction in resting MAP was similar to those reported in a recent meta-analysis which assessed the overall effectiveness of isometric exercise training (Carlson et al., 2014) and similar to previous studies involving isometric bilateral leg extension exercise training in healthy young men following 4 weeks of exercise training (Wiles et al., 2010; Devereux et al., 2010). It is widely believed that even small decreases in RBP could significantly reduce the likelihood of hypertension-related morbidity and mortality (Whelton et al., 2002; Cook et al., 1995). It has been estimated that a 5 mmHg reduction in resting SBP would result in a 14% overall reduction in mortality due to stroke, a 9% reduction in mortality due to cardiovascular heart disease and a 7% decrease in all-cause mortality (Chobanian et al., 2003). Furthermore, it has been suggested that even a 3 mmHg reduction in resting SBP has prevented or postponed more than 11,000 deaths from cardiovascular heart disease in England over the past 7 years (Public Health England, 2014; Bajekal et al., 2012). Therefore, the small but significant reductions in RBP observed in the present isometric exercise training study could be advantageous in contributing to improved cardiovascular health outcomes.

It is evident that performing isometric bilateral leg extension exercise training at an intensity equivalent to between 24 and 33% MVC can induce significant reductions in RBP after just 4 weeks in healthy young men (Baross et al., 2012; Devereux et al., 2010; Wiles et al., 2010). Wiles et al. (2010) previously reported that individuals who performed an 8-week bilateral leg isometric exercise training had statistically significant reductions in RBP at week 8, whereas in the present study significant reductions in RBP were only observed at week 4. Despite a similar trend, reductions in RBP at week 8 were not statistically significant between control and experiment condition. Based on previous work, this finding was not anticipated, a potential explanation for the time course of adaptation in RBP observed in the present training study, may relate to the altered exercise-induced physiological stimulus at week 5 since absolute exercise intensity was increased at this time point. The increase in absolute exercise intensity during the latter 4 weeks of the training intervention, and this is likely to have caused an exaggerated pressor response, heightened intramuscular pressure and a heightened exercise-induced haemodynamic and metabolic stimulus. The latter part of the exercise training intervention (from weeks 5 to 8) may have presented an insufficient period to permit adaptation to the imposed exercise stimulus. Further work on the time course of changes in RBP associated with isometric exercise therefore is warranted in the future. Any intervention that induces potentially beneficial adaptations needs to be investigated over a longer time course to ensure that the intervention continues to promote the desired outcomes. To date there is limited long-term >12 weeks data on isometric exercise training, and the data presented here would indicate longer term studies need to be conducted before this method of training is utilised as a long term intervention to reduce RBP.

Nonetheless, the reductions observed in resting SBP and MAP may translate into a reduced likelihood of hypertension-related morbidity and mortality (National Institute of Clinical Excellence, 2011; Chobanian et al., 2003). Albeit the observed reductions were evident in

healthy young normotensive men, exercise training of this modality is capable of consistently lowering RBP in a population already within the normal ranges. This is a beneficial outcome given that in a population with pre-existing BP of as low as 115/75 mmHg the risk of developing cardiovascular disease doubles with each 20/10mmHg increase (Pescatello et al., 2004). Moreover, an SBP reduction of just 2 mmHg could reduce the risk of cardiovascular events mortality by 10% and the risk of ischaemic heart disease by 7% in a middle aged population without cardiovascular disease (Lewington et al., 2002). Therefore, the main findings from this research thesis add further support to the growing body of evidence indicating that isometric exercise training is an effective lifestyle intervention and a non-pharmacological alternative to reduce RBP.

6.3 The effects of isometric exercise training on peripheral vascular adaptations in healthy young men

Vascular endothelial function

The findings from the present exercise training study are the first to demonstrate a clinically relevant and significant reduction in RBP following 4 weeks of high intensity isometric bilateral leg exercise, accompanied by an improvement in systemic vascular endothelial function. Following 4 weeks of isometric exercise training, the vascular endothelium may have an increased ability to balance the synthesis and release vasoactive molecules in favour of vasodilatation. The results from the present training study infer that those individuals who experienced the largest reductions in resting MAP and DBP exhibited higher resting brachial artery FMD following 4 weeks of training. This provides additional evidence that isometric exercise training-induced reductions in RBP are inversely associated with improvements vascular endothelial functions, which may be reflective of a reduction in TPR (Bronas and Leon, 2013).

According to Poiseuille's Law, a small change in vessel diameter has a large effect on resistance to flow, as represented by improved vascular endothelial function. Thus, this improvement in endothelium-dependent vasodilatation of the conduit artery is likely to be a mechanism responsible, at least in part, for the observed RBP reduction with isometric exercise training (Smith and Fernhall, 2011; Fagard and Cornelissen, 2007). Despite the clear theoretical links presented above, improved endothelial function cannot be unequivocally confirmed as the sole mechanism responsible for lowering RBP since neither TPR, $\dot{Q}$, indices of cardiac autonomic function or neural regulation of vascular tone were measured at week 4 of the exercise training intervention. Nevertheless, an improvement in systemic vascular endothelial function in theory would allow enhanced vasodilatation of the peripheral arteries, and hence a decrease in peripheral vascular resistance leading to a reduction in RBP. This is in agreement with previously reported findings within the literature whereby exercise training has been shown to lower RBP and decrease TPR at rest (Hambrecht et al., 2000; Clausen, 1977). The current study measured the indices of cardiac autonomic function pre and post 8-week intervention, it is suggested that these measures are performed more frequently during future isometric training intervention studies.

There are few studies which have directly examined the relationship between vascular endothelial function and exercise training-induced RBP reductions in humans (Scholten et al., 2014; Cotie et al., 2014; Swift et al., 2012; Beck et al., 2013; Badrov et al., 2013; McGowan et al., 2007a; 2007b) and animal models (Mota et al., 2014). Two studies have demonstrated that improvements in vascular endothelial function are associated with beneficial reductions in RBP in healthy young individuals (Beck et al., 2013; Badrov et al., 2013). The results from the present training study are in agreement with previous research from Beck et al. (2013) who reported concomitant reductions in RBP (SBP: 10 mmHg and DBP: 8 mmHg) and brachial artery FMD by 34% when expressed as a percentage change from pre to post an 8-week

resistance exercise training intervention in young prehypertensive women. The present training study showed a comparatively similar magnitude of improvement in conduit artery vascular endothelial function (26%) when expressed as a percentage change from pre to week 4 of isometric exercise training. It is unclear from the results presented by Beck et al. (2013) whether concomitant reductions in RBP and improvement in conduit artery vascular endothelial function occurred at week 4 since these parameters were only measured at pre and post (8-weeks) training. Brachial artery FMD the present study increased by 2.27% (training: 8.65% vs. control: 6.38%), and the extent of this improvement may have positive implications for vascular health. As mentioned in Chapter 5, for every 1% improvement in brachial artery FMD the likelihood of the occurrence of cardiovascular events is reduced by 13% (Inaba et al., 2010). Isometric bilateral leg extension exercise training may be an effective lifestyle intervention to alter future cardiovascular outcomes and reduce the risk of cardiovascular disease since this study demonstrated an improvement in brachial artery FMD of 2.27%.

Endothelial dysfunction is a significant prognostic indicator of increased likelihood of experiencing an adverse cardiovascular event (Yeboah et al., 2007). In a previous study examining the relationship between endothelial function and risk of adverse cardiovascular events (Gokce et al., 2002a), those individuals who had experienced no prior events (7.3%) had a larger resting FMD than those who had a history of prior cardiovascular events (4.9%). The main finding from this thesis would support the effective role of isometric lower limb training in improving endothelial function in healthy individuals. It remains to be seen to what extent isometric exercise training can improve endothelial function in those populations with endothelial dysfunction, further research should examine if small improvements similar to that observed in this thesis may have the potential reduce long-term health risk.

Badrov et al. (2013) also reported exercise training-induced reductions in resting SBP following 4 weeks of isometric handgrip exercise training, and resistance vessel function was improved by 57% relative to baseline following 8 weeks of exercise training. This improvement is higher in magnitude compared to the improvement reported in conduit artery vascular endothelial function in the present isometric exercise training study. It is problematic to compare the time course and magnitude between the findings from Badrov et al. (2013) because of the location of the measurement at the resistance vessel and conduit vessel respectively. There is evidence that the mechanisms differ by which conduit and resistance vessels endothelial function are altered following exercise training in hypertensive rats (White et al., 1996). More specifically, resistance vessels have been proposed to be highly variable to mediators of endothelial- dependent vasodilatation other than nitric oxide such as endothelium derived hyperpolarising factor (Houghton et al., 1998; White et al., 1996) and this may explain the relatively greater increases in resistance vessel function in the study by Badrov et al. (2013).

There is limited research investigating the effectiveness of isometric exercise training to improve vascular endothelial function therefore direct comparisons between the same exercise modality are difficult to achieve, however research involving dynamic resistance exercise training is more readily available. Brachial artery FMD increased (mid training: 8.65 ± 1.02 % vs. mid control: 6.38 ± 1.14 %) compared to the non-exercising control condition in the present study. This magnitude of improvement is comparable to previous improvements in brachial artery FMD ranging between 1.70% and 2.07% following dynamic resistance exercise training and combined aerobic and dynamic resistance training interventions (Ashor et al., 2015; Spence et al., 2013). The improvement in brachial artery FMD following 4 weeks of isometric exercise training were also similar to those improvements observed at week 4 of an 8-week intervention (8.4%) (Tinken et al., 2008) and at week 2 of an 8-week intervention (8.6%) (Birk et al., 2012); both of these studies used a lower limb cycling exercise training intervention. In

summary, the overall magnitude of improvement in conduit artery endothelial function is comparable with dynamic resistance and combined exercise training modalities, and most importantly, the reductions in arterial blood pressure are associated with vascular functional adaptations.

There was a significant improvement in brachial artery FMD following 4 weeks of exercise training, however no such changes were evident at week 2, week 6 or week 8 of the training intervention. The observed improvements in brachial artery FMD did not occur until week 4 and therefore are in alignment with the concept that improved conduit artery vascular endothelial function typically occurs within the initial 2 to 4 weeks of exercise training (Hunt et al., 2013; Birk et al., 2012; Tinken et al., 2010). The results of the present study do not support the concept originally proposed by Laughlin (1995) that functional vascular adaptations are proceeded by permanent changes in artery structure, since no concomitant changes in artery diameter were observed during weeks 5 to 8 of the isometric exercise training intervention. In agreement with the present results, Birk et al. (2012) has also reported that lower limb cycling exercise training significantly increased in brachial artery FMD at week 2 and arterial remodelling not altered at any time point in the 8-week intervention. Although the improvement in brachial artery FMD occurred earlier in the study by Birk et al. (2012), the pattern was similar between studies i.e. there was an initial increase in FMD within the first 4 weeks, with no further changes in artery diameter from week 4 onwards.

Previous research has shown a significant improvement in vascular function within the first 2 weeks which was progressively increased or maintained until week 4 of the exercise training intervention (Hunt et al., 2013; Tinken et al., 2010; 2008). The more rapid onset and maintained improvement in vascular function in the aforementioned studies is likely to be related to the smaller amount of muscle mass involved in handgrip exercise and plantar flexion exercise. A

smaller amount of muscle mass involved in muscular contractions is considered to provide a significantly lower neurohumoral, haemodynamic and metabolic challenge compared to exercise involving large muscle groups (Atkinson et al., 2015). Arguably the traditional time course notion proposed by Laughlin (1995), that the rapid localised functional responses are surpassed by structural adaptations, may be more likely to occur in exercise training studies involving small muscle groups (Hunt et al., 2013; Tinken et al., 2010). The slower rate of improvement in vascular endothelial function in the present study may relate to the distinct exercise-induced haemodynamic changes in blood flow, shear rate and blood pressure known to vary between exercise modalities (Green et al., 2005). Furthermore, a larger amount of muscle mass involved during exercise may substantially influence over the exercise-induced haemodynamic stimuli, because the amount of muscle mass recruited has been shown to be positively related to the exercise pressor reflex (Gálvez et al., 2000; Seals et al., 1989; Mitchell et al., 1980).

The slower rate of improvement in vascular endothelial function in the present study may also be attributed to the exercise intensity, the exercise modality and the progression of the exercise stimulus over the isometric exercise training intervention. Although the same relative exercise training intensity was used throughout the isometric exercise training intervention, participants were retested at the end of week 4 using the discontinuous incremental isometric exercise test. The target training torque was significantly increased for the latter 4 weeks of the isometric exercise training intervention (See Table 16); and the level of overload may have been too great to elicit a state of vascular hormesis within the time frame investigated. In support of this theory, the measurement of electromyography has been used as an indirect index of central command during exercise (Taylor et al., 1988; Schibye et al., 1981) and the level of central command has previously been shown to be positively related to the number of motor units activated during an isometric muscular contraction (Mitchell et al., 1983; 1980). Taking this

into account, the observed decline in electromyography from the vastus lateralis during muscular contractions performed at a constant force might be reflective of improved functioning of the neuromuscular system within the first 4 weeks of present training study. Whereas, electromyography from the vastus lateralis remained elevated during muscular contractions performed at a constant force during week 5 to 8 (Section 5.5.10), which may indicate that the exercise stimulus remained a heightened neuromuscular challenge to which the body had not undergone supercompensation.

In light of this, recent evidence has demonstrated that adequate progressive overload brings about continuous and dynamic improvement in vascular endothelial function and increases in resting artery diameter over a more prolonged period (24 weeks) (Spence et al., 2013) than typically reported in previous time course studies (≤12 weeks) (Hunt et al., 2013; Birk et al., 2012; Tinken et al., 2010; 2008). Spence et al. (2013) reported that 24 weeks of dynamic resistance exercise training, that was overloaded in a progressive manner, resulted in a time course of functional and structural changes which did not lend themselves to conform with the traditional notion proposed by Laughlin (1995). Arguably, the highly dynamic process of balancing changes in vascular function and structure are particularly susceptible to a progressive overload of an exercise stimulus. The findings from Spence et al. (2013) would support the viewpoint that adaptations in vascular function and structure can occur at much later time points within an exercise training intervention than typically reported within the literature. It is widely acknowledged that vascular structural remodelling occurs in order to normalise transient repeated increases in shear stress (Green et al., 2004b). Based upon this assumption, structural adaptations may not have occurred in the present training intervention if the exercise-induced haemodynamic stimulus was still undergoing normalisation to find an optimal state of vascular homeostasis.

This is a potentially important finding that exemplifies the heightened exercise-induced stimulus may have induced functional overreaching (Kreher and Schwartz, 2012) and in combination with the inadequate recovery period prevented supercompensation (Bompa and Haff, 2009). The findings from this thesis indicate that vascular structural changes did not occur in measured variables during weeks 1 to 4 or weeks 5 to 8, and future work may elucidate as to whether the progression applied at week 5 in this study limited structural changes at week 8. It is currently unknown whether adaptations in all primary outcome variables would have occurred if measures were assessed a further 2 weeks upon cessation of the exercise training intervention, future direction of research could focus on this period (i.e. 10-week or 12-week exercise training interventions), and the application of progressive overload in these contexts.

Vascular structure

Eight weeks of high intensity isometric bilateral leg extension exercise training did not alter common femoral artery or brachial artery resting diameter at any time point throughout the intervention. These results demonstrate that structural changes, when assessed via resting artery diameter, were not essential for mediating RBP reductions following 4 weeks of exercise training. Interestingly, superficial femoral artery resting diameter was significantly increased at week 2 of training alongside increased antegrade blood flow and shear and lower retrograde shear rate. It could be implied form these results that skeletal muscle blood flow capacity had increased and that improved vascular reactivity had occurred downstream from the exercising limb because of the very early time these improvements occurred in the exercise training intervention. Previous studies have not reported changes in vascular structure to occur this early in an exercise training intervention (Tinken et al., 2010), substantiating the notion that these changes in superficial femoral artery diameter may in fact be functional as opposed to structural. The significant improvement in superficial femoral artery resting diameter at week

2 may be representative of a functional improvement, yet this finding may suggest that this conduit artery is more responsive to lower limb training and therefore future studies should consider assessment of vascular function in the superficial femoral artery. Future studies may also benefit from measuring alternative markers of vascular structural adaptations such as intima media thickness/lumen ratio as this may permit clearer differentiation from vascular functional changes (Dinenno et al., 2001; Bots et al., 1997).

There were no significant changes in resting artery diameter in either the brachial, common femoral or superficial femoral artery during the latter 4 weeks of the exercise training intervention which does not support the original hypothesis by Laughlin (1995) that structural remodelling of the vasculature takes over from earlier functional improvements. The lack of observable change in vascular structure (and function) between weeks 5 to 8 would coincide with the findings that RBP was unaltered following 8 weeks of isometric exercise training. To date, the findings are equivocal with reports of increased localised or systemic artery diameter (Stebbings et al., 2013; Spence et al., 2013; Baross et al., 2012; Tinken et al., 2010; Thijssen et al., 2007; Rakobowchuk et al., 2005; Miyachi et al., 2001; Dinenno et al., 2001). The lack of change in resting brachial and common femoral artery diameter reported in the present study are in alignment with other studies have reported no change in localised or systemic artery diameter (Thijssen et al., 2013; Hunt et al., 2013; Birk et al., 2012; Tinken et al., 2008). A possible explanation for the unaltered common femoral resting artery may relate to the largely unresponsive nature of conduit arteries with large diameters, particularly in the lower limbs, to a shear stress stimulus in healthy individuals (Walther et al., 2008; Wray et al., 2005).

The discrepancy between the results of the present study and those reported by Baross et al. (2012), who reported increased resting common femoral artery diameter after 8-weeks of training, are likely to be attributed to factors including differences in resting artery diameter at

baseline, initial training status, and health status of the participants (Green et al., 2014b). Previous research has shown that unhealthy populations have a smaller baseline artery diameter and undergo significant localised structural adaptations following short-term leg extension exercise training (Billinger et al., 2009). This may help to explain why common femoral artery diameter was higher at baseline in the present training study compared that reported in the study by Baross et al. (2012) in middle aged pre hypertensive men (9.34 mm vs. 8.10 mm respectively), which arguably reduces the likelihood for adaptation due to encroachment on a ceiling diameter.

Furthermore, a key consideration that may help to explain the present findings may reside in the progressive overload of the exercise stimulus. It is important to acknowledge that although significant reductions in RBP were observed at week 4 in the present study in the presence of improved brachial artery FMD, it is not possible to eliminate the prospect of delayed improvements in structural vascular adaptations (i.e. beyond 8 weeks). The present exercise training intervention might have been insufficient in total duration to balance the highly dynamic nature of adaptation to the exercise stimulus. This is feasible given that the modification of the isometric exercise stimulus introduced at week 5 could have adjusted the dynamic balance of functional versus structural adaptations, which is in alignment with the concept proposed by Spence et al. (2013).

Vascular blood flow patterns

Previous research has attempted to investigate the relationship between exercise training-induced adaptations in resting blood flow, shear rate, vascular endothelial function and RBP (Scholten et al., 2014). The results from the present study showed a trend for improved brachial artery antegrade blood flow at rest at week 4 and significantly improved superficial femoral

artery antegrade blood flow at rest at week 2 (Section 5.5.7). Previous research has shown that 12 weeks of aerobic exercise training significantly reduces resting retrograde shear rate (Scholten et al., 2014). Furthermore, reductions in resting retrograde shear rate have been associated with enhanced conduit artery vascular endothelial function and reductions in RBP in healthy and unhealthy populations (Scholten et al., 2014). The data from the current study demonstrates a trend between improvements in brachial artery FMD and reductions in retrograde shear rate (P =0.058) (Section 5.5.11). This could indicate that there are favourable changes in shear stress patterns may be a key physiological stimulus that could contribute to improvements in vascular health.

It has been suggested that improvement in antegrade blood flow is indicative of enhanced skeletal muscle blood flow capacity to the non-exercising limbs and arterial compliance, this may contribute to a reduction in peripheral vascular resistance (Bronas and Leon, 2013; Rippe, 2012). Favourable changes in blood flow patterns and total blood flow have been implicated in the beneficial anti-atherogenic effect of exercise training on vascular endothelial cell phenotype (Whyte and Laughlin, 2010). Regions of sufficient levels of blood flow, which is predominantly laminar in nature may offer protection against pro-atherogenic conditions of low and turbulent flow (Augustin et al., 2009). Whereas in vitro and in vivo animal studies have demonstrated that resting shear stress patterns characterised by high levels of retrograde shear rate can increase pro-atherogenic gene expression (Laughlin et al., 2008). Resting antegrade blood flow and antegrade shear rate increased and retrograde shear rate decreased in the superficial femoral artery at week 2, this would infer that blood flow moved in a predominantly forward direction and potentially became more laminar in nature. These findings may have potential implications for promoting an anti-atherogenic state of flow within the vasculature, and therefore may contribute to reduced risk of developing atherosclerosis and the risk of adverse cardiovascular events.

6.4 The effects of an acute bout of isometric bilateral leg extension exercise at high and low intensities on peripheral vascular endothelial function

The primary aim of the acute exercise study within this thesis was to ascertain the precise antegrade and retrograde components of blood flow and shear rate patterns during an acute bout of high and low intensity isometric bilateral leg exercise. To date, research investigating the blood flow and shear rate patterns induced during an acute bout of isometric lower limb exercise is sparse. The acute exercise study within this thesis demonstrates novel findings with regard to the patterns of arterial blood flow and the influence this exerts upon endothelium-dependent vasodilatation within the brachial artery.

The concomitant elevation in antegrade and retrograde components of blood flow and shear rate during isometric bilateral leg extension muscular contractions resulted in repeated forward and backward oscillations of flow across the surface of the vascular endothelium. This in turn may act as a potent modulator for long-term adaptations in endothelial cell membrane deformation and signalling events in favour of nitric oxide production (Dimmeler and Zeiher, 2003). There is a clear difference in the stimuli created during the isometric bilateral leg extension exercise when performed at a high compared to low intensity, which may go some way to help explain why the transient reduction was observed only post high intensity exercise. Increased antegrade shear rate during isometric muscular contractions has previously been attributed to elevated exercising SBP (Thijssen et al., 2009b), and increased retrograde shear rate during isometric muscular contractions has been attributed to the compression of blood vessels within the exercising skeletal muscle (Sadamoto et al., 1983). It could be implied that whilst an increase in intramuscular pressure and/or sympathetically mediated vasoconstriction is likely to have caused the increased retrograde shear rate (Padilla et al., 2010; Creuder et al., 2009), there was an elevation in exercising SBP and heart rate during high intensity exercise

in the present study (see Table 16). This was likely to have occurred in order to off-set increased retrograde shear rate during muscular contraction via increased antegrade shear rate to maintain or attempt to maintain adequate blood flow perfusion to the exercising muscle.

The present study also revealed more pronounced post exercise hyperaemia following high versus low intensity isometric exercise. This is arguably an integral aspect of the exercise-induced haemodynamic stimulus for subsequent alterations in vascular endothelial function. The magnitude of post exercise hyperaemia is considered to reflect the extent of metabolite accumulation (Bangsbo et al., 1998; Kagaya and Homma, 1997; Walløe and Wesche, 1988) as well as the magnitude of oxygen debt and the subsequent level of the stimulus for the pressor reflex response to exercise (Osada et al., 2003; Taylor et al., 1988). The significant increase in blood flow upon release of high intensity isometric muscular contractions may be reflective of a higher magnitude of oxygen debt and metabolite accumulation compared to low intensity isometric muscular contractions. An intensity-dependent increase in blood lactate accumulation has been documented during sustained isometric bilateral leg exercise (Devereux et al., 2012). Furthermore, an intensity-dependent increase in blood lactate accumulation during isometric muscular contractions has been positively associated with the magnitude of post exercise hyperaemia (Taylor et al., 1988). The intensity-dependent increase in the magnitude of post exercise hyperaemia may also have been due to heightened downstream flow-mediated vasodilatation in the profunda femoral artery that has been previously reported to occur (Koller and Kaley, 1991). In addition, factors including increased intramuscular pressure, mechanical compression of the vasculature, and subsequent ischemic conditions during muscular contractions as well as local metabolite accumulation within the quadriceps are likely to have contributed to the significantly heightened post exercise hyperaemia in the high versus low isometric exercise intensity.

An important finding from the present study is that oscillatory shear index was lower in comparison to baseline values during both high and low intensity isometric muscular contractions despite the increase in retrograde blood flow. This finding is in alignment with the results of Padilla et al. (2010) whereby a decrease in oscillatory shear index compared to baseline values was reported to occur during rhythmic handgrip exercise in healthy young individuals. Moreover, oscillatory shear was reduced to greater extent upon cessation of high intensity muscular contractions compared to low intensity isometric muscular contractions. This is likely to be attributed to the concomitant reductions in retrograde shear rate being offset by greater increases in antegrade shear rate. The results from the present study are in alignment with previous reports of low or temporarily eliminated retrograde blood flow in peripheral vessels during periods of reactive hyperaemia (Mahler et al., 1977) and this has been considered to be caused by reduced pressure from wave reflections most likely due to increased vasodilatation downstream (Nichols et al., 2011). It could be inferred from the noticeable absence of retrograde blood flow and retrograde shear rate during the recovery period, that the arteries and arterioles downstream from the profunda femoral artery such as in the superficial femoral or popliteal artery, resistance vessels and microvasculature, may undergo vasodilatation in order to normalise the heightened exercise-induced shear stress stimulus although this requires further investigation.

The Starling-Resistor Theory might provide another possible explanation for the observed significant increases in post exercise blood flow immediately following cessation of isometric muscular contraction (Halliwill and Minson, 2010). The change in antegrade and retrograde blood flow from contraction to cessation of contraction, and the resultant shear rate patterns, are likely due to the balance between central arterial pressure and the microvasculature critical closing pressure. As such, increased vasodilatation downstream would theoretically lower the critical pressure of resistance. This may explain the larger increase in antegrade shear rate and

decreased retrograde shear rate and oscillatory shear index upon cessation of high intensity isometric muscular contractions. Elevations in oscillatory shear and retrograde shear rate *in vitro* are considered to exert a negative impact upon vascular health via increased upregulation of endothelin-1, increased adhesion molecules and increased superoxide production by xanthine oxidase from the mitochondria (Takabe et al., 2011; McNally et al., 2003; Ziegler et al., 1998b). It would therefore appear advantageous for vascular health that isometric bilateral leg extension exercise can elicit a temporary reversal in retrograde shear rate and oscillatory shear index during the recovery period in between muscular contractions. According to *in vitro* studies outlining the potential detrimental impact of oscillatory and turbulent flow behaviour upon the vascular endothelium (Jenkins et al., 2013), the transient reductions in retrograde shear rate may have a favourable long term influence over the vascular endothelium although further research is required to substantiate this concept.

This is the first study of its kind to demonstrate that high intensity isometric bilateral leg extension exercise elicited a greater increase in antegrade shear rate and reduction in retrograde shear rate upon release of muscular contractions compared to that induced by low intensity isometric bilateral leg extension exercise (see Chapter 4). Furthermore, only an acute bout of high intensity isometric exercise increased retrograde shear rate during isometric muscular contractions and decreased retrograde blood flow and retrograde shear rate upon cessation of isometric muscular contractions compared to baseline values. In marked contrast, acute bout of low intensity isometric bilateral leg extension exercise did not alter retrograde shear rate at any point in comparison to baseline values.

The exercise-induced haemodynamic responses are particularly notable when the change in blood flow and shear rate are expressed as a percentage change from muscular contraction to the recovery period in between muscular contractions. High intensity isometric exercise

resulted in a larger percentage increase in antegrade shear rate from contraction to recovery (Δ 41%); there was no change in antegrade shear rate from contraction to recovery following low intensity isometric exercise. High intensity isometric exercise also resulted in a larger percentage decrease in retrograde shear rate from contraction to recovery (Δ -50%) compared to low intensity isometric exercise (Δ -13%). A recent study showed that isometric bilateral leg exercise performed at a high intensity as part of graded exercise test brought about a lessor increase in antegrade shear rate (Δ 15%) and lessor decrease in retrograde shear rate (Δ -18%) from contraction to recovery (Smith, 2014) compared to the present study findings. This evidence supports the notion that isometric bilateral leg extension exercise performed at a constant force may produce a different exercise-induced haemodynamic response, potentially more effective at promoting antegrade shear rate and reducing retrograde shear rate upon cessation of muscular contractions, in comparison to isometric exercise performed at a constant level of electromyography where cardiovascular responses are more steadily controlled (Schibye et al., 1981). Therefore, it could be implied that the method of prescribing isometric exercise intensity according to a sustained torque versus electromyography in healthy young individuals is likely to alter blood flow perfusion within the exercising limbs. It is also likely to affect the degree of mechanical compression of the blood vessels, intramuscular compression of the blood vessels and the net balance between centrally mediated vasoconstriction and local metabolic vasodilation (Hansen et al., 1993; Gaffney et al., 1990).

In summary, increased antegrade and retrograde blood flow during isometric muscular contractions is likely to occur in order to overcome the level of intramuscular pressure and degree of mechanical compression (Rowell, 1993). This heightened blood flow response is likely to permit adequate muscle blood flow perfusion, which is arguably also achieved by substantial increases in exercising blood pressure (Smolander et al., 1998; Petrofsky et al., 1975; Goodwin et al., 1972). Isometric bilateral leg exercise performed at a constant force

(equivalent to 32 % MVC) caused a significant increase in post exercise hyperaemia, and it is likely to be reflective of the magnitude of the oxygen debt and accumulation of locally released metabolites (Osada et al., 2003; Bangsbo et al., 1998; Kagaya and Homma, 1997; Gaffney et al., 1990; Walløe and Wesche, 1988). A sustained elevation in exercising blood pressure and slow rate of wash out of accumulated metabolic factors within the exercising skeletal muscle upon cessation of isometric muscular contractions may help to explain the greater post exercise hyperaemia following high versus low intensity isometric bilateral leg extension exercise. The finding that brachial artery FMD was immediately and temporarily reduced following an acute bout of high intensity, but not low, isometric exercise (see Chapter 4). This finding may indicate the presence of sympathetic vasoconstriction within the non-exercising limbs in order to direct increased blood flow to exercising limbs during the recovery period in between isometric muscular contractions. In support of this proposal, sympathetically meditated vasoconstriction has been shown to occur in active areas in order to redirect more blood flow to the fatigued exercising muscle beds (Frances et al., 2008; Shoemaker et al., 2007; Rowell and O'Leary, 1990).

The magnitude and patterns of blood flow and shear rate, blood pressure-mediated differences across the vessel wall, increased oxidative stress and inflammatory markers may be associated with decreased FMD (Carter et al., 2013; Hunt et al., 2013; Birk et al., 2012; Newcomer et al., 2011). Based upon the findings from Peters et al. (2006) who reported increased oxidative stress following acute isometric exercise, it could be speculated that the physiological stimulus produced by an acute bout of isometric bilateral leg extension exercise may have caused transient heightened levels of oxidative stress. The downregulation of oxidative stress, increased antioxidant capacity and pressure-mediated haemodynamic responses within the peripheral vasculature may be potential precursory mechanisms for beneficial adaptations observed in vascular function thus mediating exercise training-induced RBP reductions. These

temporary elevations in oxidative stress during an acute isometric exercise bout may play a crucial role in the normal vascular response to exercise with prolonged exercise training (Suvorova and Kojda, 2007).

Repeated exposure to an acute exercise bout has been shown to produce excess hydrogen peroxide, a reactive oxygen species molecule produced in the mitochondria of endothelial cells, which diffuses through the vascular wall increasing the expression and activity of eNOS (Lauer et al., 2005; Cai et al., 2001; Drummond et al., 2000). A reduction in oxidative stress and/or an upregulation of antioxidant pathways may be a likely precursory mechanism for enhanced endothelium-dependent vasodilatation following isometric bilateral leg extension exercise training. Most recently, improvements in brachial artery FMD have been associated with lower nitrotyrosine (a critical marker of endothelial oxidative stress) content in venous endothelial cells (Gurovich et al., 2014). Endothelial cells are exposed to cyclic strain from distention of the arteries caused by an increase in blood pressure. Cyclic strain has been shown to upregulate endothelial nitric oxide synthase mRNA expression (Ziegler et al., 1998b; Harrison et al., 1996), endothelial derived hyperpolarising factor synthase, vascular endothelial growth factor and reduce vascular smooth muscle cell proliferation in cultured cells (Schad et al., 2011). Isometric exercise performed at a high intensity elicited a higher exercising blood pressure, heart rate and distinct blood flow and shear rate pattern compared to the lower intensity (see Sections 4.5.2 and 4.5.3). It is plausible that vascular adaptations may be pressure mediated (Newcomer et al., 2011), not solely shear stress-mediated, and this is supported by recent evidence which has demonstrated that exercising blood pressure is positively associated with improved resting vascular endothelial function (Lambaise et al., 2014). The extent to which cyclic strain signals the upregulation of pro-atherogenic gene expression or anti-atherogenic gene expression *in vivo* remains unclear and warrants further investigation, this may have a potentially important role in the exercise-induced haemodynamic stimulus for beneficial

vascular adaptations and ultimately favourable effects on RBP. This thesis has highlighted that the isometric exercise-induced increases in blood flow and shear rate upon cessation of muscular contraction may be an important modulator contributing to the immediate reduction in endothelium-dependent vasodilatation in an acute exercise model.

6.5 Specific exercise-induced blood flow and shear stress patterns as a potential stimulus for vascular adaptations and resting blood pressure reductions

The important mechanistic role of the exercise-induced haemodynamic stimulus in bringing about beneficial vascular adaptations has been highlighted (Padilla et al., 2014; Newcomer et al., 2011; Laughlin et al., 2008). However the exploration of the association between acute exercise-induced haemodynamic stimuli and chronic exercise training-induced adaptations in RBP is a concept described as rarely explored in humans (Raven and Chapleau, 2014; Green et al., 2011c; Whyte and Laughlin, 2010). The haemodynamic alterations that occur during an acute bout of exercise have been described as having a major role in modifying the expression of genes linked to alterations in vascular phenotype (Newcomer et al., 2011; Padilla et al., 2010). To date, there has been no investigation into the optimal blood flow and shear rate magnitude and/or pattern responsible for alterations in peripheral vascular function and/or structure and the overlying impact this has upon TPR and RBP.

It could be inferred from the acute exercise study within this thesis (Chapter 4) that the distinct blood flow and shear rate patterns induced as a consequence of a high intensity bout of isometric bilateral leg extension exercise were sufficient to improve resting brachial artery FMD, resting blood flow patterns within peripheral conduit arteries and lowered RBP after 4 weeks of training. Although the specific component of blood flow and shear rate responsible for modulating favourable adaptations in vascular function have not been isolated, heightened levels of antegrade blood flow and shear rate, reduced levels of retrograde blood flow,

retrograde shear rate and/or oscillatory shear index during the recovery period in between contractions may play an important role. Moreover, a combination of stimuli including the amalgamated effect of increased antegrade and retrograde shear rate during muscular contractions, diminished retrograde shear rate as well as increased antegrade shear rate during the recovery period may be key modulators for subsequent adaptations. These may be important exercise-induced haemodynamic stimuli given that only the high intensity exercise condition (and not the low intensity exercise condition) caused the aforementioned acute haemodynamic response.

Previous research has alluded to a possible antegrade shear rate threshold during exercise bouts, which may positively influence the magnitude and rate of vascular adaptation (Atkinson et al., 2015; Tinken et al., 2009). The heightened antegrade blood flow and antegrade shear rate observed was of a sufficient magnitude to initiate an effect on vascular endothelial function. However, these components were elevated to a similar extent between high and low intensity exercise conditions, leading to the notion that aspects of the exercise-induced haemodynamic profile other than antegrade blood flow and shear rate may be of more interest when trying to establish the mechanism responsible for vascular adaptations. Only one study has attempted to investigate the impact of elevations in retrograde blood flow pattern using an external compression cuff upon vascular endothelial function over a prolonged period of time (Thijssen et al., 2015). Two weeks of continuously applied external compression was reported to impair localised vascular endothelial function and this was attributed to elevated retrograde shear rate during exercise (Thijssen et al., 2015). Arguably, the continuous application of an external compression cuff is not representative of repeated bouts of acute exercise and therefore the specific acute exercise-induced haemodynamic stimuli responsible for mediating subsequent beneficial adaptations in vascular function and blood pressure require clarification.

The acute exercise-induced haemodynamic stimuli induced by high intensity isometric exercise have been shown to have an immediate down regulatory effect on systemic vascular endothelial function (see Chapter 4). The results from the present study support the existence of a biphasic response; brachial artery FMD was immediately reduced and returned almost to baseline values within 30-minutes post high intensity isometric exercise. This biphasic response has been proposed to be an essential catalyst for favourable vascular adaptations in function and structure, a process referred to as *vascular hormesis* (Dawson et al., 2013). This initial down regulation of vascular function may be a key modulator of subsequent chronic upregulation of resting vasodilator function, and therefore reductions in TPR and lowered RBP. The main findings from the isometric exercise training study within this research thesis would tend to support the vascular hormesis theory given that both RBP and brachial artery FMD were significantly improved following 4 weeks of isometric exercise training (see Chapter 5) performed at the same high intensity as utilised within the acute exercise study in this thesis.

To date, only one study has combined the assessment of vascular endothelial function in an acute exercise model and chronic exercise training adaptations in vascular endothelial function and RBP (McGowan et al., 2006). Whilst McGowan et al. (2006) did not investigate the haemodynamic responses caused by an acute bout of isometric handgrip exercise, brachial artery FMD was attenuated immediately post-acute isometric handgrip exercise at the start of an 8-week training intervention. Interestingly, when the same acute isometric handgrip exercise protocol was performed, as part of a training intervention three times per week for a total of 8 weeks, resting brachial artery FMD was improved and non-significant reductions in resting MAP were evident. Furthermore, when brachial artery FMD was assessed immediately post isometric handgrip exercise the transient reduction in FMD was less noticeable (McGowan et al., 2006). This is the only study of its kind to demonstrate such translation of changes in vascular endothelial function from an acute exercise model into an 8-week exercise training

model. This finding, in conjunction with the results from the present thesis, are likely to have implications for the way that acute responses in vascular function are measured to inform the effectiveness and progression of an exercise training programme. It is currently unknown what the transient reduction in brachial artery FMD changes in the response to cumulative bouts of acute isometric bilateral leg exercise over an 8-week exercise training intervention.

6.6 Interpreting the physiological mechanism(s) responsible for isometric exercise training-induced reductions in resting blood pressure

The physiological mechanism(s) by which isometric exercise training-induced reductions in resting blood pressure have been proposed to occur include alterations in cardiac function and structure, vascular function and structure, autonomic regulation of the cardiovascular system, neural regulation of vascular tone, oxidative stress and ischemia. Improvement in systemic vascular endothelial function and regional blood flow patterns are likely to have contributed to a reduction in TPR and are seemingly valid mechanisms for the RBP lowering effects of isometric exercise training as evidenced in the present training study results (Section 5.5). The improvement in vascular endothelial function as assessed via brachial artery FMD (Corretti et al., 2002; Celermajer et al., 1992) in the present thesis may be attributed to the upregulation of endothelial nitric oxide synthase and nitric oxide bioavailability (Hambrecht et al., 2003; Delp and Laughlin, 1997; Sessa et al., 1994). Increased endothelial nitric oxide synthase substrate (L-arginine), reduction in endothelial nitric oxide synthase inhibitor, enhanced vascular regeneration through endothelial progenitor cells (Giannotti et al., 2010) and enhanced expression of vascular endothelial growth factor (Servos et al., 1999) may play a role in the mediating improved vascular endothelial function. There is some evidence to suggest that alteration in early endothelial progenitor cell phenotype is responsible for enhanced repair capacity, and this may be a potential mechanism responsible for the upregulation of endothelial nitric oxide synthase activation and nitric oxide bioavailability (Giannotti et al., 2010).

Furthermore, the improvement in brachial artery FMD at week 4 may be explained by improved shear stress-induced signal transduction within endothelial cells including the PI3 Kinase signalling pathway and endothelial cell membrane channel activation via mechanisms such as deformation and cytoskeleton reorientation (Johnson et al., 2011).

Recent research has suggested that additional biomarkers of vascular endothelial function, including increased plasma concentrations of prostaglandin and decreased endothelin-1, might play a role in the improved vascular function (Beck et al., 2013). Enhanced nitric oxide endothelium-dependent vasodilatation is likely to have been responsible for the observed increase in systemic resting antegrade blood flow, however the possibility that an attenuation in vasoconstrictor function via either neural or hormonal factors cannot be excluded. In addition, the endothelium is a primary sensor for changes in shear stress which has been proposed to mediate changes in artery function and the arterial remodelling process (Green et al., 2011c). It is not possible to ascertain from the present study whether the observed improvements in brachial artery FMD were mediated via a shear stress-dependent mechanism and therefore this mechanism requires clarification in future studies involving isometric exercise. This observed improvement in systemic vascular endothelial function is considered to be mediated through a nitric oxide and endothelium dependent mechanism (Green et al., 2004b; Corretti et al., 2002), blockade experiments were not performed as part of this thesis to substantiate that FMD changes were endothelium- or nitric oxide-dependent.

There is a large body of evidence demonstrating that endothelium-independent vasodilatation, an indicator of sensitivity of vascular smooth muscle cells to nitric oxide, is unchanged in healthy individuals following exercise training (Birk et al., 2012; Llewellyn et al., 2012; Tinken et al., 2009; Goto et al., 2003). There is evidence to suggest this mechanism plays an important role in enhanced vasodilator capacity in healthy humans and this could be confirmed in future

studies involving isometric exercise training. There are a number of intrinsically mediated vasoactive substances other than nitric oxide produced by the vascular endothelium such as prostaglandins, which elicit hyperpolarisation of the vascular smooth muscle cells. Endothelium derived hyperpolarising factors such as hydrogen peroxide elicit vasodilatation through stimulation of the potassium channels within the vascular smooth muscle cells (Durand and Gutterman, 2013). Evidence has recently emerged which demonstrates that heightened intramuscular pressure can shift the vasodilator mediator of FMD from nitric oxide to hydrogen peroxide in healthy human cannulated tissue (Beyer et al., 2014). Nonetheless, this should not detract from the wealth of accumulated evidence that brachial artery FMD is an indicator of nitric oxide-mediated and endothelium-dependent dilatation under normal resting conditions in human conduit arteries (Green et al., 2014a; Doshi et al., 2001). Therefore, the improvements in resting brachial artery FMD in the present exercise training study are most likely to be mediated by a nitric oxide endothelium-dependent mechanism.

All measures of conduit artery endothelial function and structure were unaltered following 8 weeks of exercise training whereas TPR was significantly increased; this may be due to excessive downstream vasodilatation within the active skeletal muscle beds. In support of this theory, systemically mediated splanchnic vasoconstriction has been shown to occur during isometric exercise in order to help redirect blood flow (Shoemaker et al., 2007), most likely to areas activated by accumulation of metabolites via the pressor reflex and group III muscle afferents (Rowell and O'Leary, 1990). An elevation in TPR following 8 weeks of the isometric exercise training intervention may have diminished the previously observed significant reduction in RBP observed at week 4. Although speculative, this may have occurred in an attempt to maintain functional blood pressure homeostasis.

Recent evidence from animal models suggests that control of arterial blood pressure via baroreflex regulation is predominantly determined at the level of the vasculature (Sakamoto et al., 2015). A mild to moderate reduction in central blood volume, known to deactivate cardiopulmonary baroreflex receptors, can induce sympathetically mediated peripheral vasoconstriction in order to maintain sufficient arterial blood pressure during reduced $\dot{Q}$ (Levick, 2010; Saitoh et al., 2008). This explanation would fit with the proposed hypotheses that heightened downstream peripheral vasodilatation have occurred downstream from the exercising muscle beds. An increase in downstream vasodilatation would trigger an increase in sympathetic mediated vasoconstriction within the inactive muscle beds, or elsewhere in the peripheral circulation, required an increase central blood volume and venous return in order to maintain RBP within normal ranges. This underlying mechanism appears to coincide with the statistically significant increase in TPR, decreased SV and unchanged $\dot{Q}$ following 8-weeks of high intensity isometric bilateral leg extension exercise training in normotensive young men. Individuals within the present training study possessed optimal RBP *a priori* in accordance with the Joint National Committee Evaluation and Treatment of High Blood Pressure Guidelines (Chobanian et al., 2003). It is plausible that negative feedback via stimulation of the baroreflex receptors occurred in order to elevate sympathetic outflow to the vasculature to increase TPR in response to excessive peripheral vasodilatation (Wehrwein and Joyner, 2013) in order to maintain RBP within optimal levels.

There is limited evidence to support change in the function of the autonomic nervous system as a mechanism for isometric exercise training-induced reductions in RBP (Millar et al., 2013; Badrov et al., 2013; Wiles et al., 2010; Ray and Carrasco, 2000). Cardiac autonomic activity remained unaltered from pre to post 8 weeks of isometric exercise training (Section 5.5.5). Although this finding does not support its role as a mechanism for training-induced reductions in RBP, heart rate variability was not measured at week 4 of training and therefore an

improvement in cardiac autonomic regulation in favour of parasympathetic dominance or reduced sympathetic outflow to the heart cannot be disregarded. It is widely accepted that heart rate variability as a measure is subject to high variability and alterations within the autonomic regulation of the cardiac muscle are not necessarily indicative of end-organ effects (Agapitov et al., 2008). In light of this point, sympathetic nervous activity to skeletal muscle was also not assessed in the present exercise training study and therefore it is not possible to eliminate augmented sympathetic vasoconstrictor responsiveness (α1-adrenoreceptor mediated vasoconstriction) within skeletal muscle at rest (Just and DeLorey, 2016) as a mechanism for isometric exercise training-induced reductions in RBP. It is plausible that peripheral sympathetic modulation within the skeletal muscle might have been attenuated at week 4 and augmented at week 8, thus contributing to the significant reduction in RBP and non-significant reduction in RBP at week 4 and week 8 respectively. It is possible that alterations in autonomic nervous system function via increased in peripheral sympathetic vasoconstrictor activity may have been present elsewhere in the vasculature which may also help to explain the observed increase in TPR at week 8 of isometric exercise training.

Research has demonstrated that the sympathetic nervous system is likely to exert a significant influence over the acute exercise-induced haemodynamic stimulus and therefore the role of the sympathetic nervous system in regulating long-term adaptations within the peripheral vasculature should not be overlooked. Recent research has shown that muscle sympathetic nerve activity exerts a significant influence over conduit artery shear rate patterns, which in turn influence the redistribution of blood flow, blood vessel capacitance, reflex pathways and stroke volume (Padilla et al., 2010). It was not possible to determine from the results of the present study whether reductions in sympathetic nervous system activity were, in part, responsible for the improved resting vascular function and blood flow patterns. Interestingly, alterations in vascular tone are considered to reflect the interaction between sympathetically

driven vasoconstriction and nitric oxide-mediated endothelium-dependent FMD within the peripheral vasculature (Padilla et al., 2014), and it may be that alterations in *functional sympatholysis* as a result of acute exercise help to shape vascular adaptations to chronic exercise training (Mortensen et al., 2014). Although there is much discussion surrounding the direct influence the sympathetic nervous system exerts over vascular tone, the autonomic nervous system also maintains control over the release of neurohumoral factors including circulating catecholamines and activation of renin-angiotensin-aldosterone system (Thomas, 2011). Therefore, an attenuation in circulating hormones such as angiotensin-II and noradrenaline cannot be excluded as a potential mechanism responsible for isometric exercise training-induced reductions in RBP.

7. CONCLUSION

The overlying aim of this thesis was to more clearly define the physiological mechanisms responsible for isometric exercise training-induced reductions in RBP. The primary objective of this research thesis was to investigate whether alterations in resting peripheral vascular function and structure were the physiological mechanisms responsible for isometric exercise training -induced reductions in RBP. This thesis has demonstrated that isometric bilateral leg extension exercise training significantly enhanced conduit artery vascular endothelial function and conduit artery antegrade blood flow and shear rate in healthy young men, which has implications for decreased likelihood of hypertension-related morbidity and mortality. The improvement in brachial artery vascular endothelial function was inversely associated with reductions in resting blood pressure. This is likely to have important implications for overall cardiovascular and vascular endothelial cell health. The improvements in peripheral vasculature endothelial function occurred within the initial 4 weeks of isometric exercise training, whereas indices of vascular structure remained unaltered throughout the 8-week

training intervention. A more diligent application of progressive overload of the exercise stimulus over time may be necessary when examining the physiological mechanism responsible for RBP reductions. The acute isometric exercise model should be used more appropriately to further understanding of chronic adaptations in cardiovascular parameters following exercise training. An acute exercise model is seemingly advantageous in the identification of optimal manipulation of acute programme variables based upon the exercise-induced haemodynamic stimulus it affords. This explicitly relates to exercising blood flow and shear rate pattern during and upon immediate cessation of isometric muscular contractions, which are likely to be an integral part of the physiological stimulus responsible for vascular adaptations. The main findings from this research thesis should pose as useful preliminary findings for future isometric bilateral leg extension exercise training studies to bridge the gap in existing knowledge in the translation of exercise-induced haemodynamic responses into acute cardiovascular responses and subsequently chronic cardiovascular adaptations to isometric exercise.

7.1 Future directions

Future research should investigate isometric exercise in the form that it is most likely to be performed in a real world setting. This might be in the form of isometric bilateral leg exercise used in isolation, isometric exercise as part of a home-based training method or as part of a combined exercise training intervention. This is particularly poignant given the increasingly reported advantageous effects combined exercise training has been shown to exert on vascular endothelial function and resting blood pressure. Furthermore, it is essential to investigate a range of isometric exercise intensities to obtain further mechanistic insight into the exercise-induced haemodynamic stimulus associated with the most beneficial adaptations within the peripheral vasculature and resting blood pressure. Future studies involving isometric exercise training ought to elucidate the acute exercise-induced haemodynamic stimuli responsible for

improvements in resting vascular endothelial function and resting blood pressure. Moreover, future isometric bilateral leg exercise training studies should investigate shear rate, independent of the alternative exercise-induced stimuli, as the primary physiological stimulus for responsible for bringing about exercise training-induced vascular adaptations.

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Websites

www.bmus.org www.seniam.org

APPENDIX 1

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: **……..**

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

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

8. Are you presently taking any substances which may affect your performance?

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

9. 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.

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

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

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

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

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

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

13. 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: **……**

Contact information of your general practitioner (GP)

Name of your General Practitioner: .....

Address of your General Practitioner's Surgery to which you are registered:

.....

I (the named participant above) give permission for the researcher to contact my general Practitioner (if required) to inform them of any abnormalities which may arise or to discuss your current health and fitness status if you have consulted your GP within the last six months.

Signature of Subject: ……..

Signature of Sport Scientist: ……..

Date: …….

APPENDIX 2

PARTICIPANT INFORMATION

A research study is being conducted at Canterbury Christ Church University (CCCU) as part of a PhD thesis by Lucinda Howland. This study has been approved within the University by the Faculty of Social and Applied Sciences Research Ethics Committee.

Lay Title of Project*: "The reliability of blood pressure monitoring devices and non-invasive doppler ultrasound to measure blood pressure and specific cardiovascular variables".*

Part One

The reliability and validity of non-invasive sound waves (Doppler ultrasound) to measure how fast the blood flows (blood flow velocity) in the main leg artery (femoral artery) and the main arm artery (brachial artery), how wide the femoral and brachial artery are (artery diameter), and the volume of blood being pumped from the heart in one minute (cardiac output).

Part Two

The reliability and validity of the Finometer® PRO device to measure traditional 'one-off' resting blood pressure measures.

Background

Doppler ultrasound is simply the projection of sound waves into the human body using a probe that is placed against human skin. Visual images of internal organs, blood vessels and body tissue are produced. As a result, it is possible to see inside the human body without using invasive and complicated procedures. Doppler ultrasound is commonly used in the Sport and Exercise Science setting due to its non-invasive nature and it allows the measurement of variables that would otherwise be difficult to measure accurately. Traditional 'one-off' resting blood pressure measurement is simply a method of obtaining single blood pressure measurements at a set time in the day whilst the participant is in a rested state. Before Doppler ultrasound and 'one-off' resting blood pressure measurements can be utilised in research conducted within the Sport and Exercise Science Department at CCCU, they must be checked as to whether they can measure these variables accurately over a number of occasions (i.e. reliability).

What will you be required to do?

Participants in this study will be required to:

- Visit the laboratory on a maximum number of six different days
- Visit the laboratory at approximately the same time of day on each of these six different days.

To participate in this research, you must:

- Be a male with normal blood pressure (normotensive)
- Be a non-smoker
- Have no known medical conditions
- Not be taking any medicines

Procedures

Participants will be asked to complete a Departmental Health and Fitness Questionnaire about their current health and fitness status. Written voluntary informed consent will have already been obtained and participants will be knowledgeable about the study. There are two parts to this study (see titles above).

- Firstly, the participant will be required to lie down for 20-minutes in a rested state. After the rest period, a probe will be placed against the inside of the thigh and then against the inside of the upper arm. Lastly, the probe will be placed on the chest bone and the rib cage.
- Secondly, the participant will be required to be seated in a rested state. An appropriately sized inflatable arm and finger cuff will be secured on the left upper arm and left hand index finger in order for 'one-off' resting blood pressure measurements to be taken.

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

- No food or caffeine 6 hours prior to testing
- Drink only water within the 6 hours prior to testing
- No alcohol 48 hours prior to testing
- Not to be taking any medication for 4 weeks prior to testing
- Not to have donated blood during the 6 weeks prior to testing
- Free from illness/infection during the 2 weeks prior to testing
- Not to have exercised strenuously in the 24 hours prior to testing

Please note: you will be required to wear shorts to provide access to the thigh area and you will be asked to remove your t-shirt to provide access to the chest area during the ultrasound. Please read the continuing sheets entitled '***Participant information sheet regarding the ingredients of the Aquasonic Ultrasound Transmission Gel'***, in order to ensure that you are not allergic to the ultrasound gel. If you are unsure as to whether you are allergic to the ingredients, you should check with your General Practitioner before consenting to take part in this study.

Feedback

The participant will receive feedback regarding the results of this study. Should any abnormalities be discovered the participant will be informed and with permission all information will be passed on to your General Practitioner 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 [redacted]. 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

It is anticipated that the results but no personal information from this research will be disseminated through journal articles, conference papers, conference posters and the thesis.

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.

Additional Information

The ingredients of Aquasonic Ultrasound Transmission Gel are listed below:

- Reserve osmosis (RO) water
- Humectants
- Polymer
- Preservatives
- Water soluble fragrance
- FD & C color
- Propyl paraben and methyl paraben in bacteriostatic concentration
- pH range 6.5 – 6.95

Any Questions?

Thank you for taking the time to read these instructions. The researcher can be contacted regarding any queries using the following contact details:

Lucinda Howland

Doctoral Research Student

Department of Sport Science, Tourism and Leisure
Canterbury Christ Church University
North Holmes Road
Canterbury
Kent CT1 1QU
University contact number: [redacted]
University email: [redacted]

CONSENT FORM

Title of Project: *"The reliability and validity of blood pressure monitoring devices and non-invasive doppler ultrasound to measure blood pressure and specific cardiovascular variables".*

Name of Researcher: Lucinda Howland

Contact details: [redacted]

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
Lucinda Howland	_____	_____
Researcher	Date	Signature

Copies: 1 for participant

APPENDIX 3

PARTICIPANT INFORMATION

A research study is being conducted at Canterbury Christ Church University (CCCU) as part of a PhD thesis by Lucinda Howland. This study has been approved within the University by the Faculty of Social and Applied Sciences Research Ethics Committee.

Lay Title of Project: "*The relationship between how much and how fast the blood flows (blood flow) and the amount of force the subject is producing (torque) during exercise where the muscle length does not change, but force is still produced during the contraction (isometric exercise) where it gradually gets harder as time goes on (incremental) and during recovery from exercise"*.

Background

Isometric (static) muscle contraction involves force being produced but no change in the length of the muscle and no movement at the joint occurs e.g. pushing against a wall. It has been shown that performing isometric exercise over a number of weeks can lead to a reduction in resting blood pressure. Exercising in this way may also change the structure and function of the arteries in the working muscles. Recent research has highlighted that the exercise intensity (how hard you perform the exercise) may affect how much change occurs to resting blood pressure and the arteries. During this study we want to look at the immediate effect a range of different exercise intensities has on blood pressure, artery size and artery blood flow. 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 at the Canterbury Campus on a maximum number of 8 different occasions, at least 48 hours separating each occasion
- Visit the laboratory at approximately the same time of day on each of these 8 different occasions
- Perform an exercise test where the exercise intensity gradually gets harder as time goes on

To participate in this research, you must:

- Be a healthy male aged between 18-45 years old with normal blood pressure (normotensive)
- Be a non-smoker
- Have no known medical problems that may impair your ability to participate in the study and/or have been free from illness/infection for two weeks prior to the start of testing
- Not be taking any medicines/ receiving any treatment for medical conditions
- Not be suffering from any form of musculoskeletal injury
- Not have undergone blood donation during the six weeks prior to the start of testing or during the course of the study

Procedures

After arriving at the laboratory, you will be asked to complete a Departmental Health and Fitness Questionnaire about your current health and fitness status. You will be made aware about what the study requires you to do and be asked to provide your written voluntary informed consent. You will be asked to adhere to the following criteria:

- No food or caffeine 6 hours prior to testing
- Drink only water within the 6 hours prior to testing
- No alcohol 48 hours prior to testing
- Not to be taking any medication for 4 weeks prior to testing
- Not to have donated blood during the 6 weeks prior to testing
- Free from illness/infection during the 2 weeks prior to testing
- Not to have exercised strenuously in the 36 hours prior to testing

Incremental isometric exercise test

You will be asked to complete an incremental isometric exercise test on the first visit and brief isometric exercise on the remaining visits. Firstly, you will be asked to tense your legs as hard as possible in order to establish the maximum force which can be produced (maximum voluntary contraction test). This value will allow us to set the exercise for the next stage of the test. You will then be asked to repeat the same leg tensing exercise, holding the tension for two minutes at a time. As time goes on, each two-minute exercise bout will require more effort to perform until you feel they can no longer perform the exercise.

Ultrasound imaging

The main leg artery will be scanned using Doppler Ultrasound whilst you are performing the exercise. This requires a hand-held probe being placed against the skin of the upper thigh area. Ultrasound transmission gel will be used to help obtain the images (see Ultrasound Transmission Gel Ingredients

list at the end of this information sheet). You will be required to wear loose fitting shorts in order for the researcher to access the leg area.

Blood pressure and heart rate measurement

Blood pressure will be measured continuously whilst you are performing the exercise using a machine called a 'Finometer®PRO device'. This device requires that you wear a small finger blood pressure cuff which lightly pulsates when worn. You will be required to wear an upper arm blood pressure cuff, however, this is only inflated at the start of the procedure and remains deflated for the remainder of the test. Heart rate will be measured whilst you are performing the exercise. You will be required to wear three small circular sticky pads (electrodes) on the chest and rib area in order to measure the electrical activity of the heart.

Torque and electrical activity of the muscle (EMG) measurement

Torque can be described as a twisting or turning force. The amount of torque you can produce when asked to tense your legs will be measured whilst you perform the exercise. This information will be directly transferred from the machine you exercise on to a nearby computer. The electrical activity (EMG) of the leg muscles will be measured whilst you perform the exercise. This involves placing two small sticky pads (electrodes) on each thigh and one pad on the flat part of the elbow. The area of skin where electrodes will be placed must be prepared by shaving using a normal disposable razor. It should be noted that during the leg testing exercise, you might feel some discomfort or shortness of breath. However, this is similar to the feeling when exercising at the gym or during normal physical activity.

Feedback

You will receive feedback regarding the results of this study. Should any abnormalities be discovered the participant will be informed, and with permission, all information will be passed on to your General Practitioner 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 [redacted]. 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

It is anticipated that the results, but no personal information, from this research will be disseminated through journal articles, conference papers, conference posters and the thesis.

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.

Additional Information

The ingredients of Aquasonic Ultrasound Transmission Gel are listed below:

- Reserve osmosis (RO) water
- Humectants
- Polymer
- Preservatives
- Water soluble fragrance
- FD & C colour
- Propyl paraben and methyl paraben in bacteriostatic concentration
- pH ranges 6.5 – 6.95

Please note:

If you are unsure as to whether you are allergic to the ingredients, you will be required to check with your General Practitioner before consenting to take part in this study.

Any Questions?

Thank you for taking the time to read this information. The researcher can be contacted regarding any queries using the following contact details:

Lucinda Howland

Doctoral Research Student

Department of Sport Science, Tourism and Leisure
Canterbury Christ Church University
North Holmes Road
Canterbury
Kent CT1 1QU
University contact number: [redacted]
University email: [redacted]

CONSENT FORM

Title of Project: "*The relationship between how much and how fast the blood flows (blood flow) and the amount of force the subject is producing (torque) during exercise where the muscle length does not change, but force is still produced during the contraction (isometric exercise) where it gradually gets harder as time goes on (incremental) and during recovery from exercise"*.

Name of Researcher: Lucinda Howland

Contact details: [redacted]

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
Lucinda Howland	_____	_____
Researcher	Date	Signature

Copies: 1 for participant

APPENDIX 4

PARTICIPANT INFORMATION

A research study is being conducted at Canterbury Christ Church University (CCCU) as part of a PhD thesis by Lucinda Howland. This study has been approved within the University by the Faculty of Social and Applied Sciences Research Ethics Committee.

Lay Title of Project: *"The effect of an 8-week isometric (where the muscle stays the same length throughout the contraction) double- leg training programme on resting blood pressure, Flow-mediated vasodilatation (a non-invasive technique to evaluate the endothelium nitric oxide dependent function of the arteries) and vascular remodelling (adaptations to the artery diameter) on selected conduit arteries (main arteries supplying the arm and leg with blood)".*

Background

Isometric (static) muscle contraction involves force being produced but no change in the length of the muscle and no movement at the joint occurs e.g. pushing against a wall. It has been shown that performing isometric exercise over a number of weeks can lead to a reduction in resting blood pressure. Exercising in this way may also change the structure and function of the arteries in the working muscles. Recent research has highlighted that the exercise intensity (how hard you perform the exercise) may affect how much change occurs to resting blood pressure and the arteries. During this study we want to look at changes in blood pressure and artery function and structure in two groups: those who exercise quite hard and those who do not perform any exercise at all. We predict that the group who perform harder exercise will experience beneficial changes in their blood pressure and arteries. The group not exercising at all should experience no changes. 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 will be asked to commit to a **8-week** exercise training condition and 8-week non-exercising control condition. You will complete one condition first separated by a 4-week period before completing the other condition, the order will be allocated at random. One condition requires moderate to vigorous effort exercise and the other condition is a non-exercising control condition. This exercise will involve you tensing both of your leg muscles at the same time in order to contract the muscles. You will also undergo some tests whilst in a rested state at various stages throughout the intervention. You will be asked to visit the laboratory at the same time of day for each visit and you will need to **commit to all of these visits**. You can see from the diagram below how may visits to the sport science laboratory (Canterbury campus) will be required: week 1: 2 visits, weeks 2 to 9: 3 visits per week (preferably Monday, Wednesday and Friday) and week 10: 1 visit (see **Diagram 1).**

To participate in this research, you must:

- Be a healthy male aged between 18-45 years old with normal blood pressure (normotensive)

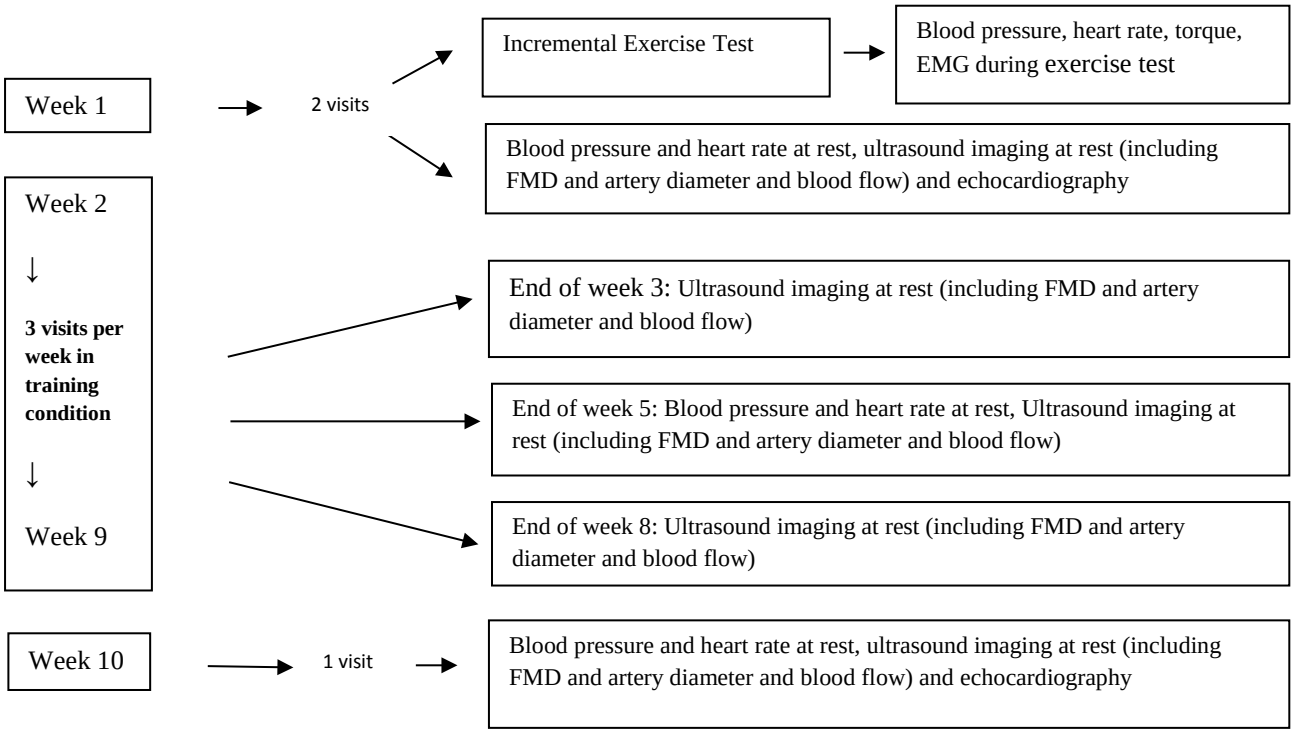
- Be a non-smoker
- Have no known medical problems that may impair your ability to participate in the study and/or have been free from illness/infection for two weeks prior to the start of testing
- Not be taking any medicines/ receiving any treatment for medical conditions
- Not be suffering from any form of musculoskeletal injury
- Not have undergone blood donation during the six weeks prior to the start of testing or during the course of the study

Procedures

Before testing can begin you will be asked to complete a Departmental Health and Fitness Questionnaire about your current health and fitness status. You will be made aware about what the study requires you to do and be asked to provide your written voluntary informed consent. You will be asked to adhere to the following criteria:

- No food or caffeine 6 hours prior to testing
- Drink only water within the 6 hours prior to testing
- No alcohol 48 hours prior to testing
- Keep your dietary intake of salt the same throughout the 10-week study
- Not to be taking any medication for 4 weeks prior to testing
- Not to have donated blood during the 6 weeks prior to testing
- Free from illness/infection during the 2 weeks prior to testing
- Not to have exercised strenuously in the 36 hours prior to testing

Diagram 1. Outlines the procedures you will undergo over the 10-week period:



Incremental isometric exercise test

You will be asked to complete an incremental isometric exercise test during your first 2 visits only. Firstly, you will be asked to tense your legs as hard as possible in order to establish the maximum force

which can be produced (maximum voluntary contraction test). This value will allow us to set the exercise for the next stage of the test. You will then be asked to repeat the same leg tensing exercising, holding the tension for two minutes at a time. As time goes on, each two-minute exercise bout will require more effort to perform until you feel they can no longer perform the exercise. From this test we can calculate a training intensity value unique to you for the 8-week isometric training programme.

Ultrasound imaging

The main leg artery will be scanned using Doppler Ultrasound whilst you are resting and exercising. This requires a hand-held probe being placed against the skin of the upper thigh area, upper arm area, chest and upper ribcage area. Ultrasound transmission gel will be used to help obtain the images (see Ultrasound Transmission Gel Ingredients list at the end of this information sheet). You will be required to wear loose fitting shorts and a t-shirt in order for the researcher to access the leg area.

Flow-mediated vasodilatation

Flow mediated vasodilatation is simply a technique where a cuff is tightened around the arm or leg when you lay down in a rested state and when it is released ultrasound imaging is used to measure the changes inside the leg muscle. It is a widely accepted technique and represents how well the arteries change in size in response to a stimulus.

Blood pressure and heart rate measurement

Blood pressure will be measured continuously whilst you are performing the exercise and at rest using a machine called a 'Finometer®PRO device'. This device requires that you wear a small finger blood pressure cuff which lightly pulsates when worn. You will be required to wear an upper arm blood pressure cuff, however, this is only inflated at the start of the procedure and remains deflated for the remainder of the test. Heart rate will be measured whilst you are performing the exercise and at rest. You will be required to wear three small circular sticky pads (electrodes) on the chest and rib area in order to measure the electrical activity of the heart. Blood pressure will also be measured at rest using a 'Dinamap' device. You will be required to wear an upper arm blood pressure cuff which is inflated for approximately 10-15 seconds.

Torque and electrical activity of the muscle (EMG) measurement

Torque can be described as a twisting or turning force. The amount of torque you can produce when asked to tense your legs will be measured whilst you perform the exercise. This information will be directly transferred from the machine you exercise on to a nearby computer. The electrical activity (EMG) of the leg muscles will be measured whilst you perform the exercise. This involves placing two small sticky pads (electrodes) on each thigh and one pad on the flat part of the elbow. The area of skin where electrodes will be placed must be prepared by shaving using a normal disposable razor. It should be noted that during the leg testing exercise, you might feel some discomfort or shortness of breath. However, this is similar to the feeling when exercising at the gym or during normal physical activity.

Feedback

You will receive feedback regarding the results of this study. Should any abnormalities be discovered the participant will be informed, and with permission, all information will be passed on to your General Practitioner for diagnosis and further investigation.

Confidentiality

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Lucinda Howland

Doctoral Research Student

Department of Sport Science, Tourism and Leisure
Canterbury Christ Church University
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CONSENT FORM

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Name of Researcher: Lucinda Howland

Contact details: [redacted]

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

Copies: 1 for participant

APPENDIX 5

Department of Sport Science, Tourism and Leisure

LOWER BODY WARM UP PROCEDURE

Prior to performing the isometric leg exercise protocol, all subjects are required to complete the following standardised warm-up procedure.

Warm-up procedure:

i. 5-minute stationary cycling (approximate heart rate 140 bpm)
ii. Static stretching (hold for 6-8 seconds and repeat 3 x each leg)
    Quadriceps
    Hamstrings
    Knee rotations
iii. 10 forward lunges (each leg)
iv. 10 body weight squats (to parallel)